SCIENCE WITHOUT BORDERS: ALEXANDER VON HUMBOLDT'S CONCEPTS IN TODAY'S WORLD

ХУМБОЛТОВ СЪЮЗ В БЪЛГАРИЯ
HUMBOLDT-UNION IN BULGARIEN

Unterstützt von / Supported by

Alexander von Humboldt
Stiftung / Foundation

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Proceedings of the Humboldt Kolleg

Varna, September 18 – 21, 2019

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ISBN 978-619-00-1217-7

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Format: 70 x 100 / 16

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Faber Publishing House
Veliko Tarnovo, 1 Gara Veliko Turnovo street
+359 62 600 650
www.faber-bg.com

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Sofia 2020

The publication of this volume was supported by
the Alexander von Humboldt Foundation
Germany

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VORWORT

Zum weltweit gefeierten 250. Jubiläum des großen deutschen Wissenschaftlers, Enzyklopädisten und Naturforschers Alexander von Humboldt hat die Humboldt-Union in Bulgarien in Zusammenarbeit mit der Botschaft der Bundesrepublik Deutschland in Sofia ein wissenschaftliches Forum unter dem Motto *Science without borders: Alexander von Humboldt's Concepts in Today's World (Varna, 18. – 21.09.2019)* veranstaltet. Die Initiative konnte dank der finanziellen Förderung der in Bonn angesiedelten Alexander von Humboldt-Stiftung realisiert werden.

Die Idee des großen deutschen Intellektuellen vom offenen, grenzüberschreitenden Charakter der Wissenschaft prägt nicht nur seine bemerkenswerte persönliche Laufbahn, sondern sie ist vorbildlich auch für die Tätigkeit der drei Stiftungen, die im Laufe von über 150 Jahren seinen Namen getragen haben. Mit den Jahren hat diese Idee nicht nur nichts von ihrer Aktualität eingebüßt, sondern sie übt eine immer stärkere Anziehungskraft auf die gegenwärtige akademische Öffentlichkeit der Welt aus: zum einen – durch diverse Aspekte der unaufhörlichen Bemühung um die Erweiterung der Forschungs- und Erkenntnisbereiche im Hinblick auf die Natur, die Menschen und die Gesellschaft, und zum anderen – durch die Partnerschaften unter Forschern aus unterschiedlichen Ländern, die sich als wesentliche und unabdingbare Charakteristik des gegenwärtigen wissenschaftlichen Prozesses etabliert haben. Die Teilnahme am Humboldt-Kolleg in Varna von rund 140 erfahrenen Forscher*innen und Nachwuchswissenschaftler*innen aus 14 Ländern war nur eine von mehreren internationalen Initiativen, durch die sich diese Kooperation präsentiert.

Der vorliegende Band vereint erweiterte Fassungen von 15 der im Forum angebotenen Beiträge. Sie alle sind mit dem Rahmenthema des Kollegs verbunden – weniger als Forschungsgegenstand denn vielmehr als dessen Verwirklichung, als Ergebnis von grenzüberschreitenden wissenschaftlichen Initiativen oder als Erfahrungsaustausch von Ideen und Methoden. Die Beiträge decken unterschiedliche Wissenschaftsgebiete ab – Gesellschaftswissenschaften, Medizin und Biologie, Chemie, Physik und Mathematik, Ingenieurwissenschaften. Der Eröffnungsartikel vom Generalsekretär der Alexander von Humboldt-Stiftung – Dr. Enno Aufderheide, vergegenwärtigt wichtige, mitunter auch wenig bekannte Tatsachen aus dem Lebenswandel des großen Wissenschaftlers und deckt die Entwicklung einer beeindruckenden Persönlichkeit auf. Drei Artikel

aus dem Bereich der Geisteswissenschaften umreißen drei von den zahlreichen Untersuchungsrichtungen auf diesem sehr umfangreichen Forschungsfeld: von der Medizin in Mesopotamien und Griechenland der Antike über den Verdienst eines Wissenschaftlers und Ingenieurs aus dem 19. Jh. zur Uferbefestigung der Donau in Bratislava bis hin zur heute sehr starken Besorgnis um die Perspektiven der Wissenschaften von der historischen Vergangenheit mit ihrer ureigenen Mehrsprachigkeit in unserer immer stärker sich durchsetzender monolingualen scientistischen Kultur. Auf dem Gebiet der Biologie und der Medizin präsentiert der Band Ergebnisse von der Anwendung innovativer Methoden in der Erforschung verschiedener Ojekte – Neuro- und Herznetzwerke; vom Varizella-Zoster-Virus verursachte Augeninfektionen; neue Polypeptiden mit antikanzerogener Wirkung und deren rationaler Synthese; das Fehlen von Toxizität bei bestimmten bulgarischen Pflanzen mit Hinblick auf ihre Anwendung als Nahrungsergänzungsmittel; die Auswertung der biologischen Vielfalt der Algen im Schwarzen Meer durch metagenetische Herangehensweise. Die naturwissenschaftlichen Texte thematisieren auch noch Phänomene aus dem Bereich der Kernphysik und deren Anwendung sowie die Rolle der digitalen Simulationen bei der Erforschung von linearen Polymeren. Vorgestellt wird eine Methodologie zur Findung genauer Lösungen von nichtlinearen Private-Differentialgleichungen. Auch über die Aufdeckung des Potentials eines mathematischen Modells zur Simulation des Migrationsstroms großer Menschengruppen wird berichtet. Der Meinungsaustausch über instumentelle und analytische Herangehensweisen, Standards und mathematische Modelle zur Bestimmung der Oberflächeneigenschaften fester Stoffe, deren Einsatz in diversen Industriezweigen wichtig ist, verdient ebenfalls Beachtung.

Zum herzlichen Dank sind wir Herausgeber des Bandes allen anonymen Gutachtern verpflichet, die mit ihrer kompetenten Bewertung der Beiträge und ihren sachkundigen Empfehlungen an die Autoren die Qualität der Publikationen wesentlich verbessert haben. Unser Dank gilt auch Frau Maya Marinova für die Redaktion der englischsprachigen Texte.

Dieses Buch setzt die Tradition der Humboldt-Union in Bulgarien fort, eine Auswahl von Beiträgen zu veröffentlichen, deren Kurzfassungen auf den wissenschaftlichen Begegnungen von bulgarischen und ausländischen Humboldt-Alumni mit Wissenschaftlern anderer Institutionen vorgetragen worden sind. So werden dort begonnener Erfahrungs- und Meinungsaustausch sowie sich anbahnende langfristige Partnerschaften auf dem endlosen Feld wissenschaftlicher Untersuchungen nachhaltig unterstützt. Wir wollen hoffen, dass der vorliegende Band ein gutes Souvenir unseres Kollegs bleibt, weil – wie schon die Alten es gewusst, und die Römer es benannt haben – *scripta manent*.

1. Juli 2020 *Die Herausgeber*

FOREWORD

In 2019, when the world celebrated the 250th anniversary of the birth of the great German scientist – encyclopaedist and nature researcher – Alexander von Humboldt, the Humboldt Union in Bulgaria, in collaboration with the Embassy of the Federal Republic of Germany, organized a scientific forum – a *Humboldt Kolleg* on *Science without Borders: Alexander von Humboldt's Concepts in Today's World*. It took place from 18 to 21 September in Varna and was convened thanks to the financial support of the Alexander von Humboldt Foundation based in Bonn.

The idea of the renowned German scientist for the open cross-border nature of science is leading both in his remarkable life and in the activities of the three foundations that have borne his name for more than a century and a half. Not only has this idea not lost its relevance over the centuries, but it has been gaining more and more weight in the modern scientific world: on the one hand, through various aspects of the constant pursuit of expanding the boundaries of knowledge about nature, man and society, and on the other hand, through the partnership between scholars from different countries, which has become an essential and intrinsic feature of the modern scientific process. The participation of approximately 140 established scientists and young researchers from 14 countries at the *Humboldt Kolleg* in Varna was just one of the manifestations of science as an international phenomenon.

This collection combines extended versions of 15 of the presented reports. All of them are related to the topic of the *Kolleg* – less in terms of research problems and more in terms of their realisation as a result of cross-border scientific initiatives or exchange of ideas and approaches. The articles cover various scientific fields – humanities, medicine and biology, chemistry, physics and mathematics as well as technical sciences. The introductory article by Dr. Enno Aufderheide, Secretary General of the Alexander von Humboldt Foundation, recalls important facts, including less known ones, from the biography of the eminent scientist and reveals the development of a remarkable figure. The three articles in the sphere of humanities outline three of the many different directions in the pursuits of scholars in this field – from medicine in Ancient Mesopotamia and Greece, through the contributions of a 19th-century scientist and engineer to the regulation of the Danube River in Bratislava, to concerns about the prospects of the sciences of the past and their inherent linguistic pluralism in today's increasingly monolingual world. In the field of

biology and medicine, the present collection offers results from the application of innovative methods in the research of various subjects, including neural and cardiac cell networks, eye infections caused by the varicella zoster virus, new polypeptides with anticancer activity and their rational synthesis, the lack of toxicity of certain Bulgarian plants with regard to their potential use in food supplements as well as assessment of algal biodiversity in the Black Sea by using a metagenetic approach. The texts related to natural sciences also thematise certain phenomena of nuclear physics and their application, and the role of computer simulations in the study of linear polymers. A methodology for finding exact solutions of nonlinear partial differential equations is presented, and the potential of a mathematical model for simulating the migration flow of large groups of people is revealed. Instrumental and analytical techniques, standards and mathematical models for determining the surface characteristics of solids, which are important for their use in various industrial fields, are discussed. The technological and technical barriers to plutonium proliferation are investigated considering the specifics of nuclear fuel cycles.

The editors of the collection acknowledge with deep gratitude all anonymous reviewers whose valuable expertise and recommendations to the authors helped to improve the proposed articles. We also express special thanks to Maya Marinova for the linguistic editing of the English texts.

This book continues the tradition of publishing selected reports from the periodic scientific forums where Humboldtians from Bulgaria and Europe meet with other established scientists and young researchers to share results, exchange ideas, and start new collaborations in the boundless field of scientific research. We hope that the present collection is a good monument to this event, because – as acknowledged way back by the ancient cultures and claimed by the Romans – *scripta manent*.

29 June 2020 *The Editors*

ALEXANDER VON HUMBOLDT: EIN LEBEN FÜR WISSENSCHAFT, MENSCHLICHKEIT UND NATUR

Enno Aufderheide

Keywords: *Humboldt, biogeography, human rights*

Abstract: *Alexander von Humboldt's father died early. His mother engaged teachers for her sons Wilhelm and Alexander, who educated them in the spirit of enlightenment and tolerance. At university, Alexander studied administration, accounting and economy. When his mother finally agreed he could study mining, he was able to reconcile her and his wishes. As a civil servant in Prussian mining, he not only quickly increased the profits of the mines, but also invested his ingenuity and private money to improve safety and living conditions of the miners. After his mother's death, Alexander meticulously prepared for a research expedition to Latin America. Here, he not only developed revolutionizing insights into the interdependence between the animate and inanimate spheres, he also developed a strong opposition to slavery. In his travel narratives, Humboldt created a novel appreciation for the beauty and perfection of nature. His transcontinental personal network was instrumental in his endeavor to collect the world's knowledge in sciences and the humanities in his opus magnum "The Cosmos". His influence on our way of thinking is probably far beyond our imagination.*

Vom „Schloss Langweil" in die Welt

> *„Hier in Tegel habe ich den größeren Teil dieses traurigen Lebens zugebracht, unter Leuten, die mich liebten, mir wohlwollten und mit denen ich mir doch in keiner Empfindung begegnete, in tausendfältigem Zwange, in entbehrender Einsamkeit, in Verhältnissen, wo ich zu steter Verstellung, Aufopferung etc. gezwungen wurde."*

Diese Schilderung seiner Jugend, die Alexander von Humboldt 1792 in einem Brief an seinen Freund Freiesleben niederschrieb, mag uns überraschen. Und doch ist sie ein Schlüssel zu Humboldts Leben.

Er wuchs in wohlhabenden Verhältnissen auf und mit intensiver Förderung durch exzellente Hauslehrer. Was seiner Kindheit aber fehlte, war der Umgang mit gleichaltrigen, gleichberechtigten Freunden außer seinem Bruder Wilhelm.

Fig. 1: *Humboldts Mutter Marie-Elisabeth, 1775, ©Stiftung Stadtmuseum Berlin.*

Dies erklärt vielleicht, warum intensive Freundschaften in seinem späteren Leben so eine große Rolle spielten und warum sein Verhältnis zu seinem Bruder bis an dessen Lebensende sehr eng blieb.

Aber auch wenn Alexander sich unglücklich fühlte, seine Erziehung und Ausbildung war außerordentlich vielseitig und auf dem modernsten Stand. Nach der Ausbildung durch Hauslehrer folgten Studien in Frankfurt an der Oder, Hamburg, Göttingen und Freiberg in Sachsen, wobei er neben Buchhaltung und Volkswirtschaft auch Naturwissenschaften und Bergbau intensiv studierte. Zudem gaben ihm diese Studienjahre die Gelegenheit, mit dem berühmten Entdeckungsreisenden Georg Forster über die Niederlande nach England zu reisen. Dort lernte er so berühmte Männer wie die Kapitäne Sir Joseph Banks und Captain William Bligh, aber auch den Physiker Henry Cavendish kennen. Das Zusammentreffen mit den Kapitänen, aber auch der Anblick der Schiffe der Ostindien-Kompanie weckten eine tiefe Sehnsucht nach der Ferne in ihm, die sicher eine der Wurzeln seiner berühmten Forschungsreise nach Lateinamerika war.

Zunächst aber fügte er sich nach dem Studium dem Wunsch der Mutter, in den öffentlichen Dienst einzutreten. Als Minendirektor in Franken vermochte er es, die seit Jahren defizitären Minen profitabel zu machen und er setzte, da der Staat hierzu nicht bereit war, sein privates Kapital ein, um in einer Berufsschule für Bergleute diese nicht nur in Mineralogie, sondern auch in Arbeitssicherheit zu unterrichten.

Im Jahr 1796 starb Humboldts Mutter. Mit einem Schlag wurde Alexander zu einem reichen Erben, gab seine Stellung im preußischen Staatsdienst auf und bereitete sich intensiv auf eine Expeditionsreise in die Tropen vor. Durch Gespräche mit Experten und Besuche botanischer und geologischer Sammlungen in Wien, der Schweiz, Paris und London, durch Anschaffung der besten verfügbaren Instrumente und intensive Übung ihrer Handhabung erwarb Humboldt die Kenntnisse, die seine spätere Reise wissenschaftlich so ertragreich machen sollte.

Der Weg, der Humboldt letztlich in die Tropen führte, war von zahlreichen Zufällen und Fehlschlägen beeinflusst. Es sollte sich aber als besonders glückliche Konstellation erweisen, dass er zum einen gemeinsam mit dem französischen Botaniker Aimé Bonpland reiste und zum anderen, nach dem sich andere Optionen zerschlagen hatten, vom spanischen König einen Reisepass erhielt, der alle spanischen Offiziellen in Lateinamerika verpflichtete, Humboldt bei seiner Forschungsarbeit zu unterstützen. Im Jahr 1799 brach Humboldt von La Coruña in Spanien über Teneriffa in die Karibik auf und ging im heutigen Venezuela von Bord des Schiffes. Auch wenn dies nicht seiner ursprünglichen Planung entsprach, nutzte er die Gelegenheit, um den Orinoco zu erkunden, der ihn sicherlich seit seiner Jugend, in der er das Buch von Robinson Crusoe gelesen hatte, faszinierte. Konkret gelang es ihm, die Verbindung zwischen den Flusssystemen des Orinoco und des Amazonas nachzuweisen. Von dieser war gerüchteweise schon berichtet worden, gleichwohl gab es erhebliche Zweifel, dass es zwischen diesen beiden großen Flusssystemen keine eindeutige Wasserscheide geben sollte. Schon auf diesem Teil der Lateinamerika-Reise zeigten sich Humboldts besondere Ansätze. Nämlich insbesondere die Erforschung des Zusammenhangs zwischen belebter und unbelebter Natur – hier besonders am Beispiel der Untersuchung von humösem „Schwarzwasser" – und mineralreichem „Weißwasser" und den unter diesen Bedingungen vorkommenden Lebewesen, aber auch seine Betrachtungen der unmenschlichen Behandlung der indigenen Bevölkerung und der schwarzen Sklaven durch die spanischen Missionare.

Der bedeutendste Teil seiner Lateinamerika-Exkursion war aber zweifelsohne die Erkundung der Anden mit der Besteigung mehrerer Vulkane. Berühmtestes Ergebnis dieser Expedition ist das „Naturgemälde der Anden" mit dem Humboldt die Geobotanik, die Lehre von der Verbreitung und den Lebensraumansprüchen der Pflanzen, begründete.

Humboldt beschloss die Reise mit längeren Aufenthalten in Mexiko, Kuba und einem Besuch der Vereinigten Staaten, wobei er auf Kuba eine bahnbrechende kritische Auseinandersetzung mit der Sklaverei formulierte und in den USA wichtige Kenntnisse über das Grenzgebiet zu Mexiko vermittelte.

Die Reisetagebücher, die Humboldt während der Reise führte, blieben für ihn sein Leben lang ein wichtiges Arbeitsinstrument, in dem er auch Beobachtungen späterer Zeiten zusammentrug und miteinander verknüpfte.

Fig. 2: *Die Route von Humboldts Lateinamerikareise 1799 – 1804.*
<https://de.wikipedia.org/wiki/Datei:Map_Alexander_von_Humboldt_expedition-de.svg#file> (30.1.2020).

Fig. 3: *Alexander von Humboldt 1807: Zeichnung von Frédéric Christophe de Houdetot.*

So überragend die Lateinamerika-Reise Humboldts war, darf über sie doch nicht die ungeheure Reise durch Russland und Sibirien 1829 vergessen werden, die Humboldt anders als seine Lateinamerika-Reise nicht selber finanzieren konnte, sondern auf deren Finanzierung durch den Zar er angewiesen war. Gleichwohl vermochte er sich Freiräume über das vom Zaren Eingeräumte hinaus zu schaffen und wichtige Beiträge zum Bergbau im Ural, zur Geographie des Altai und des Kaspischen Meeres sowie zur Klimaforschung zu formulieren.

Zur Zeit seiner Russland-Reise lebte Humboldt bereits in Berlin, nachdem er zuvor nach seiner Rückkehr aus Lateinamerika rund 20 Jahre in Paris gelebt hatte: ein Brückenbauer zwischen Frankreich und Preußen in den Zeiten der Napoleonischen Kriege. Die letzten Jahrzehnte seines Lebens wirkte Humboldt als global vernetzter Wissenschaftler, als Berater des preußischen Königs und als begnadeter Wissenschaftskommunikator in Berlin, zu dessen öffentlichen Vorlesungen Männer und Frauen aus der Aristokratie ebenso wie aus dem einfachen Bürgertum kamen. Großen Einfluss auf das Weltverständnis des gebildeten Bürgertums hatte er insbesondere mit seinem fünfbändigen Lebenswerk „Kosmos", in dem er das Wissen der Welt über die Naturwissenschaften, aber ebenso über die Ethnologie und Aspekte der Geisteswissenschaften zusammentrug.

Als Humboldt 1859 starb, hatte er nicht nur die Wissenschaft durch seine Interdisziplinarität und durch die Organisation internationaler Kooperationen verändert, er hatte durch seine klare Haltung gegen die Sklavenhaltung und den Kolonialismus auch die Entwicklung der westlichen Ethik vorangebracht.

Dr. Enno Aufderheide
Generalsekretär der
Alexander von Humboldt – Stiftung
Bonn

SECULAR SCIENCE IN MESOPOTAMIA AND GREECE

Markham J. Geller

Keywords: *Babylonian medicine, cuneiform medical treatises, Pre-Socratic philosophical doctrine, semiotics of signs and symptoms*

Abstract: *One popular perception is that Babylonian medicine was not actually a science but was dominated by religious thought, with the active presence of gods in human affairs, including disease. According to this thinking, the line between Babylonian medicine and magic was blurred, making it hard to distinguish between the activities of doctors and exorcists. The usual consensus is that the Greeks refreshed everyone's thinking about the cosmos by removing religion and the gods from theories of natural science. The common assumptions are questioned here, based on a recently published ancient catalogue of Babylonian medicine listing some 90 cuneiform medical treatises known by their opening lines. This highly unusual cuneiform tablet organises the data into sequentially numbered component chapters – a record of an ancient medical library much more systematically organised than the much later Hippocratic Corpus.*

Changing basic perceptions of ancient science represents a major challenge, particularly when fixed ideas are shared by several disciplines.[1] Since the beginning of the 19th century, an idealising vision of the so-called "Greek miracle" still persists, combined with the notion that Babylonian thought was essentially mythological and religious, an intellectual cul-de-sac which had little or no impact on the development of early Greek philosophy. One popular perception is that Babylonian medicine was not actually a science but was dominated by religious thought, with the active presence of gods in human affairs, including disease. According to this line of thinking, the line between Babylonian medicine and magic was blurred, making it hard to distinguish between the activities of doctors and exorcists.[2] In any case, it is possible to argue that Babylonians had little faith in physicians or healers, in a world in

[1] Many of the ideas in this paper were developed during the course of the ERC Advanced Grant Project BabMed (2013 – 2018), and particularly resulting from collaboration with Cale Johnson.

[2] One of the main advocates of this position is Scurlock 1999, arguing for the 'physician' (Akkadian *asû*) being considered as an apothecary, with the exorcist (Akkadian *āšipu*) taking the lead as healing professional.

which the righteous were thought to suffer while the wicked were rewarded.[3] The usual consensus is that the Greeks refreshed everyone's thinking about the cosmos by removing religion and the gods from theories of natural science. I would like to question some of these common assumptions.

This biased image of Babylonian versus Greek medicine is reinforced by Herodotus, whose remarks are still taken seriously. Herodotus insisted that his contemporary Babylonians had no real doctors or medicine, but simply reclined in the street waiting for layman's medical advice.[4] This extraordinary view is contradicted by a recently published ancient catalogue of Babylonian medicine listing some 90 cuneiform medical treatises known by their opening lines. This highly unusual cuneiform tablet organises the data into sequentially numbered component chapters (or 'tablets'). In essence, this record of an ancient medical library is much more systematically organised than the much later Hippocratic Corpus, since all anatomically based treatises are listed in a strict head-to-foot sequence, while all general pathologies are listed separately.[5] The point is this: comparisons between the Hippocratic corpus and 'Oriental' (i.e. Babylonian and Egyptian) medicine have stressed the religious component of earlier medicine which was fundamentally overtaken by the more rational approach of the Greeks. However, such a system based primarily on theology, that is, on divine or demonic personal intervention into the patient's medical condition, would not require much emphasis on *technē* and methods of treatment; the emphasis would rather be on prayer and exorcism, which is clearly not the case. The highly systematic character of Babylonian medicine, as known in later periods, requires a different kind of evaluation and analysis.

This type of evidence also runs counter to a widely-held view that ideas within Babylonian culture were transmitted in a 'stream of tradition' which did not adapt easily to change or innovation over time, combined with an inherent resistance to new modes of thinking.[6] By roughly 750 BCE, Babylonian

[3] This evaluation of the Mesopotamian world view is based on an assumption that the pessimism expressed in the Akkadian literary text, *Ludlul bēl Nēmeqi,* was highly influential within Mesopotamian society; in this composition, the speaker despairs of being healed by physicians or exorcists or diviners, but only the god Marduk can save him. For this text, see the recent edition of Oshima 2014. See Annus, Lenzi 2010: 35, 37.

[4] Herodotus (I, 197), see now Geller 2018: 29 for a discussion of this passage.

[5] See Steinert 2018 with in-depth contributions from Cale Johnson, Strahil Panayotov, Eric Schmidtchen, and the present writer.

[6] The 'stream of tradition' argument was forcefully advocated by A. L. Oppenheim in his seminal work, *Ancient Mesopotomia* (1977). Much of what Oppenheim proposed is still valuable and useful, but the argument needs to be revaluated in the light of the large number of recently published texts from the first millennium BCE, which indicate innovation and changes in views, although the stream of tradition retained strong influences, even in late

scholarship (largely in the realms of medicine and astronomy) had managed to develop various alternatives to millennia-old causal theories expressed through mythologies and theologies.[7] In the cuneiform medical treatises of the Royal Library of Nineveh, for instance, diseases that had previously been viewed in terms of ghosts, demons and deities were now viewed through observation-driven models of causation of illness.

By the 8th century BCE, Babylonian medicine served as an example of a discipline which consistently moved away from theological models in favour of analogies based on the natural and social world. Healing strategies gradually shifted their allegiances from exorcism and formal incantations towards astronomy and astrology and computation, which were essentially godless disciplines; planetary influences replaced divine intervention. The long history of observational norms eventually evolved into a system based upon clinical observation of changes in human anatomy, combined with some awareness of the properties of plants and drugs, and a systematic approach to therapy utilised a theory of natural elements (heat, cold, aridity, moisture); similar concepts were refined and developed further within Greek science and medicine (e.g. that plants had *dynameis* or powers). The fact that Hippocratic medicine was rooted in the Pre-Socratic philosophical doctrine is well known,[8] but that Babylonian medicine followed these same models has not yet been adequately recognized.

As Francesca Rochberg and others have pointed out, Babylonians recorded their observations and resulting predictions (or inferences) in the form of a loose causality of associated ideas expressed in the fixed form of a statement 'if P then Q',[9] which probably arose from the predominant influence of legal

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periods of Mesopotamian thinking. Nevertheless, late Babylonian texts adopted modes of reasoning which can be compared with early Greek science; see Rochberg 2004.

[7] The classic example of this change is the abandoning of the cosmic view that celestial bodies were guided in their movements by the personal whim of the wisdom god Ea, whose *uṣurtu* 'scheme' controlled the heavens. Similarly, the Pre-Socratics removed the gods and their mythology from their toolkit, and in doing so, various mechanical factors or processes, such as proto-elements or roots of the natural world, were pressed into service – rather than deities and myth – as pieces of their explanatory model (cf. Most 2016). Babylonian medicine (and later forms of magic) shows similar patterns of drawing analogies from the natural world.

[8] See Lloyd 1973, and for the latest edition of Pre-Socratic texts, see Laks, Most 2016.

[9] See Rochberg 2010. Babylonian science in general relied upon implicit theory which was hardly ever committed to writing, in contrast to philosophy, which was a purely Greek invention. One working assumption is that early Greek science adopted views which already existed but are not easily recognisable in Babylonian tablets, since Babylonian scholarship relied upon juridical casuistic models ('if P then Q') rather than the more didactic genres of Greek theoretical treatises known from Greek philosophy. At the same time, rhetoric (which had no place within a Babylonian curriculum) helped formulate the literary genres of Greek science.

codes within the Babylonian school curriculum.[10] The dominant influence of legal reasoning even within medical diagnosis can be seen in the following example from the Babylonian Diagnostic Handbook (cf. Scurlock 2014: 53).

symptoms **traditional diagnosis**

If (a patient) is sick in the night and healthy in the morning: It is "Hand of Urash",
because of (*ashu*) the wife of (another) man.

causal factor (borrowed from jurisprudence)

What at first appears to be assigned to a 'religious' cause (the hand of the god Urash) has a more worldly explanation based on a specific idiom borrowed from legal tradition (the word *aššum* = 'because of').[11] The term *aššum* indicates the cause of an action which will determine the verdict, such as someone being found guilty because he stole property. In this recipe, the word *aššu* points to an illness presenting itself because of the patient's sexual encounter with another man's wife. Furthermore, the common diagnostic description of disease caused by the 'hand of a god' (as in the above example, the god Urash), or alternatively, disease being frequently attributed to the 'hand of a ghost', originally no doubt indicated personal divine or demonic interference in the patient's illness. However, over the course of time, these terms had evolved into non-personal labels for specific diseases, used in conjunction with technical disease terminology. We find a later tendency to replace earlier 'religious' terms for disease, as a 'hand of a specific god', with technical disease names, like a term for a skin condition.[12] This was driven by the need for greater precision and accuracy, although various factors, including professional conservativism, did not allow for the complete abandonment of traditional terminology. This is why the expression 'hand of a god' or 'hand of a ghost' remained in usage, even though the meaning behind these terms had changed.

Francesca Rochberg has also argued forcefully that in certain regards the concept of 'nature' (*physis*) was uniquely Greek, not shared by any earlier

[10] Some 400 examples of copies of Hammurabi's Law Code have been found in school archives, making it the most often copied of any ancient Babylonian text. I am grateful to Cale Johnson for this observation.

[11] Chicago Assyrian Dictionary A/2, 466.

[12] This argument was put forward in Geller 2015: 204 – 205, with examples, but the argument has not been followed up or refuted so far.

scientific community (Rochberg 2018). In fact, the Greek term *physis* referred in earlier periods to individual 'characteristics' of an object and only later to the 'natural environment' in an abstract sense. In order to determine whether Babylonian science ever thought in these same terms, it is important to recognize that Babylonian scholarship was not adept at coining new terminology but relied upon an inherited traditional vocabulary, which could nevertheless be applied to new concepts. For example, the Babylonian term *šiknu*, 'physical state', was often applied in late explanatory texts to explain the 'properties' of medical plants and stones.[13] As such, the Babylonian term *šiknu* corresponds to one meaning of *physis* as a 'characteristic' or 'nature' of something, which is not so very distant from a more abstract meaning of nature known to the Greeks.

Babylonian medicine (including recipes, etiological 'incantations', diagnostic lists of symptoms, and medical procedures) has not generally been investigated for the scientific thinking and orientation behind this vast corpus of texts. Babylonian medicine offers a comprehensive and extensive array of technical vocabulary, drawn from ancient glossaries of *materia medica* and diseases, as well as a rich array of explanatory medical commentaries.[14] On the conceptual level, diagnostics and the prognostic predictions are based upon the semiotics of signs and symptoms, which at one level apply to the human body but then by extension to the cosmos in general, as a universal feature of ancient science from this region. Moreover, compound medical recipes involve sequential ordering and combining of solid and liquid ingredients within a rudimentary chemistry (much like cooking recipes); the principles involved in creating such recipes were based upon theory and hierarchies of concepts about the physical environment, which were never effectively explained within Babylonian technical literature.

Furthermore, contrary to common perceptions in secondary literature, there is little magic within Babylonian medicine. The so-called medical 'incantations' (Akkadian *šiptu*) mostly lack the characteristic adjurations, conjurations, appeals to divinities, descriptions of demons, and other signatures of Babylonian magic; 'medical incantations' were aimed at defining the cosmos in which disease originated and was to be treated. Medical 'incantations', like

[13] See Stadhouders 2011 and Stadhouders 2012 for an edition and translation of the explanatory plant list, *Šumma šikinšu* (lit. 'a plant, its characteristic'); a model citation would read, 'if a plant's nature (*šiknu*) is similar to plant B, its name is X'. See also Schuster-Brandis 2008 for an edition of similar explanatory stone lists known as *Abnu šikinšu* (lit. 'a stone, its characteristic').

[14] A volume of Akkadian medical commentaries is soon to be published by John Wee. See also the Yale Cuneiform Commentaries Project (https://ccp.yale.edu/).

the counterpart medical 'rituals,' were medical procedures which explained the conceptual basis and function of the recipes. Here are two examples of such explanatory 'theory' imbedded in Babylonian medical texts:

1). A 7th century Babylonian text from Nimrud against skin lesions (*simmu*) reads:

> The lad being afflicted groans and the maiden being afflicted thrashes about. [The moon god] Sin noticed it by himself, [the gods] Ea, Enlil, and Belet-ili became worried by it. Sin opened his mouth speaking, 'We [the gods] placed *simmu*-lesions in the land, after we created mankind we bound up life and death with it. O universal *simmu*-legion, [the god] Anu has created you so that you seize the body of both man and god. The one bound up in flesh...., [the gods] Ea and Belet-ili have determined your destiny.'..... **[O Gula], healer among the great gods, [bring instruments] of healing, your scalpel and prescription**. (Geller 2000: 338)

The purpose of this historiola within a medical prescription is to explain the primordial (and intractable) nature of skin disease, created at the same time as man, and to press the healing goddess Gula to arrive with her recipes and scalpel in hand. This latter phrase allegorises Gula's arrival in terms of the usual tools of human therapy – recipes and scalpels – rather than incantations or magical rituals.

In fact, other medical incantations go further, warning the patient to get healed before Gula arrives with her scalpel, hinting at the inherent dangers of surgery, as in the following medical incantation for ailing eyes, our second example of explanatory 'theory' within an incantation:

2) Incantation. Eyes with the porous blood vessels, why have you been blurred by chaff, thorns,....-fruit, or river algae? Why have you been blurred by clods or twigs in recesses? Rain down here like a star, keep falling here like a meteor, **before the flint (-knife) and scalpel of Gula reaches you**![15]

The specific problem expressed here is that the eye which has become clouded with foreign matter must be cleansed by its own tears before the

[15] This text comes from a forthcoming edition of Mesopotamian eye-disease texts from S. Panayotov and the present writer, to be published by de Gruyter (Berlin), planned for 2020.

doctor arrives with his scalpel. There is nothing magical in this incantation, but it is down-to-earth in its depiction of medical practice. Both of these examples, of skin lesions and bloodshot eyes, come to the same conclusion. These passages describe physical symptoms which are exposed to the risks of surgical treatments; better to be healed before the surgeon arrives. But there is no magic in these medical incantations.[16]

The aim of this paper is to argue for a new framework for assessing the early phases of scientific thinking, which culminated in the emergence of Greek rational medicine, but this should be combined with a fresh view of systematic rationalities characteristic of cuneiform medical sources from Mesopotamia.[17] Medicine represents the most multi-faceted discipline within Mesopotamian intellectual culture, borrowing concepts and methods from jurisprudence, astronomy, mathematics, and divination, while formulating rational propositions and approaches. Future studies need to investigate the *science* behind Babylonian medicine by comparison with early Greek philosophy. This cannot be achieved by limiting the inquiry to ancient medicine (e.g. Babylonian and Hippocratic medicine), since comparisons of these two systems of medicine – while important – are insufficient to provide answers to the deeper questions about scientific method, rationality, and analogical reasoning. The overall question is whether the highly systematic and theoretical basis of Babylonian thought may have made a significant (although largely unacknowledged) contribution to early Greek philosophy, while Greek emphasis on theory may have influenced the late Babylonian curriculum.[18] The answers to this question will probably challenge long-held views of the primacy of Greek thought, which developed without reference to the broader

[16] The figurative allegories occurring within medical incantations were etiological and explanatory (in a theoretical sense) rather than magical (see the examples given in Collins 1999), in stark contrast to the older venerable tradition of Babylonian healing rituals and incantations with frequent appeals for divine intervention and assistance.

[17] One of the key debates within early Greek science involved the role of observation as opposed to sheer reasoning (Lloyd 1979, Most 2016). Although Babylonians had no category of theoretical tractates within their conservative curriculum, nevertheless they contributed detailed observations of the movements of heavenly bodies over centuries which were later incorporated into Greek astronomical theory. Babylonians also produced a *Diagnostic Handbook* listing some 15,000 observations of symptoms. Greek scholars analysed the existing data without recreating it. It may even be that precisely *because* Babylonian scholarship was known in antiquity for recording phenomena, this reputation may have spurred some Greek theorists towards new methods based upon logic and analogy and away from observation.

[18] One must, however, take into account the historical context of technical knowledge within each sphere of influence, bearing in mind the non-institutional character of Greek philosophical schools in contrast to the highly centralised academic culture and common curriculum of Babylonian academies.

intellectual context in which science and philosophy were able to develop and thrive.

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Markham J. Geller
University College London
m.geller@ucl.ac.uk
Freie Universität, Berlin
mark.geller@fu-berlin.de

DIE LETZTE BASTION DER MEHRSPRACHIGKEIT IN DEN GEISTESWISSENSCHAFTEN FÄLLT: ÜBER DIE ZUKUNFT DER ALTERTUMSWISSENSCHAFTEN IN EINER EINSPRACHIGEN WELT DES (UN-)WISSENS UND NOTWENDIGE POLITISCHE GEGENMASSNAHMEN

Alexander Rubel

***Keywords**: Multilingualism, English only, Classics, Humanities*

***Abstract**: This paper discusses the question of whether the "internationalization" of science means that students of the Humanities and especially of Classics, who have long been multilingual, must now in the 21st century have to write in English, even if not all of us can adequately express our thoughts in the language of Shakespeare. Some problems become evident in the discussion: in order to be noticed at all in the Anglo-American world, scientific publications increasingly have to appear in English, regardless of the language skills of the authors. Criticism is levelled at national research policies in European countries that carelessly abandon their own languages in favour of the dominant tongue (not always used with complete accuracy). Also criticised are the questionable but dominant bibliometric instruments that privilege contributions in English, and which manage to ignore books being limited to periodicals. This paper argues in favour of multilingualism and for new European and national policies, which endorse multilingualism and diversity.*

Ich kann die Leser dieser Zeilen gleich zu Beginn beruhigen: Bei den folgenden Bemerkungen handelt es sich keineswegs um ein weiteres Klagelied über die sprachliche Anglisierung des guten alten Europa und den Bedeutungsverlust der altehrwürdigen europäischen Kultursprachen. Unternommen wird hier eine nüchterne Analyse eines existierenden Sachverhalts und der sich daraus ergebenden Folgen für die Geisteswissenschaften im Allgemeinen und für eine traditionell international arbeitende geisteswissenschaftliche Disziplin, die in Pluralform sogenannten Altertumswissenschaften. Trotz der Aufspaltungen und Spezialisierungen in hochkomplexe Einzelwissenschaften wie klassische Philologie, Alte Geschichte und Archäologie (klassische, provinzialrömische, sowie diverse andere) kann man mit Fug und Recht im Sinne Mommsens und in der Tradition dieser Fächer, die sich mit den Hinterlassenschaften der antiken Kulturen des Mittelmeerraumss beschäftigen, von einem einheitlichen

Disziplinverbund sprechen, für das das Deutsche immerhin diesen eigenen Begriff geprägt hat (der englische Begriff „Classics" schließt die Archäologie aus und nimmt die Alte Geschichte nur zur Hälfte mit).

Diesen Aufsatz auf Deutsch zu schreiben, scheint auf den ersten Blick wie „travallier pour le roi des Prusses". Wenn es darum geht, den Verlust der Sprachenvielfalt zugunsten des Englischen in den Wissenschaften unter besonderer Berücksichtigung derer vom Altertum in einer anderen Sprache als dem Englischen zu beschreiben, taucht ein grundlegendes Problem auf. Diejenigen unter den Altertumswissenschaftlern, die nur Englisch lesen (sind das Wissenschaftler?), werden die hier vorgebrachten Argumente nicht zur Kenntnis nehmen können. Dieser Personenkreis sei auf meine ausführliche Beschäftigung mit diesem Thema in einer amerikanischen Fachzeitschrift verwiesen (Rubel 2019). Dass ich mich erneut und in meiner Muttersprache diesem Thema widme, ist einerseits der Gelegenheit geschuldet, in einem von der Alexander-von-Humboldt-Stiftung geförderten Sammelband, der die Beiträge einer im Jahr des Humboldt-Jubiläums organisierten Tagung versammelt, publiziert zu werden. Der große Naturforscher, der selbst polyglott war und vor allem auf Französisch, aber auch in seiner Muttersprache und auf Spanisch publizierte (es gibt natürlich auch autorisierte englische Übersetzungen seiner Werke), ist gewissermaßen ein Sinnbild für Mehrsprachigkeit in der Wissenschaft (Trabant 2019). Andererseits ergibt sich so die Gelegenheit, einige Argumente, die ich im Englischen eher mit einem dieser Sprache angemessenen ironischen Ton vorgebracht habe, mit der scheinbar immer nötiger werdenden klaren Polemik zum Ausdruck zu bringen, für das das als besonders „hart" verschriene Deutsche vielleicht besser geeignet ist. Darüber hinaus habe ich viele Reaktionen und Rückmeldungen auf die Veröffentlichung von „Quo vadis" erhalten, die ich teilweise in die Argumentation einfließen lassen kann, ebenso wie einige neue, besonders himmelschreiende Beispiele von angloamerikanischer Ignoranz in den Altertumswissenschaften. Außerdem hat dieser Aufsatz, im Gegensatz zu meinem mehr fachspezifischen Dingen gewidmeten englischen Text, eine ausgesprochen politische Dimension und Zielrichtung, weil m. E. die Selbstheilungskräfte der geisteswissenschaftlichen Disziplinen an ihrem Ende angelangt sind. In diesem Sinne richtet sich der auf Deutsch verfasste Aufsatz auch idealerweise an die kultur- und wissenschaftspolitischen Entscheidungsträger in Deutschland und Österreich (ich bin mir im Klaren darüber, dass dieser Wunsch utopisch ist).

Vielleicht ist auch einmal der Zeitpunkt gekommen, sich wirklich und mit sehr deutlichen Worten zur Wehr zu setzen. Damit wird keineswegs insinuiert, die mir persönlich sehr teure Sprache Englisch, die ich selbstverständlich und gerne verwende, zu diskreditieren. Im Gegenteil: sie auch als Wissenschaftssprache vor

entstellender Verwendung durch Halbgebildete zu schützen, ist ein hohes Ziel. Ich argumentiere hier nicht gegen das Englische, sondern allenfalls gegen seine unsinnige Verwendung, vor allem aber für etwas: für die sprachliche Vielfalt und die Mehrsprachigkeit in den Geisteswissenschaften, besonders in ihrem letzten, bislang noch wenig angetasteten Refugium, den Altertumswissenschaften. Die meisten kritischen Beiträge, die über den Verlust sprachlicher Vielfalt in den Geisteswissenschaften berichten, sind bei aller Kritik recht konziliant und versuchen an Vernunft, Intelligenz und „common sense" zu appellieren, um zu einem wohlwollenden Umdenken vor allem bei denjenigen anzuregen, die nur oder bevorzugt Englisch lesen. Bei vielen Kollegen rennt man damit offene Türen ein, weil diese ohnehin nicht zu den Verächtern der Mehrsprachigkeit gehören, sondern durch eine solide Ausbildung und das eigene Forschungsinteresse ganz selbstverständlich mehrere Sprachen lesen. Die Zahl derer, die jedoch nur noch Englisch lesen, wird indes immer größer und hat eine kritische Masse erreicht. Diese kritische Masse besetzt nun auch zunehmend Lehrstühle und Forschungsstellen, und das nicht nur an drittklassigen Standorten. Das Problem wurde auch grundsätzlich bei den am meisten Betroffenen erkannt: Die Britische Akademie hat zwei Positionspapiere veröffentlicht, die den Wissenschaftsstandort Großbritannien in Gefahr sehen, wenn angehende Wissenschaftler nur noch über wenig oder gar keine Fremdsprachenkenntnisse verfügen (Language matters 2009; Language matters 2011). Im letztgenannten Papier findet sich eine bedenkenswerte Empfehlung: „Research advances not just by uncovering new material but also by creating new ways of thinking about, understanding, and analyzing the material in question. One of the best ways of freeing up and quickening the thinking process is by engaging with traditions of thought outside one's own – by confronting those other (national, discursive) traditions in their own languages. Now, more than ever, research is a global enterprise. It has been observed that if 'the research base' of UK younger educated researchers in the humanities and social sciences is increasingly monoglot in character, it runs the risk of being marginalized, and will end up, as it were, world-famous only in England".

Meine nach eingehender Beschäftigung mit dem Sachverhalt gewonnene Überzeugung ist jedoch, dass alle gutwilligen Appelle, auch wenn sie von den betroffenen Institutionen selbst kommen, zu keinerlei Veränderung dieses Trends geführt haben und im derzeitigen Stadium der Entwicklung v.a. ein Herumreißen des Ruders auf kulturpolitischer Ebene erfolgen muss, und zwar auf gesamteuropäischer Ebene. Das werde ich im Folgenden mit einem mir persönlich naheliegenden Fokus auf der deutschen Sprache als Wissenschaftssprache genauer ausführen. Zunächst aber zu einer kurzen Beschreibung des Problems (ausführlich: Trabant 2013; Mittelstraß et al. 2016; Rubel 2019).

Zu Zeiten von Alexander von Humboldt war das Lateinische nicht mehr ganz unangefochten, aber doch immer noch *die* Sprache der Wissenschaft. Dissertationen und wissenschaftliche Publikationen für ein Fachpublikum wurden generell in der keineswegs toten alten Sprache abgefasst. Jedoch waren schon längst die Nationalsprachen auf dem Vormarsch, besonders wenn auch – wie etwa bei den erfolgreichen Schriften Humboldts über seine Forschungsreisen – ein größeres Publikum angesprochen war. Diese Tendenz zu den Nationalsprachen in den Wissenschaften wurde schon von den französischen Enzyklopädisten beklagt, die sich darüber beschwerten, dass Wissenschaftler anstatt sich auf ihre Forschungen (und deren Publikation im gewohnten Latein) zu konzentrieren nun auch mehrere europäische Sprachen erlernen müssten (Trabant 2013: 158f). In der Folge wurde die moderne europäische Wissenschaft (andernorts fand sie zu Beginn des 19. Jh. noch nicht statt) mehrsprachig und Alexander von Humboldt ist geradezu das Kardinalbeispiel für den selbstverständlichen Umgang mit mehreren Traditionen von Wissenschaftssprachen, wobei er das Französische etwas bevorzugte (Trabant 2019). Jedenfalls waren die modernen Wissenschaften bei aller Abkapselung durch die Nationalismen des 19. Jh. eine mehrsprachige europäische Symphonie, und Sprachkenntnisse in den Idiomen derjenigen Nationen, die sich in den Wissenschaften hervortaten, waren unabdingbar für den wissenschaftlichen Austausch. So schrieb Alexander von Humboldt an den jungen Kollegen Jean-Baptiste Boussingault 1832: „[S]chreiben Sie mir, ob sie Deutsch können, [...] ich sage nicht in der Art einiger Gelehrter in Paris, die vom Nachschlagen im Wörterbuch ächzen, sondern frei, indem Sie ein deutsches Buch über Geologie lesen, wie Sie das Englische lesen“ (Humboldt/Boussingault 2015: 305).

Je nach Disziplin hatten bestimmte europäische Sprachen zu gewissen Zeiten eine besonders wichtige Rolle. Zunächst das Französische, aber im Verlauf des 19. Jh. dann besonders auch das Deutsche (das Spanische und das Russische hatten jeweils auch ihre Hochzeiten bis in die erste Hälfte des 20. Jhs., siehe hierzu auch Ammon 2001: 344; Gordin 2015: 7 – 9, 303 – 315). Gerade in den Altertumswissenschaften (aber nicht nur dort) war das Deutsche eigentlich zum Leitmedium der internationalen Forschung geworden. Darüber ist viel geschrieben worden und hier ist nicht der Ort in glorreicher Vergangenheit zu schwelgen (ausführlich Rubel 2019 mit weiterer Literatur). Nach dem ersten Weltkrieg kam es erstmalig in der Geschichte der Wissenschaften zu einem Sprachboykott, dem deutsche und österreichische Wissenschaftler aller Disziplinen ausgesetzt waren (Reinbothe 2019) und der durch das Naziregime und die Ermordung oder Vertreibung der jüdischen Wissenschaftler zu einer irreversiblen Entwicklung wurde. Jedoch waren die Altertumswissenschaften lange Zeit noch ein Refugium für Mehrsprachigkeit, weil die antike

Zivilisation, die sie erforschen, aufgrund des Alexanderzugs und v.a. aufgrund der dauerhaften Ausbreitung des römischen Reiches *per definitionem* ein Unterfangen ist, an dem ganz Europa nicht nur kulturell, sondern auch geographisch und damit letzten Endes aus nationalstaatlichen Interessen Teil hat. So war es bis vor einer Generation noch ganz selbstverständlich, dass mindestens vier Sprachen, nämlich das Deutsche, das Französische, das Italienische und das Englische die allgemein in Publikationsorganen akzeptierten und auf Kongressen problemlos präsentierten Sprachen dieser Disziplinen darstellten, bisweilen um das Spanische und das Neugriechische erweitert, je nach geographischer Orientierung auch gelegentlich das Russische mit einschließend.

Diese selbstverständliche Vielsprachigkeit und die einvernehmliche Forderung an die Ernsthaften unter den Fachvertretern, zumindest diese Sprachen zur Lesefertigkeit zu beherrschen und Publikationen entsprechend wahrzunehmen, ist heute nicht mehr gegeben. Auf studentischem Niveau war das schon vor vielen Jahren illusorisch. In meinem ersten Semester an einer Universität im äußersten Süden Deutschlands wurde ich Zeuge, wie sich eine Studentin beim Professor für Altorientalistik über die Literaturliste für ihr Referat beschwerte. Sie beklagte sich in breitestem Schwäbisch: „Des isch jo alls auf Italjeenisch!“. Pikiert antwortete der Professor, der selbst rund 20 Sprachen, darunter ein Dutzend tote, beherrschte: „Sie wollen mir doch nicht gerade zu verstehen geben, dass Sie die europäischen Dialekte nicht wenigstens bis zur Lesefertigkeit beherrschen?“. Das war bereits 1991 eine kühne Forderung an eine Studentin. Mittlerweile sind aber auch die passiven Sprachkenntnisse der Lehrkörper in den Disziplinen betroffen, die sich mit den Hinterlassenschaften der Antike beschäftigen. Besonders dramatisch ist dieser Befund in denjenigen Ländern, in denen Englisch gesprochen wird. Mangelnde Sprach- und damit auch mangelnde Literaturkenntnisse sind nicht nur bei Vertretern zweitklassiger Bildungseinrichtungen zu beobachten, sondern zunehmend sind auch Leuchtturminstitutionen, besonders renommierte Verlagshäuser der angloamerikanischen Welt, von diesem Trend betroffen. Neben der Nachlässigkeit von Gutachtern muss hier auch der ökonomische Faktor als ein Grund angenommen werden. In meinem ausführlichen Aufsatz zu dem Thema habe ich eine Reihe beschämender Beispiele von Unwissenheit und Ignoranz aus den letzten Jahren aufgelistet (Rubel 2019: 200, 205 – 207), die ich hier nicht durch Wiederholung ehren möchte. Ein ganz neues Beispiel möchte ich aber dennoch nennen, weil es sich in diesem Falle nicht etwa um versehentliche Auslassung von Forschungsergebnissen aus Unkenntnis (zurückzuführen auf mangelnde Sprachkenntnisse) handelt. Wir haben es vielmehr mit einem Fall von bewusstem Verschweigen trotz Sachkenntnis zu tun, was mit dem

fadenscheinigen Argument, ein (vermeintlich) sprachunkundiges Publikum nicht mit fremdsprachiger Sekundärliteratur belästigen zu wollen, begründet wird. Ich musste das betreffende Buch, bei dem es sich um eine nur vorgeblich innovative Studie zum besseren Verständnis der athenischen Demokratie handelt, für die Historische Zeitschrift besprechen (ausführliche Kritik bei Rubel 2020). Der amerikanische Autor schlägt einen „ontological turn" vor, da in den Altertumswissenschaften die Griechen nach wie vor über Gebühr idealisiert würden. Unter Verzicht auf Nennung von vielen Arbeiten, die bereits seit über einer Generation eine „Anthropologisierung" der alten Welt eingeleitet haben und die antike Kultur in ihren eigenen Bedingungen sehen, konstruiert der Autor einen nur vermeintlichen und daher fadenscheinigen Neuansatz. Schlimmer als die inhaltliche Mittelmäßigkeit ist jedoch die nicht-englischsprachige Literatur ausschließende Vorgehensweise. Mit der bewussten Entscheidung des Autors, auf die Erwähnung von Forschungsergebnissen zu verzichten, die nicht in englischer Sprache abgefasst wurden, ist eine neue, geradezu unfassbare Dimension von Ignoranz und Arroganz erreicht, die den einfachsten Grundlagen von Wissenschaftlichkeit Hohn spricht. Der explizite Verzicht auf die Angabe nicht-englischsprachiger Literatur, um den intendierten Laienleser, für den das Buch zu voraussetzungsreich sein dürfte, nicht zu überfordern, ist für diesen eine Beleidigung, denn er würde kaum das Buch aus der Hand legen, wenn er erführe, dass manche nicht ganz dumme Ideen von Italienern, Franzosen und Deutschen zuerst gedacht wurden. Aus Sicht einer wirklich internationalen Wissenschaft ist dies gar ein Skandal: „And because the study is expressly written to appeal to as many non- specialist readers as possible, the great majority of the works included are Anglophone" – Anderson 2018, S. xiv). Das versehentliche Übersehen von konkurrierender Forschung ist entschuldbar, wenngleich peinlich. Das bewusste Übergehen jedoch eine bodenlose Frechheit; eben nicht verzeihliche Ignoranz, sondern kalkulierte Arroganz. Das Buch ist in der „Oxford University Press" erschienen.

Dieser Tiefpunkt wissenschaftlicher „Internationalität" verschlägt einem fast die Sprache. Rufen wir uns noch einmal den Titel der Tagung in Varna nochmal in Erinnerung: „Science without borders" im Sinne Alexander von Humboldts, der polyglott Welten erschlossen hat, meint eben eine der wenigen positiven Dimensionen der Globalisierung, eben die freie Zirkulation von Ideen und Innovation. Im 21. Jahrhundert werden jedoch trotz der scheinbaren Grenzenlosigkeit durch autistische Selbstbeschränkung auf eine Sprache wieder Grenzen aufgebaut, wo lange gar keine mehr waren, gerade in den traditionell mehrsprachigen Altertumswissenschaften. Aber selbst wenn in aktuellen Publikationen auf anderssprachige Literatur verwiesen wird, weil offenbar kein Weg daran vorbei führt, wird das dem Leser gewissermaßen

entschuldigend mitgeteilt, wie zuletzt in einem von einem Amerikaner verfassten Einführungsbändchen über die griechische Kriegsführung, das sich an ein studentisches Publikum richtet. Darin verweist der Autor mehrfach darauf, dass bestimmte Bücher relevant seien, „obwohl" sie in einer anderen Sprache verfasst seien (horribile dictu!). „Though in German, this book was groundbreaking" heißt es etwa von einem Buch von J. Latacz (Sears 2019: 29; weitere Beispiele S. 116 und 142). Hinter solchen Aussagen stehen unterschwellig sogar herabwürdigende Denkmuster, die Studenten nahelegen, dass a) eigentlich keine besonderen wissenschaftlichen Leistungen in anderen Sprachen erbracht werden und b) es nicht nötig ist, für solche offenkundigen Ausnahmen auch noch diese Sprachen zu erlernen. Wahrscheinlich sollte man jedoch dem Verfasser unter den heutigen Bedingungen dankbar sein, dass er überhaupt auf die fremdsprachigen Werke verwiesen hat (den Hinweis auf dieses Buch verdanke ich Kai Brodersen).

Wo soll das hinführen? Im Prinzip gibt es nur zwei mögliche Lösungen des Problems. Eine wäre, die Suprematie des Englischen, die bereits in den Naturwissenschaften voll und ganz akzeptiert wird, auch für die Geisteswissenschaften und auch für das Nischenfach Altertumskunde zu akzeptieren und den lästigen Widerstand endlich aufzugeben. Was die Niederländer und in Ansätzen auch die Skandinavier bereits vorgemacht haben, könnte auch vom Rest Europas nachgeholt werden: Die Aufgabe einer eigenen Wissenschaftstradition in der Landessprache. Es gibt bereits in Bayern (ausgerechnet dort) Bestrebungen, auf den Unterricht in der Landessprache bei bestimmten Masterprogrammen zu verzichten und um der „Internationalität" willen, nun auch die Lehre auf Englisch abzuhalten. Welch ein ausgemachter Unsinn dies aus lernpsychologischer und hochschulpädagogischer Sicht ist, hat der dem „Verein für Deutsche Sprache" angeschlossene „Arbeitskreis für Deutsch als Wissenschaftssprache" mehrfach dargelegt (siehe dazu die Mitteilungen des Arbeitskreises auf <www.adawis.de/aktuelles>).

Würde man jedoch nur die Forschungspublikationen „internationalisieren", indem man Englisch als Publikationssprache zwingend vorgibt, würde dies eine ganze Reihe durchaus erheblicher Nachteile für die Qualität dieser Forschung mit sich bringen, wie ich ausführlich beschrieben habe (Rubel 2019: 207 – 211). Die Naturwissenschaftler haben offenbar recht schmerzfrei auf nationalsprachliche Forschungstraditionen verzichtet (Ammon 2015). Warum das in den Geisteswissenschaften schlechterdings unmöglich ist, ist ausführlich beschrieben worden (am besten bei Mittelstraß et al. 2016). In kurzer Zusammenfassung wäre die Fachwelt unter anderem dem Problem ausgesetzt, dass Fachvertreter, die Englisch als Muttersprache beherrschen einen ungerechtfertigten Vorteil im Kampf um Ressourcen und

Publikationsmöglichkeiten erhielten. Wesentlich bedeutsamer ist jedoch die Tatsache, dass die Präzision des Ausdrucks und die argumentative Entwicklung von Ideen nur bei ganz außergewöhnlich hoher Sprachkompetenz und auch dann nur annähernd möglich ist, niemals jedoch die gleiche Qualität erreicht, wie bei der Verwendung der Muttersprache. Wissenschaftler, die nicht über Englisch als Muttersprache verfügen, „sagen nicht das, was sie sagen wollen, sondern das, was sie sagen können" (Il s'avère que les non-anglophones ne disent pas ce qu'ils veulent, mais ce qu'ilspeuvent. Seguin 2015). Das ist besonders in den Geisteswissenschaften ein Problem, weil hier die Sprache selbst Gegenstand und Teil des Forschungs- und Denkprozesses ist, im Gegensatz etwa zur abstrakten Formelsprache in den Naturwissenschaften, die auch über weitere formalisierte und auch bildhafte Ausdrucksmöglichkeiten verfügt (Diagramme, Graphiken etc.). Die Produktion von Wissenschaft in einer erlernten Sprache kann im Falle der Geisteswissenschaften immer nur minderwertiger sein als bei Verwendung der Muttersprache. Argumentation, Idiomatik und Stringenz bleiben bei Verwendung einer Fremdsprache, selbst wenn diese auf hohem Niveau beherrscht wird, immer defizitär. Darunter leidet auch die inhaltlich-fachliche Seite von geisteswissenschaftlichen Publikationen.

Übersetzungen ins Englische auf hohem Niveau könnten hier nützlich sein, erfordern aber einen erheblichen ökonomischen Mitteleinsatz, der erneut diejenigen, die Englisch nicht als Muttersprache beherrschen, über Gebühr benachteiligen würde.

Eine generelle Übernahme des Englischen als Forschungssprache (oder gar in der Lehre) kann also keine Alternative sein, auch wenn dies bisweilen sogar von Fachvertretern gefordert wird, von Politikern ohnehin. Besonders absurd erscheint dies in solchen Disziplinen, die sich wissenschaftlich mit Sprache selbst befassen. So verdanke ich dem Romanisten Hans Goebl die erschreckende Erkenntnis, dass Romanisten (!) fordern, man möge Forschungsbeiträge zu romanischen Sprachen, die sich an Spezialisten wenden, die gewöhnlich sämtliche romanischen Sprachen fließend lesen (und meist auch sprechen) können, nur noch auf Englisch publizieren (Goebl 2010 und persönliche Korrespondenz). Dies ist in etwa so absurd wie der Fall eines österreichischen Forschungsantrags zur deutschen Sprache, der auf Englisch gestellt werden musste (<http://adawis.de/fileadmin/user_upload/Seiten/Aktuelles/FWF_5 – 2016.pdf>). Der Sachverhalt, dass Forschungsanträge in Österreich ausschließlich auf Englisch gestellt werden können, hat zu heftigen Reaktionen seitens der wissenschaftlichen Gemeinschaft geführt (etwa Schmitt 2015), jedoch wurde eine Petition, die von mehreren Tausend Unterstützern unterschrieben wurde, von den österreichischen Entscheidern abgetan (Initiative 2015). Im Gegensatz zu Frankreich etwa, wo das Festhalten an der

Sprachtradition auch in den Wissenschaften kulturpolitische Unterstützung erfährt (siehe auch den Appell der Gräzistin Jaquelin de Romilly 2007), hat die deutsche Wissenschaftslandschaft mit einem Komplex zu kämpfen, der sich nur teilweise mit den Nachwirkungen der Nazizeit und dem damit verbunden Schamreflex erklären lässt (dazu Trabant 2005: 101).

Entscheidend für die Ursachenbeschreibung ist die Erkenntnis, dass nicht in erster Linie die Ignoranz einer immer größer werdenden Gruppe von Geisteswissenschaftlern aus anglophonen Ländern für diese Situation verantwortlich ist. Denn diejenigen, welche nur noch Literatur in ihrer eigenen Muttersprache zur Kenntnis nehmen spielen deshalb schon *per definitionem* auch in ihrer Heimat nur in der zweiten Liga. Für den Niedergang der Vielsprachigkeit in den Geisteswissenschaften und auch in der letzten Bastion, den Altertumswissenschaften, ist vielmehr ein wesentlich dramatischeres Element verantwortlich: Die vehementen Verfechter einer falsch verstandenen Internationalisierungspolitik in Europa, welche Internationalisierung als die Verengung des sprachlichen Ausdrucks in den Wissenschaften (und sogar der Lehre!) auf das Englische versteht, tragen die Hauptschuld an der Misere. Dieser politische Irrweg wird von opportunistischen Wissenschaftlern flankiert, die aus verschiedenen, bisweilen individuell nachvollziehbaren Gründen auf den englischsprachigen Zug aufspringen. Der nachvollziehbarste Grund ist indes derjenige der Notwehr: Auch ich bekenne mich schuldig, bisweilen auf Englisch publiziert zu haben, um überhaupt wahrgenommen zu werden. Andernfalls erlebt man immer wieder, dass in englischsprachigen Publikationen die eigenen (bisweilen ziemlich alten) Forschungsergebnisse als Neuentdeckungen bedeutender anglo-amerikanischer „Wissenschaftler" auftauchen, die in ihrer sprachautistischen Blase gefangen sind. Daraus ergibt sich weiteres Frustrationspotential, das bereits der Mediävist Hermann Heimpel in einer berühmten Rezension verspürt hat, als er ein geflügeltes Wort prägte: „Literaturkenntnis schützt vor Neuentdeckungen" (Heimpel 1954: 210). Einerseits ist keinesfalls garantiert, dass diejenigen, die anderssprachige Literatur nicht zur Kenntnis genommen haben, ihr Versäumnis einräumen, wenn die Ergebnisse auf Englisch (Notwehr) vorgelegt werden. Möglicherweise ignorieren sie auch die englische Variante, weil sie andernfalls ihr Versäumnis eingestehen müssten. Andererseits setzt man sich, selbst wenn man von Muttersprachlern beim Korrekturlesen unterstützt wird, der Gefahr aus, dass Manuskripte von Gutachtern von Zeitschriften aus sprachlichen Gründen zurückgewiesen werden. Bei Verwendung einer Fremdsprache ist auch nach Korrektur durch Muttersprachler die Gefahr nicht gebannt, dass die Argumentation nicht im gleichen Maße stringent ist, wie beim Gebrauch

der Muttersprache, und so Manuskripte indirekt aus sprachlichen Gründen zurückgewiesen werden.

Der einzige Ausweg, wenn man die europäische Vielsprachigkeit fördern oder, um es alarmistisch zu formulieren, sogar retten will, zielt auf die wissenschaftspolitischen Verteilungsmechanismen. Will man es ironisch formulieren, so ist dies gar ein „neomarxistischer" Weg, denn es geht um die Umverteilung der Produktionsmittel im Wissenschaftsbetrieb, also vereinfacht gesagt, ums liebe Geld. Dies muss auf Grundlage der Erkenntnis geschehen, dass Internationalisierung der Wissenschaften eben ganz im Sinne Humboldts die Förderung von Mehrsprachigkeit und Sprachkompetenz bedeutet und nicht die Selbstbeschränkung auf eine „lingua franca". „Lingua franca", die Sprache der Franken, meint jedoch die romanische Pidginsprache der arabischen Händler des Mittelalters, die Sprache der Hafenhuren und Seeleute. Das freimütige Bekenntnis, dass das weltweit – auch in der Wissenschaft – verwendete Englisch eben nur eine „lingua franca" ist, trifft den Kern des Problems in den Geisteswissenschaften (zu Englisch als lingua franca siehe Seidlhofer 2011): Tarzan kann eben keine philosophischen Diskussionen über Heidegger in seinem Idiom führen.

Die bisherigen nationalen und europäischen Förderungsformate für Wissenschaft dekretieren für Antragsteller in ihrer überwältigen Mehrheit „English only". Die Schweden haben die teure aber sehr hilfreiche Lösung entwickelt, dass Anträge in Landessprache geschrieben werden können und *ex officio* übersetzt werden, ein guter Kompromiss (<http://www.formas.se/en/Financing/General-instructions>). Hauptargument für diese Vorgehensweise ist der Verweis auf die internationalen Gutachter, die in der Lage sein sollen, auf europäischer Ebene Anträge aus allen Mitgliedsländern evaluieren zu können. Das ergibt bei Naturwissenschaften und medizinischer Forschung wohl Sinn, ist aber bei den Geisteswissenschaften aus den genannten Gründen, die mit dem Problem inhaltlicher Stringenz und argumentativer Klarheit zu tun haben, wenig zielführend. Hoch dotierte Forschungsprojekte (etwa aus dem Bereich der ERC grants) sind ein ganz bedeutender Faktor für die Karriere und den Prestigegewinn von Wissenschaftlern und sollten daher besonders nach Kriterien der Chancengleichheit ausgerichtet sein. Die Tatsache, dass von über 500 geisteswissenschaftlichen Forschungsprojekten der europäischen Förderlinie Horizon 2020, FP 7 und ihrer Vorgänger jährlich zwischen mindestens 20 % und fast die Hälfte nach Großbritannien ging (siehe ERC: Annual Reports-Statistics), lässt sich nicht schlüssig damit erklären, dass die Forschungseinrichtungen des Vereinigten Königreiches in einem diese Differenz legitimierenden Ausmaße besser seien, als die in den

restlichen 27 EU-Mitgliedstaaten. 2019 stammten sogar 43 % aller erfolgreichen geisteswissenschaftlichen Anträge aus Großbritannien (<https://www.thebritishacademy.ac.uk/news/uk-humanities-and-social-science-researchers-secure-almost-half-prestigious-european-research>).

Ich erlaube mir an dieser Stelle (bis zum gerne akzeptierten Gegenbeweis) eine nicht ganz wissenschaftliche Vermutung zu äußern: Die Verpflichtung auf das Englische als Antragssprache hängt wohl auch mit der Tatsache zusammen, dass ein großer Teil der Gutachter, unter denen auch viele Wissenschaftler aus der neuen Welt sind, Englisch als Muttersprache spricht (30 % der Gutachter von ERC grants kommen aus dem Vereinigten Königreich oder den USA, siehe König 2016: 158). Ihnen wie auch den übrigen Gutachtern werden von Muttersprachlern verfasste Anträge für geisteswissenschaftliche Projekte aufgrund sprachlicher Eleganz und Perfektion überzeugender erscheinen als andere.

Sollte meine etwas gewagte aber nicht unwahrscheinliche Vermutung zutreffen, wäre die exklusive Verwendung des Englischen bei der Forschungsförderung auf europäischer Ebene ein dem Grundsatz der Chancengleichheit widersprechendes Kriterium. Ohne den Iren, deren gälische Volkssprache über Jahrhunderte von einer Kolonialmacht unterdrückt und fast ausgelöscht wurde, hier zu nahe treten zu wollen, könnte gerade vor dem Hintergrund des nun unabwendbaren „Brexit" an dieser Stelle mit dem richtigen politischen Willen und entsprechenden Geldmitteln nachjustiert werden, um die nationalen Wissenschaftssprachen und -traditionen zu fördern und gleichzeitig Chancengleichheit herzustellen. Nach dem 31.01.2020 (bzw. endgültig ab 2021) sprechen nur noch 5 Millionen der 447 Millionen EU-Bürger Englisch als Erstsprache, das entspricht in etwa der Situation des Slowakischen. Den Unsinn, dass Forschungsprojekte zu nationalhistorischen oder nationalsprachlichen Themengebieten in einer anderen als der Landessprache geschrieben werden müssen, könnte und sollte man sofort mit einem Federstrich beenden, da vernünftigerweise kein internationaler Spezialist und potentieller Gutachter etwa für polnische Geschichte oder westslawische Dialekte des Polnischen nicht mächtig sein sollte (andernfalls müsste er aus fachlichen Gründen als Gutachter ausscheiden). Selbiges gilt natürlich auch für das germanistische Forschungsprojekt, das trotz kontroverser parlamentarischer Debatte in Österreich auf Englisch eingereicht werden musste.

Eine besonders haarige Angelegenheit, die eigentlich eine ausführliche separate Diskussion verdient hätte (es gibt bereits einige kritische Studien zu dem Thema, etwa: Huang & Chang 2008, Ochsner et al. 2016, Sivertsen 2016), ist die Verwendung fragwürdiger bibliometrischer Instrumente zur Leistungsbewertung in den Geisteswissenschaften. Zitatenindices, allen voran das Web of Science, das nach mehreren Wechseln der Eigentümer nun im Besitz

der Firma Clarivate Analytics ist (ehemals Thomson Reuters), haben in den Naturwissenschaften einen vertretbaren Sinn und ermöglichen aufgrund der kurzen Halbwertszeit der Forschungsergebnisse und der raschen Durchdringung der Wissenschaften nach Neuerkenntnissen oder gar Paradigmenwechseln auch die Erhebung von messbaren und signifikant relevanten Daten (obwohl auch hier kritische Stimmen laut werden, Mocikat 2009). In den Geisteswissenschaften sieht das ganz anders aus. Der sogenannte „Arts and Humanities Citation Index" besagter Firma – es muss nicht extra betont werden, dass es sich hier nicht um ein Peer-Netzwerk von Wissenschaftlern sondern um ein profitorientiertes Unternehmen handelt – ist gar kein richtiger Zitatenindex, sondern lediglich eine Liste von Zeitschriften, die von besagtem Unternehmen (auf Antrag der betreffenden Verlage) aufgenommen werden und bestimmte Ansprüche erfüllen (u.a. peer-review Prinzip, englische Abstracts und Titel). Im EU-Land Rumänien etwa ist es von entscheidender Bedeutung für die Karriere von Wissenschaftlern, in Zeitschriften zu publizieren, die in diesem Index aufgenommen sind (bzw. allgemein im Web of Science, für alle Disziplinen). Mit derartigen Publikationen erhält man höhere Punktresultate bei Berufungen auf Professuren, für die bestimmte quantifizierbare Mindestkriterien erforderlich sind. Auch werden Publikationen in Zeitschriften, die im „Arts and Humanities Index" dieser Privatfirma gelistet sind, staatliche Prämien ausgezahlt (etwas über 400 Euro pro Publikation, Naturwissenschaftler können bei Publikationen in Zeitschriften mit hohem Impact-Faktor bis zu 9.000 Euro Prämie einstreichen, letzteres etwa bei Veröffentlichungen in Science oder Nature).

Im Bereich Archäologie ist eine einzige deutsche Zeitschrift im „Arts and Humanities Index" gelistet (in der natürlich auch auf Deutsch publiziert werden kann, aber auch auf Englisch): Das in Mainz erscheinende „Archäologische Korrespondenzblatt" des Römisch–Germanischen Zentralmuseums (RGZM), eine qualitativ ausgezeichnete Fachzeitschrift mit Tradition (die Zeitschrift erscheint seit 1971). Der seit 1888 ununterbrochen erscheinende „Archäologische Anzeiger" etwa taucht wie viele andere herausragende und in der Fachwelt hoch geschätzte Publikationen in dem Index nicht auf, weil die Herausgeber wahrscheinlich – mit gutem Recht – keinen Sinn in der wenig aussagekräftigen Auflistung sehen und keinen entsprechenden Antrag gestellt haben. Jedoch findet sich die österreichische Zeitschrift „Literatur und Kritik" (aus Salzburg) in diesem Index. Sollten rumänische Kollegen in diesem Organ publizieren, würden sie Prämien erhalten und ihre Chancen auf höhere Weihen im nationalen akademischen System erhöhen. Indes werden dort nur kleine Essays und Gedichte publiziert, jedoch keine wissenschaftlichen Studien mit so lästigem Beiwerk wie Fußnoten und Literaturangaben. Das muss den Rang dieser Literaturzeitschrift nicht schmälern, bietet aber zugleich die Chance für poetisch begabte aber wissenschaftlich mittelmäßige Rumänen, zu

prestigeträchtigen Veröffentlichungen zu kommen. Aber es geht auch noch einfacher: Mittlerweile haben viele rumänische Zeitschriften (aus allen Feldern der Wissenschaften), über deren wissenschaftlichen Rang man zumindest streiten könnte, deren internationale Relevanz jedoch erwiesenermaßen tendenziell gegen null geht, in diesen ja so bedeutenden „Index" Eingang gefunden. Das erleichtert nun den Aufstieg derjenigen Wissenschaftler im System, die in ihrem Fachgebiet bislang nur in nationalen oder gar regionalen Publikationsorganen veröffentlicht haben (oder nur dort und nicht andernorts veröffentlichen konnten). Sie können so bei ihren liebgewordenen Gewohnheiten bleiben und können, ohne ihre Arbeiten von international renommierten Fachleuten begutachten lassen zu müssen, dennoch höchste Stellen besetzen.

Sogar die irreführenderweise so genannten „exakten" Wissenschaften haben darunter zu leiden. So wurden in der Großstadt Jassy Mediziner als Universitätsprofessoren berufen, die in einer Bukarester Chemiezeitschrift mit Titel „Revista de Chimie" veröffentlicht haben. Diese Zeitschrift zeichnet sich vor allem dadurch aus, dass sie bei Clarivate Analysis im Web of Science gelistet ist. Allerdings haben bislang keine Nobelpreisträger oder international renommierte Wissenschaftler in dieser Zeitschrift publiziert. Unter den Autoren der neusten Nummer 70, 1, 2019, immerhin mit fast 80 Artikeln in einem einzigen Faszikel, sind ausschließlich rumänische Autoren (<https://www.revistadechimie.ro/pdf/CUPRINS%20RC%201%202019.pdf>). Damit hatten die Kandidaten das Kriterium erfüllt, entsprechend „internationale" und in dem prestigeträchtigen Index gelistete Publikationen vorweisen zu können. Dass es sich dabei weder um ein in der internationalen Fachwelt anerkanntes Organ mit wirklich unbeeinflussbarer peer-review, noch um eine Fachzeitschrift für Medizin handelt, war sekundär, hat jedoch unter Kollegen und in der Presse für Wirbel gesorgt (über den Skandal an der medizinischen Fakultät in Jassy <https://www.ziaruldeiasi.ro/stiri/profesori-umf-specialisti-in-sociologie-filiera-avansarii-legale-dar-imorale- – 223801.html>). Selbst die Naturwissenschaften, in denen das Zitatezählen und Indizieren als einigermaßen sinnvoll erachtet wird, sind also nicht gefeit vor problematischen Auswirkungen der Bibliometrie.

Wie zweifelhaft der Zweck solcher Zeitschriftenlisten (es wird ja nichts gemessen), aber auch im hypothetischen Falle funktionierender Zitatenindices (mit impact-factor Messung) in den Geisteswissenschaften ist, mag das Beispiel des Soziologen Norbert Elias zeigen. Sein Hauptwerk „Über den Prozeß der Zivilisation", erschienen 1939. Es gehört zu den bedeutendsten Werken der Sozial- und Geisteswissenschaften des 20. Jahrhunderts und bescherte dem Autor bis heute traumhafte bibliometrische Werte, versuchte man, die Flut der Zitate, die ihn und sein Werk nennen, auch nur ansatzweise zu

berücksichtigen. Sein Werk wurde aber erst rund dreißig Jahre nach dem Erscheinen gewissermaßen „entdeckt", so dass er unmittelbar (oder auch 10 Jahre) nach der Veröffentlichung keine Chance auf eine Berufung gehabt hätte, wenn bibliometrische Kriterien die Grundlage von akademischen Karrieren bilden würden. Denn die „impact factor"-Berechnung bei Clarivate Analytics arbeitet mit einem Zweijahresfenster (Mocikat 2009: 102). Würde man in den Geistes- und Sozialwissenschaften wirklich mit dem Zitatezählen anfangen, wäre das angesichts der langsamen Durchdringung mit neuen Paradigmata ein komplexes und sicher ungerechtes Unterfangen.

Im besagten „Arts and Humanities Citation Index", dieser unsystematischen Zeitschriftenliste, die weitgehend ohne jede Aussagekraft ist, finden sich wenige Zeitschriften, die nicht exklusiv in englischer Sprache publizieren. Wenige deutschsprachige und fast überhaupt keine französischen Periodika haben in diese Liste Eingang gefunden, die in keiner Weise repräsentativ die Wissenschaftslandschaft abbildet oder, wenn auch unsystematisch, nur hochwertige Publikationsorgane versammelt. Entsprechend wird auch durch die Nötigung europäischer Geisteswissenschaftler in bestimmten recht willkürlich indizierten Zeitschriften zu publizieren ein Druck aufgebaut, auf Englisch zu publizieren. Untersuchungen haben ergeben, dass in den Datenbanken von Clarivate Analytics (grob: Web of Science) gemessen am reellen wissenschaftlichen „output" in einschlägigen und international anerkannten Zeitschriften, die peer-review betreiben, das Deutsche um 50 % unterrepräsentiert ist, das Spanische gar um 70 % (Archambault et al. 2006: 337). Dies sorgt für einen das Englische bevorzugenden Trend, der durch nichts gerechtfertigt werden kann.

Das Fragwürdigste an der neuen Manie, bibliometrische Instrumente, dazu solche von profitorientierten Privatunternehmen, als Bewertungsgrundlage für akademische Leistungen heranzuziehen, ist jedoch, dass es sich eben gar nicht um Bibliometrie im Wortsinne handelt! Wenn es doch so wäre und es um Bücher ginge, um *bíbloi*, und nicht nur um Zeitschriften, dann könnte man sich als Geisteswissenschaftler vielleicht mit diesem Kriterium anfreunden. Das Buch, die Monographie, ist und bleibt nun einmal *das* Fundament geisteswissenschaftlicher Forschung. Bücher, nicht mal solche auf Englisch, werden nun von diesen bibliometrischen Instrumenten überhaupt nicht berücksichtigt. Geisteswissenschaftlern wird darüber hinaus auf diese Weise ein ganz neuer Arbeitsmodus aufgezwungen, wenn Prestige und Förderung sich an (englischen) Zeitschriftenartikeln und nicht an der Publikation von Büchern bemessen, die oftmals jahrelange Arbeit erfordern.

All diese Beobachtungen scheinen, gefördert von der allgemeinen Tendenz auch in der Altertumswissenschaft nur noch Englisch zu lesen, einen

unumkehrbaren Trend zu markieren. Wie sollte sich diese Entwicklung zur Einsprachigkeit, zu „English only", noch aufhalten lassen? In der Tat scheint trotz der Tatsache, dass viele Kollegen aus Großbritannien und aus Übersee, besonders die anerkannten Größen ihres Fachs, noch viele Sprachen lesen, der Zug bereits abgefahren zu sein. Das System wird sich – auch angesichts der Verkürzung des Studiums durch die Bolognareformen – wohl nicht selbst wieder ins Lot bringen können. Einzig kulturpolitische Maßnahmen zur Förderung der Mehrsprachigkeit könnten dem zunehmenden Autismus in den Geisteswissenschaften noch Einhalt gebieten, auf nationaler wie auch auf europäischer Ebene. Umso bedenklicher ist es indes, feststellen zu müssen, dass zumindest in Deutschland die meisten Bildungspolitiker im Sinne einer völlig fehlverstandenen „Internationalisierung" der Hochschulen in allen Bereichen auf die Förderung und Bevorzugung des Englischen setzen. Ein Panoptikum des Grauens in dieser Hinsicht, gekrönt von den Bemühungen der TU München, Englisch als exklusive Unterrichtssprache in den höheren Studiengängen (auch für Landeskinder) einzuführen, wird mit entsprechenden Belegen und Verweisen auf weitere Literatur vom ADAWIS auf seiner Internetseite präsentiert (<http://adawis.de/aktuelles/>).

Doch genau an dieser Stelle müsste ein Umdenken einsetzen und die vollmundig geforderte Mehrsprachigkeit auf europäischer Ebene wirklich gefördert und nicht nur gefordert werden (zur EU-Sprachenpolitik <https://www.europarl.europa.eu/factsheets/de/sheet/142/sprachenpolitik>; kritische Hinweise zur notwendigen Sprachenförderung bietet der Tagesspiegel <https://www.tagesspiegel.de/wissen/mehrsprachigkeit-in-der-eu-englisch-und-viel-mehr/10926958.html>). Es geht keineswegs darum, nun das Englische etwa durch das Französische oder das Deutsche zu ersetzen; jedoch müssen die Bewertungskriterien für akademische Leistungen in den Geisteswissenschaften sowie die Kriterien zur Forschungsförderung neu überdacht werden. Auf nationaler Ebene gäbe es eine Vielzahl von Ansätzen, die verfolgt werden könnten. Der Arbeitskreis Deutsch als Wissenschaftssprache hat eine Reihe konkreter Vorschläge formuliert und diese auch in die politische Diskussion auf Landes- und Bundesebene gebracht (aufgelistet hier <http://adawis.de/politik/>). Diese hier im Einzelnen zu besprechen würde zu weit führen.

Entscheidend ist aber meiner Meinung nach die Förderung der Mehrsprachigkeit und Sanktionierung von Einsprachigkeit auf nationaler und auf europäischer Ebene durch die einzig effektive Stellschraube der Hochschul- und Wissenschaftspolitik: das liebe Geld. Nur über die Umverteilung von Fördergeldern und die gezielte Unterstützung von Mehrsprachigkeit wird sich an der beklagenswerten und hier beklagten Situation etwas ändern können. Einerseits muss Mittelbewilligung für ERC Forschungsprojekte nach von

geisteswissenschaftlichen Peer-Netzwerken ausgearbeiteten Kriterien erfolgen, die Internationalität von Antragstellern nicht mit dem Vorhandensein von englischsprachigen Arbeiten begründen, sondern mit der Relevanz im Fach und der Qualität der italienischen, französischen, deutschen, spanischen, griechischen oder russischen Beiträge. Als Fußnote mag das Beispiel eines emeritierten rumänischen Kollegen dienen, dessen nationales Forschungsprojekt zunächst mit dem Verweis auf die nicht ausreichende Internationalität seiner Forschungsarbeiten abgelehnt wurde, um dann nach Protest doch akzeptiert zu werden. Der international bekannte Epigraphiker Ioan Piso publiziert in der Tat beklagenswerterweise in internationalen bedeutenden Publikationen „nur" auf Deutsch, Französisch und natürlich in seiner Muttersprache, bisweilen auf Italienisch und selten sogar auf Englisch, letzteres für die Gutachter offensichtlich nicht in ausreichendem Maße (Publikationsliste hier <http://hiphi.ubbcluj.ro/personal/ioan_piso/publicatii.html>).

Dieser Unsinn muss aufhören! Politische Institutionen, die von den Steuerzahlern ihrer Länder finanziert werden, sowie europäische Organe die aus Beiträgen von 28 (ab dem 31.1.2020 aus 27) Mitgliedsstaaten finanziert werden, müssen Strategien zur Förderung ihrer Landessprachen als Wissenschaftssprachen entwickeln. Gerade Deutschland als größter Nettozahler in der EU ist hier in der Pflicht und muss Konzepte liefern. Für ein Land, das rund 25 % des EU-Haushalts finanziert, kann die derzeitige Sprachpolitik in der Wissenschaftsförderung nicht befriedigend sein. Dabei geht es keineswegs darum, das Englische nun auszurotten und Maßnahmen *gegen* die Verwendung dieser wunderbaren Sprache zu treffen, sondern vielmehr Geld in die Hand zu nehmen, um umfassende Übersetzungsleistungen im Bereich der Wissenschaften, vor allem der Geisteswissenschaften zu finanzieren. Im europäischen Bereich müsste das auch Übersetzungen von und in andere Sprachen als dem Englischen einschließen. Im Bereich der schönen Literatur (und der Sach- und Kinderbücher) gibt es bereits gelungene Beispiele von Förderinstrumenten für Übersetzungen (z.B. www.traduki.eu). In der Tat wird man aber von Asiaten kaum verlangen dürfen, europäische Dialekte studieren zu müssen, um geisteswissenschaftliche Forschungen zur Kenntnis nehmen zu können. Obwohl man den Lernwillen asiatischer Studenten nicht unterschätzen sollte – einer meiner Kommilitonen, ein japanischer Doktorand aus Osaka, musste auf Geheiß seines Doktorvaters noch das große Latinum nachholen, um eine Doktorarbeit über die Bauernkriege Anfang des 16. Jahrhunderts in Süddeutschland anfertigen zu können (er stellte danach als stolzer Inhaber des Latein-Diploms fest, dass die Studienordnung ausländische Doktoranden ausdrücklich von dieser Pflicht entband) – ist der Gebrauch des Englischen in der außereuropäischen Kommunikation sicher gerechtfertigt.

Im Bereich der europäischen Forschungsförderung könnte aber eine Finanzierung von Übersetzungen der Förderungsanträge aus den Amtssprachen (zu denen sogar das Maltesische und das Gälische gehören) ins Englische (kontrolliert von Fachexperten) eine große Hilfe sein. Die Schweden (siehe oben) bieten auf nationaler Ebene für ihre Forschungsförderung einen solchen Service an. Auch die Förderung zweisprachiger Fachzeitschriften, die durch Übersetzung und Verdoppelung des Umfangs enorme Ressourcen verschlingen würden, wäre eine sinnvolle Ergänzung und würde vor allem in Ländern, deren Sprache aufgrund der Wissenschaftsgeschichte nicht zu den am weitesten verbreiteten Sprachen gehört, zum Erhalt der jeweils eigenen Wissenschaftstradition beitragen. In meinem Fachgebiet sind mir die kroatische Zeitschrift „*Vjesnik za arheologiju i historiju dalmatinsku*" (Bulletin for the Archaeology and History of Dalmatia) und das rumänische Periodikum „*Istros*" bekannt, die eine solche Lösung des Dilemmas anbieten (inwieweit und in welcher Form hier die Autoren selbst zu den englischen Übersetzungen beitragen müssen, ist mir nicht ersichtlich).

Hinsichtlich der Bewertung wissenschaftlicher Leistungen muss ebenfalls auf europäischer Ebene vorgegangen werden. Wenn man schon bibliometrische Kriterien einführen und benutzen möchte, so darf das nicht kommerziellen Interessen von Firmen überlassen werden, sondern muss auf europäischer Ebene mit viel Geld und viel Expertenwissen angegangen werden. Das gilt übrigens genauso für die Natur- und Lebenswissenschaften. „Damit nicht-englischsprachige Arbeiten honoriert werden, ist es erforderlich, eine europäische, mehrsprachige Zitatdatenbank zu schaffen" (Mocikat 2009: 103). Die noch etwas ungeschlachte und wenig strukturierte Datenbank ERIH PLUS (European Reference Index for the Humanities and the Social Sciences <https://dbh.nsd.uib.no/publiseringskanaler/erihplus/about/index>) könnte die Grundlage für eine solche Unternehmung liefern.

In jedem Falle ist nun die Politik am Zuge. Die Wissenschaft kann und muss aber auch in Zukunft im Rahmen ihrer Möglichkeiten mitwirken und sollte krasse Fälle von Ignoranz immer offenlegen und in Rezensionen bedingungslos für die grundlegenden Standards der Mehrsprachigkeit eintreten, die die Geisteswissenschaften und besonders die Altertumswissenschaften definieren. „Das Mittel sollte daher sein, weiterhin auf Deutsch zu publizieren, hier und da zur guten Verständigung auch auf Englisch oder einer anderen Sprache, und im internationalen Diskurs jene Arbeiten offenzulegen, die wegen offensichtlicher Ignoranz anderssprachiger Forschung minderwertige Ergebnisse präsentieren" (von Rummel, Busch s.a.).

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Alexander Rubel
Institute of Archaeology
Romanian Academy, Iasi
Romania
rubel@arheo.ro

Jesus College
Oxford
Great Britain
alexander.rubel@jesus.ox.ac.uk

LANFRANCONI – THE SCIENTIST WHO CHANGED THE SHAPE OF THE DANUBE

Jana Magdaléna Májeková

Keywords: *Lanfranconi, Danube, regulation, Bratislava*

Abstract: *Enea Grazioso Lanfranconi (1850 – 1895) was a scientist born in the town of Peglio (Lombardy). After his studies in the University of Milan, he settled down in Bratislava (Pozsony/Pressburg), where he spent his productive years. This paper relates to Lanfranconi's most valuable scientific asset – his work in the field of hydrology. Lanfranconi was interested in the regulation of the Danube, as he knew that floods were a serious problem. He regularly published articles on this matter in Bratislava periodicals, and in 1880 he wrote a book on this topic. The purpose of the present paper is to enlighten the regulation plan of the Danube proposed by Lanfranconi in 1880 and to identify his contribution. The research has shown that, thanks to Lanfranconi's regulation, the Danube gained a new shape, which led to the creation of the waterfront and, respectively, to the ship transport improvement on the river.*

Enea Grazioso Lanfranconi was a scientist, engineer, and inventor, who influenced hydrology, the culture of Hungary, and the society of Bratislava – at that time called Pozsony or Pressburg – in an irrevocable way. In order to better understand his scientific ideas, decisions, aims, and personality, we need to know the background of his life. Why did he settle down in Bratislava, what were his activities there, and how did he take his place in history?

Lanfranconi was born in the town of Peglio in Lombardy, in 1850, in a prosperous family. Following the example of his father, who worked as a railway engineer, he developed a passion for technical sciences. In 1870 the family settled down in Bratislava, where Lanfranconi Senior continued his work on the construction of the railway network in Austria-Hungary. At that time, twenty-year-old Enea Lanfranconi finished his studies in Milan and started a new period of life in Bratislava, a dynamically developing city in the Hungarian part of the monarchy (Zólyomi 1969: 437).

Lanfranconi spent in this city his productive years. He came from a different cultural environment, but he immediately fell in love with Hungarian culture. Testimony of it can be found in his activities and statements as well as in memoirs of his contemporaries. Over time, he became an integral and important part of the local society.

Observing the personality of Lanfranconi, we can say that it had renaissance features. He had a wide range of interests, such as technology, archaeology, art, languages, and philanthropy, which opened doors everywhere and to everyone. He was a member of the Hungarian Historical Society, the prestigious Toldy Circle, the Cultural Association, the Firefighters Association, the Bratislava Rowing Club, where only the most influential and the richest men were associated, and many others (*Pressburger Zeitung* 03.08.1885: 2; 22.01.1888: 3 – 4; 24.03.1891: 5). He also tirelessly presented Bratislava abroad, mainly through art. He possessed a palace in the city, where he maintained his art collection: about 300 paintings of Italian, French, Spanish, Dutch, and other artists (Zólyomi 1969: 437).

However, the main reason due to which he could devote himself to all of these activities was his property and financial certainty. He managed to become rich thanks to his profits from the Devín quarries (nowadays a borough of Bratislava; at the time called Dévény/Theben), an inheritance from his father as well as renting estates he owned there, and from his job as an engineer (Bokor 1895: 233). In 1875, Enea even constructed a cableway in his quarry in Devín and later, in 1890, he invented and patented a self-loading ramp (Szinnyei 1900: 704).

Lanfranconi devoted himself to hydrology – more precisely, to the regulation of the Danube from Devín to Gönyű (a village near Győr; at the time called Gönyő). He considered this section to be the largest inundation area of the Danube River in general. Lanfranconi's work in this field was of great value particularly for Bratislava and for the whole area between Devín and Gönyű, through which the Danube flows. Floods posed a serious problem and were persistently reducing the quality of life and development. As he wrote: *"The inhabitants of the Pressburg* [Bratislava]*, Wieselburg* [Moson], *Raab* [Győr], *and Komorn* [Komárom] *Counties, where the fields are flooded almost every year and often even the dwellings themselves are afflicted with floods, know it all too well."* (Lanfranconi 1880: 35 – 36.)

The city of Bratislava alone was afflicted by six devastating floods during the 19th century. The largest of them was in 1850, in which half of the city was under water. This is evidenced by many memorial tables and water level signs, scattered throughout the city centre, which are reminiscent of this natural disaster. One of them can be found on the facade of the Primate's Palace in the heart of the city. However, the cellars in the lower parts of this city were filled with water every year. On annual average, the summer floods in Bratislava were 5 metres, and in Gönyű – 3,5 metres above the zero point of the ordinary water level (Lanfranconi 1880: 53 – 54).

Moreover, Lanfranconi wanted to regulate the river not just to protect this area from floods, but also to enable ship transport, in which he saw large

potential for trade and also for military purposes, because of the extensity of waterways (Lanfranconi 1880: 66).

His intentions of regulation can be found mainly in his articles in Bratislava periodicals and in his books on this matter, which were the first works about the regulation of this section of the Danube. In his works he appealed to the Hungarian government, referring to the economic principles and ideas of Count István Széchenyi (1791 – 1860), to make all the necessary steps and investments to this goal (Lanfranconi 1880: 65).

His first and most influential book, published in 1880, was named *On the Waterways of Central Europe and the Importance of the Regulation of the Danube River with special Consideration for the Section between Theben* [Devín] – *Gönyő* [Gönyű]. The book was written in German, but in the same year was also translated into Hungarian and French, and each version was published in the Karl Angermayer publishing house in Bratislava.

In the book, Lanfranconi described the basin, the length, the width, and the depth of the river, the flooded areas, the transverse profile and the speed, as well as the amount of water of the Danube. In the first part of the book he summarized the benefits which the regulation could bring with regard to nature, citizens, protection of the land, and transportation, but also to the monarchy itself, as it would provide progress and prosperity. According to him: *"Water is the most important element of the soil culture."* (Lanfranconi 1880: 13). What does it mean? If the waterways are well laid out, an amount of water enough to irrigate the surrounding lowlands could be released. The correct use of water for irrigation purposes would then also allow prevention of flooding. This particular aspect was the most important for Lanfranconi. The Danube in its normal state fertilized the waterfront and promoted the vegetation and animal life. Nevertheless, floods destroyed everything created with the aid of water by then, so he wanted to find a proper solution to change this.

Lanfranconi tried to identify causes, to name consequences, and to find ways to prevent floods. In his opinion, the main factors governing the flow of water were width, gradient and depth. He was focusing mainly on the depth of the river and its unsuitability in case of shallows, because the depth of the river between Devín and Gönyű was on average 2 metres, making it shallower than the other sections. He considered the shallowness a barrier to the flow, which made it impossible for ships to sail and prevented the smooth outflow of ice blocks during winter. It was therefore necessary to obtain the correct depth, which was one of the main purposes of the Danube regulation for him. *"In order to well regulate a river,"* he wrote, *"it should, wherever possible, be buried deep in the ground, as deep as permissible..."* (Lanfranconi 1880: 63).

The cardinal point of the regulation was based on a definitive fixation of the main flow. To this end there was a need of excavation works, stabilisation of the tips of important islands and cutting off all side branches. Such branches in Bratislava were largely weaned and backfilled with soil, and today they are covered by residential areas. One of the most significant parts of the regulation was the elevation of the banks and the creation of bank-protecting structures. Lanfranconi also wanted to remove the sandbanks which had been deposited on this section for centuries in the stream. He supposed that the costs of a definitive fixation of the Danube between Devín to Gönyű would be ten million guilders (Lanfranconi 1880: 90 – 91).

In the map (Fig. 1), we can see the new canal and the situation after the regulation, and at the same time the wild Danube before the regulation, as this map was made by altering the former one by updating a lithographic cliché of the old Danube. This map, made by shrinking of larger base maps, contained the small details of the old Danube upon which the course of the new canal was drawn from a newer source. In this way, the original, complicated, and curved shape of the river and its tributary streams, as well as its new shape and tamed banks are evident.

Lanfranconi had been preparing these designs for more than ten years. After his first work was released in 1880, he also published two other books in German: *Rescue of Hungary from Floods* in 1882 and *Floods in Hungary and the Regulation of the Lower Danube* in 1887. In them, he further developed the ideas set out in his first work.

Systematic river canalizing in Bratislava lasted ten years, from 1886 to 1896, so the project of regulation continued even after Lanfranconi's death in 1895 (Pišút 2018: 186). The result of his regulation was that the Danube became deeper and narrower. The banks of the river were aligned and stone-faced, which led to the creation of the waterfront and to the improvement of ship transport on the Danube. For his activities, he also received an award from the monarch Franz Joseph and became a holder of the Commander Order (*Pressburger Zeitung* 09.12.1887: 2).

Lanfranconi died prematurely in 1895 in Bratislava at the age of 45, when he committed suicide by shooting himself with a rifle (*Pressburger Zeitung* 09.03.1895: 1 – 2; Fig. 2). The Lanfranconi family tombstone is located at the St. Andrew's Cemetery in Bratislava. Lanfranconi's body was temporarily placed there. A few days after the funeral, the body was taken and buried in the Italian town of Varese in Lombardy at the request of the family.

His legacy is still alive among Bratislava citizens. One of the campuses of the Comenius University in Bratislava and one of the most important bridges

Fig. 1: Map of Bratislava (Pozsony/Pressburg) County, 1910 (Osztrák–Magyar Monarchia vármegyéi a XX. szd. elején.) <http://lazarus.elte.hu/hun/maps/1910/vmlista.htm>.

Die Unterzeichneten geben hiemit tiefbetrübt die trauervolle Nachricht von dem Ableben ihres innigstgeliebten, unvergeßlichen Gatten, Sohnes, Bruders, Schwiegersohnes, Schwagers und Onkels, des Herrn

Enea Lanfranconi,

Ingenieur, Bauunternehmer, Mitglied des großen technischen Rathes im k. u. Ackerbauministerium, Comthur des Franz Josefs-Ordens, Commandeur des serbischen Takova-Ordens und Ritter des k. rumänischen Kronen-Ordens 2c.,

welcher Samstag, den 9. März l. J., Früh 7 Uhr, nach kurzem Leiden, im 45. Lebensjahre, selig in dem Herrn entschlafen ist.

Die irdische Hülle des theueren Verblichenen wird Montag, den 11. März l. J., Nachmittag 4 Uhr, in der St. Andreas-Friedhofskapelle feierlich eingesegnet und sodann im Friedhofe daselbst zeitweilig beigesetzt.

Die heil. Seelenmessen werden Donnerstag, den 14. März l. J., Vormittag 10 Uhr, in der Kirche der W. E. P. P. Franziskaner gelesen.

Preßburg, den 9. März 1895.

Clodi Lanfranconi, geb. **Bogen**, als Gattin. **Marie Lanfranconi** geb. **de Romeri**, als Mutter. **Romeo Lanfranconi**, **Luigi Lanfranconi**, als Brüder. Ober-Ingenieur **Albert Bogen**, **Klotilde Bogen**, geb. **v. Hinleder**, als Schwiegereltern. **Emilie Lanfranconi** geb. **Schoch**, **Eugenie Ocskay v. Ocsko** geb. **Bogen**, als Schwägerinnen. **Stefan Ocskay v. Ocsko**, als Schwager. **Theodor** und **Hilda Edl**, **Stefan** und **Ladislaus Ocskay v. Ocsko**, als Neffen und Nichte.

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Das Leichenbegängniß findet durch die „Erste Preßburger Leichenbestattungs-Anstalt" statt.

Fig. 2: Funeral notice of Enea G. Lanfranconi (Pressburger Zeitung 132 (68), 10.03.1895: 5).

over the Danube in Bratislava bear his name, although the first letter "n" has been omitted, and so the bridge, as well as the campus, are called "Lafranconi", instead of "Lanfranconi".

The personality of Lanfranconi and his work in every field of his interests were very special. He proved that he was able to develop the environment in which he lived and to support the local people by honest work, effort, and dedication to his profession.

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Jana Magdaléna Májeková
Institute of History
Slovak Academy of Sciences
Bratislava, Slovak Republic
janka.majekova@gmail.com

THE APPLICATION OF NEURONAL NETWORKS TO STUDY THE TOXICOLOGICAL EFFECTS OF DIFFERENT COMPOUNDS

Evgeni Kirazov, Ludmil Kirazov

Keywords: *neuronal networks, amyloid β-peptides, microelectrode arrays, Alzheimer's disease*

Abstract: *Microelectrode arrays (MEAs) are devices that contain multiple (tens to thousands) microelectrodes through which neural signals are obtained, essentially serving as neural interfaces that connect neurons to electronic circuitry. MEAs have been applied to explore the pharmacological and toxicological effects of numerous compounds on spontaneous activity of neuronal and cardiac cell networks. The MEA system enables simultaneous extracellular recordings from multiple sites in the network in real time, thereby providing a robust measure of network activity.*

During the early 1970s, the young neurobiologists Gunther Gross and Dieter Weiss, who worked under the guidance of the renowned neuropathologist Georg Kreuzberg at the Max-Planck-Institute for Psychiatry in Martinsried, Munich, shared the idea of "eavesdropping" on the "conversations" between cultured neuronal cells. However, they lacked the appropriate "spyware" for this purpose.

In 1979 Gross reported the creation of the predecessor of the contemporary MEAs (Gross 1979: 273 – 279). In 1985 the scientist published an article (Gross et al. 1985: 243 – 252) where he described the newly created MEA, with transparent electrodes from indium tin oxide on a 5x5 cm glass plate. Monolayer tissue cultures are grown on MEA from dissociated tissues that are prepared from certain areas of the brain or the spinal cord of 14 – 15-day-old mouse embryos (Fig. 1).

The spatiotemporal pattern of electrical activity provides important information regarding network structure and function, which is difficult or impossible to obtain using other electrophysiological techniques. The electrical activity of individual neurons is recorded on the tip of an electrode and is fed to its terminus at the periphery of the chip and then, through an external amplifier, into a computer with specialized software.

Around the end of the first week after seeding, the cultures develop spontaneous electrical activity, which is stabilized around the third week. Each

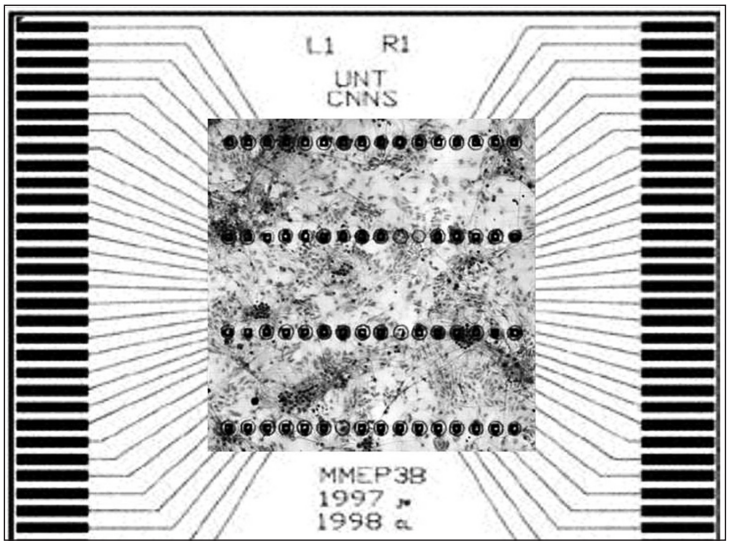

Fig. 1: Microelectrode array with neuronal network cultured on the microelectrodes.

tissue culture, which is a network of interconnected neurons, acquires characteristic electrical activity, which is considered native. This native activity is used as a basis for comparison in experimental treatments of the cultures with various agents.

Up to four different single neuron activities can be distinguished from the electrical activity derived from one electrode. Action potentials (impulses), also called spikes, are integrated according to certain criteria, such as frequency, maximum and minimum interval between single spikes, etc., resulting in defined groups of pulses termed bursts. These two parameters – spikes and bursts – characterize the electrical activity of every culture.

The neurotoxin trimethyl-tin (TMT) is one of the first substances whose effect was investigated on neurons cultured on MEA, as it is known that TMT causes acute changes in the function of nerve cells, which directly affects the transmission of signals (signal transduction) between the neurons (Gramowski et al. 2000: 331 – 341). It can be seen that the cells begin to react at low concentrations (2 – 3 μM), and at 4 μM one signal disappears completely. Removing TMT from the culture by medium change results in recovery of the native activity (Fig. 2). When the amplitude and the form of the action potential remain unchanged upon treatment with a particular agent, it can be concluded that the metabolism of the cell is not impaired, and the effect is directly on the synapse. No morphological changes in the cells have been observed at these concentrations. At higher concentrations (50 – 100 mM), there was total inhibition of electrical activity, which cannot be restored with

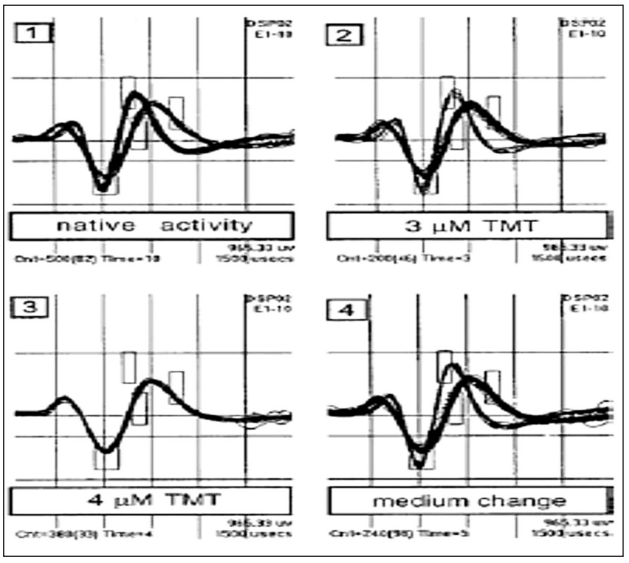

Fig. 2: Reversible inhibition of electrical activity by TMT.

repeated medium changes. Such concentrations of TMT are in the range of concentrations that are lethal to mice when administered *in vivo*. This and many other examples demonstrate the comparability of the results obtained *in vitro* with MEA to those obtained *in vivo*.

This experimental model proved to be very suitable for testing the toxicity of various substances, as it provides information about the chemical effects leading to disruption of the function of nerve cells, which allows revealing the mechanisms of action of a particular compound.

Employing the MEA model system, we investigated the effect of the amyloid β-peptides on the electrical activity of neuronal networks cultured on MEA. The amyloid β-peptides are aberrant metabolic products of the amyloid precursor protein (APP) and are involved in the etiology of Alzheimer's disease. There are various assumptions about the mechanism of their toxicity. There are numerous publications that describe different effects of amyloid β-peptides on neuronal cells. Some of the major ones are: inhibition of cell adhesion in cultures; inhibition of the outgrowth of neurites; disruption of calcium homeostasis; inducing oxidative stress; causing abnormal phosphorylation of the τ-protein; initiation of apoptotic mechanisms; participation in inflammatory mechanisms; effects on electrophysiological processes, etc.

In our initial experiments with the peptide, which is the carrier of the biological activity, consisting of the amino acid sequence of Aβ25 – 35 (Kirazov et al. 2002: 103 – 108), we found a concentration-dependent inhibition of the electrical activity of cultured neuronal networks (Fig. 3).

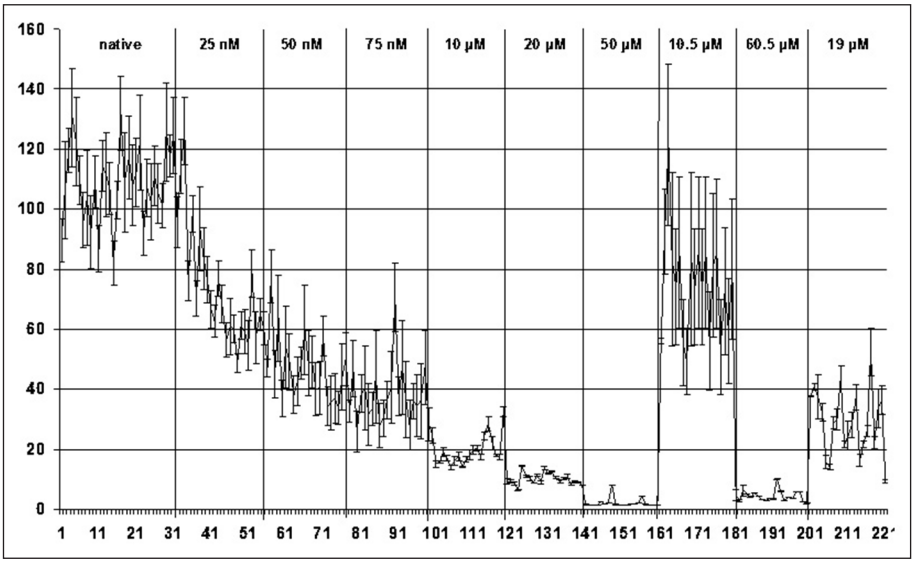

Fig. 3: Effect of Aβ25 – 35 on the spike rate of a spinal cord network.

The effect is rapid, taking place in the first three to five minutes after the addition of the peptide, and reversible. Upon lowering of the concentration of the peptide by medium changes, the electrical activity of the neurons recovers.

Treatment with Aβ25 – 35 influences neither the amplitude nor the shape of action potentials, which indicates that the energy metabolism of neurons is not affected.

In summary, the effects of Aβ25 – 35 on the electrical activity of the neuronal networks are as follows: fast – 3 – 5 min after addition of the peptide; concentration-dependent; reversible; not influencing the amplitude or the shape of the action potentials; Central Nervous System region-specific.

Having established the inhibitory effect of Aβ25 – 35, we naturally had to think about the mechanism of action of this peptide. One of the most frequently reported effects of Aβ-peptides is the induction of oxidative stress. Therefore, we compared the effect of Aβ25 – 35 with that of a well-known agent causing peroxidation of membrane lipids – divalent iron ions (Fe2+) (Kirazov et al. 2002: 91 – 94).

We found that in comparison to the effect of the amyloid peptide, the effects of Fe2+ ions were slower (90 min after addition) and irreversible (Fig. 4). On the basis of these results, we can suggest that, unlike the non-specific general toxic effect of lipid peroxidation, the effect of Aβ has a specific site of action.

This conclusion has been further supported by additional experiments, in which the effects of Aβ25 – 35 on the redox activity and viability of cultured

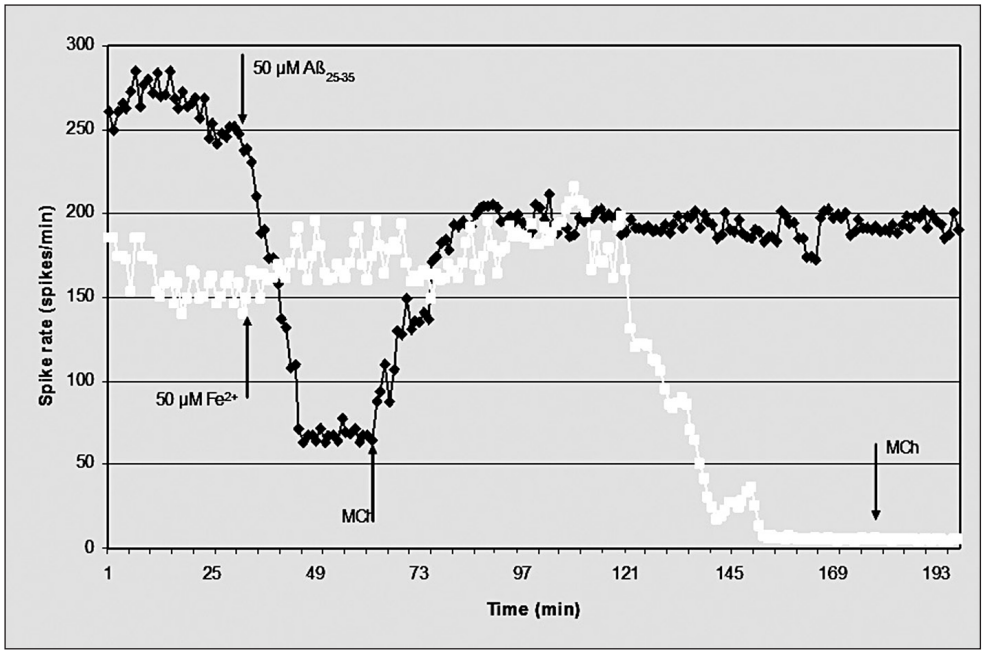

Fig. 4: Differential effects of Aβ25 – 35 and Fe2+ ions on the spike rate of cultured neuronal networks.

neuronal cells were compared with those of the well-known oxidants divalent iron ions and hydrogen peroxide. The effect of Aβ is always the fastest and the most prominent one. It is not affected by the antioxidants vitamin E and propyl gallate, unlike the protective effect these antioxidants exhibit against the effects of the iron ions and hydrogen peroxide. Almost identical results were obtained in studies of the effect of two Aβ-peptides with different amino acid chain length – Aβ1 – 40 and Aβ1 – 42 (Kirazov et al. 2004: 75 – 80).

Starting in 2001, colleagues from the Universities in Rostock and Leipzig have taken upon themselves the laborious task of creating a database encompassing the effects of numerous neuroactive substances, with a known mechanism of action, on neuronal networks cultured on MEA. They record about 100 characteristics of the effects of these substances on the electrical activity of cultured neuronal networks. In this way, they create a kind of pharmacological "fingerprint" of each substance. These features are introduced in the database, which is then used to compare the effects of unknown substances with those of well-known and characterized ones. This provides directions to search for the mechanism of action of the unknown substance and significantly shortens the period of experimental search (Gramowski et al. 2006: 455 – 465; Gramowski 2006: 410 – 415).

Using this approach, the characteristics of the effects of the Aβ-peptides investigated by us have been introduced in the database and compared with those of neuroactive substances with known mechanisms of action (Kirazov et al. 2008: 905 – 910). The observed effects are illustrated with 4 characteristics of neuronal electrical activity:

– Burst shape: the average duration of plateaus in bursts (burst plateau);
– Synchronization: the number of coordinated spikes within 1 ms (simplex);
– General activity in bursts: mean burst amplitude (burst amplitude);
– General spiking activity: mean spike rate (spike rate).

Employing the data analyzing tool for pattern recognition – Pattern Expert, we compared the influence of four "fingerprinted" compounds to the effects of Aβ-peptides. The compounds were as follows: baclofen (a direct agonist of GABAB receptors), levetiracetam (opposes the activity of negative modulators of GABA and glycine-gated currents and partially inhibits N-type calcium currents in neuronal cells), fentanyl (a strong agonist at μ- and kappa-opiate receptors), and diazepam (a GABAA receptor agonist). The effects of the first three compounds on cultured neuronal networks are shown on Fig. 5.

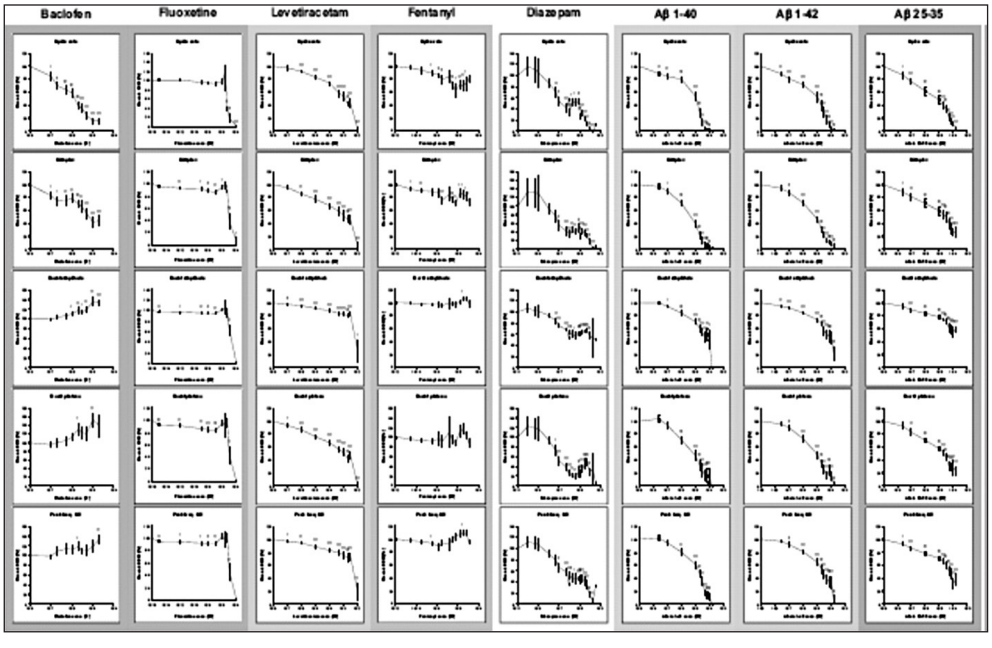

Fig. 5: Selected spike train parameters for 5 compounds and the 3 Aβ peptides.

Pattern recognition and classification experiments indicate a similarity of the effects of Aβ-peptides to those of GABAA receptor agonists such as diazepam, clonazepam, and propofol (Fig. 6). For example, we can observe a course of cessation which is known from GABAA agonists. This allows us to suggest the involvement of at least one ion-channel receptor mechanism, as controversially discussed in literature.

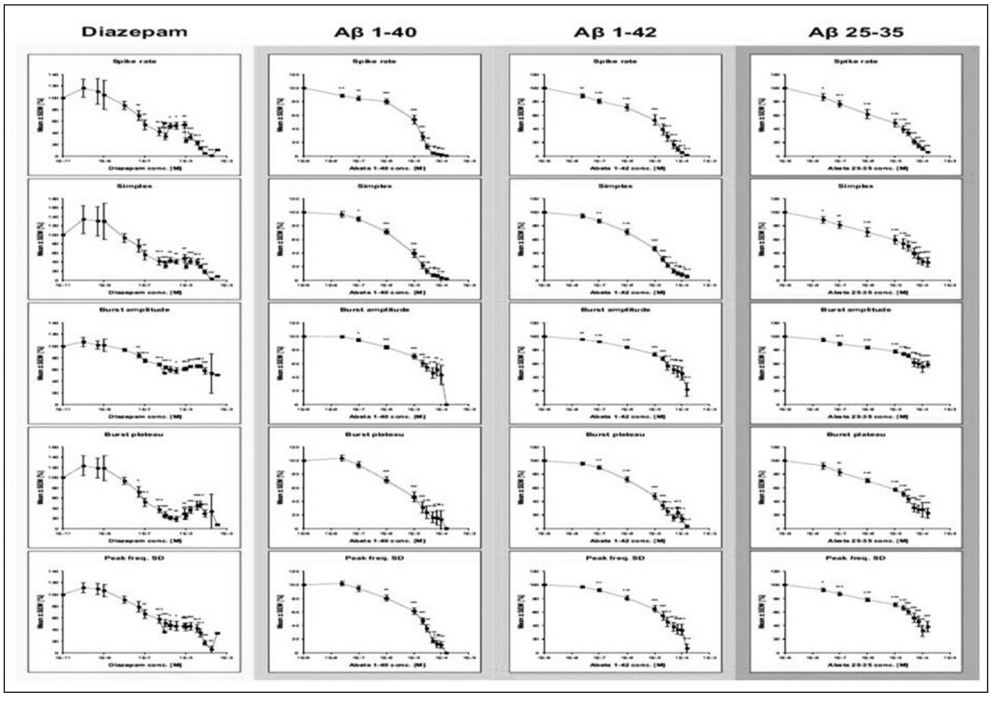

Fig. 6: Selected spike train parameters for diazepam and the 3 Aβ peptides.

These results led us to the hypothesis that the rapid neurotoxic effect of increased Aβ-peptide concentrations on the synaptic function could cause the early memory loss in Alzheimer's disease, preceding the synaptic loss and neuron degeneration. By hypothesis, this mechanism seems to be similar to that described in literature in cases of amnesia caused by benzodiazepine treatment.

At present, modern MEA systems have different applications. There are numerous attempts for MEA chips to be used for monitoring of toxic substances in the environment – air and water. For instance, water samples could be constantly fed to MEA chips. The presence of toxic substances in the water would change immediately the electrical activity of the cells in the chip and will be reported instantly.

MEA systems offer greater flexibility in terms of biological tissue and experimental design. The electrodes of MEA can be placed flat – for monolayer

cultures, as the example discussed above. A wide variety of electrically excitable biological tissues can be placed on MEA. This includes tissue from the heart muscle, primary cultures of nervous system tissues from various regions of the central nervous system, whole tissue pieces – e.g., a slice of the hippocampus or the retina. MEA chips specialized for a particular tissue can be purchased from commercial suppliers.

MEAs may also have a three-dimensional structure, which allows the penetration of the electrodes through the outer layer of the damaged cells in tissue sections, thereby allowing the electrode to make contact with the neighbouring healthy cells. The spatial arrangement of the electrodes can be changed, e.g., by placing more electrodes in specific regions of the hippocampal slice or the retina. After use, the biological tissue can be removed from the MEA and can be used repeatedly.

The added value of this experimental model is significant. The approach to determining the toxicity is in the transition state from intensive *in vivo* testing of the toxicity of various agents on animals, to approaches based on *in vitro* screening, which also allows the processing of large numbers of samples within a short time. The requirement for a better understanding of the potential hazards of tens of thousands of substances currently used in everyday life and the need to increase the number of substances whose potential toxicity is characterized are the main driving forces behind this change. In addition, the need to reduce the time, cost and number of animals used in modern methods for testing toxicity also highlights the need for changes in the approach to hazard assessment.

Acknowledgements: The work of E. Kirazov on the effects of amyloid β-peptides on the electrical activity of cultured neuronal networks was supported by grants from the Alexander von Humboldt Foundation.

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Evgeni Kirazov
Institute of Experimental Morphology, Pathology and Anthropology
Bulgarian Academy of Sciences
Sofia, Bulgaria
ekirazov@yahoo.com

Ludmil Kirazov
Institute of Experimental Morphology, Pathology and Anthropology
Bulgarian Academy of Sciences
Sofia, Bulgaria

EYE INFECTION IN VARICELLA ZOSTER VIRUS NEW EPIDEMIC

Petja Vassileva, Yordanka Kirilova

Keywords: *varicella zoster, herpetic infection, viral inflammation, antivirals*

Abstract: *Human herpes infections are a major cause of morbidity worldwide. Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV) are frequent causes of ocular pathology. Ocular herpes is a recurrent disease, and its complications may lead to blindness. Recently, the incidence of VZV has been increasing for unknown reasons. Based on a studied series of 159 patients, we have distinguished typical signs and symptoms of VZV eye infection. The applied therapeutic approach applied depends on the clinical form and stage of the disease, and includes new anti-viral drugs (AVD), resurfacing procedures and ocular surgery: amniotic membrane transplantation (AMT), and perforating keratoplasty (PK). Common clinical observations are misdiagnosis and delayed treatment. We demonstrate a new form of VZV – chronic panuveitis with good prognosis after AVD treatment. Most of our patients were in the advanced stage, and visual impairment was a result of a cumulative effect of consecutive disease attacks. Prolonged treatment is recommended for prevention of recurrences, severe complications, and systemic involvement. Ocular herpes is a disastrous disease, causing suffering to millions of people, and representing an important economic burden.*

Introduction

The recent interest in human viral infection is due to many reasons. Some important causes are the advances in etiological studies, the development of efficient antivirals (AVD), and the growing interest in the phenomena of latent infection. Clinical studies demonstrate the leading role of viruses in general human pathology. Evidences of a severe intraocular inflammation due to a herpetic infection are published (Forghani 2000: 351 – 387). Environmental factors, misuse of antibiotics and steroids, air pollution, and unhealthy lifestyle play an important role. Viruses are one of the most exciting groups of pathogens. As highly adaptable opportunistic agents, viruses causing ocular herpetic infection have evolved in a symbiotic relation with their hosts. Major improvement after a specific anti-viral therapy makes prompt diagnosis essential.

Herpetic viruses

Human herpes virus infection is a main cause of morbidity worldwide. It is a recurrent disease, and eye complications may lead to blindness.

The ocular viral infection is caused most commonly by HSV type 1 and 2 (HSV1, HSV2) in patients under 60 years of age, while VZV affects mostly people over 60. The decline in Cell Mediated Immunity (CMI) is associated with Cytomegalovirus (CMV), and may develop in young immunocompromized patients. Epstein-Barr-Virus (EBV) is a rare cause of eye infection, mostly in immunodeficient patients (Buchbinder et al 1992: 1153 – 6). The main risk factors for the human herpetic eye disease (HED) include advancing age, immunocompromized status, or immunosuppressive therapy. An important group are patients with the acquired immune deficiency syndrome (AIDS) and congenital immunity disorders. Recent observations have drawn attention to hereditary disposition, especially to chronic VZV infection and shingles. Trauma and stressful lifestyle are considered to play a role in endogenous reactivation of HSV.

Pathogenesis of herpetic infection/sequence of events

The primary HSV infection affects the orofacial area (Fig. 1a, 1b). It is transmitted digitally by contact, such as caressing in the early childhood, and passes mostly subclinically. The virus enters the mucous membrane of the host, and its particles move along the nerve fibers. HSV latency establishes in the trigeminal ganglion. Endogenous neuronal viral recurrences may develop later in life (Fatahzadeh 2007: 737 – 763). A relative immunity against superinfection exists in healthy epithelial surfaces. As long as the eye remains non-irritated and the epithelial surface – intact (no rubbing or trauma), there is efficient protection, and no superinfection is possible. After the so-called "signal" (usually stress-induced), about 3 days are needed to overcome virus latency control, and an endogenous recurrence may occur. Fortunately, only few people, harbouring HSV in the ganglia, will experience the recurrent disease (Gaynor et al 2000: 85 – 109).

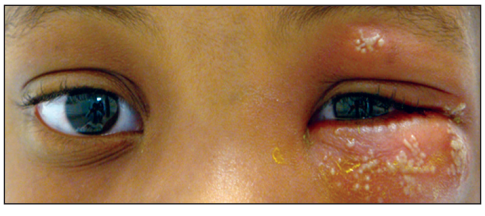


Fig. 1a, 1b: HSF on lips and eues.

There are many studies on the most important pathophysiologic characteristics of HSV. Virus particles have the ability to gain access to the peripheral sensory nerve, travelling with the endoneuronal plasma flow. They reach and hide in the nuclei of the ganglion cells, and a nuclear replication may follow. Virus particles are stopped by immune defense and retreat into its ganglion cells nuclei. Here, they disappear as "nake" virus DNA attached to the host cell DNA in a status of a neuronal latency. For a peripheral disease to occur, at "signals", viral DNA starts to replicate complete viruses, which are retransported to the site of primary infection, and various forms of the peripheral herpes disease develop.

Risk factors

Pathological stress is the most common and generally accepted cause of the HSV recurrence. It is considered that about one third of the population is at risk. Stress is varied, and even patients themselves cannot define it. Important sources of stress include fever, emotional shock, exhausting situations, sunburn, and jet-lag. The "signals" are transmitted through the blood/lymph circulation, but their biochemical nature is still unknown. There is also no molecular explanation of the individual resistance to recurrent infection yet.

There are many similarities and differences between most common human herpetic infections caused by HSV and VZV. The differential diagnosis is a very important issue. Transmission of the diseases differs – HSV infection is acquired in childhood and has lifetime latency. Almost all children are infected with VZV during varicella, but for shingles to develop, superinfection plays an additional role. Therefore, it is very important for adults to avoid contact with infected children. On the other hand, adults with shingles can infect people who have not been sick with varicella.

Eye complications

There are various challenges of the herpetic eye disease: it is a recurrent disease which affects the whole eye and may lead to severe complications.

Classification is very important in such a chronic disease. It has two main stages: a herpetic corneal stage with an active viral replication and metaherpetic eye disease – non-healing disorders after the viral corneal disease. Typical for the viral stage are blepharitis, conjunctivitis, keratitis (superficial and deep), trabeculitis, iritis, and scleritis (Fig. 2a, 2b). During the second, metaherpetic stage, a superficial bullous keratopathy usually develops, and it can progress to deep ulcers with a corneal perforation.

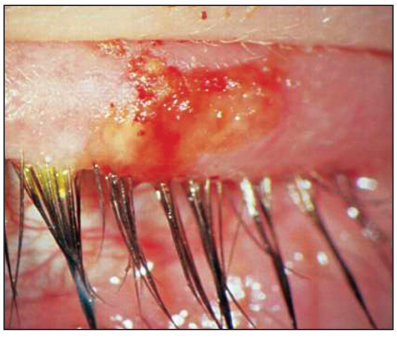


Fig. 2a, 2b: Child with herpetic blepharitis.

Differential diagnosis HSV/ VZV

The treatment approaches to HSV and VZV are quite different, mostly because topical AVD treatment is not efficient for VZV. Mainstream therapy involves systemic application of different purine nucleoside analogues acting by interfering viral DNA synthesis during transcription of the viral genome. These drugs inhibit virus replication and are recommended as mandatory as early as possible after the initial symptoms and signs of the disease. They are included in a continuous combined treatment depending on the form and stage of the disease (Table 1). HSV1 and HSV2 affect only orofacial, ocular, and genital

Group	Subgroup	Therapy
I	A viral superficial (epithelial)	Antiviral agents
	B viral deep	Antiviral agents plus steroids*
II	C metaherpetic superficial (epithelial)	Conservative resurfacing
	D metaherpetic deep	Surgical reconstruction

Table 1. Therapy Subgroups According to Classification.

areas, while VZV has various manifestations in the eye and the whole body, including internal organs (liver, kidney, brain) and can endanger life. HSV has a course with attacks and local activation, but VZV takes a chronic course with exacerbations, viremia, and superinfection (Wilson et al 1992: 119 – 126). Latency in HSV is established in the sensory ganglion of the face and genitalia, and that in VZV – in the dorsal and sensory ganglia of the whole body.

New epidemic of VZV

VZV is a DNA virus with one serotype. It infects only people, and humans are its natural reservoir. It is endemic in all human communities, from primitive tribes to megapolicies. VZV is one of the oldest known diseases and has coexisted with humans for over a million years. Nearly 25 centuries ago, Hipocrates called its most common form – intercostal shingles – "belt of fire".

In 2016 in the USA, 1 000 000 new cases of VZV were registered, with about 4 000 000 patients in treatment. Two years later, during winter 2018/19, adults diagnosed with shingles in Sofia, Bulgaria, reached about 30 people per day. It is believed that 1 in 3 persons will have VZV in their lifetime. The most threatened are people over 75 – 80 years of age. Recent data have demonstrated that for unknown reasons there has been a rapid increase in VZV patients of younger age (30 – 40 years old!).

VZV cannot be destroyed, and the only option for treatment is suppression of its replication with AVD. The group of purine nucleoside analogues includes: Aciclovir, Ganciclovir, Valaciclovir, and Valganciclovir – for oral, intravenous, and intraocular application.

Impaired quality of life with VZV infection

VZV complications cause a decreased visual acuity, resulting in a poor quality of life. Numerous disabilities of different kind can be observed. Physical problems include pain, vomiting, fatigue, weight loss, and insomnia. Some people may experience anxiety or depression. Social isolation and limited communication are very common. People suffer from impaired everyday function, including hygiene, travel, shopping, cooking, etc.

Most adults become infected with VZV by the age of 40. The clinical picture is varied as the whole body is affected. Eye diagnosis is often delayed because of atypical manifestation of intraocular inflammation (Vrcek et al 2017: 21 – 26). Very challenging is the fact that treatment is the most effective if it starts within 72 hours after the first symptoms occur.

VZV disease scenarios – clinical experience

In case of a VZV disease, several scenarios may develop. Congenital uveitis is very rare. Usually, the eye involvement occurs during chickenpox in childhood or right after, affecting conjunctiva, eyelids, and sclera. Rarely, an optic neuritis with a good prognosis is observed. Years later, shingles may develop with headache, fever, paraestesia, and skin lesions. This attack is a result of an activation of the latent infection (Forghani 2000: 351 – 382). For ophthalmologists, the most important is the involvement of the ophthalmic nerve in the trigeminal ganglion – Herpes Zoster Ophthalmicus.

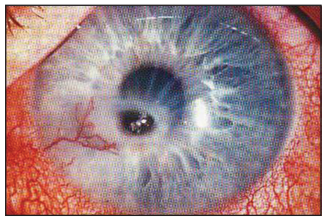
The most mysterious and difficult to diagnose is the ocular involvement without signs of shingles – *herpes sine herpete*, with only data of varicella history, usually at adult age.

Patients with delayed diagnosis and at an advanced stage of the herpetic disease are a majority – above 70 % (Fig. 3). In our retrospective review of medical records of 159 consecutive patients with VZV eye infections over a period of two years, we studied the clinical characteristics of the herpetic eye disease. We demonstrated that the herpetic infection recurrencies consisted of viral replication, chronic pathologic immune response, trophic disturbancies, and, in some patients, a secondary infection. We considered the following signs as a pathognomonic for the herpetic disease: an unilateral involvement (in about 90 % of the cases), a chronic course with exacerbations, an increased intraocular pressure in 65 % (Fig. 4), a sectoral iris atrophy (Fig. 5), and a secondary sight-threatening glaucoma.

We faced a lot of challenges during the management of such advanced pathology. The epithelial and deep stromal keratitis should be treated urgently, as a corneal perforation may occur, necessitating corneal transplantation in order to save sight (Fig. 6a, 6b). Another dangerous complication is the development of uveitis, often associated with superinfection, evidence of vitritis and vasculatis, with predominantly affected arterial vessels. We present a new form of VZV – chronic panuveitis with good prognosis as a result of AVD treatment (Fig. 7a, 7b).

The diagnosis of the herpetic eye disease is mainly clinical and is one of the most unrecognized. An important feature is the significant increase of anti-VZV IgG during the reactivation stage.

Severe complications often occur after use of steroids. Our therapeutic approach depends on the clinical form and stage of the disease (Василева 2013: 40 – 47) and includes new anti-viral drugs, resurfacing methods – AMT (Fig. 8), and surgery – PK (Fig. 9).



3. Fig. 3: Advanced stage of ulcerating interstitial HSV keratitis.

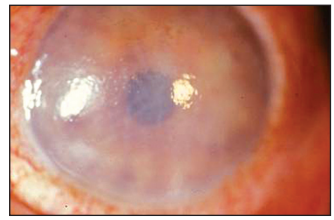
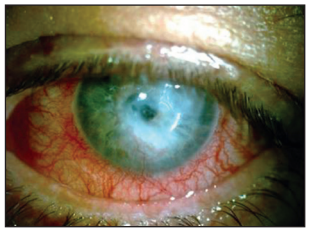


Fig. 4: Hypertensive herpetic keratouveitis.

Fig. 5: Sectoral atrophy of Iris stroma in VZV.

a) 

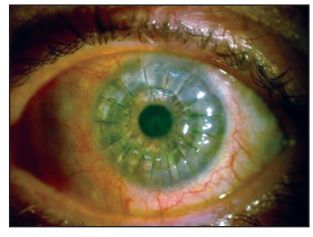
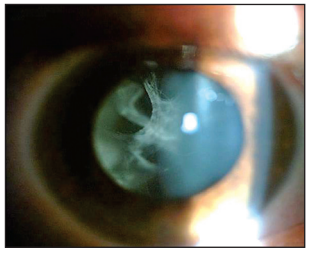
b) 

Fig. 6: Central corneal perforation after longterm Herpes Zoster Ophthalmicus (a), successful treatment with Penetrating Keratoplasty (PK a chaud) (b).

a) 

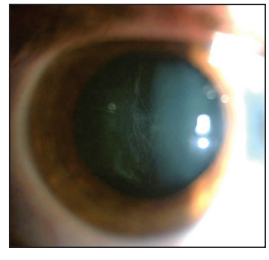
b) 

Fig. 7: Chronic panuveitis (VOD= 0,02 (a), improvment post AVD treatment: VOD= 0,6 (b).

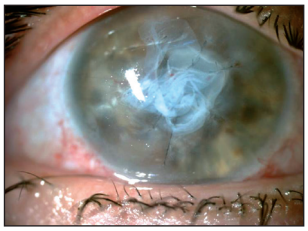


Fig. 8: AMT in central corneal perforation.

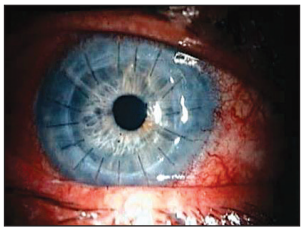


Fig. 9: Saving postherpetic keratitis with PK.

There is no consensus on the benefit of vaccination against VZV. Decreased immunity against VZV occurs with aging. A live vaccine was approved by FDA in 2011, but it is still not widely used (Oxman et al 2005: 2271 – 2284).

Conclusions

The human eye is an important target for infection of herpetic viruses. Diagnosis is often overlooked. There are still controversies regarding current therapy. Most data are still experience-based, not evidence-based (Herpetic Eye Disease Study Group (2001) Cornea; 20 123 – 128).

It is very important to differentiate between HSV and VZV. The attack is caused by different triggers: stress for HSV, and decreased immunity for VZV. Treatment is also different, as topical medications are not efficient for VZV.

VZV causes pain and suffering to millions of people worldwide each year. As of now, it is impossible to predict if or when VZV will reactivate, who may develop shingles, how severe an individual case may be, and how long the pain may last. It is important to know that almost all adults aged over 50 are at risk for this often debilitating disease, and the risk increases with age. Latency control is an extremely important issue, but it is still not elucidated, and there has been no progress in this field. Some studies point to the role of interferone gamma in maintaining latency. It has been suggested that a release of interleukins during systemic infection can activate VZV (Forghani 1986: 267 – 284, Kriesel JD 2002)

Early and correct diagnosis and timely treatment are the most important for managing this potentially sight-threatening disease. At present, we have at our disposal efficient antiherpetic medications, and vaccination seems promising to prevent or modify the course of VZV infection. Systemic application of AVD during follow-up, depending on the disease activity, reduces the incidence of recurrencies and improves patients' life.

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Petja Vassileva
Specialized Eye Hospital
Medical University
Sofia, Bulgaria
centersight@sobalpashev.com

Yordanka Kirilova
Specialized Eye Hospital
Medical University
Sofia, Bulgaria

IN VITRO EVALUATION OF AVPI-PEPTIDES AS ANTICANCER AGENTS

Maya G. Georgieva and Tamara I. Pajpanova*

Keywords: *anti-cancer agents, AVPI-peptidomimetics, MTT assay*

Abstract: *The programmed cell death, or apoptosis, regulates the elimination of unhealthy and unnecessary cells without formation of undesired by-products. The apoptotic dysfunction is linked to various types of cancer. In particular, the suppression of apoptosis is associated with enhanced survival of cancer cells. In this context, inhibitor-of-apoptosis proteins (IAPs) play an essential role in regulating cell death by direct inhibition of caspases or modulation of different receptor signalling pathways. The activity and levels of IAPs are regulated by the second mitochondria-derived activator of caspases (Smac) via its N-terminal tetrapeptide, Ala^1-Val^2-Pro^3-Ile^4 (AVPI). Therefore, the inhibition of the X-chromosome-linked IAP-baculoviral IAP repeat 3 motif (XIAP-BIR3) using Smac peptides is a promising therapeutic approach in cancer therapy. In the present work, we have examined a series of newly synthesized AVPI and AVPI-RGD peptidomimetics as potential anti-cancer agents. In the course of our investigations, we have performed in vitro screening of selected AVPI mimetics, using different cell lines.*

The programmed cell death, or apoptosis, plays a crucial role in regulating cell number and tissue homeostasis (Scharma et al. 2006; Mannhold et al. 2010). Apoptosis represents a complex sequence of events leading to the elimination of unnecessary or damaged cells from normal tissues without formation of undesired or toxic substances in the surrounding milieu (Mannhold et al. 2010; Lockshin et al. 2007). There is a wide range of stimuli that trigger apoptosis within normal and pathophysiological conditions, including receptor agonists, DNA damaging agents, reactive oxygen species (ROS), growth factors, pro-cytokines, accumulation of misfolded ERPs (endoplasmic reticulum proteins), and others (Georgieva et al. 2013: 147). Two pathways of apoptosis are well-known, extrinsic and intrinsic, both leading to the activation of caspases (**c**ysteine-**a**spartate **s**pecific **p**rote**ases**) – a family of protease enzymes that essentially contribute to apoptosis and inflammation (Georgieva et al. 2013: 147).

Many human diseases are linked to dysregulated cell death (Mannhold et al. 2010). While excessive apoptosis is mainly associated with strokes and neurodegenerative disorders, insufficient apoptotic rates are among the main hallmarks of cancer (Mannhold et al. 2010; Lockshin et al. 2007). Inhibition of

apoptosis is linked to the enhanced survival rates of cancer cells and therefore aggravates the cytotoxic therapies (Lowe et al. 2004). In this context, inhibitor-of-apoptosis proteins (IAPs) are major contributors that suppress cell death by interacting with several modulators of apoptosis through different receptor signaling pathways and inhibiting the activity of caspases (Mannhold et al. 2010; Wu et al. 2000). In regards to their regulatory function, IAPs are strong modulators of receptor signaling pathways including transcription nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and tumour-necrosis factor (TNF) (Mannhold et al. 2010). The human IAP family comprises eight functionally and structurally related proteins, all of them containing at least one baculoviral IAP repeat (BIR) motif, while four possess three BIRs (Wu et al. 2000). The eight IAPs differ in their amino acid size, ranking from 102 (Survivin) to 4845 (Bruce) amino acids (Mannhold et al. 2010). The human IAPs, including X-chromosome-linked IAP (X-IAP), cellular IAP1 and IAP2 (cIAP1 and cIAP2), neuronal apoptosis inhibitory protein (NAIP), Survivin, Bruce, Livin, and testis-specific IAP (Ts-IAP), are presented in Fig. 1.

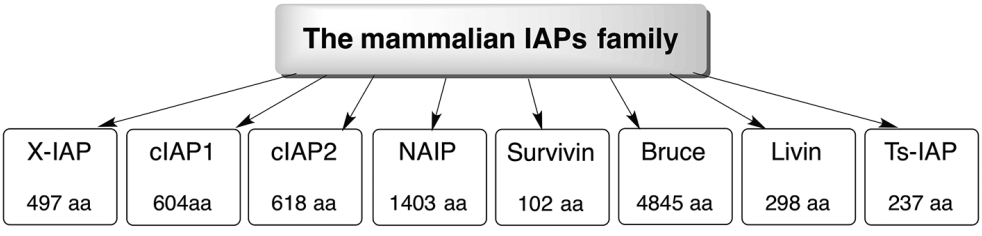

Fig. 1: Representation of the eight human IAPs.

The activity and levels of IAPs are regulated by the second mitochondria-derived activator of caspases (Smac) through the Smac *N*-terminal tetrapeptide, Ala^{1}-Val^{2}-Pro^{3}-Ile^{4} (AVPI) (Mannhold et al. 2010; Wu et al. 2000; Wang et al. 2011; Sun et al. 2008; Balakrishnan et al. 2016). Among all IAPs, the X-chromosome-linked IAP (XIAP) is a well-characterized member of the IAP family, being the most effective toward caspases (Sharma et al. 2006). While the second BIR domain (BIR2) as well as the linker between BIR1 and BIR2 is a potent inhibitor of caspase – 3 and – 7, the third BIR domain (BIR3) of XIAP (XIAP-BIR3) interacts with the active caspase – 9 (Sharma et al. 2006; Balakrishnan et al. 2016; Takahashi et al. 1998; Deveraux et al. 1999). Therefore, the inhibition of XIAP-BIR3 using Smac peptides mimicking the AVPI structure (AVPI peptidomimetics) and nonpeptide small molecules is considered as a promising therapeutic approach in cancer therapy (Mannhold et al. 2010; Balakrishnan et al. 2016). Structural details of AVPI, including

its amino acid sequence, are shown in Fig. 2A, while its structure-activity relationships (SARs) and pharmacophore model (Sharma et al. 2006; Sun et al. 2008) are depicted in Fig. 2B. The hydrophobic regions are indicated in light grey, whereas the hydrophilic part is showed in dark grey (Sharma et al. 2006; Sun et al. 2008) (Fig. 2B). The Smac core motif, bound to the BIR3 domain of XIAP, is shown in Fig 2C (Protein Data Bank code: 2OPY). The green dashed lines represent the hydrogen-bond interactions within the BIR3 active site. The hydrophobic surface area of the BIR3 domain is indicated in yellowish colour, whilst the hydrophilic part is presented in blue. The amino acid sequence of the tetrapeptide AVPI follows the same orientation as indicated for its 2D structure (cf. Fig. 2A). Structures of selected monovalent non-peptidic Smac mimetics and their binding affinities to the XIAP-BIR3 domain (Wang et al. 2011) are represented in Fig. 2D. Their binding affinities comply with the estimated SARs for small-molecule-based AVPI mimetics (Mannhold et al. 2010).

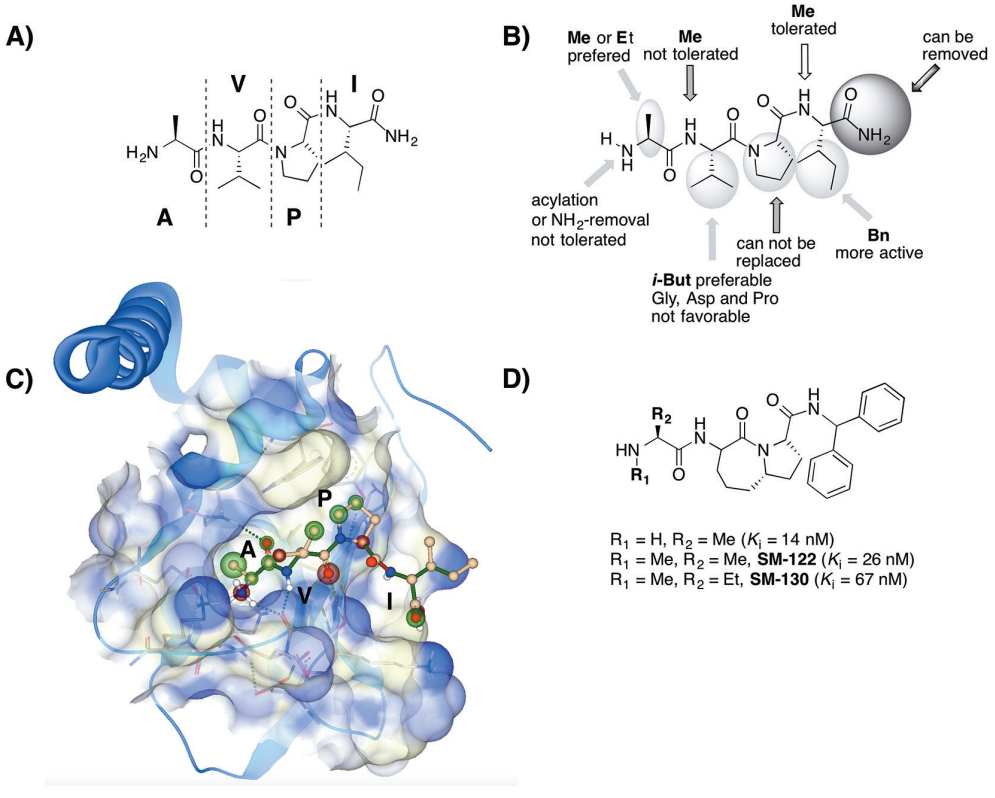

Fig. 2: Amino acid sequence and docking model of Ala-Val-Pro-Ile (AVPI) as well as structures of selected Smac mimetics.

In the present work, we focus on investigation of the anti-proliferative effects of a series of newly synthesized AVPI peptidomimetics and AVPI-RGD peptidomimetic conjugates (double modified hybrids), alone or as a combination of their single subunits as anti-cancer agents. Using an Fmoc synthetic strategy, facile structural modifications of the AVPI key core structure were performed to replace proline (Pro) by a hydroxyproline (HyP) amino acid, followed by a conjugation with RGD (Arg-Gly-Asp sequence) analogues. As a result, a series of AVPI mimetics and their conjugated hybrids with RGD analogues were obtained. The performed structural modification towards double-modified AVPI-RGD and AVPI-CavGD conjugates (hybrids) and single-modified AVPI peptidomimetics are shown in Fig. 3.

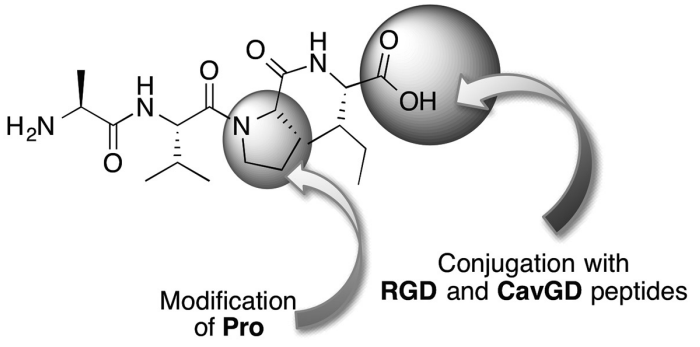

Fig. 3: Intended structural modifications towards AVPI-RGD and AVPI-CavGD conjugates and AVPI peptidomimetics.

To evaluate the anti-proliferative effects of these new peptidomimetic structures, we performed an extensive *in vitro* screening, using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction assay as an experimental technique (Lobner 2000). In this assay, the tetrazolium salt (MTT, yellow colour) is reduced in viable cells to an insoluble formazan structure (purple), which is used for spectrophotometric quantification of the obtained effects (Lobner 2000). The experiments were conducted by determination of the cellular viability (in %) in four human cell lines – namely, epithelial breast cancer cell line (MDA-MB-231 cell line), breast cancer cell line (Michigan Cancer Foundation-7, MCF-7), hepatocellular carcinoma cells (HepG-2 cell line), and colon cancer cells (HT-29 cell line). The cellular viability of all cell lines was estimated after 72 h incubation period at concentrations ranking from 8.0 to 2000 μM of the respective AVPI-RGD parent conjugate or its peptidomimetics, alone or as a combination of the respective modulated subunits (e.g., combinative use of AVPI and RGD analogues).

In general, the anti-proliferative profile of the tested peptidomimetics, alone or as a combination of their subunits, followed the same trend in all cell lines when compared to the control groups (considered as 100 % cell viability) at concentrations lower or equal to 250 μM (e.g., 250, 125, 62, 31, 16, and 8.0 μM). With the exception of one AVPI-RGD peptidomimetic, showing a decrease on cellular viability of approximately 45±2.0 % at 250 μM, no other representatives had noticeable anti-proliferative effects at this or lower test concentrations. Furthermore, the parent AVPI-RGD conjugate or a single modification of AVPI toward AVHyPI did not lead to significant anti-proliferative effects at the tested concentrations and cell lines. However, all double-modified hybrid structures of AVPI-RGD showed a pronounced anti-proliferation at higher concentrations of 1000 and 2000 μM in all cell lines, or even at 500 μM in MCF-7, MDA-MB-231, and HepG-2 cell lines (approximately 50±3.0 %). In addition, it is worth mentioning that all double-modified hybrids exhibited IC_{50} values ranking from 350 to 700 μM, when considered all tested cell lines. The strongest anti-proliferative activity was observed for the double-modified AVHyPI-AgpGD hybrid at concentrations of 250 (45±2.0 %), 500 (22±2.0 %), 1000 (20±1.0 %), and 2000 (12±1.0 %) μM in MCF-7 cell line, being between 2- and 6-fold more active than its parent AVPI-RGD conjugate. The anti-proliferative effect of AVHyPI-AgpGD is presented in Fig. 4. The effect was measured on a human breast cancer line (MCF-7) after 72 h exposure to different concentrations of compound (8.0–2000 μM). Untreated cells were used as positive control and the results were expressed as the mean % of untreated controls ±SD from at least three independent experiments.

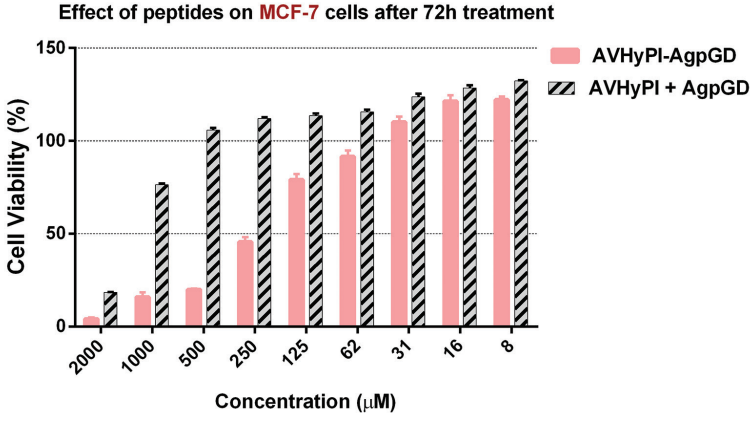

Fig. 4: Anti-proliferative effect of a double modified hybrid in comparison to its subunits used in combination.

The double-modified conjugates (hybrids) exhibit higher activity toward the MDA-MB-231 cell line (at a concentration of 500, 1000, and 2000 μM) than their parent AVPI-RGD conjugate or single-modified AVPI subunit. In the experiments with HepG-2 and HT-29 cells, the same trend could be observed at higher concentrations (e.g., 1000 and 2000 μM). As mentioned above, only one AVPI-RGD conjugate showed noticeable anti-proliferative effect against breast cancer cell line MCF-7, with the lowest effective concentration of 250 μM. Compared to the combinatorial use of the modified AVPI and RGD subunits, the tested double-modified hybrids exhibited increased anti-proliferative activity against MDA-MB-231, MCF-7, and Hep2G cells at concentrations higher than 500 μM. In the case of colon cancer cells HT-29, the conjugated peptidomimetics did not show significant anti-proliferative effects.

In conclusion, we performed an extensive *in vitro* evaluation of newly designed and synthesized single-modified AVPI and RGD analogues as well as AVPI-RGD double-modified conjugates (hybrids) as anti-cancer agents. In the course of our study, the anti-proliferative effects of all compounds were tested on four human cell lines and at different concentrations after 72 hours incubation, including their efficacy against breast, liver, and colon cancer. It could be summarized that: (1) single modifications of AVPI towards AVHyPI or AVPI-RGD itself did not lead to a significant improvement of anti-proliferative effects against all tested cell lines and at all concentrations; (2) double-modified hybrids showed significant improvement in anti-proliferative activity (between 2- and 3-fold) compared to their parent AVPI-RGD at concentrations that were equal or higher than 500 μM (or even at 250 μM for AVHyPI-AgpGD on MCF-7 cells); and (3) the double-modified hybrid AVHyPI-AgbGD showed increased activity against MDA-MB-231, MCF-7, and HepG2 cells, being 3- or even 6-fold more active compared to the combination of the single-modified subunits (e.g., AVHyPI + AgbGD). However, the effective concentration of about 1000 or even 2000 μM could be a limitation factor for further development of such compounds, when considering the effective doses needed to achieve a maximal effect against identified cancer cell lines (e.g., MCF-7 or MDA-MB-231 cells) without observation of side effects. Therefore, further structural modifications at different or the same positions of AVPI-RGD, using shorter amino acids and/or conjugates with improved physicochemical behaviour (e.g., stability and solubility), need to be performed and investigated for their cytotoxic activity. In this context, a next series of differently substituted AVPI analogues with extended amino acid size or other modified substituents is under exploration.

Acknowledgments: *This study was supported by the National Program "Young Scientists and Postdoctoral Students", Module "Young Scientists", 2019.*

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Maya G. Georgieva
The Roumen Tsanev Institute of Molecular Biology
Bulgarian Academy of Sciences
Sofia, Bulgaria
mgeorgieva@bio21.bas.bg; m.geo@abv.bg

Tamara I. Pajpanova
The Roumen Tsanev Institute of Molecular Biology
Bulgarian Academy of Sciences
Sofia, Bulgaria
tamara@bio21.bas.bg

LACK OF ACUTE *IN VIVO* TOXICITY OF ETHYL ACETATE EXTRACT FROM AERIAL PARTS OF *GEUM URBANUM* L. ON INTESTINAL EPITHELIAL TISSUE AND PEYER'S PATCHES

Lyudmila Dimitrova, Stanislav Philipov, Maya Zaharieva, Lilia Tserovska, Galina Zhelezova, Milena Popova, Vassya Bankova, Hristo Najdenski

Keywords: *Ethyl acetate extract of Geum urbanum L., acute in vivo toxicity, intestinal epithelial tissue, Peyer's patches*

Abstract: *Geum urbanum L. (genus Geum, family Rosaceae) is a herbaceous perennial plant species, commonly known as wood avens or St. Benedict's herb and widespread in temperate regions. It has been used since ancient times in the European and particularly in the Bulgarian traditional medicine for treating gastro-intestinal diseases as well as for reducing bleeding and inflammation sequelae in mucous membranes. Decoctions and infusions from the aerial parts, roots, and rhizomes were recommended for treatment of diarrhea, dysentery, dyspepsia, gastroenteritis, leucorrhoea, hemorrhages, infections, and fever. Despite the long and widespread use of different G. urbanum L. preparations in traditional medicine, little is known about the toxicological profile of the plant. The toxicity of the active plant's extracts is of critical significance for their future use as food additives with favourable effects against the diseases listed above. The main objective of our study was to characterize the acute toxicity of an ethyl acetate extract obtained from the aerial parts of G. urbanum L., regarding its effects on intestinal epithelial tissue and Peyer's patches after 14-day application and considering the 3R requirements. The pathomorphological evaluation of specimens from these tissues evidenced the lack of toxic effect in biologically active concentrations. Our findings contribute to the better pharmacological characterization of the tested extract and evaluation of its potential for future development as a food additive.*

Introduction

The genus *Geum* (family *Rosaceae*) consists of about 70 plant species, widespread in the temperate regions of Europe, Asia, North and South America, Africa, and New Zealand (Cheng 2011: 203 – 222). Eight of these species also belong to the Bulgarian flora, including the medicinal plant *Geum urbanum* L.

It is a perennial herbaceous plant that can be found at altitudes between 200–1500 m above the sea level on the territory of the country (Assyov 2012: 212) and is also known as wood avens, herb Bennet, colewort, and St. Benedict's herb (Piwowarski 2014: 46 – 50). It has been used since ancient times in traditional medicine for treatment of gastrointestinal disorders, liver, biliary tract, and uterus disorders, as well as against hemorrhoids (Tita 2009: 141 – 156, Yordanov 1972: 347). Tinctures from the roots and aerial parts of *G. urbanum* L. have been applied against rheumatism, gout, infections, and fever (Vogl 2013: 750 – 771). More than 200 compounds (monoterpenoids, sesquiterpents, triterpenoids, flavonoids, hydrolysable tannins, phenylpropanoids, etc.) have been isolated from the genus *Geum* after 1920 (Cheng 2011: 203 – 222) until now. However, the chemical composition of *G. urbanum* L. regarding secondary metabolites, their biological activity, and their potential for medical use have been less studied. According to the literature data, a total of 49 compounds have been isolated until now. Among them, gemin A, gallic and ellagic acids, and epicatechin are in the largest amount (Paun 2015: S110, Znaharia 2017). Gallic and ellagic acids are the result of hydrolysis of ellagotanins in the gastrointestinal tract, and this is the reason for the beneficial effects of *G. urbanum* L. extracts on gastrointestinal disorders, while gemin A has a positive effect in gum inflammation (Znaharia 2017). In our previous study, we isolated seven individual compounds from the ethyl acetate (EtOAc) extract from roots of the plant, and their structures were elucidated by comparison of their spectral characteristics (^{1}H and ^{13}C NMR, MS) with literature data. The compounds *3-O*-methylellagic acid-*3′-O-α-3″-O*-acetylrhamnopy-ranoside and *3-O*-methylellagic acid-*3′-O-α--2″-O*-acetylrhamno-pyranoside were found for the first time in the genus *Geum*, and compounds tormentic acid, *3,3″*-di-*O*-methylellagic acid-*4-O-β-D*-glucopyranoside, and niga-ichigoside F1 were new for the species *G. urbanum* L. We investigated their antimicrobial activities against a panel of Gram-(+) and Gram-(-) bacteria, but they showed lower individual effect than the tested extracts. The EtOAc extract from aerial parts (EtOAc-AP) was the most active, with high radical-scavenging ability (Dimitrova 2017: 113). In addition, we proved for the first time its quorum sensing (QS) inhibiting effect against *Pseudomonas aeruginosa* PA01 on two QS-regulated phenotypes: swarming motility and pyocyanin production (Dimitrova 2019: 341 – 349). However, the development of this active extract as a dietary supplement requires an in-depth toxicological evaluation, which has not been done so far. The purpose of our study was to investigate the acute toxicity of the extract on intestinal epithelium tissue (IET) and Peyer's patches (PP) of H-albino mice after oral administration of an EtOAc-AP extract at increasing doses, up to ten times higher than those with antimicrobial activity.

Materials and methods

Plant material. As described before, the dry roots and aerial parts of *G. urbanum* L. used in this study were commercial products purchased from Sunny-Yambol, Ltd® in April 2014 (Dimitrova 2017: 113). The extraction was performed immediately after the delivery of the products.

Preparation of extracts. The extract was prepared as described previously (Dimitrova 2017: 113). Briefly, 500 g aerial parts of *G. urbanum* L. were macerated twice in 3 l methanol (MeOH) at room temperature. The total MeOH extract was filtered and concentrated in a vacuum evaporator. It was extracted successively with petroleum ether and EtOAc. The extract was evaporated to dry residue.

Acute toxicity test. The test was performed according to Guideline 423 of the Organization for Economic Co-operation and Development (OECD). The animal study was approved by the Institutional Ethics and Animal Care Committee (Permit No. 125/07.10.2015 – 07.10.2020). The H-albino male and female mice (age 8 – 12 weeks, weight 21 ± 2 g) were delivered by the National Breeding Centre (Slivnitza, Bulgaria) and housed in the Animal Care Facility of the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences (Permit No. 352/06.01.2012, No. 11130005). Considering the lack of previous investigations on the toxicological profile of this extract in published scientific literature, estimates for both sexes were performed in our study. The growth conditions were specified in the Guide for the care and use of laboratory animals (National Research Council, 2010). The accommodation period prior to the beginning of the experiment was 14 days at 12 h light/dark cycles and *ad libitum* access to food and water. Each animal was marked individually to allow identification during the experiment. The animals were separated in four groups, each including three male and three female mice – a control group and three groups treated orally for 14 days with 210 mg/kg (Group I), 70 mg/kg (Group II) and 20 mg/kg (Group III) of the extract. The doses were prepared *ex tempore* by sonication of the extract in distilled water (dH_2O). Animals were monitored daily for changes in behaviour and nutrition. They were euthanized on the 14th day, and the probes of IET and PP were subjected to preparation of specimens for pathomorphological evaluation.

Pathomorphological analysis. The paraffin sections prepared from the specimens were stained with Hematoxylin and Eosin (H&E) and were deparaffinized with xylene. The slides were passed through decreasing concentration of ethanol (EtOH) (100 %, 90 %, 80 %, 70 %) and rinsed in water to remove the xylene and to be hydrated. Thereafter, they were stained for 5 min with filtered solution of the nuclear stain hematoxylin (1 gm hematoxylin

in 10 ml EtOH, 20 mg ammonium alum, boiled in dH_2O and supplemented with 0.5 g of mercuric oxide). The non-specific background was removed using a weak acid alcohol (5 min, 1 % HCl in 70 % EtOH). An aqueous solution of eosin (1 g yellow eosin, 80 ml dH2O, 320 ml EtOH, 2 drops glacial acetic acid, 0.5 % HCl) was applied as further staining for 10 min and washed away with tap water (5 min). The eosin-stained slides were dehydrated with increasing concentrations of EtOH and rinsed several times with xylene in order to become completely transparent. The ready slides were covered with a thin layer of polystyrene and a glass cover slip.

Results

Pathomorphological changes on IET specimens and PP in female animals. The three treated groups did not report an increased number of lymphatic follicles compared to the control group. The changes showed a sinus response (an increased number of intrasinusoidal macrophages) at 20 and 70 mg exposure doses. The tissue macrophages formed aggregates in sinusoids (individual variation). In all study groups, we observed a lack of granulomatous lymphadenitis, large lymphoblasts or apoptotic lymphocytes, as well as a presence of a small number of corpus macrophages. Findings of plasma cells in the medullary zones were present. None of the cell populations showed extensions with infiltration into the cortical and capsule zones. A dominant population of medullary macrophages in all treated groups was visible. Their cytoplasm was filled with optically empty vesicles. Changes were reported at average exposure doses (20 and 70 mg). The treated groups of animals were characterized by a lack of complete lymphoid necrosis profile and intrasinusoidal erythrocytes (sinus erythrocytosis). Isolated signs of cellular edema and karyorexis and sinus ectasia were observed in the treated groups. Pigment deposits (hemosiderin, melanin, and ceroid/lipofuscin) were visible in the cytoplasm of sinusoidal macrophages in both control and treated animals. No dose-dependent progression of deposits was observed. The density of leukocytes on the area of the own plate (evaluation on the fields of high magnification) was below 10 % (grade 1). In individual animals, the density was up to 12 %, but without the dispersion of inflammatory cells (grade 2). Increasing the exposure dose showed similar results and low variability. Regarding the assessment of epithelial changes, no value above 25 % was present in any of the groups, and the estimate for hyperplastic changes was 1. The minimum degree of Goblet cell loss was grade 1. No cryptitis, crypt-abscesses, and erosion were reported. The evaluation of mucosal architecture showed isolated changes in the architecture of the crypts. Recurrent findings

of bifurcations and branched crypts were observed in none of the groups. Regarding the villous blunting in the treated groups, the villous-crypt ratio was most often 2:1. The ratio was higher in individual animals but did not exceed 3:1. Rating of 1 and a low (minimum) degree of villous blunting with no findings of villous atrophy were found in the groups.

Pathomorphological changes on IET specimens and PP in male animals. The changes showed a sinus response (an increased number of intrasinusoidal macrophages) at 70 mg exposure doses. A low number of tissue macrophages formed aggregates in sinusoids. Lack of granulomatous lymphadenitis in the study groups and no finding of stimulated (reactive) follicles was observed. Isolated paracortical areas with no increased size relative to the control group and isolated findings of plasma cell precursors (immunoblasts and plasmablasts) were found. Separate macrophage aggregates in the paracortical and medullary regions were detected. Changes were reported at average exposure doses (20 and 70 mg/kg) and were absent after application of the highest dose (210 mg/kg). A lack of lymphoid necrosis and no histologic evidence of scars (accumulation of chromatin, karyolysis, and eosinophilic cell debris) were reported. Vessels changes in the treatment groups, including sinus hyperemia (congestion) and sinus erythrocytosis, were observed. The lack of hemosiderin deposits was accompanied by intracytoplasmatic pigment deposits (hemosiderin, melanin, and ceroid/lipofuscin). Findings of limited deposition of amorphous, eosinophilic, and hyalinized extracellular material were present. The topography of the changes was isolated in the subcapsular sinuses with no progressive extension of deposits. Regarding the evaluation of the inflammatory changes, the estimate of the density in the treated groups relative to the control was minimal (below 10 % and grade 1). The expansion of the inflammatory infiltration included mucosal involvement (grade 1) and mucosal and submucosal layer involvement (grade 2). The assessment of the epithelial changes showed an increase in the epithelial cells in longitudinal crypts compared to the baseline number of epithelial cells in the crypt in the treated groups (variations of 14 % to 21 %). Considering the loss of Goblet Cells, the limit of 20 % was not exceeded (grade 1). A score of 0 on cryptitis, crypt-abscesses, and erosion (erosion changes) was calculated. Evaluation of the mucosal architecture revealed non-parallel crypts in each of the treated groups. Regarding the villous blunting, in the treated groups the villous-crypt ratio was most often 2:1. In the high-concentration groups, the ratio was higher in individual animals but did not exceed 3:1. A rating of 1 and a low (minimum) degree of villous blunting with no findings of villous atrophy in the groups was calculated.

Discussion

In this study, we evaluated the acute *in vivo* toxicity of an EtOAc-AP extract on the IET and PP after oral administration for 14 days in order to better characterize its pharmacological profile and *in vivo* tolerance. The results are shown of Fig. 1 and Fig. 2.

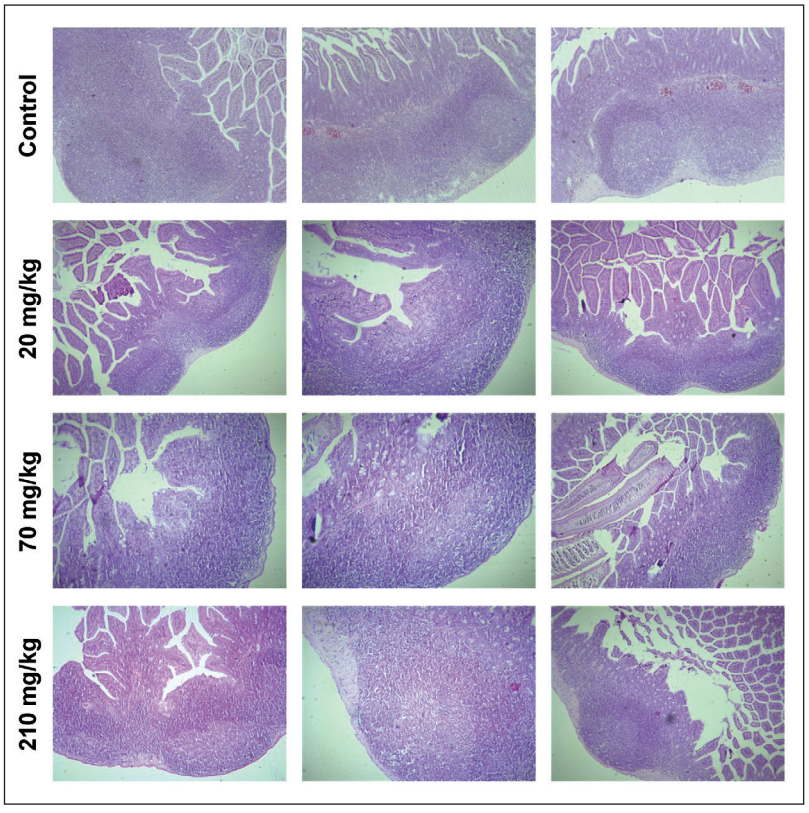

Fig. 1: Pathomorphological changes on IET specimens and PP in female animals after oral (per os) treatment with EtOAc-AP extract of G. urbanum L. Legend: The changes show a sinus response (increased number of intrasinusoidal macrophages) at 20 and 70 mg exposure doses. The tissue macrophages form aggregates in sinusoids (individual variation) wherein the dominant population of medullary macrophages with optically empty vesicles (light appearance in the slides) is visible in all treated groups. Changes are visible at average exposure doses (20 and 70 mg). Isolated signs of cellular edema and karyorexis and sinus ectasia in the treated groups are found. Pigment deposits (hemosiderin, melanin and ceroid/lipofuscin) are in the cytoplasm of sinusoidal macrophages in both control and treated animals. A lack of increased number of lymphatic follicles, granulomatous lymphadenitis, large lymphoblasts, apoptotic lymphocytes and complete lymphoid necrosis profile, as well as intrasinusoidal erythrocytes (sinus erythrocytosis) is observed.

We observed a decrease in ***intestinal lymphoid tissue lymphocytes***, associated with mucosal invasion by opportunistic microbes and/or parasites which can lead to local mucosal erosion, ulcerative defects, and the systemic spread of pathogens. Even small outbreaks of acute PP inflammation may allow microbes to enter regional mesenteric lymph nodes (MLN). The proliferation (hyperplasia) of resident macrophages may show a morphological finding with

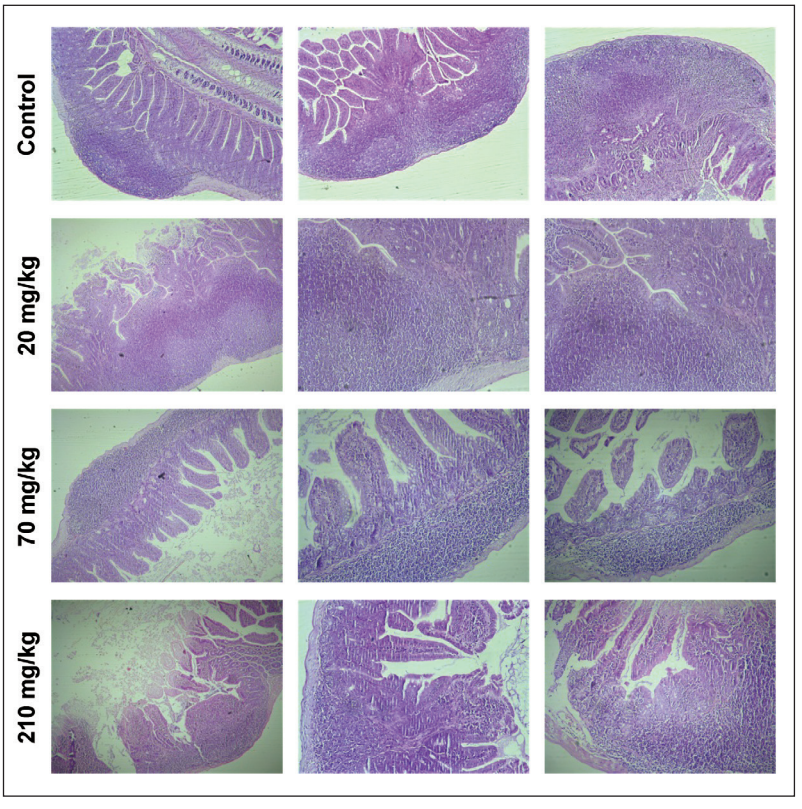

Fig. 2: Pathomorphological changes on IET specimens and PP in male animals after oral (per os) treatment with EtOAc-AP extract of G. urbanum L. Legend: The changes show a sinus response (increased number of intrasinusoidal macrophages) at 70 mg exposure dose and low number of tissue macrophages form aggregates in sinusoids. Isolated paracortical areas with no increased size relative to the control group and plasma cell precursors (immunoblasts and plasmablasts) are found. Separate macrophage aggregates are visible in the paracortical and medullary regions at average exposure doses (20 and 70 mg). Other changes include vessels changes in the treatment groups including sinus hyperemia (congestion) and sinus erythrocytosis, and intracytoplasmatic pigment deposits (hemosiderin, melanin and ceroid/lipofuscin). Findings of limited deposition of amorphous, eosinophilic and hyalinized extracellular material are also presented. A lack of granulomatous lymphadenitis, reactive follicles, lymphoid necrosis, and accumulation of chromatin, karyolysis and eosinophilic cell debris is observed. No hemosiderin deposits as well as progressive extension of deposits is found.

an increased number of intrasinusoidal macrophages entering through the efferent lymph, which drains a large number of macrophages. Comparing lesions in the LN with organs and tissues is an approach that enables providing sufficient evidence of pseudohyperplastic changes. MLN are continuously stimulated by intranodal antigens and can therefore have a large number of intrasinusoidal macrophages as well as multifocal macrophage aggregates in the cortex and paracortex. Significant individual variations in animals may exist. ***Lymphocytic hyperplasia*** includes both follicles rich in B cells and rich in T cells from the paracortex (paracortical areas) and is indicative of humoral or cell-mediated response. Lymphoid hyperplasia is usually a reactive or immune response and is not considered a preneoplastic lesion in the LN. ***Stimulated (reactive) follicles*** (secondary follicles) are usually larger than not stimulated primary follicles and have a paler staining of the germinal centre, with large lymphoblasts, an increased number of apoptotic lymphocytes, and a reduced number of corpus macrophages. ***Hyperplastic follicles*** are identified by increasing the number and size of the follicles and converting them into secondary follicles. ***Paracortical hyperplasia*** is characterized by an increase in cell density and, depending on the degree of hyperplasia, an enlargement of the paracortical area. The three treated groups in our study did not show an increased number of lymphatic follicles compared to the control group. The changes included a sinus response (increased number of intrasinusoidal macrophages) at 20 and 70 mg exposure doses. The degree of reaction did not reach granulomatous lymphadenitis in any of the study groups. There were no findings of stimulated and reactive large lymphoblasts or apoptotic lymphocytes. A small number of corpus macrophages were present. Isolated paracortical areas showed no increased size in comparison with the control group. The cell density of the treated groups remained lower than that of the control group.

Plasma cells increase their number in response to antigenic stimulation and following the production of antibodies. B-cell hyperplasia can occur concurrently with plasma cell hyperplasia. Plasma cell hyperplasia or plasmacytosis is a common finding in rodents, especially in submandibular LN. The medullary zones usually contain plasma cells and their precursors as dominant cell types, and these connections are the major sites of plasma cell hyperplasia. In the treated groups, only the presence of plasma cells was reported in the medullary zones. Plasma cell precursors (immunoblasts and plasmablasts) were isolated. None of the cell populations showed extensions with infiltration into the cortical and capsule zones.

Macrophage accumulations are usually the result of proliferation of resident sinusoidal macrophages but can also be considered as a collection

of macrophages in each region of the LN. Macrophage aggregates may be peripherally located around the paracortex (paracortical areas) or in the cortical, paracortical, and medullary regions. A dominant population of medullary macrophages was found in all treated groups. No proliferative activity of residual sinusoidal macrophages was reported in the study groups. Separate macrophage aggregates were found in the paracortical and medullary regions. Changes were reported at average exposure doses (20 and 70 mg), being absent at the highest exposure dose.

Lymphoid necrosis can be focal, multifocal or diffuse within a LN. Some differences in the manifestations and severity of lymphoid necrosis could be found between the LN of the same animal, depending on the initiator factor and the effectiveness of the immune response. Lymphocytic necrosis is characterized by cell edema, accumulation of chromatin, karyorexis or karyolysis, and in more severe lesions – by profuse eosinophilic cell debris. Necrosis is accompanied by inflammatory cells, including neutrophils and activated macrophages with intracytoplasmatic cell debris. A complete lymphoid necrosis profile was not reported in the treated groups of animals. Isolated signs of cellular edema and karyorexis were detected. The changes affected isolated cells in the treated groups. No histologic evidence of the remaining scars (accumulation of chromatin, karyolysis, and eosinophilic cell debris) was found.

This form of necrosis must be distinguished from ***apoptosis***, in which there is an individual cell death characterized by cell contraction, nuclear pycnosis, apoptotic fragmentation, and reduced macrophage numbers. This type of cell death usually occurs in the germinal centres of the secondary follicles, where it is an important homeostatic mechanism. Apoptosis can also be induced by a variety of harmful stimuli, when given in low doses, but the same stimuli may be able to induce necrosis at higher doses. In the treated groups, isolated apoptotic bodies were found in areas with reduced macrophage numbers. The zones corresponding to germline (germinative) centres were observed. The formation of secondary follicles was not reported in the groups and the findings were not repeatable. The findings made in all three exposed groups support the possibility that this process may be due to a stimulus or stimuli. The same stimuli initiated the process at higher exposure. No evidence of massive necrosis or concomitant severe circulatory changes was found in the groups. The lack of chronic necrosis and the low apoptotic levels can be associated with a lack of evidence of lymphatic depletion in the treated groups. Paracortical lymphocytes were present in number and density close to those in the control group.

Lymphatic sinus (sinusoidal) ectasia can include both the medullary and subcapsular sinuses. Diffuse sinus ectasia is usually associated with lymphoid atrophy. This lesion could be detected in control animals, especially in the mesenteric and mediastinal LN of aging mice. The lesion was characterized by the presence of enlarged or cystic sinuses, lined by a lymphatic endothelium, and filled with pale eosinophilic/amphophilic material (probably lymph). Lymphocytes, plasma cells, and macrophages mixed with lymph could be found. Isolated signs of sinus ectasia were detected in the treated groups.

Angiectasis is most commonly seen in the MLN of rats and mice, sometimes accompanied by hemorrhage. This lesion can be distinguished from hematoma by the presence of mucosal endothelial cells and from early hemangiomas by the absence of large neoplastic endothelial cells. Intrasinusoidal erythrocytes (sinus erythrocytosis) can be the result of an LN draining the area of bleeding. In these sites there may also be intrasinusoidal erythrocytes, but, depending on the chronicity, they may be accompanied by a variable number of macrophages loaded with hemosiderin, erythrophagocytosis, and inflammatory cells. Restricted vessels changes were reported in the treatment groups, including sinus hyperemia (congestion) and sinus erythrocytosis. They showed similar values to those of the control group. Sinus erythrocytosis (intrasinusoidal erythrocytes) was not accompanied by hemorrhages. This means that changes are an artifact that results from euthanasia or tissue dissection during necropsy. The lack of abundant hemosiderin deposits further supports such an interpretation of the results obtained. Pigment deposits (hemosiderin, melanin, and ceroid/lipofuscin) were in the cytoplasm of sinusoidal macrophages in both control and treated animals. The findings in the experimental groups were close to those in the control groups.

Amyloidosis occurs in many strains of mice and other rodents but is not observed in the LN of rats, being low in most mouse strains. LN amyloidosis is more common in female mice, and the rate of amyloidosis increases with age, with 20 – 30 % of LN affected at 24 months of age. In them, amyloid deposition occurs in various tissues, including the LN. MLN is most commonly affected and the amyloid accumulates predominantly in the subcapsular sinuses, with progressive expansion in the paracortical areas of the LN. In the treated groups, limited deposition of amorphous, eosinophilic, and hyalinized extracellular material was reported. No changes and no dose-dependent progression of deposits were detected in any of the treated animals. The topography of the changes was isolated in the subcapsular sinuses with no findings of progressive extension of deposits in the paracortical areas of the LN.

Conclusions

Our study contributes to the better pharmacological characterization of the EtOAc-AP extract of *G. urbanum* L. and evaluation of its *in vivo* acute toxic effect on intestinal epithelial tissue and Peyer's patches. No pathological changes were observed in both examined tissues after oral administration of biologically active concentrations of the tested extract. The extract did not lead to histological changes of intestinal epithelial tissue and Peyer's patches, and, therefore, it is perspective for future development as a food additive.

Acknowledgements:

The present study was conducted in the frame of grant №80-10-13/09.04.2019 for Assist. Prof. Lilia Tserovska, Ph.D. The authors are grateful to the Alexander von Humboldt Foundation for the "Equipment subsidies" grant to Maya M. Zaharieva, Ph.D. We are grateful to Assist. Prof. Yana Ilieva for proofreading the manuscript and correcting the English.

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Contributions: Assist. Prof. L. Dimitrova prepared the plant extracts of *G. urbanum* L., with the help of Asoc. Prof. M. Popova, participated in the *in vivo* study design and experiments, and wrote the manuscript. Assoc. Prof. S. Philipov prepared the pathomorphological specimens, evaluated them, and participated in the writing of the manuscript. Assist. Prof. M.M. Zaharieva participated in the study design, the *in vivo* experiments and the writing of the manuscript. Assist. Prof. L. Tserovska and Prof. G. Zhelezova participated in the study design and the writing of the manuscript. Prof. V. Bankova determined the extraction design with solvents of different polarity and participated in the preparation of the extracts. Prof. H. Najdenski determined the final study design, led the *in vivo* experiments and corrected the manuscript.

Lyudmila Dimitrova
Stephan Angeloff Institute of Microbiology
Bulgarian Academy of Sciences
Sofia, Bulgaria
lus22@abv.bg

Stanislav Philipov
Faculty of Medicin
St. Kliment Ohridski University of Sofia
Sofia, Bulgaria
stanislav_philipov@abv.bg

Maya Margaritova Zaharieva
Stephan Angeloff Institute of Microbiology
Bulgarian Academy of Sciences
Sofia, Bulgaria
zaharieva26@yahoo.com

Lilia Tserovska
Faculty of Medicine
St. Kliment Ohridski University of Sofia
Sofia, Bulgaria
l_tserovska@abv.bg

Galina Zhelezova
Faculty of Medicine
St. Kliment Ohridski University of Sofia
Sofia, Bulgaria
gzhelezova@gmail.com

Milena Popova
Institute of Organic Chemistry with Centre of Phytochemistry
Bulgarian Academy of Sciences
Sofia, Bulgaria
popova@orgchm.bas.bg

Vassya Bankova
Institute of Organic Chemistry with Centre of Phytochemistry
Bulgarian Academy of Sciences
Sofia, Bulgaria
bankova@orgchm.bas.bg

Hristo Najdenski
Stephan Angeloff Institute of Microbiology
Bulgarian Academy of Sciences
Sofia, Bulgaria
*hnajdenski@abv.bg, hnajdenski@gma*il.com

MOLECULAR TAXONOMY – NEW INSIGHTS FOR POTENTIALLY TOXIC PHYTOPLANKTON SPECIES IN THE BLACK SEA

Nina Dzhembekova, Snejana Moncheva, Petya Ivanova, Nataliya Slabakova, Satoshi Nagai

Keywords: *metabarcoding, microalgae, resting cysts, Black Sea biodiversity*

Abstract: *The innovative metagenetic approach was applied to both water column and sediment samples to explore "realized" and "hidden" microalgal diversity. The results revealed much higher microalgal species diversity in the Black Sea than previously described. In planktonic communities, 13 operational taxonomic units (OTUs) associated with potentially toxic species were detected, based on amplifying 18S V4-V5 rRNA gene regions from some of them (e.g. Aureococcus anophagefferens, Karenia bicuneiformis, Karlodinium veneficum, and Pfiesteria piscicida) hereby reported for the first time in the Black Sea. Among the resting stages in the benthic communities assessed by using 18S V7-V9 rRNA gene regions, 21 OTUs were assigned to harmful microalgae, most of them not yet identified morghologically in Black Sea sediments (e.g. Azadinium dexteroporum, A. poporum, Amphidoma languida, Karenia papilionacea, and Fibrocapsa japonica). The new approach provides a perspective for more precise identification of species and, in particular, for detection of harmful algal bloom species and their resting stages, which is instrumental for the implementation of robust monitoring programs and ecological risk assessment studies.*

Introduction

Biodiversity of phytoplankton as a key primary producer and a sensitive indicator of ecosystem perturbations is essential for the assessment of the state and functioning of the marine ecosystem (Ptacnik et al. 2008; Strong et al. 2015; WFD, 2000/60/EC; MSFD, 2008/56/EC).

The Black Sea hosts over 1600 phytoplankton species (Moncheva, Parr 2015), among which 49 have been listed as harmful. Many microalgae (including harmful species) form resting stages as a part of their life cycle. Although microalgal diversity in the Black Sea has been extensively studied since the end of XIX century, traditional identification methods may result in underestimation of species diversity due to methodological constraints on capturing the high spatial and seasonal heterogeneity of phytoplankton communities.

Molecular techniques are more universal and have a higher potential to decode microbial diversity (Logares et al. 2014). A source of innovation in marine monitoring techniques is metagenetics (metabarcoding), emerging as a powerful tool for accurate, rapid, and cost-efficient phytoplankton species composition analysis based on community DNA signatures directly from environmental samples. This approach has been successfully applied for monitoring phytoplankton composition and harmful algal bloom (HAB) species in other marine basins, including detection of novel species not reported previously from the area of investigation (Piredda et al. 2016; Nagai et al. 2017, 2018; Sildever et al. 2019). Metabarcoding has also been effective for studying microbial diversity in sediment samples (Forster et al. 2016; Pirreda et al. 2017; Jung et al. 2018). The aim of the present study is to assess the suitability of metabarcoding for evaluating the biodiversity of microalgae in water column and sediment samples, exploring "realized" and "hidden" diversity of the Black Sea, with a focus on HAB species."

Materials and Methods

Seawater samples (N=13) collected during 2013 – 2015 in Varna Bay (Bulgarian Black Sea Coast) were analyzed by applying V4-V5 18S rRNA gene and MiSeq sequencing (Dzhembekova et al. 2017). Surface sediment samples from 13 stations in different areas across the Black Sea were analyzed by amplification of V7–9 hypervariable regions of the 18S-rRNA gene and MiSeq sequencing (Dzhembekova et al. 2018) (Fig. 1).

Results and Discussion

A total number of 1,154 operational taxonomic units were detected from 2,935,830 raw sequence data in the water samples. It should be noted that 51 % of all OTUs and 70 % of all MPSs (massive parallel sequences) scored >0.980 BLAST top hit similarity and were used for further taxonomic analyses.

In the sediment samples, 2,251 OTUs were recovered from a total number of 2,483,047 sequences. Approximately 39 % of the OTUs and 63 % of the MPSs were assigned on a 98 %-sequence similarity value to the reference database and used for taxonomic identification.

Altogether, 306 OTUs from water samples and 348 OTUs from sediment samples were assigned to microalgal taxa with similar composition at the class level in the two datasets (Fig. 2).

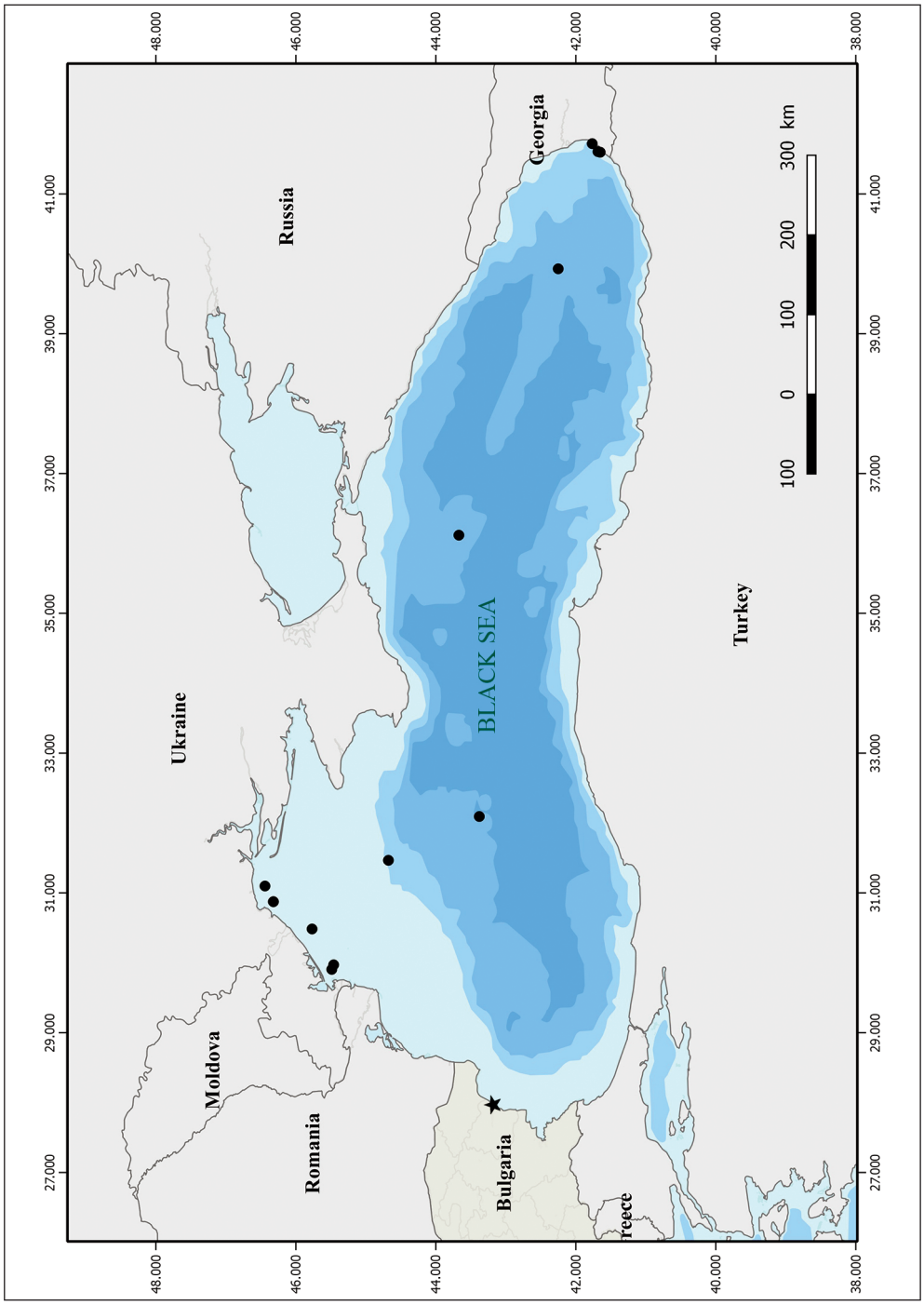

Fig. 1: Map of the sampling stations
(water sampling station ★; sediment sampling stations •).

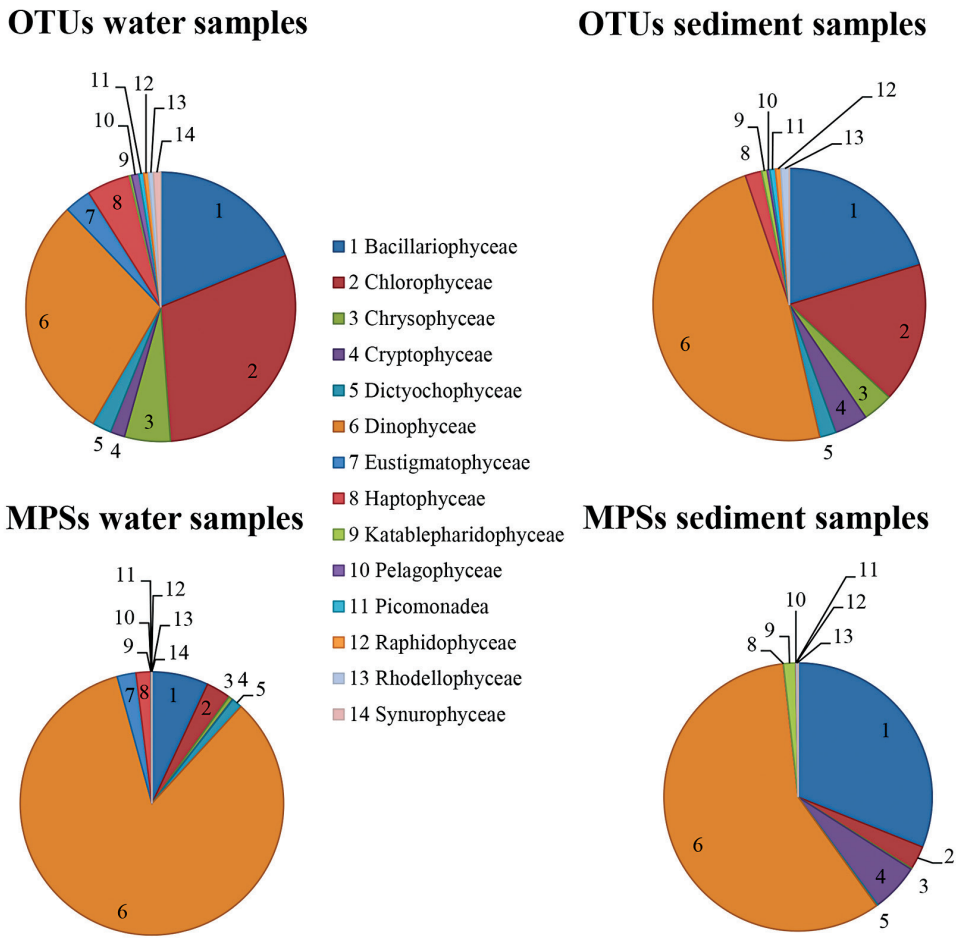

Fig. 2: Relative abundances of the numbers of OTUs and MPSs at the class level
Table 1. Potentially toxic microalgal species detected by metabarcoding.

Among the detected OTUs, 27 phytoplankton species listed as harmful in Moestrup et al. (2009) were unambiguously identified (Table 1).

Most of the potentially toxic species belong to the dinoflagellates, some of which are represented by a significant total sequence number. Within genus *Alexandrium* (associated with the production of paralytic shellfish poisoning toxins), we identified four potentially toxic species – one in the water samples and three in the sediment ones. *A. ostenfeldii* has been repeatedly found in the phytoplankton in different regions. Although usually not reaching high abundance, sporadic blooms of *A. ostenfeldii* have been reported in Bulgarian waters (Moncheva et al. 2001). *A. andersonii* and *A. catenella* have not been

	Class	Number of water samples where detected	Number of sediment samples where detected
Alexandrium andersonii	Dinophyceae	0	1
Alexandrium catenella	Dinophyceae	0	1
Alexandrium minutum	Dinophyceae	0	5
Alexandrium ostenfeldii	Dinophyceae	2	0
Amphidinium carterae	Dinophyceae	0	1
Amphidoma languida	Dinophyceae	0	10
Azadinium dexteroporum	Dinophyceae	0	3
Azadinium poporum	Dinophyceae	0	2
Cochlodinium polykrikoides	Dinophyceae	1	8
Gymnodinium catenatum	Dinophyceae	0	13
Gonyaulax spinifera	Dinophyceae	10	10
Karenia bidigitata	Dinophyceae	11	0
Karenia papilionacea	Dinophyceae	0	6
Karlodinium veneficum	Dinophyceae	8	13
Lingulodinium polyedrum	Dinophyceae	4	4
Pfiesteria piscicida	Dinophyceae	8	0
Pheopolykrikos hartmannii	Dinophyceae	4	9
Prorocentrum minimum	Dinophyceae	0	11
Protoceratium reticulatum	Dinophyceae	2	10
Pseudo-nitzschia delicatissima	Bacillariophyceae	0	2
Pseudo-nitzschia pseudodelicatissima	Bacillariophyceae	9	0
Pseudo-nitzschia pungens	Bacillariophyceae	1	0
Fibrocapsa japonica	Raphidophyceae	0	2
Heterosigma akashiwo	Raphidophyceae	7	4
Prymnesium polylepis	Prymnesiophyceae	0	9
Aureococcus anophagefferens	Pelagophyceae	1	1

Table 1: Potentially toxic microalgal species detected by metabarcoding.

reported in the Black Sea, whereas *A. minutum* has been observed in different areas of the basin (Moncheva, Parr 2015). *A. andersonii* is a species with questionable toxicity (Sampedro et al. 2013), but for the other two species toxin production has been proved (Anderson et al. 2012). In addition to potentially toxic representatives of the genus, we also identified several non-toxic species (*A. affine*, *A. margalefii*, *A. mediterraneum*, and *A. tamutum*). The inability to reliably distinguish morphologically toxic and non-toxic *Alexandrium* species may hamper light microscopy based HAB monitoring programmes; therefore, the application of molecular methods is crucial.

In the course of this work, a number of ichthyotoxic dinoflagellates were also detected by metabarcoding, constituting different portions of the total sequence number.

Cochlodinium polykrikoides is an ichthyotoxic dinoflagellate, widely distributed in eutrophic coastal waters and often forming blooms associated with fish kills (reviewed in Kudela, Gobler 2012). Interestingly, in Bulgarian waters it was registered for the first time as resting cysts from sediment samples (Rubino et al. 2010). In our study, the species was detected by next generation sequencing (NGS), represented with just a few sequences in only one water sample, while represented with a high number of sequences in some sediment samples.

Pfiesteria piscicida has never been identified morphologically in Black Sea samples, probably because of its complex life cycle including multiple stages (Steidinger et al. 1996). Metabarcoding, however, proved to be very effective – in our study, the species was detected in 9 water samples, and the OTU revealed complete identity with a reference sequence in GenBank. Species strains with a broad temperature and salinity tolerance have been reported to cause mass fish mortality in different environments (Place et al. 2008); thus, the presence of *Pfiesteria piscicida* in the Black Sea calls for a special investigation on the local strain's toxicity.

Pheopolykrikos hartmannii is another ichthyotoxic brackish species identified both in our water and sediment samples, with a varying MPSs number.

Karenia was represented by two species not reported previously in the Black Sea. *Karenia bidigitata* (synonym of the accepted name *Karenia bicuneiformis*) was represented only in the water samples, and *K. papilionacea* was detected only in the sediment ones. The high morphological similarity of the species within this genus as well as with some *Gymnodinium* species could be a reason for missing records in the routine monitoring in the Black Sea.

Karlodinium is a comparatively new genus separated from *Gymnodinium* on the basis of molecular data (Daugbjerg et al. 2000). Our metabarcoding

approach allowed the detection of *Karlodinium veneficum* in all sediment samples – with significant sequence reads – and also in almost all water samples, while it had not been reported previously in the Black Sea. It is considered toxic for many marine invertebrates and fishes, distributed globally (Place et al. 2012).

Some species known as yessotoxin producers were also identified. *Gonyaulax spinifera*, a typical species for the Black Sea, was registered in many samples in both datasets, whereas *Lingulodinium polyedrum* was detected in a few samples only. *Protoceratium reticulatum* was more frequently identified in the sediment samples than in the water column. The detection of yessotoxins in cultivated and wild mussels from Bulgarian waters (Peteva et al. 2018) set the need for a further study to disclose the source species.

Genus *Dinophysis* is common for the Black Sea and is represented by numerous species. The low intragenus variability in 18S rRNA genes limits the taxonomic resolution based on such molecular markers (Raho et al. 2013), as also reflected in the results of this study. Eight different species showed the same similarity (0.990) with the OTU sequence detected in six water samples, making species determination impossible. Similar is the case with *Prorocentrum*. Two OTUs, annotated as *Prorocentrum* (one found in all water samples and the other one detected in the sediment), revealed complete identity in the target regions with several species. Only *Prorocentrum minimum* was clearly distinguished, with a significant number of reads, in most of the sediment samples.

Azaspiracids are recently identified marine biotoxins (Twiner et al. 2008), reported in shellfish from numerous geographical sites. In our samples, three species that are a possible source of these toxins were detected (*Azadinium poporum*, *Azadinium dexteroporum*, and *Amphidoma languida*), none of them reported previously in the Black Sea.

Potentially toxic diatoms were represented by OTUs assigned to *Pseudo-nitzschia* genus, found predominantly in the water samples, with high frequency (Dzhembekova, Moncheva 2014). The NGS approach allowed for detection of three species (*P. delicatissima*, *P. pseudodelicatissima* and *P. pungens*). Even though domoic acid has already been reported in cultivated mussels and plankton samples from the Bulgarian Black Sea Coast (Peteva et al. 2018), no special investigations for the source species have been conducted yet.

The raphidophytes were represented by two OTUs, one assigned to the cosmopolitan toxigenic *Heterosigma akashiwo*, detected in both datasets, and the other, *Fibrocapsa japonica,* found only in the sediment samples. Globally, *H. akashiwo* often causes harmful blooms with negative effects on the ichtyofauna (Kempton et al. 2008), but there are no data of a high abundance in the Black Sea.

Last but not least, we detected *Aureococcus anophagefferens*, which is a picoplanktonic member of the Pelagophyceae that causes harmful brown tides in estuarine waters (reviewed in Gobler et al. 2005), whereas the species is missing in the Black Sea records so far.

Conclusions

Our results suggest that metabarcoding is of high potential for analyzing the microalgal biodiversity both in water column and in sediment. Generating high-throughput data, the method provides information about community composition and allows accurate identification of multiple species, including potentially toxic microalgae, not reported previously in the Black Sea. Among the advantages of metabarcoding, especially in the case of HAB monitoring, is the precise identification of species that cannot be differentiated by light microscopy, such as cryptic species (e.g. *Alexandrium*, *Karenia*, and *Pseudo-nitzschia*), small-sized phytoplankton (e.g. *Azadinium*m, *Aureococcus*), species with multiple stages (e.g. *Pfiesteria piscicida*), or cells with morphology affected by fixatives (e.g. *F. japonica* and *H. akashiwo*).

Although metabarcoding is a sensitive and powerful instrument for monitoring of HABs, the method also has some limitations. This molecular approach provides only relative abundance data that still cannot be directly related to cell concentration, which is essential in the case of monitoring harmful algae. The resolution of target regions and, accordingly, their taxonomic identification power is still insufficient in some cases (e.g. *Dinophysis* and *Prorocentrum*), which can be improved by application of multiple markers (Sildever et al. 2019). The public nucleotide databases are incomplete in terms of effective number of sequences available. Nonetheless, molecular-based identification broadens our knowledge on phytoplankton species diversity in the Black Sea and is a promising tool for improving HABs monitoring programs.

Acknowledgements:

This work was supported by the National Science Fund, Ministry of Education and Science (MES), Bulgaria under the project "Phytoplankton cysts – an intricacy between a "memory" or a "potential" for Black Sea biodiversity and algal blooms" (Grant number DN01/8, 16.12.2016) and the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", supported by MES of Bulgaria (Agreement № D01 – 230/06.12.2018).

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Nina Dzhembekova
Institute of Oceanology / Marine Biology and Ecology
Bulgarian Academy of Sciences
Varna, Bulgaria
sonata_bg@yahoo.com

Snejana Moncheva
Institute of Oceanology / Marine Biology and Ecology
Bulgarian Academy of Sciences
Varna, Bulgaria
snejanam@abv.bg

Petya Ivanova
Institute of Oceanology/Marine Biology and Ecology
Bulgarian Academy of Sciences
Varna, Bulgaria
pavl_petya@yahoo.com

Nataliya Slabakova
Institute of Oceanology/Marine Biology and Ecology
Bulgarian Academy of Sciences
Varna, Bulgaria
n.slabakova@abv.bg

Satoshi Nagai
National Research Institute of Fisheries Science/ Research
Center for Aquatic Genomics
Fisheries Research Agency
Yokohama, Japan
snagai@affrc.go.jp

IMPACT OF GEOMETRIC CONFINEMENT ON SYSTEMS OF SEMIFLEXIBLE POLYMERS

Andrey Milchev, Kurt Binder, Arash Nikoubashman, Sergey Egorov

Keywords: *soft matter, phase transitions, computer simulations*

Abstract: *In a series of studies using Molecular Dynamics (MD) computer simulations of semiflexible linear polymers along with Density Functional Theory (DFT), we have explored the structure and phase transitions between differently ordered states induced by confinement of such systems in different geometry. In cases when the contour length of the polymer chains is comparable to the slit thickness or to the radius of the spherical capsule in which such systems are enclosed, we find qualitative deviations from bulk behavior with varying system density or degree of polymer stiffness.*

I. Introduction

Semiflexible macromolecules find various applications as versatile materials – especially, owing to their possible liquid crystalline order (Ciferi 1983), and they are also important constituents of living matter (Alberts 2007). The interplay of stiffness on the local scale and flexibility on larger scales along the contour of macromolecules is a crucial aspect in understanding their conformations in solutions and melts as well as their resulting physical properties. On a coarse-grained level, the most important characteristic of a semiflexible polymer is its persistence length l_p, characterizing its stiffness. In fact, l_p describes the length along the chain contour over which the orientations of subsequent bonds are strongly correlated. In particular, for biopolymers, l_p is typically much larger than the size of monomeric units, ranging, for example, from about *50 nm* for double-stranded (ds) DNA to about *10 μm* for filamentous (F) actin; these linear dimensions are comparable to cell sizes or sizes of nanofluidic / microfluidic devices.

Hence, confinement of semiflexible macromolecules in various geometries has recently found widespread attention, particularly for the case of cylindrical or slit pores (Reisner 2012: 106601, Ye 2016: 2948). Also, the confinement of single semiflexible polymers inside spheres has been extensively studied (Gao 2014: 4674), e.g., with the motivation of understanding the packaging of very long ds-DNA in bacteriophage capsids (Marenduzzo 2013: 20081). However, much less attention has been paid to the confinement of many (shorter)

semiflexible macromolecules inside spherical containers. These systems may be of interest for problems such as the self-organization of actin filaments in cell-size confinement, drug delivery from spherical vesicles, etc.

II. Brief Description of the Model

We use a coarse-grained model whereby several building units of the macromolecule are combined into a single monomer so that polymer chains are modeled as N spherical beads of diameter σ and mass m, which are connected via massless springs – Fig. 1. Both bonded and non-bonded pairs of effective monomeric units of the semiflexible polymers *repel* each other (for $r<r_c$) with the standard Lennard-Jones potential

$$U^{LJ}(r) = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} + \frac{1}{4}\right], \quad r < r_c\,, \tag{1}$$

with $r_c = 2^{1/6}\,\sigma$ and $U^{\mathrm{LJ}}(r > r_c) = 0$. Here, r is the distance between the beads, and the energy scale ε of this excluded volume-type interaction is taken as $\varepsilon=k_BT=1$ (where k_B is the Boltzmann constant and T denotes temperature). Its range σ can be identified based on the thickness of the chain. Neighboring beads along a chain are bound together by the finitely extensible nonlinear elastic (FENE) potential (it is harmonic at $r \to 0$)

$$U^{FENE}(r) = -\frac{1}{2}kr_0^{\,2}\ln\left[1 - \left(\frac{r}{r_0}\right)^2\right], \quad r < r_0\,, \tag{2}$$

with $r_0 = 1.5\sigma$ and $k = 30\varepsilon/\sigma^2$. These standard choices prevent the unphysical crossing of bonds, and the effective distance between neighboring beads (bond length) is $l_b = 0.97\sigma$. The bending potential $U_{\mathrm{bend}}(\theta_{ijk})$ has been chosen as

$$U_{bend}(\theta_{ijk}) = \varepsilon_b\left[1 - \cos\left(\theta_{ijk}\right)\right], \tag{3}$$

where ε_b controls the strength of the bending potential, and θ_{ijk} is the angle between two successive bonds. In the case of *attractive* confining walls, we use the so-called Mie-potential:

$$U_{Mie}(z) = \varepsilon_{Mie} \frac{5}{3}\left(\frac{5}{2}\right)^{2/3}\left[\left(\frac{\sigma}{z}\right)^{10} - \left(\frac{\sigma}{z}\right)^{4}\right], \qquad (4)$$

where z is the distance perpendicular to the wall, and $U_{Mie}(z_{min}) = -\varepsilon_{Mie}$ at $z_{min} = (5/2)^{1/6}\sigma$, while for *repulsive* walls we use the potential, Eq. (1). The range of U_{Mie} is 5σ. The persistence length l_p is easily determined from the autocorrelation function of the bond angles θ from the relation $l_b/l_p = -\ln\langle\cos\theta\rangle$, which yields for stiff chains (i.e., $\theta \to 0$) simply $l_p/l_b \approx \theta^2/2$. It can be shown that $l_p = \varepsilon_b$ for $\varepsilon_b > 2$.

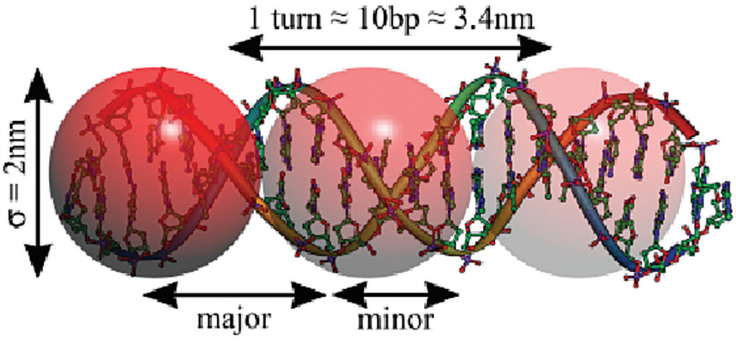

Fig. 1: Schematic illustration of the mapping from double-stranded DNA to a bead-spring model.

Apart from typical properties of the polymer chains such as the mean squared radius of gyration, $\langle R_g^2\rangle$, and the end-to-end distance, $\langle R_e^2\rangle$, the collective properties of the system are described by the (nematic) order parameter S, given by the largest eigenvalue of the Saupe order tensor, $\boldsymbol{Q}^{\alpha\beta}{}_{ni} = \frac{1}{2}(\langle 3\boldsymbol{u}^{\alpha}{}_{ni}\,\boldsymbol{u}^{\beta}{}_{ni}\rangle - \delta_{\alpha\delta},$ where the index n labels a chain of the system, and the index i (running from *1* to $N-1$) labels the bonds of a chain. $\boldsymbol{u}_{ni}$ is the unit vector in the direction of the bond, and α, β denote Cartesian coordinates. Bond orientation with respect to a given wall is measured by the second Legendre polynomial, P_2, which reads $P_2(r) = 3/2\langle\cos^2\vartheta\rangle - 1/2$, where ϑ is the angle with the surface normal. $P_2(r)$ takes on the value -0.5 if bonds are parallel to the wall, *1* if perpendicular, and *0* if randomly distributed. An important quantity, derived in the simulations, is the excess surface energy (surface tension) γ,

$$\gamma = \frac{1}{2}\int_0^{L_z}\left[P_{zz}(z) - \frac{1}{2}\left(P_{xx}(z) + P_{yy}(z)\right)\right]dz, \qquad (5)$$

where the diagonal elements of the pressure tensor, $P_{\alpha\beta}$, are determined from the virial theorem, $P_{\alpha\beta} = \rho k_B T\delta_{\alpha\beta} - (3V)^{-1}\langle\Sigma_n\, r^{\alpha}{}_n F^{\beta}{}_{tot}(\mathbf{r}_n)\rangle$, with the density ρ, and $F^{\beta}{}_{tot}$ is the total force acting on monomer n within the volume V of the system. L_z in (5) denotes the width of the slit between the two walls.

The Newtonian equations of motion have been integrated in the Molecular Dynamics (MD) simulations using a velocity-Verlet-algorithm in a NVT statistical ensemble a with Langevin thermostat. Our systems, containing about 5.10^5 particles, are equilibrated for ~10^8 integration steps, followed by production runs of ~10^9 steps. In view of the large demand of CPU time, we use Graphics Processing Units (GPUs) with the HOOMD-Blue software (Anderson 2008: 5342). In our studies we also complement the MD results with those from the Density Functional Theory (DFT), thus exploiting the advantages of each approach. Our implementation of DFT (Egorov 2016: 174902) is based on the expression for the Helmholtz free energy as a functional of the nonuniform molecular density $\rho_{mol}(\mathbf{r},\omega) = \rho_{iso}(\mathbf{r})f(\mathbf{r},\omega)$, where $\rho_{iso}(\mathbf{r})$ is the isotropic (orientationally independent) part, and $f(\mathbf{r},\omega)$ is the spatially and angularly dependent orientational distribution function. Here ω is a compact notation for the polar angles θ and φ, and $f(\mathbf{r},\omega)$ is defined as an average over all the bonds. For the isotropic phase in the bulk, $f_{iso}(\omega) = 1/(4\pi)$ everywhere. The Helmholtz free energy is then decomposed, as usual, into ideal and excess terms, $F(\rho_{mol}(\mathbf{r},\omega)) = F_{id}(\rho_{mol}(\mathbf{r},\omega)) + F_{exc}(\rho_{mol}(\mathbf{r},\omega))$, where $F_{exc}(\rho_{mol}(\mathbf{r},\omega))$ depends essentially on the excluded volume between chain segments, for more see (Egorov 2016: 174902).

III. Confinement in a Planar Slit

III. A. Slit with Repulsive Walls

A comprehensive investigation of semiflexible polymers in a solution under good solvent conditions interacting with a repulsive flat wall (Fig. 2) indicates that both the spatial density distribution is inhomogeneous ($\rho(z)$ depends on the distance z from the wall), and the angular distribution of the orientation of the bonds is both spatially dependent and anisotropic, unlike the bulk isotropic solution.

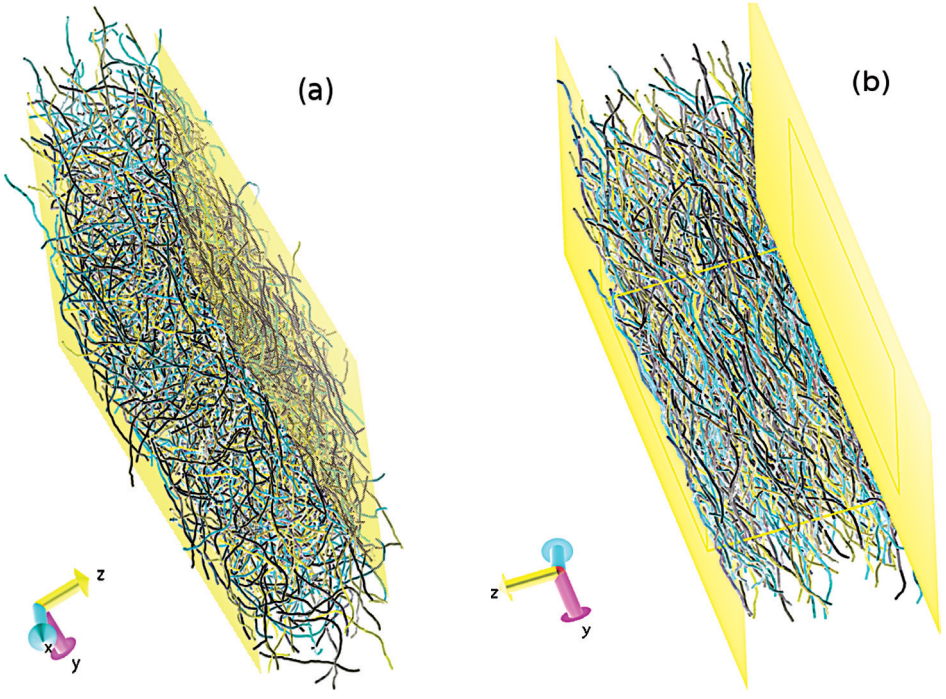

Fig. 2: Typical snapshot configurations in a slit of width L_z = 40, containing chains of stiffness κ=32 with N=32 monomers each, at densities (a) ρ = 0.1- isotropic, and (b) ρ = 0.3 – nematically ordered (Egorov 2016: 1600036).

This happens at densities ρ smaller than the density ρ_i where in the bulk the two-phase coexistence region between isotropic (I) and nematic (N) phases begins. In a solution of semiflexible polymers, one finds that the end-to-end vector of a chain is oriented parallel to the wall, $P_2(z)\approx 0.5$, when the center of mass of the chain is close to the wall. Related to this, nontrivial profiles $R^2_{g\parallel}(z)$, $R^2g^{\perp}(z)$ of the parallel and perpendicular parts of the mean-square gyration radius of the chains occur depending on the distance z from the wall, and chain ends get enriched at the wall, leading to enhanced surface tension γ (Egorov 2016), see Fig. 3.

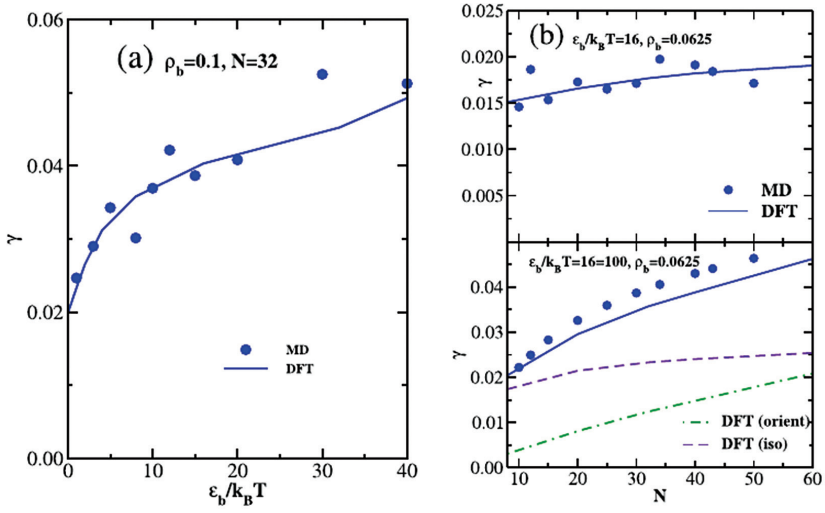

Fig. 3: (a) Surface tension γ as a function of the chain stiffness, comparing MD results (dots) with Density Functional Theory (DFT) predictions (lines). (b) γ as a function of the chain length N.

In the left panel there, one can see how γ varies with chain stiffness ϵ_b/k_BT for concentration $\rho_b = 0.1$ and chain length $N = 32$, while the right panel displays γ as a function of the chain length (for $\rho_b = 0.0625$ for $\epsilon_b/k_BT = 16$ – top, and $\epsilon_b/k_BT = 100$ – bottom). The profile of the nematic order parameter, S, as a function of z is shown in Fig. 4, indicating the gradual onset of nematic order in the slit with growing density ρ, kept below the bulk I-N transition density (Milchev 2016: 174902).

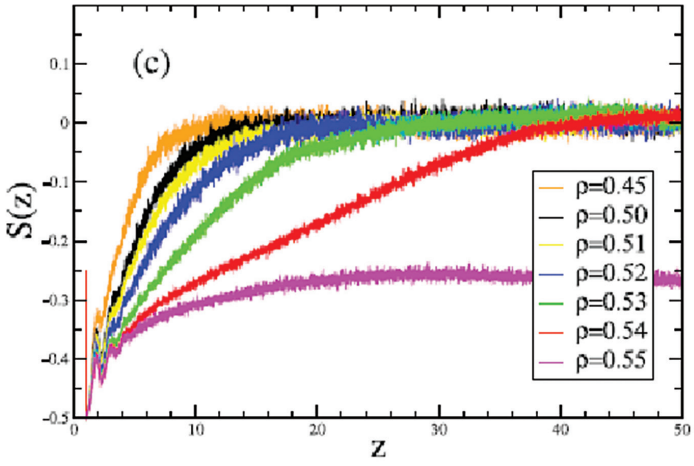

Fig. 4: Mean orientation of bonds $S(z) = P_2(z)$ plotted versus the distance z of a bond from the nearest wall, for the case $N = 8$, $\epsilon_b/k_BT = 100$, $L_z = 100$.

This is a demonstration of the phenomenon of *capillary nematization* when the finite width of the slit, L_z, where the polymer chains are confined, determines the wall-induced nematic order (see Fig. 5) before it occurs in the bulk.

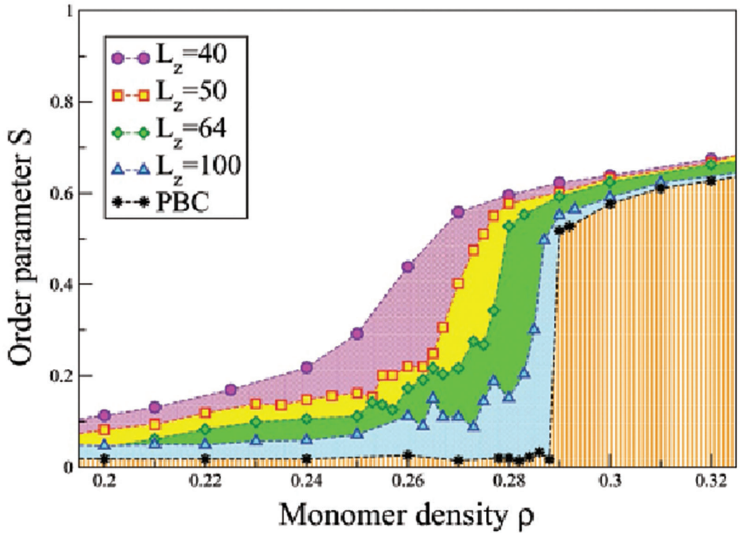

Fig. 5: Variation of the average nematic order parameter in the slit with density for several choices of L_z, as indicated. The bulk system is included for comparison. All data refer to N=32, ε_b=32.

The extended range over which stiff polymers "feel" the effect of a surface even in a dilute solution can be expected to have interesting consequences for the interaction of biopolymers (which are often rather stiff, e.g., DNA and actin) with biological membranes.

III. B. Slit with attractive walls

Molecular dynamics simulations of semiflexible polymers in a good solvent reveal a dense *adsorbed* layer when the solution is exposed to an attractive planar wall. This layer exhibits both a nematic and a smectic phase (*smA* for short, and *smC* for longer chains) with bond vectors aligned strictly parallel to the wall. The tilt angle of the *smC* phase increases strongly with the contour length of the polymers. This isotropic-nematic transformation is a Kosterlitz-Thouless transition, and similar to the nematic-smectic transition (Fig. 6), it is continuous. Our finding thus demonstrates a two-dimensional realization of different liquid crystalline phases, ubiquitous in three dimensions, that occurs in a *single* monomolecular layer ordered at least over mesoscopic scales.

As shown in Fig. 6, the tilting angle between the polymers and the boundary of the smectic layer increases gradually with the growing chain length N (apparently due to the beads along the stiff chains clicking into adjacent free places nearby). Fig. 6 exhibits the onset first of nematic and subsequently of smectic order with growing density of the adsorbed layer for semiflexible polymers of different length N whereby shown cases refer to the choice (top, from left to right): $N = 16$, $\epsilon_b = 128$, and $T = 3.2$, $T = 2.0$, $T = 0.86$; (bottom): $N = 24$, $\epsilon_b = 144$, $T = 0.80$, $N = 32$, $\epsilon_b = 128$, $T = 1.0$, and $N = 64$, $\epsilon_b = 128$, $T = 1.0$.

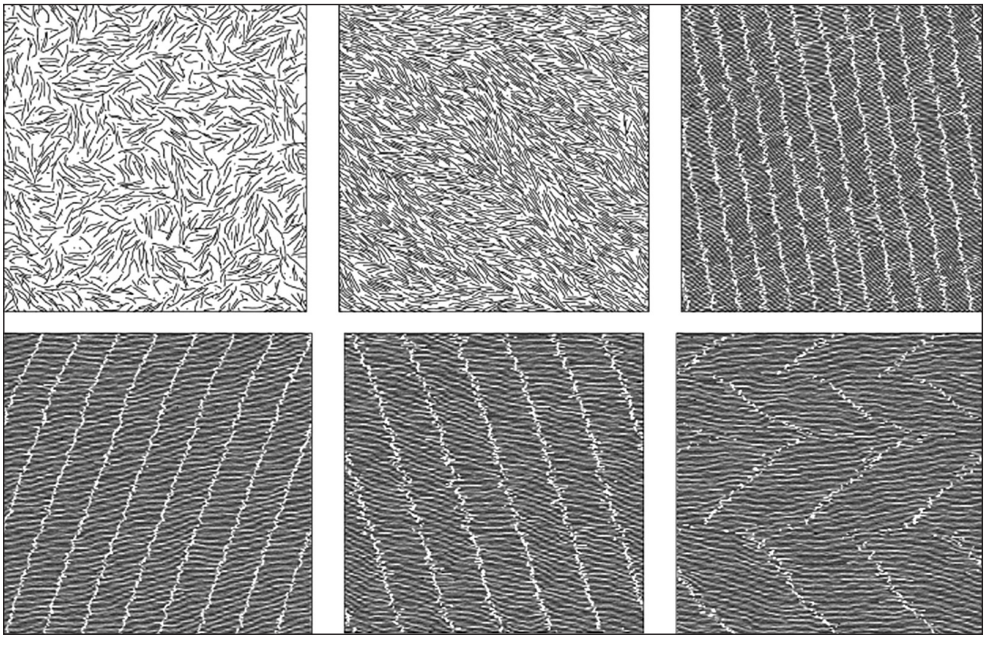

Fig. 6: Snapshots of semiflexible polymers confined in the adsorbed-layer for changing temperature (top), and chain length (bottom).

The same sequence of phase transformations, as in Fig. 6, can also be induced by growing stiffness of the polymer chains as demonstrated in Fig. 7 (Milchev 2017: 4924).

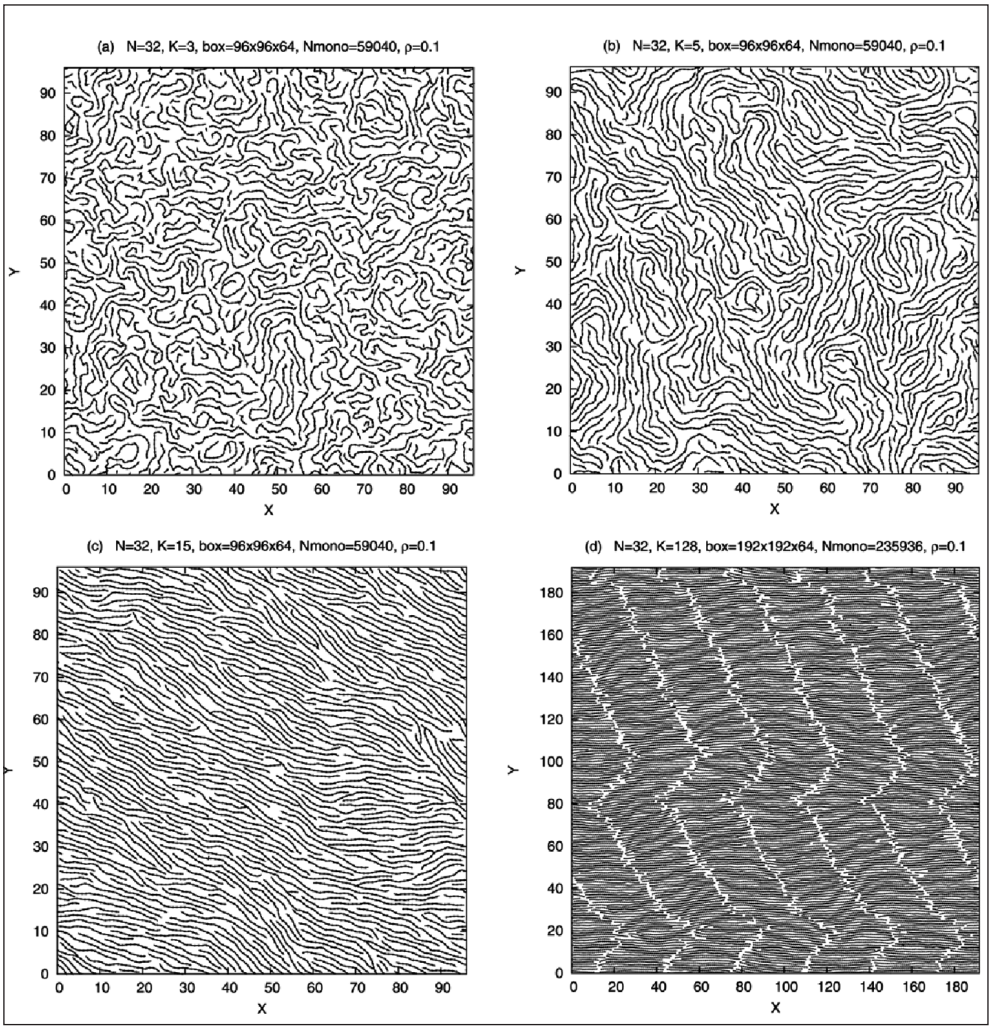

Fig. 7: Phase transitions from isotropic to nematic to smectic C phases in the first layer of polymer chains with N=32 monomers, adsorbed at the planar wall of a slit with growing stiffness $\kappa = \varepsilon_b$ *(Milchev 2017: 1888).*

The models with strongly adsorbing walls do not enhance the nematic order in the bulk, since for thin slits the density in the bulk is clearly reduced when many chains are adsorbed at the attractive walls. No "capillary nematization" effect at higher bulk densities is found in this system, unlike systems with repulsive walls. This finding is attributed to the reduction of the bulk density (in the center of the slit pore) due to polymer adsorption on the attractive wall, provided the system is studied in the canonical ensemble (fixed number of chains). Consequently, in a system with two attractive walls, nematic order in

the slit pore can occur only at a higher density than for the bulk system (e.g., at fixed chemical potential in a Grand canonical ensemble).

IV. Confinement in a Spherical Shell

IV. A. Confinement in a Sphere with Repulsive Walls

Densely packed semiflexible polymers with contour length L confined in spheres with radius R of the *same order* as L cannot exhibit uniform nematic order. Depending on the chain stiffness (which we vary over a wide range), highly distorted structures form with characteristic topological defects on the sphere surface. These structures are completely different from previously observed ones of very long chains winding around the inner surface of spheres and from nematic droplets too. At high densities, a thin shell of polymers close to the sphere surface exhibits a *tennis ball* texture due to the confinement-induced gradual bending of polymer bonds. In contrast, when the contour length of the chains is significantly smaller than the radius of the confining sphere, a few bent smectic layers form in the sphere, as shown in Fig. 8. Molecular dynamics simulations demonstrate these complex structures, and suitable order parameters characterizing them as well as the respective phase transitions are proposed (Milchev 2018: 2002).

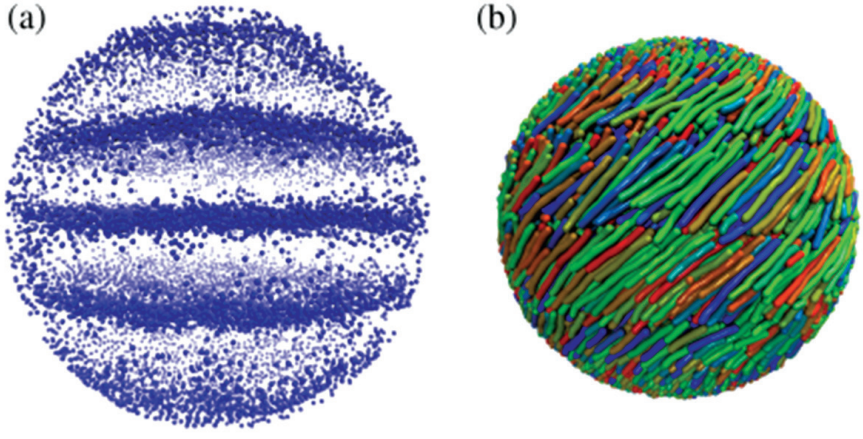

Fig. 8: (a) Chain ends positions for short chains with $N = 16$, $\kappa = 48$, $\rho = 0.7$, and $T = 0.6$. Three distinct layers of ends are visible. (b) Chains demonstrating smectic order in the inner part ($r < 0.85R$) of the sphere (Nikoubashman 2017: 217803).

Since the sphere surface massively disturbs the quasismectic arrangement, the reduction of the average nematic order parameter happens predominantly in the outer region of the sphere, where increasing chain rigidity enforces more beads to follow an orientation perpendicular to the director and, hence, parallel to the sphere surface, as is evident from the radial distribution of the bond orientations $P_2(r)$ – Fig. 9.

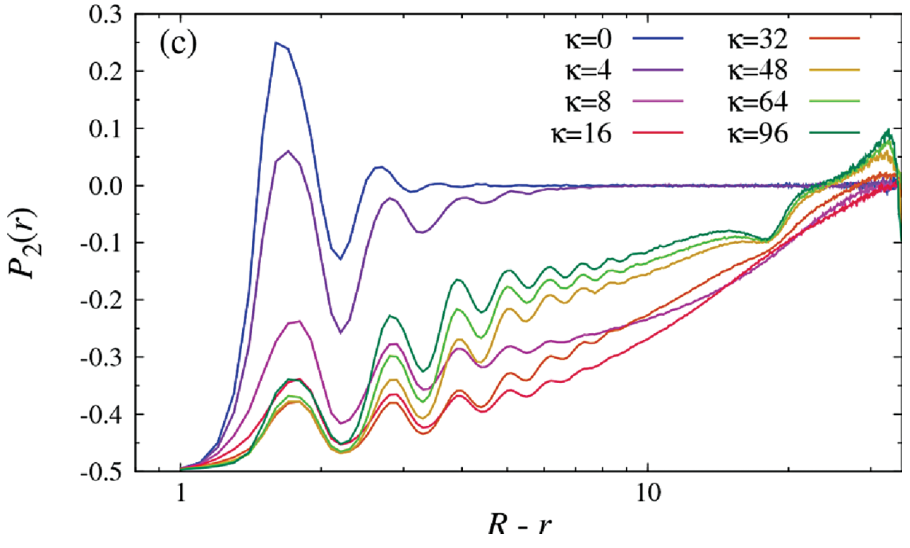

Fig. 9: Local bond orientation $P_2(r)$ plotted vs the distance from the walls, $R - r$, for the case $N = 16$, $R = 35$, $\rho = 0.7$, and eight values of κ, as indicated. A logarithmic scale for the abscissa is chosen in order to display the oscillatory structure near the confining sphere surface more clearly.

For large enough stiffness κ, the need of the bonds to orient parallel to the sphere surface (perfect parallel orientation would mean $P_2(r) = -0.5$) wins in comparison with the tendency of the system to develop an uniform long-range order inside the sphere.

The inner structure of the system of semiflexible polymers in a spherical capsule is revealed in Fig. 10 (Nikoubashman 2017: 217803), where one can see the formation of a nearly empty equatorial plane for chains with contour length $N = 32$, close to the sphere radius $R = 35$.

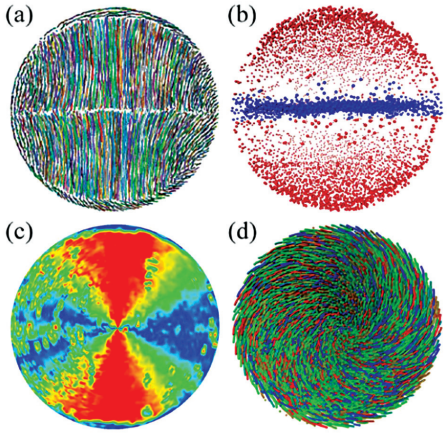

Fig. 10: (a) Slice perpendicular to the equatorial plane. (b) Snapshot of chain ends only. (c) Projection of bonds along director. (d) View from the center of the sphere to one of the poles. All data for $N = 32$, $\kappa = 96$, and $\rho = 0.7$.

Apparently, each chain that has one end close to the equatorial plane has the other one close to the sphere surface, which requires a considerable splay deformation of the nematic order field. The radial component of the nematic director field in the *z-y* plane perpendicular to the equatorial plane is shown with color gradient indicating the degree of bond-vector orientation along this field (red: full; blue: zero). The chains are twisted around the polar axis which coincides with the nematic director of the system. Immediately under the spherical shell, a typical ordering, known as "polar" and "tennis ball" topological defects, is demonstrated in Fig. 11.

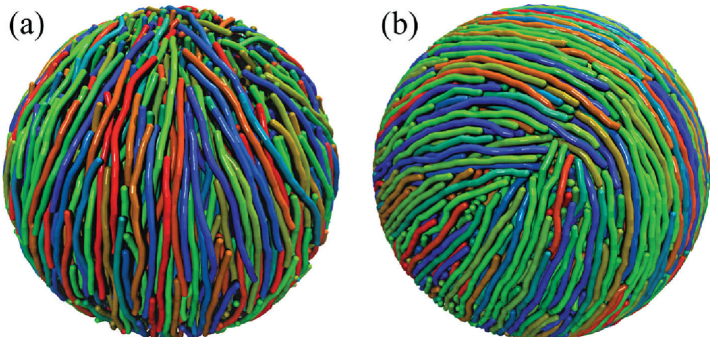

Fig. 11: (a) A snapshot of a system with $N = 32$, $\kappa = 96$, and $\rho = 0.4$, showing bipolar nematic order in a spherical cavity with radius $R = 35$. (b) Tennis ball order for $N = 32$, $\kappa = 32$, and $\rho = 0.7$.

Understanding all these unconventionally ordered structures can be helpful for suitable applications of such strongly confined polymeric systems and could give insight into related phenomena in biological systems. In addition, the "patchiness" provided by the topological defects on the sphere surface can have important implications for the use of anisotropic nanoparticles as building blocks for colloidal self-assembly. For example, the tennis ball structure implies a possible usage of functionalized tetravalent colloids with defects located at the vertices of a tetrahedron which could induce diamond-like arrangement of capsules (Nelson 2002: 1125).

IV. B. Confinement in a Sphere with Attractive Walls

Due to the attractive vesicle surface, phase separation occurs, leading to the formation of a thin shell of adsorbed monomers at the surface and a rather dilute, and therefore, disordered polymer solution in the sphere interior, see Fig. 12.

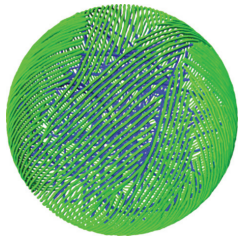

Fig. 12: A spherical shell with chains adsorbed at the inner surface (green) as distorted smectic, and with a random orientation in the interior (blue).

While at a planar attractive surface the semiflexible polymer chains would exhibit liquid crystalline (nematic and smectic) order; the curvature of the sphere frustrates this order, causing the existence of defects in the orientational order – Fig. 13 (Milchev 2018: 463). For sufficiently long ($N = 32$) and stiff ($\kappa = 128$) chains, one can observe an unexpected pattern: chain ends arrange in locally ordered domains covering the sphere surface, Fig. 13 c, f. The chain stiffness is (a, d) $k = 1$, (b, e) $k = 16$, and (c, f) $k = 128$. Only chains adsorbed at the inner surface of the sphere are shown with monomers in the range $32 < r < 35$, where r is the radial distance from the sphere center.

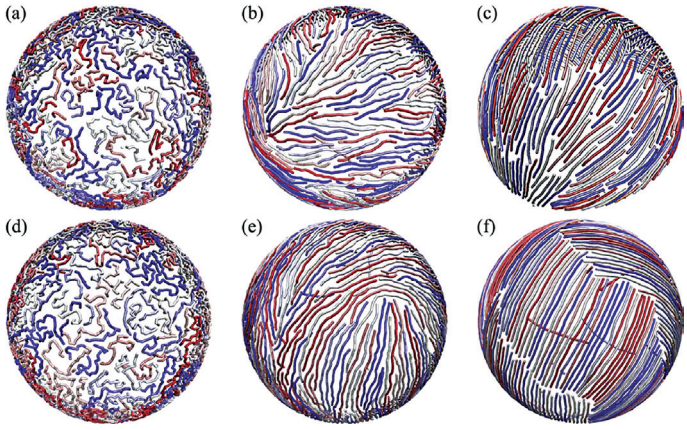

Fig. 13: Snapshots of semiflexible polymers with chain length (a-c) N = 16 and (d-f) N = 32, confined in a sphere with radius R = 35 and an attractive surface.

Evidently, the competition between the steric repulsion among monomers, the repulsion at the hard wall of the confining spherical surface, and the energy gained by adsorption, leads to a subtle interplay between the translational and orientational entropy of the stiff chains.

It is interesting to see how the surface tension γ varies with shell curvature, i.e., the radius of spherical confinement R, Fig. 14 (Milchev 2018: 463).

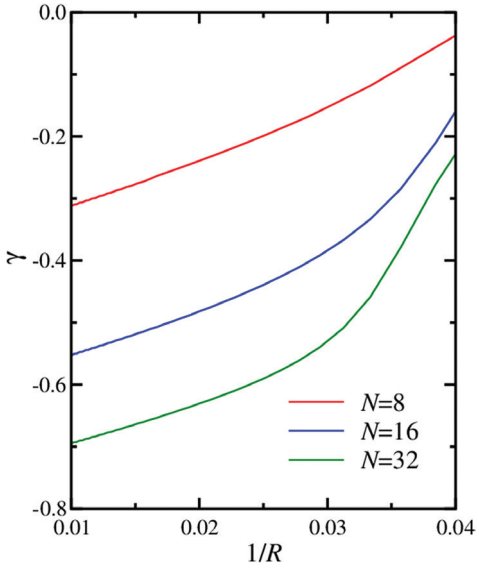

Fig. 14: Surface tension γ at an attractive spherical wall for a Mie potential of strength ε_{Mie} = 3.0, average density in the sphere ρ = 0.0625, k = 16, plotted vs the inverse radius of the sphere. Three choices of chain length N are included, as indicated. The negative sign of γ is due to wall attraction.

While in a capsule with adsorbing walls, γ varies with decreasing the capsule's radius qualitatively similar to Tolman's prediction (Tolman 1949: 333) for a drop coexisting with vapor, see also (Tsekov 2000: 3502). However, our DFT-based studies of γ in a capsule with repulsive walls (Milchev 2017: 194907) reveal non-monthonic behavior both with respect to chain length N as well as with regard to curvature R whereby this depends on the chain stiffness κ in a nontrivial way too.

V. Summary

In this paper, we have given a brief overview of our recent investigations of semiflexible polymers in geometrical confinement, using a combination of molecular dynamics (MD) simulations of a coarse-grained bead-spring model with DFT (Density Functional Theory), since these two methods complement each other favorably. We have explored only semiflexible polymers in lyotropic solutions where the effective interactions between the monomeric units are short-ranged and repulsive in character, representing effectively the excluded volume of these units. Solvent molecules are not explicitly considered, and atomistic details are sacrificed for the sake of simplicity and tractability.

In the case of a slit with repulsive walls, one observes wall-induced inhomogeneities in the density $\rho(z)$, which occur at densities ρ smaller than the density ρ_{cr} where, in the bulk, the two-phase coexistence region between isotropic (I) and nematic (N) phases begins. It is of interest to study the range over which these wall-induced inhomogeneities extend. In a solution of semiflexible polymers, additional phenomena occur: the end-to-end vector of a chain is oriented parallel to the wall when the center of mass of the chain is close to the wall. Related to this, nontrivial profiles $R_{g\parallel}{}^2(z)$, $R_{g\perp}{}^2(z)$ of the parallel and perpendicular parts of the mean-square gyration radius of the chains are observed (here z denotes the distance of the chain center of mass from the wall). Also, chain ends get enriched, affecting the surface tension of the polymer solution.

While the model with repulsive walls yields clear evidence in favor of capillary nematization, in slits with attractive walls no such effect at higher bulk densities has been found. Instead, we have found dense layers of strongly adsorbed semiflexible polymers and have demonstrated that various liquid crystal orderings take place: nematic (with quasi-long rangle order), smectic *smA* (for relatively short chains such as $N = 16$), and *smC* (for longer chains). Analyzing bond orientational correlations, we can locate both the *I*–*N* Kosterlitz-Thouless-transition and the (apparently continuous) *N*–*smC* transition.

The ordering of semiflexible polymers confined in a sphere with radius R and purely repulsive walls is carried out at densities for which the corresponding bulk lyotropic solution or melt is a well-ordered nematic. It has been found that polymers close to the walls are bent according to the curvature of the confining spheres with all their monomers placed in a few layers parallel to the sphere surface, whereas the remaining macromolecules closer to the sphere center have one chain end and their center of mass far from the surface. The latter chains are responsible for the average nematic order of the system for sufficiently stiff chains. If chain length L exceeds R, a single transition from isotropic to nematic states is found when l_p increases. However, if R is close to an even multiple of L, a second transition occurs where chain ends are enriched near the equatorial plane (and two further planes parallel to it, if $R \approx 2L$); i.e., the system develops a distorted smectic structure. The order in the surface shell is then characterized by characteristic defects with total topological charge 2, e.g., $2 \times +1$ (bipolar) or $4 \times ½$ (tennis ball – tetrahedral).

In contrast, when the system of semiflexible polymers is placed in a capsule with attractive surface, phase separation occurs between a thin shell of adsorbed monomers at the surface and a rather dilute, and therefore, disordered polymer solution in the sphere interior. The curvature of the sphere frustrates the tendency of nematic order, causing the existence of defects in the orientational order. In the dense monolayer of surface-attached polymers, the stiff chains acquire a curvature corresponding to banana-like shapes for rather small contour length, causing a *local* nematic order of *biaxial* type. An interesting phenomenon that needs further exploration is the formation of defect lines when chain ends accumulate (reminiscent of smectic liquid crystalline phases), as well as the overall pattern of the defect lines on the sphere surface.

Clearly, a full exploration of the parameter space of the coarse-grained model (radius R, contour length L, persistence length l_p, monomer density ρ, strength of adsorption potential ε_{Mie}, and solvent quality) could not be attempted, but we feel that the chosen examples are representative for the phenomena that can be expected. Our study can only be seen as a first step towards the understanding of the adsorption of biopolymers on real curved membranes, yet the latter are compressible rather than strictly rigid, chemically heterogeneous rather than homogeneous and structureless, and also our semiflexible bead-spring chains are only an approximation of real polymers. Nonetheless, one may hope that our findings will stimulate the search for suitable experimental realizations of appropriate systems that can be used for testing our predictions.

Acknowledgments:

A.M. acknowledges partial support from the German Research Foundation (DFG) under Project No. BI 314/24e1, from the COST-Action 17139 European Topology Interdisciplinary Action and from the Bulgarian Ministry of Education under project FNI / KOST-11-2018.

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Andrey Milchev
Institute of Chemical Physics
Bulgarian Academy of Sciences
Sofia, Bulgaria
milchev@ipc.bas.bg

Kurt Binder
Institute of Physics
Johannes Gutenberg University Mainz
Mainz, Germany

Arash Nikoubashman
Institute of Physics
Johannes Gutenberg University Mainz
Mainz, Germany

Sergei A. Egorov
Department of Chemistry
University of Virginia
Charlottesville, Virginia 22901, United States

SCIENCE WITHOUT BORDERS: EXOTIC NUCLEI FOR PHYSICS, ASTROPHYSICS AND APPLICATIONS

Giacomo de Angelis

Keywords: *Nuclear Astrophysics, Nuclear reactions, Radioactive ion beams*

Abstract: *The SPES facility, at the Legnaro National Laboratories, is one of the most promising radioactive ion beam (RIB) facilities presently under construction in Europe. Dedicated to basic nuclear science, nuclear astrophysics, and nuclear physics based applications, it will provide high-intensity and high-quality beams of unstable, mainly neutron-rich, isotopes at energies which allow nuclear transmutation for all combinations of projectiles and targets. SPES, fully integrated in a network of complementary European RIB-facilities, named EURISOL, is a nice example of science without borders. It makes use of an high intensity proton cyclotron, such as BEST – 70p, for the production of radioactive species. The high intensity proton beam is used to induce fission in an uranium carbide target able to sustain a maximum power of 8 kW. The aim of the project is to reach a fission rate in the target of the order of 10^{13} fissions per second. Exotic nuclei, after extraction from the target, ionization, and isotopic separation, are re-accelerated by the superconductive linear accelerator of the Legnaro National Laboratories. Reaccelerated radioactive ion beams will be used to test the reaction rates for the production of heavy elements, allowing us to probe the predictive power of the models of elemental nucleosynthesis in the Universe.*

Nuclear physics research is at the dawn of a new era. After the Big Bang and billions years of evolution, the Universe has provided us around 2000 nuclei. Based on the information from these systems, nuclear theory has been established in order to understand the structure of the nucleus. The steady progress over the past twenty years in the development of high intensity stable beams and of beams of radioactive isotopes has allowed scientists to vastly expand the objectives of experimental nuclear research. It is also becoming possible to study in the laboratory a range of nuclear reactions that take place in exploding stars, thus providing crucial information to understand the chemical elements that we find on the Earth were formed (Broggini 2019). With more than 2000 nuclei produced artificially and around 6000 expected to be produced in the facilities in operation or under construction in China, Europe, Japan, and the USA, the conventional nuclear theory meets serious challenges. For example, the discovery of the neutron halo has led to the re-examination of

several assumptions widely used in the nuclear models. The disappearance and appearance of the so-called "magic numbers" (particularly stable combinations of protons and neutrons) is presently leading to the re-examination of the nuclear shell model and also of the path of the nucleosynthesis in the Universe.

To achieve this ambitious goal, one needs to study the characteristics of unstable (radioactive) nuclei through their decays and the various nuclear interactions. Such unstable nuclei also have a wide range of applications (nuclear medicine, monitoring of climate changes, etc.).

The lightest elements (hydrogen, helium, deuterium, and lithium) were produced in the Big Bang nucleosynthesis. According to the Big Bang theory, the temperatures in the early Universe were so high that fusion reactions could take place. This resulted in the formation of light elements: hydrogen, deuterium, helium (two isotopes), lithium, and trace amounts of beryllium. Heavier elements are synthesized in the stars. Nuclear fusion in stars converts hydrogen into helium. In stars less massive than the Sun, this is the only reaction that takes place. In stars more massive than the Sun (but less massive than about 8 solar masses), further reactions that convert helium to carbon and oxygen take place in successive stages of stellar evolution. In the very massive stars, the reaction chain continues to produce elements like silicon up to iron.

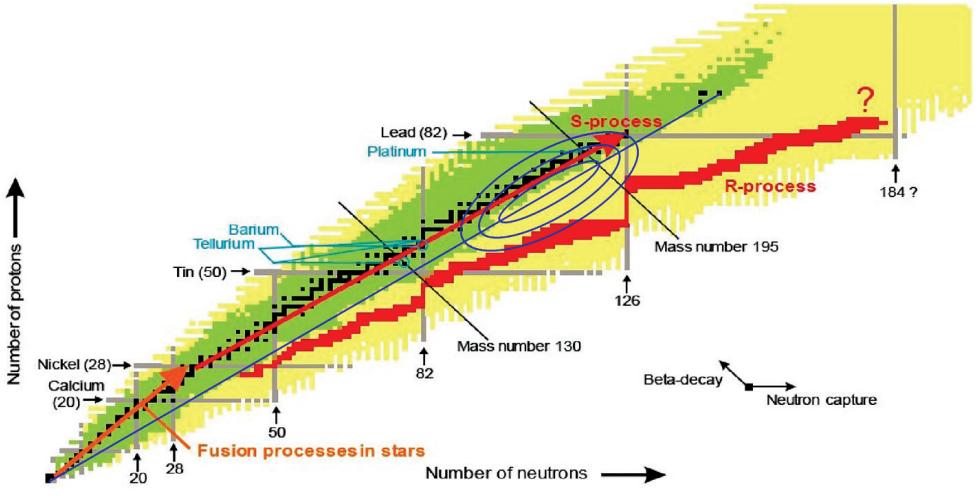

Fig. 1: Chart of the nuclides indicating various pathways for astrophysical nucleosynthesis: thermonuclear fusion reactions in stars (orange arrow), s-process path (red arrow), and r-process path generating heavy nuclei (red pathway). The nuclei marked in black are stable.

Elements with an atomic number higher than that of iron cannot be formed through fusion reactions, as one has to supply energy for the reaction to take place. However, we are made of elements with atomic numbers larger than that of iron. So, how were these elements formed? Neutron capture reactions taking place in supernova explosions or in neutron star mergers are supposed to lead to the formation of heavy elements. Two different neutron capture processes, called the slow process (s-process) and the rapid process (r-process), are leading to different element formation (Fig. 1).

Fig. 2: Artistic view of a kilonova following the merging of two neutron stars. Heavy elements are synthesized in such events.

On 17 August 2017, the LIGO/Virgo collaboration detected a pulse of gravitational waves, named GW170817, associated with the merger of two neutron stars in NGC 4993, an elliptical galaxy in the constallation Hydra (Abbott 2017). The event seemed also related to a short, 2-second long, gamma-ray burst, detected 1.7 seconds after the gravitational wave merger signal and to a visible light burst observed 11 hours afterwards. The correlation in space and time of such events provided the first strong evidence that neutron star mergers do create short gammay-ray bursts in an event which has been called kilonova, an event which may be much more common in the Universe than previously believed (Fig. 2 and Fig. 3).

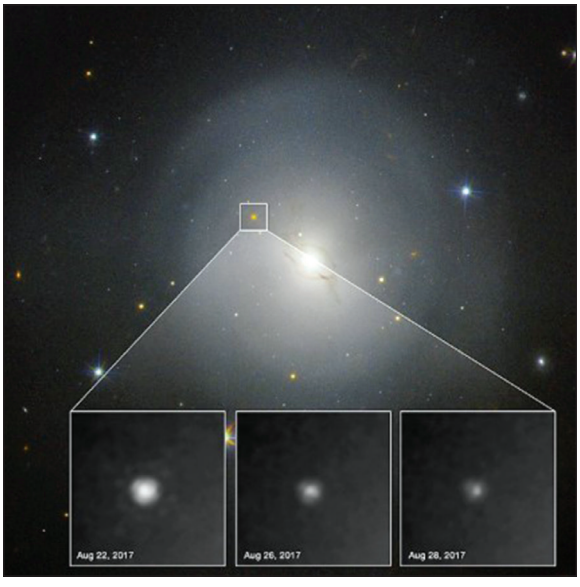

Fig. 3: First kilonova observations by the Hubble Space Telescope.

All this was made possible by the detection of gravitational waves. Gravity, in the general theory of relativity, is originated by a curvature of spacetime. This curvature is caused by the presence of mass (Fig. 4).

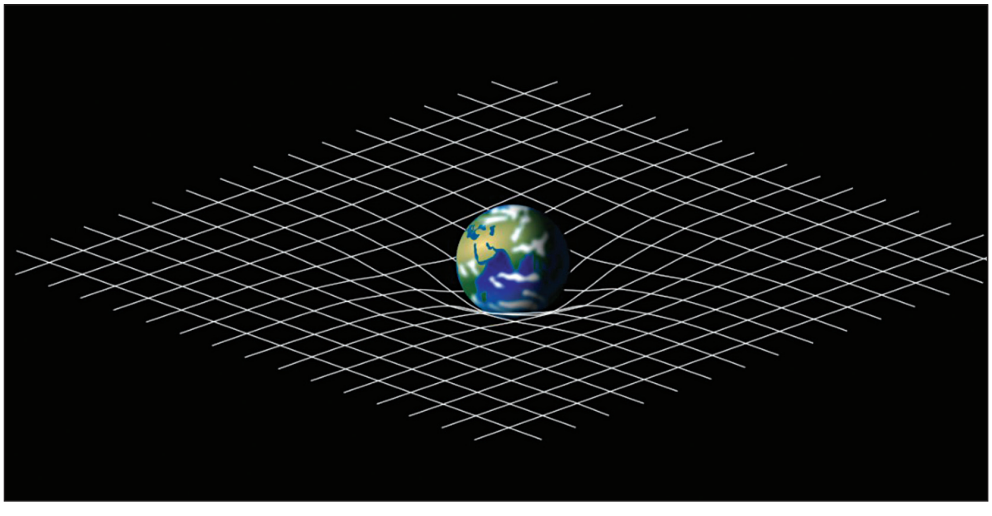

Fig. 4: Distortion of spacetime due to a massive object.

As objects with mass move around in spacetime, the curvature changes to reflect the changed location of those objects. Accelerating objects generate

changes in this curvature, which propagate outwards at the speed of light in a wave-like manner. Such propagating phenomena, known as gravitational waves, distort the spacetime. Distances between objects increase and decrease as the wave passes at a frequency equal to that of the wave.

Inspiraling binary neutron stars are predicted to be a powerful source of gravitational waves as they coalesce, due to the very large acceleration of their masses as they orbit close to one another.

Hovewer, due to the astronomical distances to these sources, the effects, when measured on the Earth, are predicted to be very small, at the level of 1 part in 10^{20}. In 2015, the use of laser interferometry allowed for the first time the discovery of gravitational waves, accomplished by the LIGO and VIRGO observatories (Abbott 2016). The waves were generated by the merger of two black holes (Fig. 5).

Fig. 5: Gravitational waves generated by the coalescence of two neutron stars (artistic view).

Gravitational waves can penetrate regions of space which electromagnetic waves cannot, giving new insights into the working of the Universe. In particular, they could be of interest to cosmologists, as they offer a possible way of observing the very early Universe. Conventional astronomy cannot do it, since, before recombination, the Universe was opaque to electromagnetic radiation.

The connection between the properties of atomic nuclei and the properties of planets, stars, and galaxies is the field of nuclear astrophysics. Open questions include: "How did the Universe create the elements?", "How can extremely dense and hot astrophysical environments be used to learn about

fundamental properties of matter?", and "How is the energy that powers stars and stellar explosions created?" Accelerator laboratory experiments using beams of stable and radioactive nuclei, neutrons, and gamma-rays are necessary for the determination of the reaction rates of astrophysically important reaction processes which allow testing of the predictive power of theoretical models. These are needed for the necessary extrapolations in the nuclear regions of interest, mostly inaccessible experimentally at present. Large scale computer simulations of stellar explosions and of nuclei as well as theoretical work in nuclear physics and astrophysics are therefore essential. In order to explore the ever-more exotic regions of the nuclear chart, towards the limits of stability of the nuclei, European nuclear physicists have built several large-scale facilities in various countries of the European Union. Today, they are collaborating in planning a new radioactive ion beam facility which will permit them to investigate hitherto unreachable parts of the nuclear chart. This European ISOL (isotope-separation-on-line) facility is called EURISOL. The latter represents at the same time the joint operation of the national radioactive ion beam ISOL facilities, comprising Belgium, France, Italy, Switzerland, and Poland, as well as a future dedicated facility with unprecedented performances. The ion yields delivered by the current ISOL facilities, or those under construction, will be exceeded by at least a factor of 100. This will open a wide field of research for physicists. The primary research goals are in nuclear structure physics and nuclear astrophysics. Other important programmes involve studies of fundamental symmetries and applications. The national (Italian) ISOL radioactive ion beam facility is called SPES (de Angelis 2015; de Angelis, Fiorentini 2016).

Fig. 6: The SPES radioactive ion beam facility, presently under construction at the Legnaro National Laboratories of INFN. The production cyclotron is visible (blue structure) on the right of the figure.

Fig. 6 shows the SPES cyclotron, the main accelerator of the SPES facility. Installed at the Legnaro National Laboratories, it is one of the most advanced nuclear installations for the production and acceleration of radioactive isotopes in Europe. It will provide high-intensity and high-quality beams of unstable, mainly neutron rich, isotopes at energies around the Coulomb barrier, allowing nuclear reactions for all combinations of projectile and targets. The technical solutions developed for its realisation define it as an intermediate step towards the future European facility EURISOL. SPES makes use of a high intensity production cyclotron such as BEST – 70p as a primary accelerator (Fig. 6). The high intensity proton beam is used to induce fission in an uranium carbide direct target (UCx) able to sustain a maximum power of 8 kW. The aim of the project is to reach a fission rate in the target of the order of 10^{13} fissions per second. Exotic nuclei, after extraction from the target, ionization, and isotopic separation, are re-accelerated by the superconductive linac of the Legnaro National Laboratories. Energies above 10 MeV/A for masses up to A = 200 will be provided with expected rates at the secondary target of the order of $10^8 – 10^9$ particles per seconds for the most prolific species. Dedicated mainly to basic nuclear science and nuclear physics based applications, SPES will also produce novel isotopes for medical use, both for cancer treatments and diagnostics.

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Giacomo, de Angelis
INFN Legnaro National Laboratory
Legnaro, Italy
giacomo.deangelis@lnl.infn.it

SIMPLE EQUATIONS METHOD (SESM) AND ITS APPLICATION FOR OBTAINING EXACT SOLUTIONS OF NONLINEAR PARTIAL DIFFERENTIAL EQUATIONS

Nikolay K. Vitanov, Zlatinka I. Dimitrova, Holger Kantz

Keywords: *nonlinear partial differential equations, simple equations method, exact solutions, traveling waves, solitons*

Abstract: *We discuss a methodology for obtaining exact solutions of nonlinear partial differential equations. The methodology is based on representation of a solution of a solved equation as an appropriate function of solutions of simpler differential equations. In such a way, the solved nonlinear partial differential equation is reduced to a nonlinear algebraic system which connects coefficients of solved equation and coefficients of solutions of simpler differential equations. Any nontrivial solution of this algebraic system leads to an exact solution of the solved nonlinear partial differential equation.*

1. Introduction

Evolution of scientific research has happened in such direction that in the course of time many researchers have started to study systems with larger complexity (several examples are Ausloos, 2000; Vitanov and Yankulova, 2006; May et al. 2008; Vitanov et al., 2012; Jordanov and Nikolova 2013, Ausloos et al., 2014; Vitanov and Ausloos, 2015; Vitanov, 2016; Borisov and Vitanov, 2019; Vitanov and Vitanov, 2019, and Nikolova et al. 2019). Such systems have many intriguing features and one of the most interesting characteristics of many complex systems is their nonlinearity (Drazin, 1992; Boeck and Vitanov 2002; Samoilenko and Petryshyn 2004; Panchev et al. 2007). The nonlinearity of complex systems can be studied, e.g. by means of time series analysis or by means of models based on differential equations (Grossberg 1981; Vitanov 2000; Kantz et al. 2004; Verhulst 2006; Ashenfelter et al. 2009; Vitanov and Dimitrova 2010). In many cases the corresponding model equations are nonlinear partial differential equations. The research on methodology for obtaining exact solutions of nonlinear partial differential equations began by study of transformations which transform a solved nonlinear partial differential equation to a linear differential equation. One example is the Hopf-Cole transformation,

which transforms the nonlinear Burgers equation to the linear heat equation. The attempts for obtaining similar transformations led to the development of methodologies such as the *Method of Inverse Scattering Transform* (see, e.g. Ablowitz and Clarkson 1991), the *Hirota method* (Hirota 2004), and others (e.g. Taylor 1989). As a branch of this research, Kudryashov (Kudryashov 2005) described the *Method of Simplest Equation* (MSE) based on determining the singularity order of solved nonlinear partial differential equations and searching for a particular solution of this equation as a series containing powers of solutions of a simpler equation called *simplest equation* (see also Kudryashov and Loguinova 2008). In this article we shall discuss a methodology called *Simple Equations Methodology* (SesM). Our way to the methodology of SEsM was long. Some elements of the methodology can be observed in our articles written 25 – 30 years ago (Martinov and Vitanov 1992a, 1992b, 1994, Vitanov 1998). What followed started in 2009 (Vitanov et al. 2009a, 2009b) and continued in 2010 by the use of the ordinary differential equation of Bernoulli as the simplest equation (Vitanov 2010) and by application of the methodology to ecology and population dynamics (Vitanov and Dimitrova 2010). In these publications we used the concept of the balance equation. This version of the methodology has been called *Modified Method of Simplest Equation* – MMSE (Vitanov et al. 2010, Vitanov 2011), and it is based on determination of the kind of the simplest equation and truncation of series constructed from solutions of the simplest equation by means of application of a balance equation. For several applications of the methodology see (Vitanov 2011a, Vitanov et al. 2011, 2013, 2015, 2017). We note especially the article (Vitanov et al. 2015) where we extended the methodology to simplest equations of the class

$$\left(\frac{d^k g}{d\xi^k}\right)^l = \sum_{j=0}^{m} d_j g^j \tag{1}$$

where $k = 1,...,$ $l = 1,...,$ and m and d_j are parameters. The solution of Eq. (1) defines a special function that contains as particular cases: (i) trigonometric functions; (ii) hyperbolic functions; (iii) elliptic functions of Jacobi; (iv) elliptic functions of Weierstrass, etc.

In the course of time, we proceeded to extend the methodology of the *Modified Method of Simplest Equation*. The last version of the methodology is connected to the possibility of using more than one simplest equation. This modification may be called MMSEn (Modified Method of Simplest Equation based on n simplest equations) but it is better to call it *SEsM – Simple Equations Method*. The reason for this is that the used simple equations are simpler than

the solved nonlinear partial differential equation but in fact these simple equations can be quite complicated (Vitanov 2019, Vitanov and Dimitrova 2019). A variant of SEsM based on two simple equations was applied in (Vitanov and Dimitrova 2018), and the first description of the methodology was made in (Vitanov 2019a) and then in (Vitanov 2019b).

The text below is organized as follows. We discuss the SEsM in Sect. 2 and show that SEsM is capable of leading to multisoliton solutions of nonlinear partial differential equations. The example will be based on the Korteweg-de Vries equation. In Sect. 3 we demonstrate that SEsM keeps the property of MMSE to lead to exact solutions of nonitegrable nonlinear partial differential equations. Several concluding remarks are summarized in Sect. 4.

2. Description of the Simple Equations Method (SEsM) with application for obtaining multisoliton solutions

The schema of SEsM is as follows. We have to solve a nonlinear partial differential equation

$$\mathcal{D}(u, \ldots) = 0 \tag{2}$$

where the left-hand side of (2) is a relationship containing the function u(...) and some of its derivatives (u can be a function of more than 1 spatial coordinate). The methodology of SEsM includes 7 steps.

1.) We apply a transformation

$$u(x, \ldots, t) = Tr(F(x, \ldots, t)) \tag{3}$$

where *Tr(F)* is a function of another function *F*. In general, *F(x,...,t)* is a function of the spatial variables as well as of the time. The transformation has to transform the nonlinearity of the solved differential equation to a more treatable kind of nonlinearity, or the applied transformation may even remove nonlinearity. Several examples of the form of transformation *Tr(F)* are:

- the Painleve expansion,
- $u(x,t) = 4\ tan^{-1}[F(x,t)]$ for the case of the sine-Gordon equation,
- u(x,t) = $4\ tanh^{-1}$[F(x,t)] for the case of sh-Gordon (Poisson-Boltzmann equation),
- another transformation.

In many particular cases one may skip this step (then we have just $u(x,...,t) = F(x,...,t)$), but in numerous cases this step is necessary for obtaining a solution of the studied nonlinear PDE. The application of Eq. (3) to Eq. (2) leads to a nonlinear PDE for the function $F(x,...,t)$. We note that the general form of the transformation $Tr(F)$ is not known.

2.) The function $F(x,...,t)$ is written as a function of other functions $f_1,...,f_N$. These functions are connected to solutions of some differential equations (these equations can be partial or ordinary differential equations) that are simpler than Eq. (2). The possible values of N are N=1,...,∞. The form of function $F(f_1,...,f_N)$ is not prescribed and can be given by different relationships, for example,

$$F = \alpha + \sum_{i_1=1}^{N} \beta_{i_1} f_{i_1} + \sum_{i_1=1}^{N} \sum_{i_2=1}^{N} \gamma_{i_1,i_2} f_{i_1} f_{i_2} + \ldots + \sum_{i_1=1}^{N} \ldots \sum_{i_N=1}^{N} \sigma_{i_1,\ldots,i_N} f_{i_1} \ldots f_{i_N} \qquad (4)$$

where α, β, γ, and σ are parameters. Of course, $F(f_1,...,f_N)$ can have another form too. We shall use the form given by Eq. (4) below. Note that the relationship (4) contains as a particular case a relationship used by Hirota. In addition, the power series of f used in the previous versions of the methodology based on 1 simple equation (and called Modified Method of Simplest Equation) are a particular case of the relationship (4) too.

3.) In general, each of the functions $f_1,...,f_N$ is a solution of a partial differential equation. These equations are simpler than the solved nonlinear partial differential equation. There are two possibilities: one may use solutions of simple partial differential equations if such solutions are available, or one may transform the more simple partial differential equations by means of an appropriate ansatz, for example, a travelling-wave ansatz such as

$$\xi = \hat{\alpha}x + \hat{\beta}t;\ \zeta = \hat{\mu}y + \hat{\nu}t \ldots$$

Then the solved differential equations for $f_1,...,f_N$ may be reduced to differential equations E_l, containing derivatives of one or several functions

$$E_l\left[a(\xi), a_\xi, a_{\xi\xi}, \ldots, b(\zeta), b_\zeta, b_{\zeta\zeta}, \ldots\right] = 0; \quad l = 1, \ldots, N \qquad (5)$$

In many cases (e.g. if the equations for the functions f_1,... are ordinary differential equations) one may skip this step, but the step may be necessary if the equations for f_1,... are complicated partial differential equations.

4.) We assume that the functions *a, b,* etc., are functions of other functions, for example, *v, w*, etc., in other words:

$$a(\xi) = A[v(\xi)]; \quad b(\zeta) = B[w(\zeta)]; \dots \tag{6}$$

The kinds of functions *A, B,...* are not prescribed. Often, one uses a finite-series relationship, e.g.:

$$a(\xi) = \sum_{\mu_1=-\nu_1}^{\nu_2} q_{\mu_1}[v(\xi)]^{\mu_1}; \quad b(\zeta) = \sum_{\mu_2=-\nu_3}^{\nu_4} r_{\mu_2}[w(\zeta)]^{\mu_2}, \dots \tag{7}$$

where *q, r,,...,* are parameters. However, other kinds of relationships may be used too.

5.) The functions *v, w,...* are solutions of simple ordinary differential equations. For several years we have used a particular case of the described methodology that was based on use of one simple equation. This simple equation was called simplest equation and the methodology based on one equation was called *Modified Method of Simplest Equation*. *SEsM* contains the *Modified Method of Simplest Equation* as a particular case (as one of the numerous particular cases of the SEsM methodology).

6.) The application of the steps 1.) – 5.) to Eq. (2) transforms its left-hand side. We consider the case when the result of this transformation is a function which is a sum of terms where each term contains some function multiplied by a coefficient. This coefficient contains some of the parameters of the solved equation and some of the parameters of the solution. In most cases a balance procedure must be applied in order to ensure that the abovementioned relationships for the coefficients contain more than one term (e.g. if the result of the transformation is a polynomial, then the balance procedure has to ensure that the coefficient of each term of the polynomial is a relationship that contains at least two terms). This balance procedure may lead to one or more additional relationships among the parameters of the solved equation and the parameters of the solution. These relationships are called *balance equations*.

7.) We may obtain a nontrivial solution of Eq. (2) if all coefficients mentioned in Step 6.) are set to *0*. This condition usually leads to a system of nonlinear algebraic equations for coefficients of the solved nonlinear PDE and for coefficients of the solution. Any nontrivial solution of this algebraic system leads to a solution the studied nonlinear partial differential equation. Usually, the aforementioned system of algebraic equations contains many equations that have to be solved with the help of a computer algebra system. Sometimes the system is quite complicated and even the computer algebra systems are unable to solve it.

Let us now show examples of the application of the methodology.

2.1. The multisoliton solution of the Korteweg-de Vries equation

Let us show that the SEsM methodology allows obtaining of a multisoliton solution of the Korteweg-de Vries equation for a particular case when *N* simple equations for exponential functions are used, and the solution is constructed by use of the particular case of the function (4). In order to do this, we consider the following version of the Korteweg-de Vries equation

$$u_t + \sigma u u_x + u_{xxx} = 0 \tag{8}$$

where σ is a parameter. We consider the particular (classical) case $\sigma = -6$ and use the transformation

$$u(x,t) = -2\frac{\partial^2}{\partial x^2} \ln F(x,t) \tag{9}$$

Let us consider the following particular case of Eq. (4)

$$\begin{aligned} F &= 1 + \sum_{i=1}^{N} f_i + \sum_{(i\neq j),i,j=1}^{N} \gamma_{i,j} f_i f_j + \sum_{(i\neq j\neq k),i,j,k=1}^{N} \delta_{i,j,k} f_i f_j f_k + \ldots + \\ &\quad \sigma_{1,\ldots,N} f_1 \ldots f_N \end{aligned} \tag{10}$$

We shall use *n* simple equations of the kind

$$\frac{df_i}{d\xi_i} = f_i, \quad \xi_i = p_i x - \omega_i t - \xi_{i0} \tag{11}$$

where the solution is

$$f_i(\xi_i) = \exp(\xi_i).$$

We substitute the solutions of Eqs. (11) in Eq. (10) and select the coefficients γ, δ,... in an appropriate way in order to obtain the relationship

$$F = 1 + \sum_{n=1}^{N} \sum_{\binom{N}{n}} \left[\exp(\xi_{i_1} + \ldots + \xi_{i_N}) \prod_{k<l}^{(n)} \frac{(p_{ik} - p_{il})^2}{(p_{ik} + p_{il})^2} \right] \tag{12}$$

Here, the notation below the second sum means the sum over all combinations of n elements taken from the set of N elements, and the notation below and above the sign for the product means the product of all possible combinations of the n elements with the condition $k < l$. The substitution of

$$u(x,t) = -2\frac{\partial^2}{\partial x^2} \ln \left\{ 1 + \sum_{n=1}^{N} \sum_{\binom{N}{n}} \left[\exp(\xi_{i_1} + \ldots + \xi_{i_N}) \prod_{k<l}^{(n)} \frac{(p_{ik} - p_{il})^2}{(p_{ik} + p_{il})^2} \right] \right\} \tag{13}$$

in Eq. (8) ($\sigma = -6$) reduces this equation to the system of algebraic relationships

$$\begin{aligned} \sum_{l=0}^{n} \sum_{\binom{l}{n}} \left[\prod_{k<m}^{(l)} \frac{(p_{ik} - p_{im})^2}{(p_{ik} + p_{im})^2} \right] \left[\prod_{k>l,k<m}^{(n)} \frac{(p_{ik} - p_{im})^2}{(p_{ik} + p_{im})^2} \right] \Big[(-p_{i_1} - \ldots - p_{i_l} + p_{i_{(l+1)}} + \ldots + \\ p_{i_n})(-p_{i_1} - \ldots - p_{i_l} + p_{i_{(l+1)}} + \ldots + p_{i_n})^3 - (-p_{i_1}^3 - \ldots - p_{i_l}^3 + p_{i_{(l+1)}}^3 + \ldots + \\ p_{i_n}^3) \Big] = 0 \\ \omega_i = p_i^3 \end{aligned} \tag{14}$$

for $n = 1,\ldots,N$. The nontrivial solution of this algebraic system leads to a solitary or a multisoliton solution of the Korteweg-de Vries equation.

In order to make the example clear, we shall obtain the two-soliton solution of the Korteweg-de Vries equation. Let us consider Eq. (8), and let σ be a parameter. The seven steps of the *SEsM* are as follows:

1.) *Transformation*

We set $u = p_x$ in Eq. (8). The result is integrated, and we apply the transformation

$$p = \frac{12}{\sigma} (\ln F)_x$$

The result is

$$FF_{tx} + FF_{xxxx} - F_tF_x + 3F_{xx}^2 - 4F_xF_{xxx} = 0 \qquad (15)$$

2.) *Relationship between F(x,t) and two functions $f_{1,2}$ that will be connected below to two simplest equations*

We shall use two functions $f_1(x,t)$ and $f_2(x,t)$, and the relationship for F is assumed to be a particular case of Eq. (4), namely:

$$F(x,t) = 1 + f_1(x,t) + f_2(x,t) + cf_1(x,t)f_2(x,t) \qquad (16)$$

where c is a parameter. The substitution of Eq. (16) in Eq. (15) leads to

$$\begin{aligned}
&f_{1xxxx} + f_{2xxxx} + 3f_{1xx}^2 + cf_{1xt}f_2 + f_{1xt} - f_{1t}f_{1x} - f_{1t}f_{2x} - \\
&4f_{1x}f_{1xxx} - f_{1x}f_{2t} - 4f_{1x}f_{2xxx} + 6f_{1xx}f_{2xx} - 4f_{1xxx}f_{2x} - f_{2t}f_{2x} - \\
&4f_{2x}f_{2xxx} + f_2f_{1xt} + f_2f_{1xxxx} + f_2f_{2xt} + f_2f_{2xxxx} + f_1f_{1xt} + \\
&f_1f_{1xxxx} + f_1f_{2xt} + f_1f_{2xxxx} + f_{2xt} + c^2f_2^2f_1f_{1xt} + c^2f_2^2f_1f_{1xxxx} - \\
&c^2f_2^2f_{1t}f_{1x} - 4f_2^2c^2f_{1x}f_{1xxx} + c^2f_2f_1^2f_{2xt} + c^2f_2f_1^2f_{2xxxx} - \\
&12c^2f_2f_{1x}^2f_{2xx} - c^2f_1^2f_{2t}f_{2x} - 4c^2f_1^2f_{2x}f_{2xxx} - 12c^2f_1f_{1xx}f_{2x}^2 + \\
&2cf_2f_1f_{1xt} + 2cf_2f_1f_{1xxxx} + 2cf_2f_1f_{2xt} + 2cf_2f_1f_{2xxxx} - 2cf_2f_{1t}f_{1x} - \\
&8cf_2f_{1x}f_{1xxx} + 12cf_2f_{1xx}f_{2xx} + 12cf_1f_{1xx}f_{2xx} - 2cf_1f_{2t}f_{2x} - \\
&8cf_1f_{2x}f_{2xxx} + 12c^2f_{1x}^2f_{2x}^2 - 12cf_{1x}^2f_{2xx} - 12cf_{1xx}f_{2x}^2 + 3c^2f_2^2f_{1xx}^2 + \\
&3c^2f_1^2f_{2xx}^2 + cf_2^2f_{1xt} + cf_2^2f_{1xxxx} + 6cf_2f_{1xx}^2 + cf_1^2f_{2xt} + \\
&cf_1^2f_{2xxxx} + 6cf_1f_{2xx}^2 + cf_1f_{2xt} + cf_1f_{2xxxx} + 6cf_{1xx}f_{2xx} + 4cf_{1x}f_{2xxx} + \\
&cf_{1x}f_{2t} + cf_{1xxxx}f_2 + 12c^2f_2f_1f_{1xx}f_{2xx} + cf_{1t}f_{2x} + 4cf_{1xxx}f_{2x} + 3f_{2xx}^2 = 0
\end{aligned} \qquad (17)$$

3.) *Equations for the functions $f_1(x,t)$ and $f_2(x,t)$*

The structure of Eq. (17) allows us to assume a very simple form of the equations for the functions $f_{1,2}$:

$$\begin{aligned}
\frac{\partial f_1}{\partial x} &= \alpha_1 f_1; \quad \frac{\partial f_1}{\partial t} = \beta_1 f_1; \\
\frac{\partial f_2}{\partial x} &= \alpha_2 f_2; \quad \frac{\partial f_2}{\partial t} = \beta_2 f_2;
\end{aligned} \qquad (18)$$

This choice will transform Eq. (17) to a polynomial of f_1 and f_2. Further, we assume that

$$\xi = \alpha_1 x + \beta_1 t + \gamma_1 \text{ and } \zeta = \alpha_2 x + \beta_2 t + \gamma_2$$

as well as

$$f_1(x,t) = a(\xi); \quad f_2(x,t) = b(\zeta) \tag{19}$$

Above, α, β, and γ are parameters.

4.) *Relationships connecting functions a and b to functions v and w which are solutions of the simplest equations*

In the case discussed here the relationships are quite simple. On the basis of Eq. (7), we obtain

$$a(\xi) = q_1 v(\xi); \quad b(\zeta) = r_1 w(\zeta) \tag{20}$$

5.) *Simple equations for v and w*

The simple equations are

$$\frac{dv}{d\xi} = v; \quad \frac{dw}{d\zeta} = w \tag{21}$$

and the corresponding solutions are

$$v(\xi) = \omega_1 \exp(\xi); \quad w(\zeta) = \omega_2 \exp(\zeta) \tag{22}$$

Below, we shall assume that the parameters $\omega_{1,2}$ are included in the parameters q_1 and r_1, respectively. We shall also assume that q_1 and r_1 can be included in χ and ζ.

6.) *Transformation of Eq. (17)*

Let us substitute Eqs. (18) – (21) in Eq. (17). The result is a sum of exponential functions, and each exponential function is multiplied by a coefficient. Each of these coefficients is a relationship containing parameters of the solution, and all relationships contain more than one term. Thus we do not need to perform a balance procedure.

7.) *Obtaining and solving the system of algebraic equations*

The system of algebraic equations is obtained by setting of above-mentioned relationships to *0*. Thus we obtain the following system:

$$\begin{aligned}
&\alpha_1^3 + \beta_1 = 0,\\
&\alpha_2^3 + \beta_2 = 0,\\
&(c+1)\alpha_1^4 + 4\alpha_2(c-1)\alpha_1^3 + 6\alpha_2^2(c+1)\alpha_1^2 + [(4c-4)\alpha_2^3 + (\beta_1+\beta_2)c +\\
&\beta_1 - \beta_2]\alpha_1 + [(c+1)\alpha_2^3 + (\beta_1+\beta_2)c - \beta_1 + \beta_2]\alpha_2 = 0.
\end{aligned} \tag{23}$$

The non-trivial solution of this system is

$$\beta_1 = -\alpha_1^3; \quad \beta_2 = -\alpha_2^3; \quad c = \frac{(\alpha_1 - \alpha_2)^2}{(\alpha_1 + \alpha_2)^2} \tag{24}$$

and the corresponding solution of Eq. (8) is

$$\begin{aligned} u(x,t) &= \frac{12}{\sigma}\frac{\partial^2}{\partial x^2}\Big[1 + \exp\left(\alpha_1 x - \alpha_1^3 t + \gamma_1\right) + \exp\left(\alpha_2 x - \alpha_2^3 t + \gamma_2\right) + \\ &\quad \frac{(\alpha_1 - \alpha_2)^2}{(\alpha_1 + \alpha_2)^2}\exp\left((\alpha_1 + \alpha_2)x - (\alpha_1^3 + \alpha_2^3)t + \gamma_1 + \gamma_2\right)\Big] \end{aligned} \tag{25}$$

The use of simpler equations for exponential functions will lead to solutions containing more solitons. Thus we have proven that the methodology of *SEsM* is capable of searching for complicated solitary wave solutions of nonlinear PDEs. This capability is acquired on the basis of the possibility of use of more than one simple equation. The relationship (4) can also be used for obtaining exact solutions of nonintegrable nonlinear PDEs. This will be demonstrated in the following section.

3. Exponential functions and nonintegrable equations

Let us consider the simple equation

$$\frac{dv}{d\xi} = v^2 - v \tag{26}$$

It has the solution

$$v(\xi) = \frac{1}{1 + \exp(\xi)} \tag{27}$$

We can search solutions of the solved nonlinear partial differential equations

$$\mathcal{D}u(\xi) = 0$$

as

$$u(\xi) = \sum_{i=0}^{N} a_i v^i \tag{28}$$

This was proposed by Kudryashov in 2012. Now let us show that the method of Kudryashov above is a particular case of the SEsM methodology for the case of use of one simple equation and representation of the searched solution as a polynomial of the solution of the simple equation. In order to do this, we consider the SEsM methodology for the case of lack of transformation, one simple equation (Eq. (26)) and the polynomial representation (28) of the solution of the equation by the solution of the simple equation. Then, the SEsM methodology is reduced to the method of Kudryashov. Hence, the method of Kudryashov is a particular case of the SEsM methodology.

Let us now use this particular case to obtain an exact solution for a particular case of the equation

$$u_t + \left(\sum_{k=0}^{l} \alpha_k u^k \right) u_x + \beta u_{xxx} + \gamma u^m u_{xxxxx} = 0 \tag{29}$$

The steps of the methodology are as follows:

1.) *Transformation*
We use a particular case of transformation (3), i.e. $u(x,t) = F(x,t)$.

2.) *Relationship between F(x,t) and the functions* $f_k(x,t)$
The function $F(x,t)$ will be searched as a function of another function $f(x,t)$, and the corresponding relationship is a particular case of the relationship (4)

$$F(x,t) = \sum_{i=0}^{N} \gamma_i f(x,t)^i \tag{30}$$

where γ_i denotes parameters.

3.) *Equation for the function f(x,t)*
The function $f(x,t)$ is assumed to be a traveling wave

$$f(x,t) = a(\xi); \quad \xi = \mu x + \nu t \tag{31}$$

where μ and ν are parameters.

4.) *Representation of the function a by another function that is a solution of a simplest equation*

There is no need to express further the function *a* through another function *v*, and instead of this we shall assume that *a* is a solution of a simple equation of the class (8).

5.) *Simple equation*

Below, we shall use a particular case of the following simple equation:

$$\frac{da}{d\xi} = \sum_{j=0}^{p} d_j a^j, \tag{32}$$

6.) *Transformation of Eq. (29)*

The substitution of Eqs. (30) and (32) in Eq. (29) leads to a polynomial of the function *a* that contains the following maximum powers of the terms of Eq. (29): *N+p – 1*; *N+3(p – 1)*; *Nm+n+5(p – 1)*; *Nl+N+p – 1*. In order to obtain the system of nonlinear algebraic equations, we have to write balance equations for these powers, i.e. in this case we have to balance the largest powers:

Nm+n+5(p – 1) and *Nl+N+p – 1*. This leads us to the balance equation

$$N(l-m) = 4(p-1) \tag{33}$$

We note that *l, m, p,* and *N* have to be integers or *0*. We have $p > 1$ and $l > m$. Then, from Eq. (33),

$$N = 4\frac{p-1}{l-m} \tag{34}$$

which means that the equations of the class Eq. (29) may have solutions of the kind

$$u(x,t) = \sum_{i=0}^{4\frac{p-1}{l-m}} \gamma_i a(\xi)^i \tag{35}$$

where

$$\xi = \mu x + \nu t$$

and the function a is a solution of the simple equation

$$\frac{da}{d\xi} = d_0 + \ldots + d_p a^p. \tag{36}$$

Let us set $m = 1$. The smallest possible value of l in this case is $l = 2$. Then, the equation (29) is reduced to

$$u_t + \left(\alpha_0 + \alpha_1 u + \alpha_2 u^2\right) u_x + \beta u_{xxx} + \gamma u u_{xxxxx} = 0 \tag{37}$$

Next, we shall use Eq. (32) as a simple equation. From Eq. (34) we obtain for the balance equation

$$N = 4(p-1)$$

which means that the equations of the class Eq. (37) may have solutions of the kind

$$u(x,t) = \sum_{i=0}^{4(p-1)} \gamma_i a(\xi)^i \tag{38}$$

where the function a is a solution of the simple equation

$$\frac{da}{d\xi} = d_0 + \ldots + d_p a^p. \tag{39}$$

We shall consider the case $p = 2$ below. In this case the application of the steps of the methodology leads to a system of 13 nonlinear algebraic equations for the parameters of the solution and the parameters of the equation. As we want to use the simple equation (26), we have to set $d_0 = 0$, $d_1 = -1$, and $d_2 = 1$, which simplifies a lot the system of algebraic equations. One nontrivial solution of this system is

$$\begin{aligned}
\gamma_0 &= -\frac{1}{15}\frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}}; \quad \gamma_1 = 4\frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}};\\
\gamma_2 &= -28\frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}}; \quad \gamma_3 = 48\frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}};\\
\gamma_4 &= -24\frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}}; \quad \mu = \frac{1}{3}\frac{6^{1/2}35^{1/4}(\alpha_2^3\beta^3\gamma^2)^{1/4}}{\alpha_2\beta}\\
\nu &= -\frac{1}{3}\frac{6^{1/2}35^{1/4}\alpha_0(\alpha_2^3\beta^3\gamma^2)^{1/4}}{\alpha_2\beta}; \quad \alpha_1 = \frac{1}{10}\frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}};
\end{aligned} \tag{40}$$

and the corresponding solution of the solved equation (37) is

$$\begin{aligned}
u(\xi) = \frac{6^{1/2}35^{3/4}\beta\gamma}{(\alpha_2^3\beta^3\gamma^2)^{1/4}}\Bigg\{ &-\frac{1}{15}+4\left[\frac{1}{1+\exp\left[\left(\frac{1}{3}\frac{6^{1/2}35^{1/4}(\alpha_2^3\beta^3\gamma^2)^{1/4}}{\alpha_2\beta}\right)(x-\alpha_0 t)\right]}\right]-\\
&28\left[\frac{1}{1+\exp\left[\left(\frac{1}{3}\frac{6^{1/2}35^{1/4}(\alpha_2^3\beta^3\gamma^2)^{1/4}}{\alpha_2\beta}\right)(x-\alpha_0 t)\right]}\right]^2+\\
&28\left[\frac{1}{1+\exp\left[\left(\frac{1}{3}\frac{6^{1/2}35^{1/4}(\alpha_2^3\beta^3\gamma^2)^{1/4}}{\alpha_2\beta}\right)(x-\alpha_0 t)\right]}\right]^3-\\
&24\left[\frac{1}{1+\exp\left[\left(\frac{1}{3}\frac{6^{1/2}35^{1/4}(\alpha_2^3\beta^3\gamma^2)^{1/4}}{\alpha_2\beta}\right)(x-\alpha_0 t)\right]}\right]^4\Bigg\}
\end{aligned} \tag{41}$$

4. Concluding remarks

We discuss the SEsM methodology for obtaining exact solutions of nonlinear partial differential equations. The goal of the old versions of this methodology was to help us to obtain particular exact solutions of nonlinear nonintegrable partial differential equations of interest for mathematics, natural and social sciences. We show that by appropriate extension of the methodology it can also lead to multisoliton solutions of integrable differential equations. We are sure that the methodology will be useful for researchers who want to have a simple methodology for obtaining exact solutions of nonlinear partial differential equations.

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Nikolay K. Vitanov
Institute of Mechanics,
Bulgarian Academy of Sciences
Sofia, Bulgaria
vitanov@imbm.bas.bg

Zlatinka I. Dimitrova
"G. Nadjakov" Institute of Solid State Physics
Bulgarian Academy of Sciences
Sofia, Bulgaria
zdim@issp.bas.bg

Holger Kantz
Max-Planck Institute for the Physics
of Complex Systems
Dresden, Germany
kantz@pks.mpg.de

STATISTICAL ANALYSIS OF THE MOTION OF A SUBSTANCE IN A CHANNEL OF A NETWORK

Tsvetelina Ivanova, Nikolay K. Vitanov

Keywords: *probability distributions, networks, human migration*

Abstract: *We discuss a mathematical model of the motion of a substance through a channel of a network. For the stationary case of the motion of the substance, we obtain a class of long-tail probability distributions for the amount of the substance in the nodes of the channel. We discuss some of these distributions as well as the application of the model for description of human migration.*

1. Introduction

Mathematical models can be used for the description and understanding of various processes from many scientific fields. For example, mathematical models of flows in networks find applications in flows in computer networks, flows in financial networks, flows in electrical and communication networks, transportation problems, optimized flows in networks (maximum result with minimum resources), shortest path finding, self-organizing network flows, etc. (Vitanov, Vitanov 2019).

Another application of models of flows in networks is related to human migration flows. Population migration involves relocation of individuals, groups, and households, between geographical locations. Understanding and studying human migration is important for making decisions about the economic development of regions of different countries (Vitanov, Vitanov, 2016; Vitanov, Vitanov 2018a; 2018b).

The study of migration became a popular topic after the large migration flows directed to Europe in September 2015. Migration models can be classified as micromodels and macromodels, depending on the migrating units. Micromodels are based on the individual migrating unit (person, group or household) and on the decision whether to remain in the current location or to move somewhere else. Macromodels are for the aggregate migration flows, and many of them use non-demographic information. Some other kinds of macromodels use demographic information. With respect to their mathematical features, migration models can be classified as probability models

or deterministic models (Vitanov, Vitanov, 2016). In our current research, we develop deterministic discrete-time macromodels of flows of a substance in parts of networks. It is also possible to develop models of differential equations (Vitanov, Vitanov, Ivanova 2018). Models with linear differential equations can be solved analytically, whereas models with nonlinear differential equations often require a numerical approach.

In this current research, we study the motion of a substance through a part of a network. A network consists of nodes and edges. These edges may or may not have a direction, thus networks can be classified as directed or non-directed. In the context of human migration, the nodes represent the countries, and the edges represent the connectivity of these countries. The substance that is flowing through a part of the network represents the migrants.

2. Mathematical model of the flow of a substance through a channel with one branch

We formulate a macroscopic deterministic mathematical model of the motion of a substance in a part of a network that consists of a channel with one branch. The channel and the branch consist of chains of nodes (or, in this case, cells) that belong to the network and edges that connect the nodes. The edges form the way of the motion of the substance. The channel consists of cells numbered from *0* to *N*. The cell from the channel that is denoted by number *M*, located between cell 0 and cell *N*, is the initial cell for the branch with *R+1* number of cells.

The nodes of the channel and the branch can have different rates at which the substance is leaving the nodes. Close to the end of the channel, the nodes may be more "attractive" for design or other reason, compared to the nodes around the initial node of the channel and the branch (Vitanov, Vitanov 2018b).

The mathematical model of the flow of the substance along the channel and the branch includes and describes in mathematical terms all possible ways to exchange the substance. In the case of a finite amount of cells along the channel and along the branch, the mathematical model consists of equations that describe the quantities of the substance in each cell that belongs to the channel and the branch. The model is formulated as follows:

$$
\begin{aligned}
\chi_0(t_{k+1}) &= \chi_0(t_k) + i_0^e(t_k) - o_0^e(t_k) + i_0^n(t_k) - o_0^n(t_k) \\
&\quad + i_0^c(t_k) - o_0^c(t_k) \\
\\
\chi_j(t_{k+1}) &= \chi_j(t_k) + i_j^e(t_k) - o_j^e(t_k) + i_j^n(t_k) - o_j^n(t_k) \\
&\quad + i_j^c(t_k) - o_j^c(t_k) - i_{j-1}^c(t_k) + o_{j-1}^c(t_k), \\
j &= 1, \ldots, M-1, M+1, \ldots, N-1 \\
\\
\chi_N(t_{k+1}) &= \chi_N(t_k) + i_N^e(t_k) - o_N^e(t_k) + i_N^n(t_k) - o_N^n(t_k) \\
&\quad - i_{N-1}^c(t_k) + o_{N-1}^c(t_k) \\
\\
\chi_M(t_{k+1}) &= \chi_M(t_k) + i_M^e(t_k) - o_M^e(t_k) + i_M^n(t_k) - o_M^n(t_k) \\
&\quad + i_M^c(t_k) - o_M^c(t_k) - i_{M-1}^c(t_k) + o_{M-1}^c(t_k) \\
&\quad + i_M^b(t_k) - o_M^b(t_k) \\
\\
\chi_l(t_{k+1}) &= \chi_l(t_k) + i_l^{e_b}(t_k) - o_l^{e_b}(t_k) + i_l^{n_b}(t_k) - o_l^{n_b}(t_k) \\
&\quad + i_l^b(t_k) - o_l^b(t_k) - i_{l-1}^b(t_k) + o_{l-1}^b(t_k), \\
l &= 1, \ldots, R-1 \\
\\
\chi_R(t_{k+1}) &= \chi_R(t_k) + i_R^{e_b}(t_k) - o_R^{e_b}(t_k) + i_R^{n_b}(t_k) - o_R^{n_b}(t_k) \\
&\quad - i_{R-1}^b(t_k) + o_{R-1}^b(t_k)
\end{aligned}
\tag{1}
$$

Overall, system (1) consists of *(N+R+1)* equations. The last cell from the channel is denoted by *N*, and the last cell from the branch is denoted by *R*. The first three equations of system (1) describe the substance along the cells that belong to the channel. The last three equations of system (1) describe the substance along the cells that belong to the branch that starts from cell *M* of the channel.

System (1) describes the following processes in each node (or cell): inflow from the environment, inflow from other parts of the network, inflow from cells that belong to the channel and the branch, outflow to the environment, outflow to other parts of the network, outflow to other cells that belong to the channel and the branch. The quantities denoted by $i(t_k)$ represent the inflows to the cells. The quantities denoted by $o(t_k)$ represent the outflows from the cells. The subscripts for each quantity denote the cell number. The superscripts *e, n, c,* and *b* denote the exchange of the substance from the cells that belong to the channel with the environment, with the network, with other cells from the channel, and with the branch, respectively. The superscripts e_b, n_b, and *b*, denote the exchange of the substance from the cells that belong to the branch

with the environment, with other parts of the network, and with other cells from the branch, respectively.

A particular case of this model is studied under the following conditions:

1. There is no exchange of a substance with the environment, except in the initial cell, where there is only an inflow from the environment.

2. There is no inflow of a substance from other parts of the network to the cells of the channel.

3. There is no inflow of a substance from cell number i to cell $(i-1)$ of the channel, $i = 1,..., N$.

4. There is no inflow of a substance from other parts of the network to the cells of the branch.

5. There is no inflow of a substance from cell number k to cell $(k-1)$ of the branch, $k = 1,..., R$.

In addition, we denote the quantities in the equations in system (1) by the following expressions:

$$
\begin{aligned}
o^b_M(t_k) &= g_0(t_k)\chi_M(t_k)\\
o^b_l(t_k) &= g_l(t_k)\chi_l(t_k)\\
o^b_{l-1}(t_k) &= g_{l-1}(t_k)\chi_{l-1}(t_k)\\
o^{n_b}_l(t_k) &= \xi_l(t_k)\chi_l(t_k)\\
\\
o^{n_b}_R(t_k) &= \xi_R(t_k)\chi_R(t_k)\\
o^b_{R-1}(t_k) &= g_{R-1}(t_k)\chi_{R-1}(t_k)\\
\\
o^c_{N-1}(t_k) &= f_{N-1}(t_k)\chi_{N-1}(t_k)\\
o^n_N(t_k) &= \gamma_N(t_k)\chi_N(t_k)
\end{aligned}
\tag{2}
$$

$$
\begin{aligned}
i^e_0(t_k) &= \sigma(t_k)\chi_0(t_k)\\
o^n_0(t_k) &= \gamma_0(t_k)\chi_0(t_k)\\
o^c_0(t_k) &= f_0(t_k)\chi_0(t_k)\\
\\
o^n_j(t_k) &= \gamma_j(t_k)\chi_j(t_k)\\
o^c_{j-1}(t_k) &= f_{j-1}(t_k)\chi_{j-1}(t_k)\\
o^c_j(t_k) &= f_j(t_k)\chi_j(t_k)\\
\\
o^n_M(t_k) &= \gamma_M(t_k)\chi_M(t_k)\\
o^c_M(t_k) &= f_M(t_k)\chi_M(t_k)\\
o^c_{M-1}(t_k) &= f_{M-1}(t_k)\chi_{M-1}(t_k)
\end{aligned}
\tag{3}
$$

The model is studied for the case of time-independent and non-negative parameters and in stationary regime of the system, which means that there are exchanges of substance in each cell but the quantity of the substance in each cell remains constant over time. The described particular case of the mathematical model for a substance flow through a channel with one branch, system (1), is of the following kind:

$$\begin{aligned}
0 &= \sigma\chi_0^* - f_0\chi_0^* - \gamma_0\chi_0^* \\
0 &= f_{j-1}\chi_{j-1}^* - f_j\chi_j^* - \gamma_j\chi_j^*, \quad j = 1, \ldots, M-1, M+1, \ldots, N-1 \\
0 &= f_{N-1}\chi_{N-1}^* - \gamma_N\chi_N^* \\
0 &= f_{M-1}\chi_{M-1}^* - \gamma_M\chi_M^* - f_M\chi_M^* - g_0\chi_M^* \\
0 &= g_{l-1}\chi_{l-1}^* - g_l\chi_l^* - \xi_l\chi_l^*, \quad l = 1, \ldots, R-1 \\
0 &= g_{R-1}\chi_{R-1}^* - \xi_R\chi_R^*
\end{aligned} \tag{4}$$

From equations (4) we obtain expressions for the quantities of the substance χ^*_i in each cell from the channel, $i = 0,..., N$, and for the quantities χ^*_b in each cell of the branch, $b = 1, \ldots, R$. By dividing these quantities by the total amount of the substance along the studied part of the network χ^*, we obtain the class of distributions of the substance along the channel with one branch. The total amount of the substance along the channel and the branch is:

$$\begin{aligned}
\chi^* = \chi_0^* \Bigg[& 1 + \sum_{i=1}^{M-1} \prod_{j=1}^{i} \frac{f_{j-1}}{f_j + \gamma_j} + \sum_{k=M+1}^{N-1} \frac{f_{M-1}}{f_M + \gamma_M + g_0} \prod_{j=1, j\neq M}^{k} \frac{f_{j-1}}{f_j + \gamma_j} \\
& + \frac{f_{N-1}}{\gamma_N} \frac{f_{M-1}}{f_M + \gamma_M + g_0} \prod_{j=1, j\neq M}^{N-1} \frac{f_{j-1}}{f_j + \gamma_j} \\
& + \frac{f_{M-1}}{f_M + \gamma_M + g_0} \prod_{j=1}^{M-1} \frac{f_{j-1}}{f_j + \gamma_j} \\
& + \sum_{b=1}^{R-1} \frac{f_{M-1}}{f_M + \gamma_M + g_0} \prod_{l=1}^{b} \frac{g_{l-1}}{g_l + \xi_l} \prod_{j=1}^{M-1} \frac{f_{j-1}}{f_j + \gamma_j} \\
& + \frac{f_{M-1}}{f_M + \gamma_M + g_0} \frac{g_{R-1}}{\xi_R} \prod_{l=1}^{R-1} \frac{g_{l-1}}{g_l + \xi_l} \prod_{j=1}^{M-1} \frac{f_{j-1}}{f_j + \gamma_j} \Bigg]
\end{aligned}$$

For convenience, the amount of the substance along the channel and the branch, respectively, can be denoted by the following expressions:

$$\begin{aligned}
\chi^* = \chi_0^* \Bigg[& 1 + \sum_{i=1}^{M-1} \prod_{j=1}^{i} \frac{f_{j-1}}{f_j + \gamma_j} + \sum_{k=M+1}^{N-1} \frac{f_{M-1}}{f_M + \gamma_M + g_0} \prod_{j=1, j\neq M}^{k} \frac{f_{j-1}}{f_j + \gamma_j} \\
& + \frac{f_{N-1}}{\gamma_N} \frac{f_{M-1}}{f_M + \gamma_M + g_0} \prod_{j=1, j\neq M}^{N-1} \frac{f_{j-1}}{f_j + \gamma_j}
\end{aligned}$$

$$\chi^*_{branch} = \frac{f_{M-1}}{f_M+\gamma_M+g_0}\prod_{j=1}^{M-1}\frac{f_{j-1}}{f_j+\gamma_j} + \sum_{b=1}^{R-1}\frac{f_{M-1}}{f_M+\gamma_M+g_0}\prod_{l=1}^{b}\frac{g_{l-1}}{g_l+\xi_l}\prod_{j=1}^{M-1}\frac{f_{j-1}}{f_j+\gamma_j} + \frac{f_{M-1}}{f_M+\gamma_M+g_0}\frac{g_{R-1}}{\xi_R}\prod_{l=1}^{R-1}\frac{g_{l-1}}{g_l+\xi_l}\prod_{j=1}^{M-1}\frac{f_{j-1}}{f_j+\gamma_j}$$

The total amount of the substance can therefore be written as:

$$y_i^* = \frac{\chi_i^*}{\chi^*} \quad \chi^* = \chi_0^*\left[\chi^*_{channel} + \chi^*_{branch}\right]$$

The obtained class of probability distributions is of this kind:

$$y_0^* = \frac{\chi_0^*}{\chi^*} = \frac{1}{\chi^*_{channel}+\chi^*_{branch}}$$

$$y_i^* = \frac{\chi_i^*}{\chi^*} = \frac{\prod_{j=1}^{i}\frac{f_{j-1}}{f_j+\gamma_j}}{\chi^*_{channel}+\chi^*_{branch}}, \quad i = 1,\ldots,M-1$$

$$y_M^* = \frac{\chi_M^*}{\chi^*} = \frac{\frac{f_{M-1}}{f_M+\gamma_M+g_0}\prod_{j=1}^{M-1}\frac{f_{j-1}}{f_j+\gamma_j}}{\chi^*_{channel}+\chi^*_{branch}}$$

$$y_k^* = \frac{\chi_k^*}{\chi^*} = \frac{\frac{f_{M-1}}{f_M+\gamma_M+g_0}\prod_{j=1,j\neq M}^{k}\frac{f_{j-1}}{f_j+\gamma_j}}{\chi^*_{channel}+\chi^*_{branch}}, \quad k = M+1,\ldots,N-1$$

$$y_N^* = \frac{\chi_N^*}{\chi^*} = \frac{\frac{f_{N-1}}{\gamma_N}\frac{f_{M-1}}{f_M+\gamma_M+g_0}\prod_{j=1,j\neq M}^{N-1}\frac{f_{j-1}}{f_j+\gamma_j}}{\chi^*_{channel}+\chi^*_{branch}}$$

$$y_b^* = \frac{\chi_b^*}{\chi^*} = \frac{\frac{f_{M-1}}{f_M+\gamma_M+g_0}\prod_{l=1}^{b}\frac{g_{l-1}}{g_l+\xi_l}\prod_{j=1}^{M-1}\frac{f_{j-1}}{f_j+\gamma_j}}{\chi^*_{channel}+\chi^*_{branch}}, \quad b = 1,\ldots,R-1$$

$$y_R^* = \frac{\chi_R^*}{\chi^*} = \frac{\frac{f_{M-1}}{f_M+\gamma_M+g_0}\frac{g_{R-1}}{\xi_R}\prod_{l=1}^{R-1}\frac{g_{l-1}}{g_l+\xi_l}\prod_{j=1}^{M-1}\frac{f_{j-1}}{f_j+\gamma_j}}{\chi^*_{channel}+\chi^*_{branch}} \tag{5}$$

For the case of a single channel, the corresponding class of distributions can be obtained from (5) by assuming that all quantities directed from cell M to the branch are equal to zero, or that for the following parameters, the condition:

$$g_0 = 0, \xi_0 = 0$$

is fulfilled.

Particular cases of this distribution can be obtained by specifying the parameter values. Equations (5) describe how the substance is distributed along a channel ($N+1$ nodes) and a branch ($R+1$ nodes) that starts from cell number M that belongs to the channel. The mathematical model and the class of distributions of the substance for the case of an infinite amount of cells along the channel and along the branch is also obtained in our research group but will not be discussed in this paper. An example graphic of the obtained class of distributions along a channel and a branch is presented on Fig. 1, with specified parameter values. The x-axis represents the cell (or node) number, and the y-axis represents the probability values.

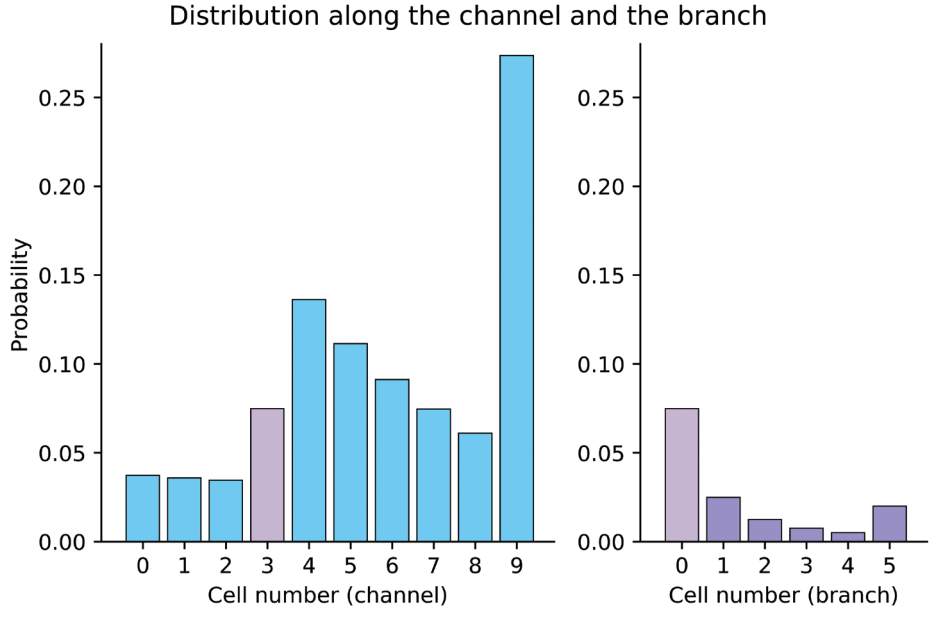

Fig. 1: Distribution of a substance along the channel and the branch. Parameter values: $\gamma_i = 0.002$, $i=0, \ldots, 9$; $f_i = 0.05 - 0.00005j$, $j= 0, 1, 2$; $f_3 = 0.002$; $f_k = 0.009 - 0.000005k$, $k=4, \ldots, 9$; $\xi_l = 0.001$; $g_l = 0.001(i+1)$, $l = 0, \ldots, 5$.

3. Mathematical model of the flow of a substance through a channel

3.1. Finite channel

As mentioned above, the mathematical model of the flow of a substance through a single channel is a particular case of the model of the motion of a substance through a channel with one branch, system (1), when the quantities that move from cell number *M* from the channel to the branch are equal to zero.

The mathematical model of a substance spreading through a channel that consists of *N* nodes is formulated in (Vitanov, Vitanov 2018b). From the point of view of human migration, the substance can represent migrants and the nodes from the channel can represent neighbouring countries. The edges among the nodes form the way of the motion of the migrants.

Analogously to the case of a channel with one branch, a particular case of the mathematical model for a finite amount of cells in a single channel is studied in (Vitanov, Vitanov 2018b) with time-independent parameters, and with expressions from equations (2). In addition, a stationary regime of the system is studied (which means that there is motion in the cells, but the quantity of the substance in each cell remains constant over time), and the motion of the substance is directed from cell i to cell *(i+1)* only, $i = 0, \ldots, N$. There is no exchange with the environment, except in the initial cell, where there is only an inflow of the substance. There is no outflow from the cells of the channel to other parts of the network. This means that conditions 1 – 3 are fulfilled. The obtained class of distributions along a finite channel in (Vitanov, Vitanov 2018/11) is of the following kind:

$$
\begin{aligned}
y_0^* &= \frac{1}{\left[1+\sum\limits_{k=1}^{N-1}\prod\limits_{j=1}^{k}\frac{f_{j-1}}{f_j+\gamma_j}+\frac{f_{N-1}}{\gamma_N}\prod\limits_{j=1}^{N-1}\frac{f_{j-1}}{f_j+\gamma_j}\right]} \\
y_i^* &= \frac{\prod\limits_{j=1}^{i}\frac{f_{j-1}}{f_j+\gamma_j}}{\left[1+\sum\limits_{k=1}^{N-1}\prod\limits_{j=1}^{k}\frac{f_{j-1}}{f_j+\gamma_j}+\frac{f_{N-1}}{\gamma_N}\prod\limits_{j=1}^{N-1}\frac{f_{j-1}}{f_j+\gamma_j}\right]}; i = 1, \ldots, N-1 \\
y_N^* &= \frac{\frac{f_{N-1}}{\gamma_N}\prod\limits_{j=1}^{N-1}\frac{f_{j-1}}{f_j+\gamma_j}}{\left[1+\sum\limits_{k=1}^{N-1}\prod\limits_{j=1}^{k}\frac{f_{j-1}}{f_j+\gamma_j}+\frac{f_{N-1}}{\gamma_N}\prod\limits_{j=1}^{N-1}\frac{f_{j-1}}{f_j+\gamma_j}\right]}
\end{aligned}
\tag{6}
$$

Two graphics from the class of probability distributions (6) with particular values of the parameters are presented in Fig. 2 (Vitanov, Vitanov 2018b). The *x*-axis represents the cell (or node) number. The *y*-axis represents the probability values.

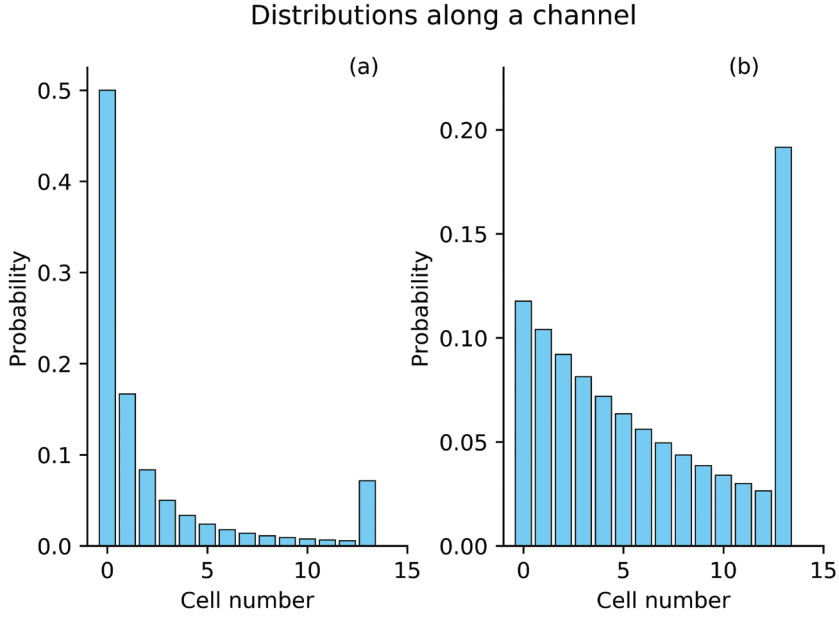

Fig. 2: Two distributions from class (6) of a substance along a channel. Parameter values (Vitanov, Vitanov 2018b): Fig. (a): $\gamma_i = 0.001$, $f_i = 0.001(i + 1)$. Fig. (b): $\gamma_i = 0.001$, $f_i = 0.0075 - 0.00002i$.

3.2. Infinite channel

The mathematical model of a substance moving along an infinite channel is discussed in (Vitanov, Vitanov 2018b). It is formulated based on similar assumptions as in the previous sections. A particular case of the mathematical model for an infinite amount of cells along a channel is studied in (Vitanov, Vitanov 2018b) with time-independent parameters with expressions from equations (2), a stationary regime of the system, and the motion of a substance directed from cell *i* to cell *(i+1)* only. Again, there is no exchange with the environment, except in the initial cell, where there is only an inflow of the substance. There is no outflow from the cells of the channel to other parts of the network. The obtained class of distributions along an infinite channel in (Vitanov, Vitanov 2018b) is of the following kind:

$$
\begin{aligned}
y_0^* &= \frac{1}{\left[1+\sum\limits_{k=1}^{\infty}\prod\limits_{j=1}^{k}\frac{f_{j-1}}{f_j+\gamma_j}\right]} \\
y_i^* &= \frac{\prod\limits_{j=1}^{i}\frac{f_{j-1}}{f_j+\gamma_j}}{\left[1+\sum\limits_{k=1}^{\infty}\prod\limits_{j=1}^{k}\frac{f_{j-1}}{f_j+\gamma_j}\right]}; i=1,\ldots,\infty
\end{aligned}
\tag{7}
$$

The class of distributions (7) is important, since it contains as particular cases many of the famous discrete probability distributions, including the Binomial distribution, the Negative-binomial distribution, the Poisson distribution, the Waring distribution, the Geometric distribution, the Yule distribution, and many others (Vitanov, Vitanov 2019).

4. Discussion

The mathematical models for a finite amount of cells can be applied in the context of human migration. As mentioned in the previous sections, we shall consider the channel as a chain of countries. Migrants can enter the channel from the entry country (initial country, denoted by *0*) and may move through the channel to its last node. The last country is called final destination country. In the general case, migrants may move in both directions: towards the final destination country or towards the entry country. In this current work, we consider movement only in one direction – towards the final destination country. It is possible for migrants to change their status to non-migrant, which is described by the parameters γ_i.

Let us discuss Fig. 2 with parameter values from (Vitanov, Vitanov, 2018/11). For the case of a migration channel and for distributions where $f_i = \alpha_i + \beta_i i$, the parameters α_i characterize the permeability of the borders between the i-th and $(i+1)$-th country of the channel. The parameters βi characterize the "attractiveness" of the i-th country of the channel. The parameters γ_i characterize the part of the migrants who obtain permission and citizenship to stay in the i-th country of the channel. For the cases visualized in Fig. 2, the parameter γ_i has the same values for the two Figs. 2a and 2b, and for all countries of the channel. In Fig. 2a, there is an increment of the permeability of the borders between the countries of the channel with increasing value of i and by lack of attractiveness of the channel ($\beta_i = 0$). In this case, the probability

connected to the final destination country is larger than the probabilities connected to countries of the channel with smaller value of *i*. This corresponds and leads to a concentration of migrants in the final destination country (Vitanov, Vitanov 2018b). Fig. 2b shows that the concentration of migrants because of the permeability of the borders can exist even if the attractiveness of the countries decreases with an increasing value of *i* (Vitanov, Vitanov 2018b).

Other applications of mathematical models of flows in networks are related to probability distributions, since new classes of distributions are obtained in our current research. Further research includes the formulation of a system of differential equations that models the processes in a particular cell (or country) from the network that contains three different types of substances with the possibility for conversion of one substance to another. The case of two different substances is discussed in (Vitanov, Vitanov 2018b).

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Tsvetelina I. Ivanova
Institute of Mechanics
Bulgarian Academy of Sciences
Sofia, Bulgaria
t.ivanova@imbm.bas.bg

Nikolay K. Vitanov
Institute of Mechanics
Bulgarian Academy of Sciences
Sofia, Bulgaria
vitanov@imbm.bas.bg

EXPERIMENTAL AND NUMERICAL STUDIES OF SURFACE PROPERTIES OF SOLIDS BY PHYSICAL ADSORPTION TECHNIQUE

Silviya Boycheva, Denitza Zgureva

Keywords: *solids, surface area, adsorption, practical experience*

Abstract: *The application of solid materials on an industrial scale requires full characterization of a number of physical, chemical, mechanical, and thermal properties to suit the specifics of their use. The detailed studies of the surface properties of solids are of great importance for their applications in adsorption, chemisorption, heterogeneous catalysis, drug delivery, hydrogen storage, fuel cells, super condensers, ceramics, etc. A standard approach for surface studies is the physical adsorption of inert gases (nitrogen, argon, and krypton) onto a solid surface at cryogenic temperature (ISO 9277:2010) and further modelling of the experimental data by the Brunauer-Emmett-Teller (BET) equation. This paper presents several practical examples and the experience gained as a result of numerous experimental studies of solid surfaces. The experimental data were measured by a volumetric adsorption analyzer donated by the Alexander von Humboldt Foundation.*

Introduction

The fundamental and industrial progress of the advanced technologies is due to the development of novel materials with improved characteristics (Fahlman 2018). The group of solids includes thousands of natural and synthetic organic and inorganic materials, as their use is widespread in many areas. Solids can be classified with respect to their origin, structure, composition, application, etc.; however, some of their main characteristics that predetermine their applications are their surface parameters. The developed surface and porosity of solids are prerequisites for their ability to support catalytic processes, drug delivery, adsorption, energy storage, etc. Many examples revealing the importance of the surface properties are provided elsewhere, while here, the most common ones from the practical and industrial point of view are reported. Regarding the application of solids in adsorption technologies, surface characteristics such as surface area, total pore volume, and pore size distribution are important in terms of industrial quality and

viability of the adsorbents. The reduced surface area as well as the low and non-uniform porosity can affect strongly the adsorption capacity and selectivity (Bonakala, Balasubramanian 2015). Many technologies for environmental protection, such as gas separation and cleaning, waste water remediation, and soil recovery, are based on adsorption, and their efficiency requires a broad contact surface and specific porosity (Dabrowski 2001).

In pharmaceutical applications, surface area and porosity are essential parameters for the purification, processing, mixing, tableting, and packaging of pharmaceutical products, as well as for their shelf life, dissolution rate, and biocompatibility (Frohberg et al. 2016). For the fast development of medicine, especially implantology, the synthesis of novel biocompatible materials is required. Controlling the porosity of the artificial bone allows it to mimic completely the natural bone so that the body can accept it and to initiate growth of tissue around (Ho-Shui-Ling et al. 2018). Another example from the practice is the influence of the surface parameters on the activity of the catalytic systems applied in different industries such as oil refining, gas purification, obtaining of biofuels, production of chemicals, etc. The extended surface area with uniformly distributed active sites stipulates the effectiveness and the rate of the catalytic processes. The pore size obeys the mass transfer of molecules through the catalytic supports, allowing control over the selectivity of the catalysts (Argyle, Bartholomew 2015). For the material development in aviation and space engineering, the surface properties are also of importance because the surface area and porosity of heat shields and insulation materials affect their weight and functionality (Paderil et al. 2005).

During the research and development stage of a new material, key properties, which are strictly inspected, are the surface characteristics. When novel solids are obtained by synthesis or restructuring, and in these processes there is more than one variable in the initial conditions, their influence on the end material is evaluated by surface analyses. Techniques for determination of specific surface area (SSA) at ambient conditions, such as methylene blue staining, ethylene glycol monoethyl ether adsorption, electro kinetic analysis of complex ion adsorption, and protein retention, have been developed, but they found limited applications (Hanaor et al. 2014; Cerato, Lutenegger 2002; Paykov, Hawley 2013). The gas adsorption method is standardized by ISO 9277: 2010 and it is applied for a wide range of solids. This standard describes the conditions for the pre-treatment of samples to release the moisture and atmospheric gases from their surface, the approaches for obtaining gas adsorption isotherms, and the application of the Brunauer-Emmett-Teller (BET) mathematical model to the experimental data. According to the standard, sampling is carried out in accordance with ISO 8213 and

ISO 14488 where prior to the measurement of an adsorption isotherm, the physically adsorbed substances from the sample surface are removed by degassing, avoiding any irreversible changes in the surface characteristics. Three approaches for sampling are available: thermogravimetric control of degassing, pressure control of degassing, and pressure-controlled heating. Often, this stage of analysis is underestimated, and the developer of new solids does not carry out a differential thermal analysis (DTA) of all samples because of the time and the costs involved. However, in many cases, any compositional or structural variation in samples with the same base leads to substantial differences in their thermal stability, thus their pre-treatment at similar conditions will cause a serious mistake in determination of their surface parameters. An indicative example is that the DTA analyses for zeolites with FAU structure, but with different compensating cations, reveal that fluorine FAU is unstable and destructs at 350 °C, while sodium and cesium forms are stable up to 800 °C (Komaty et al. 2018). Another part of surface analysis which is standardized is the obtaining of the adsorption isotherm. Four techniques are described: static volumetric method, gravimetric method, flow volumetric method, and carrier gas method. Nitrogen (N_2) at its boiling point (77.3 K) is usually the most suitable adsorbate. Very often, argon (Ar) at its liquefaction temperature (87.27 K) and carbon dioxide (CO_2) at 273.15 K are alternative adsorbates for specific surface area determination, especially in the case of graphitized carbon and hydroxylated oxide surfaces. After measuring the adsorption isotherm, the standard determines the application of the BET model in the interval of relative pressure p/p_0=0.05 – 0.35, which is only suitable for isotherms of type II according to the IUPAC classification. Most of the currently analyzed materials are microporous, and their typical isotherms are of type I, and for them the BET model in this relative pressure range gives results without physical meaning. To overcome this problem, in the research of Roquerol, an approach for individual analysis has been proposed (Roquerol et al. 2007). In practice, performing erroneous analyzes and inappropriate data processing often result in incorrect SSA values for the solids tested. Most of the commercial gas adsorption analyzers are managed by software and their automatic regimes may influence the correctness of the results.

Thanks to the donation of the Alexander von Humboldt Foundation, our laboratory was equipped with a modern volumetric gas adsorption analyzer. The present manuscript summarizes some of the challenges we have overcome and the useful experience we have gained from mastering SSA characterization techniques for various solids.

Materials and methods

Samples analyzed in this study are summarized in Table 1. Details on their synthesis are provided in the corresponding references.

Table 1. Samples subjected to analysis

Sample	Description	Ref.
Na-X	Zeolite with FAU structure prepared from pure starting materials in a molar ratio $4SiO_2$:$8.5Na_2O$:$0.5Al_2O_3$:$333H_2O$	Kalvachev et al. 2016
FAZ 1	Fly ash zeolite with FAU structure prepared by hydrothermal synthesis at 90 °C for 2 hours with prior fusion stage at 550 °C	Boycheva et al. 2019
FAZ 2	Fly ash zeolite with FAU structure prepared by hydrothermal synthesis at 90 °C for 2 hours	Popova et al. 2019
ZSM – 5	Zeolite with ZSM synthesized by seeds CBU2314, Zeolyst, SiO_2/Al_2O_3 = 23	Popova et al 2018
Biochar	Carbon black obtained by pyrolysis of sunflower husks	Commercial product
Activated carbon	Commercial carbon black SKT – 3 used in containment's ventilation system of nuclear power plants	Commercial product

The adsorption experiments were performed by a volumetric adsorption analyzer Micromeritics Tristar II 3020. Samples were preliminary degassed at appropriate temperatures and duration under a helium flow in an apparatus Micromeritics FlowPrep 60. The samples were further degassed by evacuation at a rate of 0.67 kPa/s for 1 hour, provided by a vacuum Edwards pump. The equilibrium adsorption of CO_2 onto the solids was measured in 25 experimental points at 273.15 K in the relative pressure range p/p_0 = 0.001 - 0.030, where p_0 is the saturation pressure of CO_2 (3485.6769 kPa at the temperature of the measurements). Adsorption and desorption analyses were performed with N_2, and the corresponding isotherms were built in 92 experimental points at 77 K in the pressure range p/p_0 = 0.001 - 0.995. The BET isotherms (Eq. 1) were plotted on the experimental data from the adsorption branch for the preliminary determined pressure region of monolayer formation, as follows:

$$\frac{1}{Q[(p_o/p)-1]}=\frac{C-1}{n_m C}\left(\frac{p}{p_o}\right)+\frac{1}{n_m C}, \quad (1)$$

where p/p_0 is the relative pressure (Pa), Q and n_m – the total adsorbed amount and the adsorbed amount in the monolayer (cm^3/g), and C – the BET-constant related to the adsorption enthalpy. From the linear plot parameters, a specific surface area (SSA) in m^2/g was calculated by Eq. 2:

$$S_{BET}=\frac{1}{I+A}.\sigma \ [m^2/g], \quad (2)$$

where I is the intercept of the linear BET plot, A – the slope of the plot, and σ – the adsorbate cross section area (nm^2). According to ISO 9277:2010, the molecular cross sections used for calculations are 0.162 nm^2 for N_2 at 77 K and 0.210 nm^2 for CO_2 at 273.15 K. The uncertainty of the results for SSA is calculated by Eq. 3:

$$SSA_{ERR}=\frac{SSA_{BET}\sqrt{A_{ERR}^2+I_{ERR}^2}}{I+A}, \quad (3)$$

where A is the slope of the BET isotherm, g/cm^3; I – the intercept of the isotherm, g/cm^3; A_{ERR} – the error in the slope determination, g/cm^3; and I_{ERR} – the error in the intercept determination, g/cm^3.

The sequence of the measuring procedure is presented in Fig. 1.

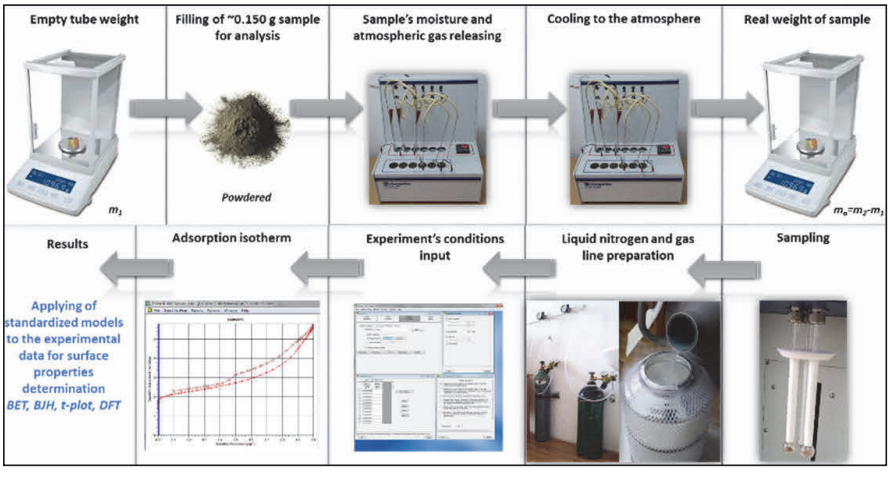

Fig. 1: Sequence of the procedures of the adsorption tests.

Results and discussion

In this section, several examples from the laboratory practice referring to the correctness of the end results of SSA calculations are provided. All presented analyses have been performed according to ISO 9277:2010. However, co-factors may have impaired the accuracy of the results obtained and this will be demonstrated by examples that will be discussed in details.

Influence of the thermal pretreatment

The influence of the preliminary temperature treatment on the SSA results was investigated on a Na-X sample, using N_2 as a working gas. Two cases were considered: Case 1 – heating at 260 °C for 2 hours and cooling down to room temperature for 2 hours under a continuous helium flow; Case 2 – thermal treatment at 380 °C for 4 hours under similar other conditions. The obtained isotherms are plotted in Fig. 2 and Fig. 3, respectively. The SSA is calculated by applying the BET model in the pressure range p/p_0=0.01 – 0.05, where the monolayer formation of adsorbed molecules is observed. The isotherms are referred to type I according to the IUPAC classification, which is typical for microporous materials. The analyses were run completely without any errors during the adsorption and desorption processes, and both isotherms are correct and suitable for further analyses. However, after the calculations of the SSA values, big discrepancies in the results were observed: 791 m^2/g in Case 1 and 892 m^2/g in Case 2. In the more detailed analysis of the isotherms, scaling them in a low pressure range, it could be seen that in Case 1 the N_2 adsorption of Na-X exhibits an S-shape form, which is attributed to the presence of moisture remaining in the micropores of the material.

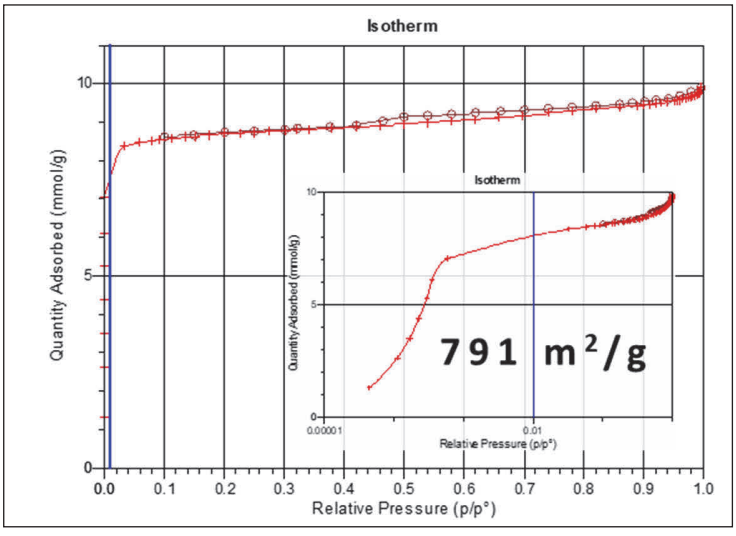

Fig. 2: Experimental isotherms measured in Case 1.

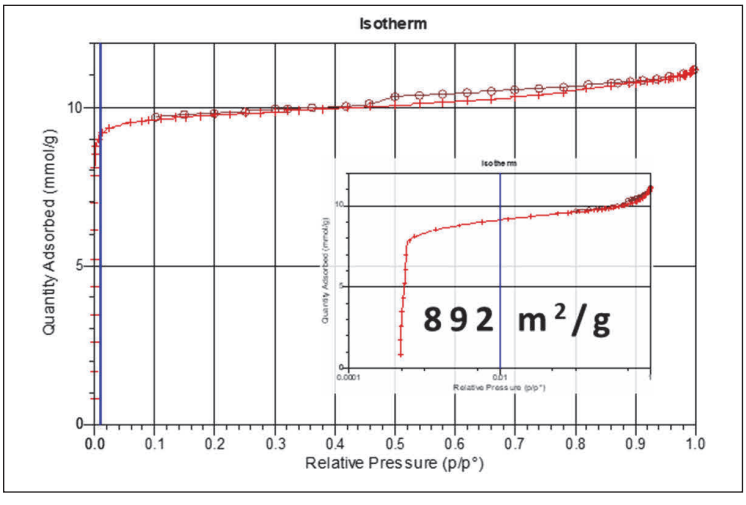

Fig. 3: Experimental isotherms measured in Case 2.

Importance of the operator's skills during the pretreatment

An evaluation of the importance of the operator's skills during the pretreatment stage regarding the SSA results was performed on a FAZ-1 sample. Two cases realized under the same conditions of degassing at 260 °C for 4 hours of heating and 2 hours of cooling under a continuous He flow were

considered. In Case 1 the experiment was performed by an inexperienced operator, while in Case 2 the measurements were carried out by an experienced specialist. The obtained N_2 isotherms are shown in Fig. 4 and Fig. 5 for Case 1 and Case 2, respectively.

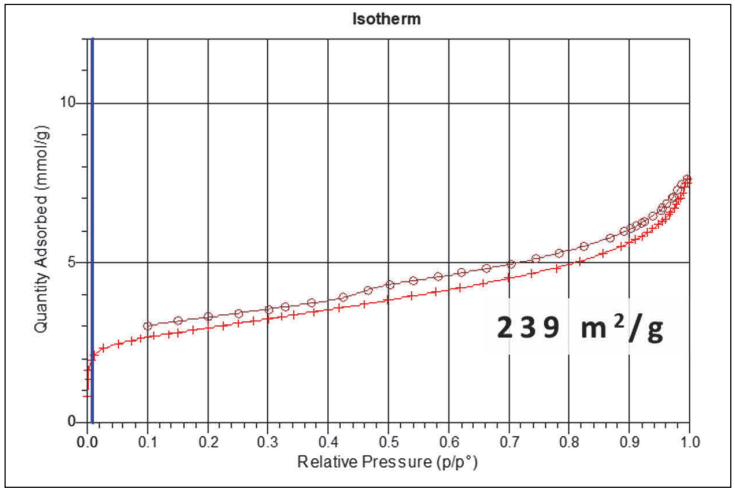

Fig. 4: N_2 isotherm of FAZ – 1 measured by an inexperienced operator.

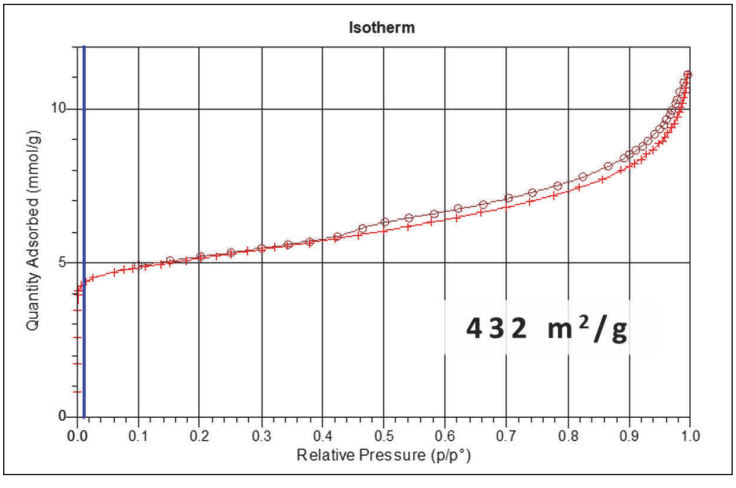

Fig. 5: N_2 isotherm of FAZ – 1 prepared by an experienced specialist.

The obtained N_2 isotherms in both cases are of type IV according to the IUPAC classification, and they have the full runs of the adsorption and desorption processes. After the SSA calculation in the pressure range p/p_0=0.01 – 0.05, significant discrepancies were observed. The calculated SSA for Case 1 was

239 m^2/g, while for Case 2 it was 432 m^2/g under the same experimental conditions. This result was unexpected because of the strict compliance to ISO 9277:2010 requirements and the absence of an S-shape as was demonstrated in the previous example. It turned out that the error came from the way the sample was placed in the degassing system. In Fig. 6 the positions of the tube, its stopper and the He flow line in the system for degassing are shown.

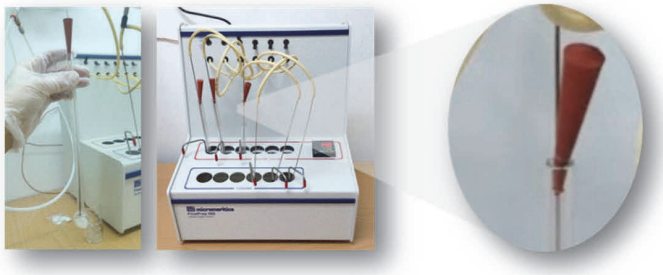

Fig. 6: Positions of tube, its stopper and the He flow line in the system for degassing.

In Case 1 of this experimental set, the stopper was deeply inserted into the tube throat, which prevented the release of the evaporated moisture, and it condensed on the tube's walls and returned back to the sample. The absence of the S-shape on the N_2 isotherm is explained by the presence of moisture on the external surface of the sample but not in its pores. The presence of moisture affects the result for SSA because of the wrong data from the measurement of the sample weight after the degassing and the further input of incorrect data to the BET model. The results in Case 2 could be considered correct, as the experiments were repeated in 5 cycles, and the obtained SSA value was verified, as the N_2 isotherms of the individual measurements overlapped, and the deviation for the calculated SSA values was less than 5 m^2/g or 1 % from the obtained 432 m^2/g.

Influence of the application of fast analyses techniques

During the design and optimization of a new material, fast analytical techniques are often used by the developers to accelerate the research. The specialized equipment for the adsorption analyses is managed by software that allows the operator to input initial and boundary conditions. For faster analyses, operators apply a shorter time for evacuation, which results again in an S-shape isotherm because of the remaining moisture in the solid. Another fast technique is the performing of the adsorption analyses in a relative pressure

region of 0.05 – 0.35, recommended by ISO 9277:2010. The time is reduced from approximately 14 hours to less than 2 hours. Here we demonstrate two SSA calculations for zeolite ZSM – 5, provided by a short N_2 adsorption procedure – Case 1 in p/p_0=0.05 – 0.35, and a long analysis – Case 2, in which the BET model is applied in p/p_0=0.003 – 0.075. In both cases, the sample is pretreated at the same conditions – namely, at 200 °C for 7 hours of heating and 2 hours of cooling under a He flow. Figures 7 and 8 represent the resultant BET plots for Case 1 and Case 2, and the calculated data for the BET model.

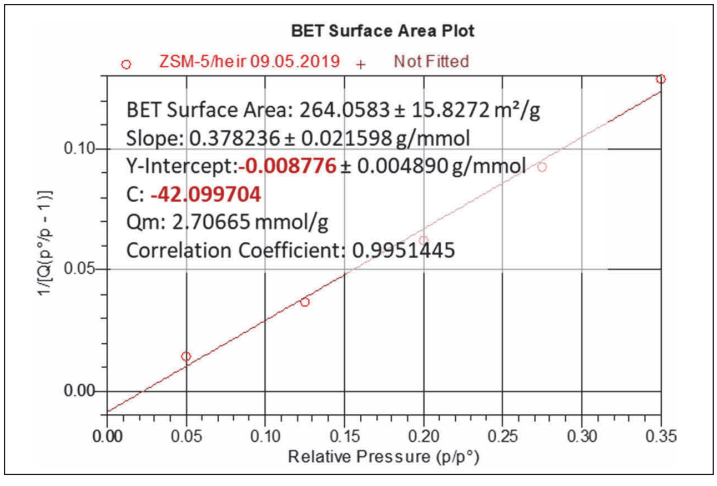

Fig. 7: Calculation of SSA for ZSM – 5 at p/p_0=0.05 – 0.3 at fast analysis conditions – Case 1.

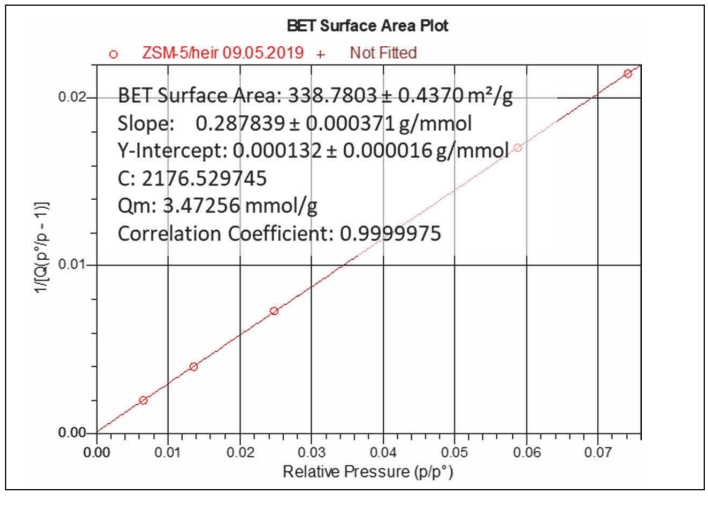

Fig. 8: Calculation of SSA for ZSM – 5 at p/p_0=0.003 – 0.075 from 92 point N_2 adsorption/desorption isotherm measured at long analysis conditions – Case 2.

In both cases, the BET graph is a straight line with satisfactory correlation between the experimental points and the model line. The calculated SSA is 264 m^2/g for Case 1 and 338 m^2/g for Case 2. The intercept of the model line in Case 1 has a negative value which has no physical meaning. The negative intercept signifies that the sample desorbs N_2 at lower pressures before adsorbing it. The recommended relative pressure region of N_2 adsorption is not appropriate for this material because the N_2 molecules are in a multilayer adsorption stage, and the BET model cannot be applied. In Case 2, the presence of micropores and the end of monolayer formation were detected by the multipoint N_2 isotherm. Thus, the BET model was correctly applied and the calculated SSA is considered as a correct value.

Influence of the adsorbate cross section

According to Equation 2, the cross section of the adsorbate (σ, nm^2) is a specific measure for the molecules of each gas and quantifies the probability for interaction between two individual particles. Databases with experimental data for this parameter exist, accounting the nature of the contacting materials and the temperature. In the commercial software for processing of experimental adsorption data, the default value for σ is set for N_2 adsorption at 77 K because of its most common use. In Fig. 9, an experimental adsorption isotherm of CO_2 at 273.15 K onto a Biochar surface is plotted together with the BET linear plot in the insert. Two calculations of the SSA are also presented below the graphs. The first calculation was made in an automatic software mode with σ value of 0.162 nm^2, which is the default parameter. The second calculation was performed after the manual input of σ value of 0.210 nm^2 corresponding to a CO_2 cross-section at 273.15 K. The BET plots for both calculations coincide, having the same intercepts and slopes, and high correlation between the model and the experimental data was observed. However, the obtained SSA values are different: 177 m^2/g and 229 m^2/g. In Table 2, the values of σ recommended by ISO 9277:2010 for the most commonly used adsorbates at different temperatures are summarized.

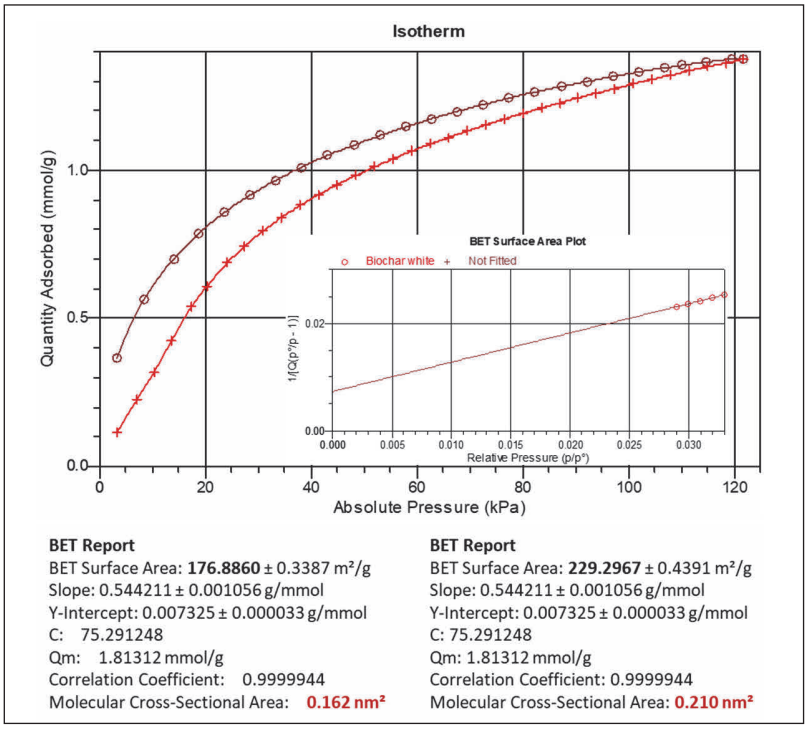

Fig. 9: CO_2 adsorption/desorption isotherms, BET linear plot, and SSA calculations in automatic and manual software modes.

Impact of the relative pressure region applied in the BET model

Until recently, it has been considered that the gas adsorption and the subsequent computation of the experimental data by the BET model is an inappropriate technique for surface analyses of microporous solids. Further, the development of the analytical equipment, the sampling improvement, and the increased accuracy of the quantitative measurements during the experimental procedures ensure good reproducibility of the calculations of the number of molecules forming a monolayer, as it is required by BET. However, the adsorption data accuracy is not the only prerequisite for reliable analyses of microporous solids because the BET plot has a wide range of linearity in low pressure regions, as discussed by Roquerol et al. in their research. The latter have proposed a self-consistency criterion for a modified BET equation for microporous materials, and this requirement has been included in the updated version of ISO 9277:2010. This criterion is related to proper determination of a relative pressure region from the adsorption isotherm for further calculation

by BET. For this purpose, the function $n_a.(1- p/p_0) = f(p/p_0)$ has to be analyzed, and its extremum has to be considered as a final point for the relative pressure region of the monolayer formation. We applied this observation, performing surface studies of microporous activated carbon via N_2 adsorption/desorption analyses. The sample was preliminary treated at 380 °C for 4 hours and was cooled for 2 hours in a He flow. The experimental N_2 isotherm is plotted in Fig. 10, and the parameters of the BET model applied to the experimental data in the default p/p_0 region of 0.05 – 0.35 are presented. The negative intercept of the linear plot confirms the irrelevance of the investigated pressure region.

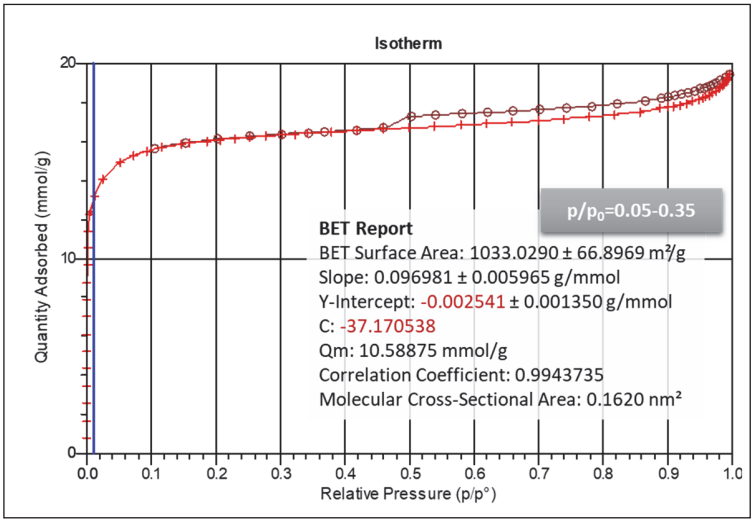

Fig. 10: N_2 isotherm onto activated carbon surface and BET data at p/p_0=0.05 – 0.35.

For determination of the proper pressure region of monolayer formation, the adsorption increase was investigated, and the results are shown in Fig. 11. The observed limit of the region for the BET model validity is p/p_0=0.06. The positive value of the intercept and the correlation of 0.99999 correspond to a reliable SSA value of 1278 m^2/g.

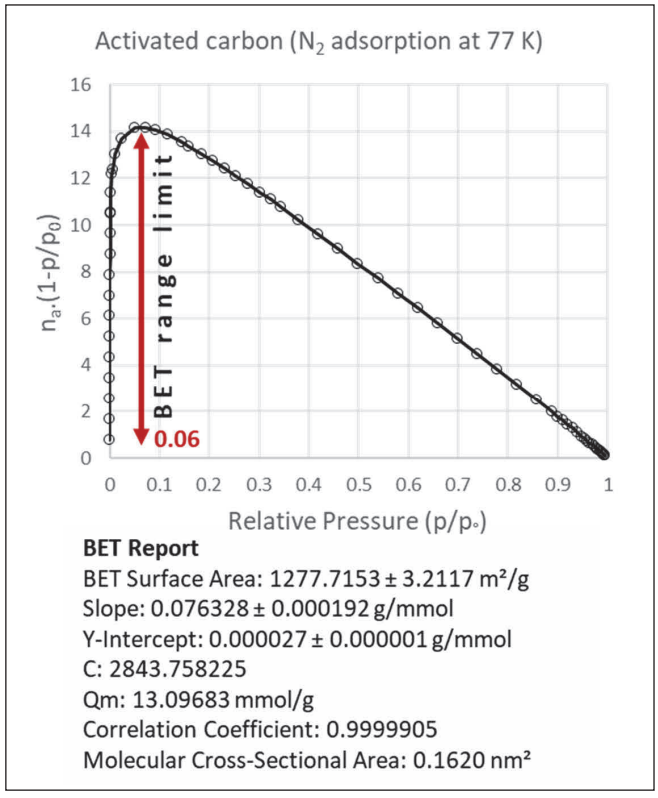

Fig. 11: BET range limit determination.

Impact of the adsorbate nature

The ISO standard admits that the results of the measurements with different adsorbates may deviate from each other because of the differences in the molecular cross sections, accessibilities to pores, and the temperatures of pressure saturation. However, as a result of our long experimental practice, we observed good agreement between the SSA values calculated from N_2 and CO_2 isotherms under the verified reproducibility of the results by repeated measurements, using fresh samples for each run. Detailed information about comparative experimental analyses was reported in our previous study (Zgureva et al. 2019). Fig. 12 presents N_2 and CO_2 isotherms for FAZ – 2 and the calculated SSA values after appropriate determination of p/p_0 regions.

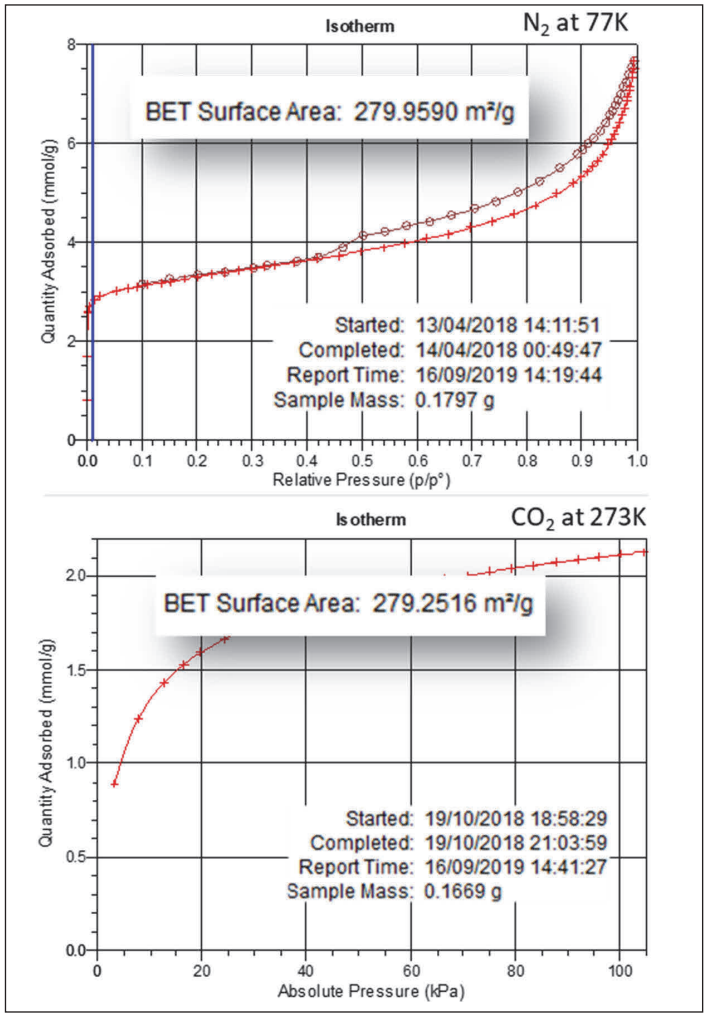

Fig. 12: Experimental N_2 and CO_2 isotherms onto FAZ – 2 surface.

The deviation between the calculated SSA values from the two isotherms is 0.25 %. The application of CO_2 as an adsorbate for SSA analysis is a faster and easily performed method, but it is limited regarding the porosity calculations.

Conclusion

Performing gas adsorption analyses and model calculations to determine the specific area of solids is not an automatic analytical process and has some special requirements. First, to maintain the accuracy of the experimental technique, a series of analyses on the same sample under equal conditions is

required. The preliminary sample treatment has a strong impact on the SSA result, thus it has to be strictly performed, and the thermogravimetric properties of the material have to be known in advance. Recourse to rapid analysis should only take place after a preliminary examination of the limitations on the applicability of the BET model. For faster analyses, CO_2 at 273.15 K could be an alternative of N_2 at 77 K, but this also has to be verified for the specific materials. There are some cases where N_2 is not applicable as an adsorbate due to the orientation of the molecules to the adsorbent surface, and then CO_2 has to be used, as shown for the Biochar sample. The microporous materials have to be carefully analyzed, especially with respect to the BET model limitation in the pressure region, which should be determined for each individual sample, based on the experimental data on the monolayer adsorption area.

Often, scientists use another laboratory for analysis, and the report received only includes the SSA value of the test sample, which is a prerequisite for hidden errors. The SSA values of solids are also often reported in scientific articles as measures without information about the experimental procedures and the selected modeling parameters. In order to understand better the magnitudes and avoid errors, it is necessary to provide SSA data with details of key analysis parameters such as: pretreatment, adsorption properties, number of experimental points, selected pressure range for the BET model, and uncertainty of the results.

Acknowledgments:

The authors are thankful to the Alexander von Humboldt Foundation for the equipment donation and to the Bulgarian National Science Fund for the financial support under Grant No. DN 17/18 (2017).

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Silviya Boycheva
Department of Thermal and Nuclear Power Engineering
Technical University of Sofia
Sofia, Bulgaria
sboycheva@tu-sofia.bg

Denitza Zgureva
College of Energy and Electronics
Technical University of Sofia
Sofia, Bulgaria
dzgureva@tu-sofia.bg

INTRINSIC PHYSICAL BARRIER QUALITY OF PLUTONIUM PRODUCED IN PRESSURISED WATER REACTORS

Ivaylo Naydenov

Keywords: *proliferation resistance, plutonium, nuclear fuel cycle*

Abstract: *In recent years, non-proliferation of nuclear materials has become an important issue, regarding civilian fuel cycle development. In addition, future advancement in nuclear energy systems and nuclear fuel cycles should comply with non-proliferation criteria. These requirements stem from the circumstance that nuclear power is considered to be a dual purpose technology. There is an on-going debate about the usability of reactor-grade plutonium in nuclear explosives manufacturing. Moreover, there is no universally recognized and verified methodology for proliferation resistance assessment. On the other hand, intrinsic material factors may play a further role in strengthening fuel cycles' proliferation resistance, since the majority of the efforts in that area so far have been focused on enhancing technical barriers. The current paper represents a synthesis of the main results obtained by applying different methodologies for proliferation properties evaluation, published separately.*

Introduction

The relevance of the problems associated with the non-proliferation of nuclear materials is defined by the following circumstances: (1) nuclear power is considered to be a dual-purpose technology (Kang 2005), and (2) the probability for proliferation creates certain obstacles for nuclear power development in terms of creating negative perception of nuclear technology (Ezoubtchenko et al. 2005). Proliferation resistance is among the core principles of nuclear power development and is an objective that should be fulfilled by advanced fuel cycles (IAEA 2008; IAEA 2013). Proliferation resistance also represents a necessary condition, set forth by the European Commission, which needs to be met in order to guarantee the future advancement of nuclear energy in the European Union (European Commission 2016). It is thought that proliferation of nuclear materials is one of the most important problems facing the future progress of nuclear energy (Kimura et al. 2011).

The main concerns about possible proliferation risk increase are based on the global plutonium inventory that is estimated to be increasing at a rate of about 60 tonnes per annum (Beller, Krakowski 1999: 3). Plutonium is considered to be among the most important materials from non-proliferation perspective (Kimura et al. 2011). In order to define the level of usability of civilian plutonium for nuclear explosive device construction, it is necessary to analyse those properties of the material that could make it suitable for non-civilian applications. Most often, the attributes linked to a material's proliferation resistance are the bare critical mass of a sphere, its decay heat, the spontaneous neutron emission, and the radiological barrier (Beller, Krakowski 1999: 3; Ezoubtchenko et al. 2005; IAEA 2010: 5 – 6). These factors compose the so-called isotopic barrier to proliferation. Some of the factors influence the material's usability, others – the probability for acquisition, transportation, and reprocessing (Skutnik, Yim 2011).

The non-proliferation of nuclear materials is defined by the International Atomic Energy Agency (IAEA) as a property of a nuclear system or facility that impedes nuclear material's diversion and/or undeclared production, as well as malicious applications of nuclear technology by a state aiming to acquire nuclear weapons or other nuclear explosive devices. This definition implies that proliferation resistance is a measure of the difficulties that should be overcome in order to develop and/or acquire nuclear weapons or nuclear explosive devices by means of a civilian fuel cycle and civilian technologies. These difficulties include, but are not limited to, technical and technological barriers, required skills, time, etc. Misusing a nuclear fuel cycle could include usage of materials, equipment, technological processes, facilities, and knowledge related to the civilian fuel cycle (IAEA 2010: 2, 4). In the case of nuclear proliferation, there is misuse of nuclear material and/or nuclear facilities by the host state (Bathke et al. 2009).

The proliferation barriers can be divided in intrinsic and extrinsic (IAEA 2010: 5; Skutnik, Yim 2011). The intrinsic barriers may be physical – related to the properties of the material, and technical – related to the properties of the nuclear facilities, the access to the facilities, and the availability of specialists with adequate knowledge and skills, and time for device development (IAEA 2010: 5). The most important factors of the intrinsic barrier are linked to the material's properties; in total, the isotopic, chemical, radiological, and weight factors have a relative share of 63.5 %. Another significant factor, with a relative weight of 18.1 %, is the available inventory (Skutnik, Yim 2011).

Methods

The presented assessments are carried out using two methodologies – the 'Figure of Merit' methodology and a multi-attribute utility analysis.

The 'Figure of Merit' methodology has been developed by a team from the Los Alamos National Laboratories, USA. It is used to assess the material's usability for the construction of a nuclear explosive device by a nation or a sub-national group. The methodology uses two criteria, FOM_1 and FOM_2, in order to assess the material. These criteria include several intrinsic factors such as bare critical mass, spontaneous neutron fraction, decay heat, and dose rate. The first criterion assesses the material's usability when the explosive yield is of little importance and pre-detonation is not an issue, while the second criterion assesses the case when nominal yield and storability are desirable. The higher the criteria value, the higher the material's usability (Bathke et al. 2009; Bathke et al. 2012). This effectively means that the FOM_1 criterion does not take into account the effects of the spontaneous neutron emission, while FOM_2 could be used to assess the impact of even plutonium isotopes on the material barrier. The present analysis has been carried out using the algorithm applied by (Naydenov, Filipov 2015). Here, the values of FOM_1 and FOM_2 are used as a metric to investigate the change in the material barrier quality.

The multi-attribute utility analysis (MAUA) evaluates 14 utility functions linked to proliferation barriers, each utility function having an assigned relative weight. The utility functions, their assigned weights, and the complete calculation algorithm are fully described by (Charlton et al. 2007). The sum of the products of the relative weights and the utility functions' values is used as a metric named 'static proliferation resistance'. The sum value belongs to the interval [0;1]. The closer the value is to unity, the better the quality of the barrier. This metric is applied in a similar fashion as the 'Figure of Merit' criteria – as a means to evaluate the change in the quality of the material barrier. The convergence of the results obtained by the two distinct methodologies is shown by (Naydenov, Filipov 2018).

Results and Analysis

The main objective of the study is to evaluate the change in the material barrier of plutonium obtained during normal operation of a reference commercial pressurised water reactor (PWR), as a function of the fuel type (uranium oxide UOX/mixed oxide MOX), the burn-up (30 – 60 GWd/tHM), and the cooling time (up to 30 years), as well as to evaluate the effect of multiple

recycling, using different mixed fuel options (MOX and mixed oxide with enriched uranium, known as MIX, fuels, 356 yrs. total length of the fuel cycle). Plutonium usability is not evaluated.

The calculated FOM_1 and FOM_2 values show a general trend of decreasing criteria values, i.e. increasing quality of the material barrier and relatively small barrier quality deterioration with cooling time. Thus burn-up has more pronounced influence on the quality of the barrier. The values of both criteria have been averaged over the 30-year spent fuel cooling period in order to analyse more thoroughly the effects of the burn-up. The results are shown on Fig. 1 and Fig. 2. Fig. 1 represents the change of the average FOM_1 value as a function of the fuel burn-up. A clear, almost linear decrease in the material's attractiveness can be observed for both plutonium from UOX and MOX spent fuels; in other terms, the quality of the material barrier visibly improves with the burn-up increase. The change in the quality of plutonium produced from uranium fuel is very well pronounced, especially when spontaneous neutron emission is not considered (FOM_1). The barrier quality of plutonium from spent MOX fuel basically remains constant with burn-up increase. The impact of the spontaneous neutron emission on the quality of the material barrier can be examined in Fig. 2. In that case, the quality of plutonium from spent MOX fuel also visibly increases with burn-up. In addition, the FOM_2 values are around two times lower than FOM_1 values. That observation is an unambiguous confirmation of the contribution of the spontaneous neutron emission to material barrier quality.

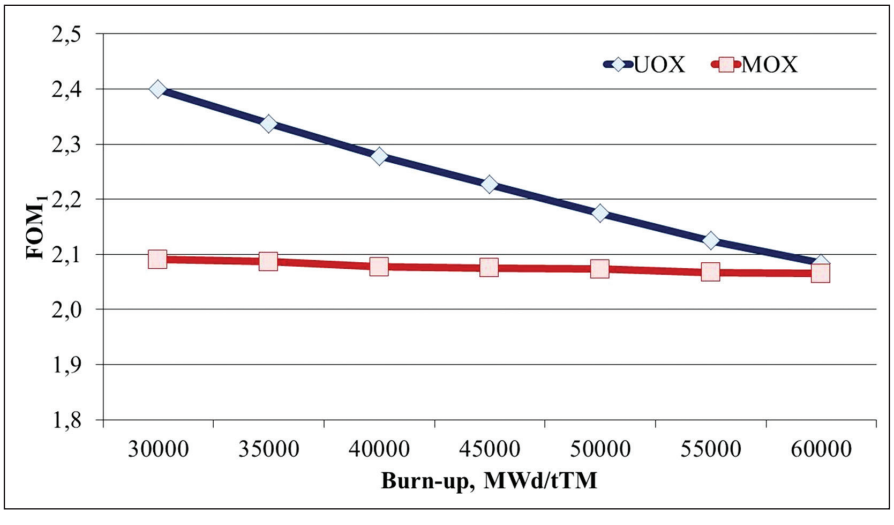

Fig. 1: Change of 30-year average FOM_1 as a function of burn-up.

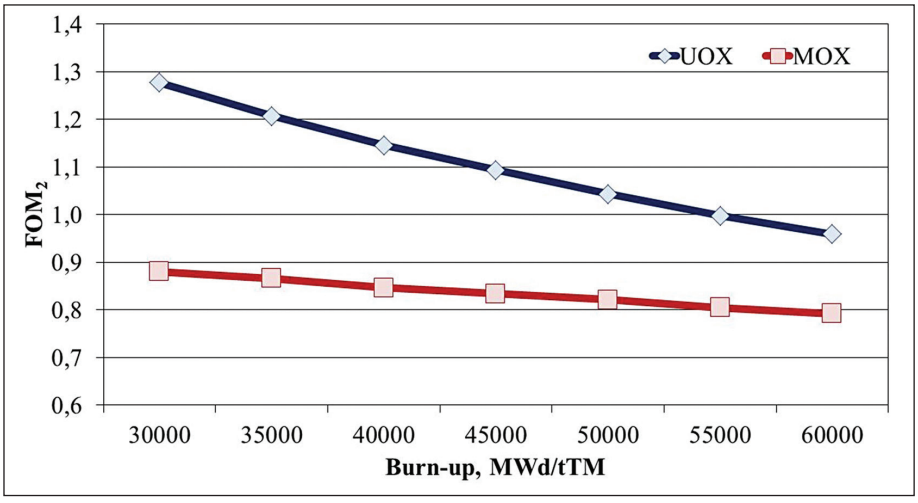

Fig. 2: Change of 30-year average FOM_2 as a function of burn-up.

The MAUA results, similarly to the 'Figure of Merit' values, are virtually independent from cooling time. Because of that, a similar approach has been undertaken and the static proliferation resistance values have been averaged over the 30-year cooling period. The dependence of static proliferation resistance on burn-up for metallic and non-separated plutonium is shown in Fig. 3a and Fig. 3b.

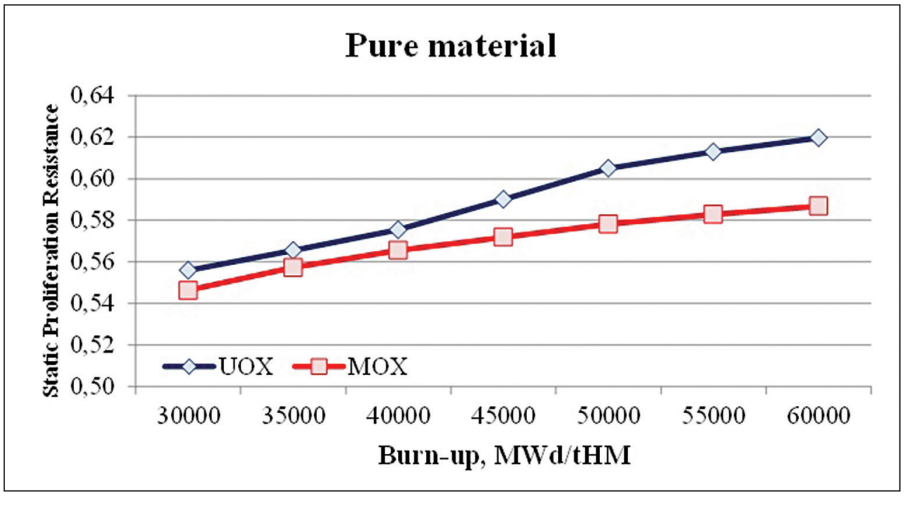

Fig. 3a: Change of 30-year average static proliferation resistance of separated plutonium as a function of burn-up.

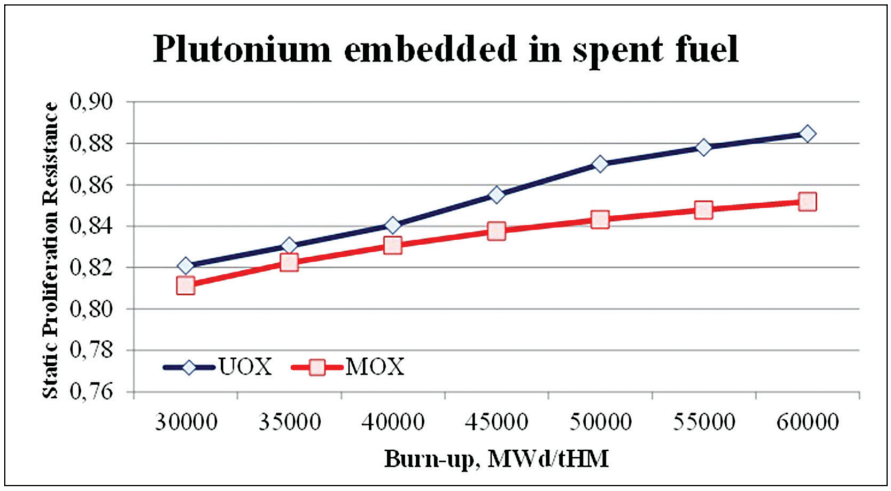

Fig. 3b: Change of 30-year average static proliferation resistance of non-separated plutonium as a function of burn-up.

The figures reveal that the character of the change of metallic plutonium barrier quality coincides with that of non-separated plutonium. Other significant observation is that, unlike the 'Figure of Merit' results, here the proliferation resistance of UOX plutonium is greater. The most likely reason for this discrepancy is the much greater plutonium inventory generated by mixed oxide fuel operation, which is considered in the MAUA analyses but is disregarded in 'Figure of Merit' assessments.

Another observation that can be made on the basis of the graphical data representation is that the improvement rate of MOX plutonium barrier quality decelerates with burn-up increase. Considering plutonium generated from uranium fuel, the graph can be divided in two approximately linear sections with inflexion point at 50,000 MWd/tHM, where the improvement rate decreases. When the material form is taken into account, a higher rate of improvement with burn-up is observed for metallic plutonium. However, the contribution of the material form to the overall static proliferation resistance is significant and is illustrated in Fig. 3c. If the plutonium is embedded within the spent fuel matrix, the relative quality of the barrier is between 42.8 % and 48.5 % higher, depending on the fuel type and the burn-up. The relative contribution is greater for plutonium from spent mixed uranium-plutonium oxide fuel and is inversely proportional to the burn-up.

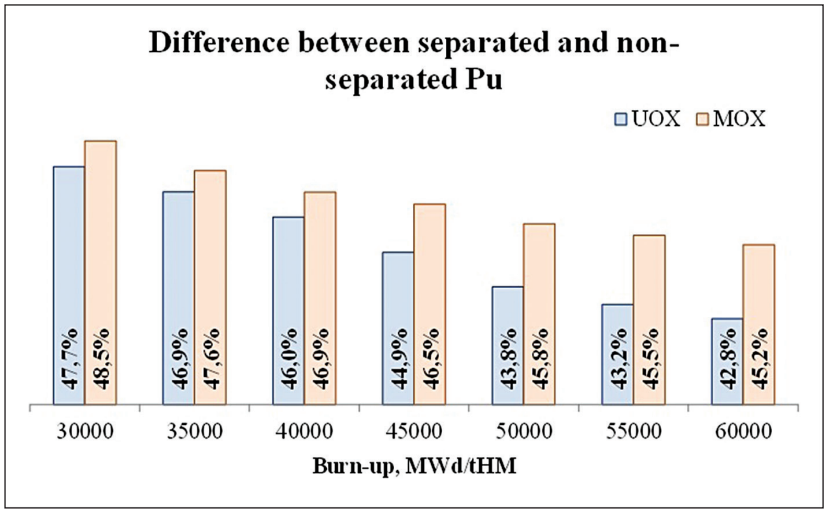

Fig. 3c: Relative increase of the static proliferation resistance of embedded plutonium compared to metallic plutonium.

The multiple recycle is analysed only by using the FOM criteria. The examined fuel cycles are shown in Fig. 4, and further details can be found in (Naydenov, Filipov 2018a). The results from the multiple recycle analysis are presented in Fig. 5 and Fig. 6. They show general improvement of the material barrier qualities, with the exception of the 'no recycle case' (when spontaneous neutron emission is not considered). If the spontaneous neutron emission is not taken into account (the main effect of even plutonium isotopes), it is evident that the properties of plutonium's material barrier remain unchanged in the case of no recycle (UOX). In the single recycle case (MOX), there is a slight improvement in barrier qualities over time, mainly due to the irradiation stage. The same observations can be made in the multiple recycle case (MIX) where the highest effect on the barrier quality is observed. Once again, the irradiation stages have a higher impact. That influence, however, is the highest after the first recycle stage and incrementally decreases with each following recycle. This is due to the deteriorating isotopic composition after each following recycle, which limits the amount of burnt odd isotopes, thus limiting the impact on the barrier quality. Unlike in MOX fuel, in MIX fuel plutonium is not the main source of fissile nuclei, which also contributes to that effect. Generally, it can be concluded that the impact of cooling time on material barrier properties is relatively low.

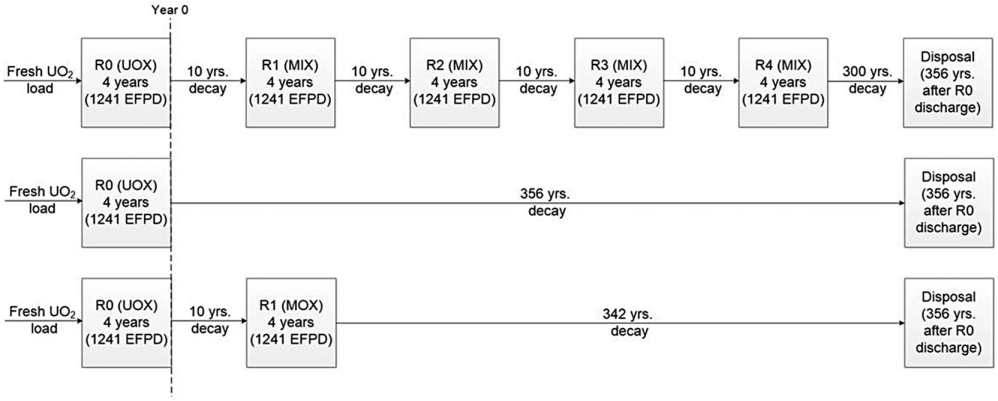

Fig. 4: Examined fuel cycles and timeline of their stages; above: multiple plutonium recycles; in the middle: no recycle; below: single plutonium recycle. R0 to R4 refers to the number of recycles with R0 – no recycle; EFPD – effective full-power days.

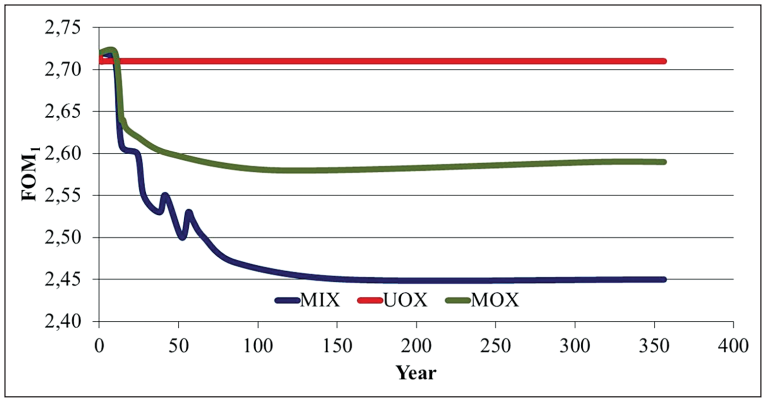

Fig. 5: Change of the FOM_1 values over time in different fuel cycles.

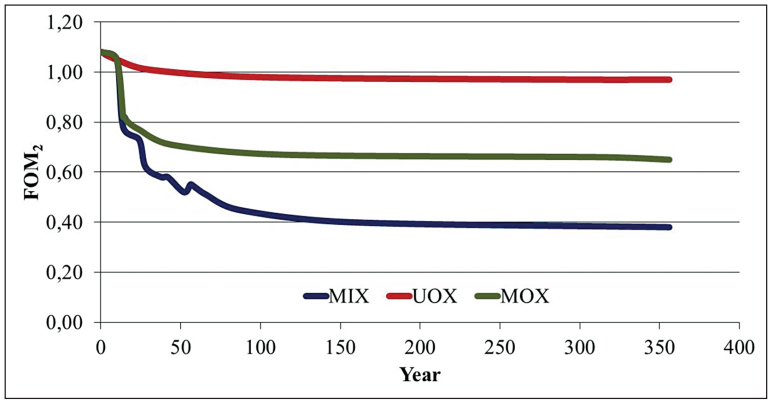

Fig. 6: Change of the FOM_2 values over time in different fuel cycles.

Main findings and conclusions

The main findings of the various analyses may be summarised as follows:

- The material barrier of plutonium obtained by spent fuel with low to medium burn-up (30 – 45 GWd/tHM) is not sufficient to render the material unattractive. However, that does not confirm plutonium's feasibility for non-civilian applications;
- The quality of the barrier of plutonium generated by UOX increases linearly with burn-up (faster isotopic vector degradation) and that adds to increased burn-up feasibility;
- If plutonium's inventory is not taken into account, the material barrier of MOX plutonium is better;
- Given the results of the multi-attribute utility analysis, the material's inventory should not be disregarded in those assessments;
- Plutonium recycling in MOX improves overall barrier quality because material's decay heat, bare critical mass, dose rates, and neutron emission increase;
- An improved material barrier contributes to the proliferation resistance of nuclear fuel cycles. However, that does not imply weaker physical protection / safeguards;
- The physical protection analyses should take into consideration the properties of the material;
- The major contribution to material barrier improvement in the recycle cases is made during the irradiation cycles. Long-term decay (interim storage) has weak influence on plutonium's properties;
- Multiple plutonium recycle has the most pronounced influence over material barrier qualities;
- Overall, the decay process has a low impact on the proliferation barrier.

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Ivaylo Naydenov
Department of Thermal and Nuclear Power Engineering
Technical University of Sofia
Sofia, Bulgaria
ivaylo.naydenov@gmail.com

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Proceedings of the Humboldt-Kolleg
Varna, September 18 – 21, 2019

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Edited by:
Lora Taseva, Radka Argirova, Dilyana Boteva,
Maria Luisa Grilli and Tăchiță Vlad-Bubulac

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Format: 70 x 100 / 16

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Faber Publishing House
Veliko Tarnovo, 1 Gara Veliko Turnovo street
+359 62 600 650
www.faber-bg.com

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Supported by
The Alexander von Humboldt Foundation
Germany