Katedra i Zakład Chemii Fizycznej i Biofizyki
Uniwersytetu Medycznego we Wrocławiu

Pharmaceutical Science:
Physical Chemistry and Biophysics for Pharmacy 2025
4th and 5th of December, 2025

BOOK OF ABSTRACTS

Conference under the scientific press patronage of
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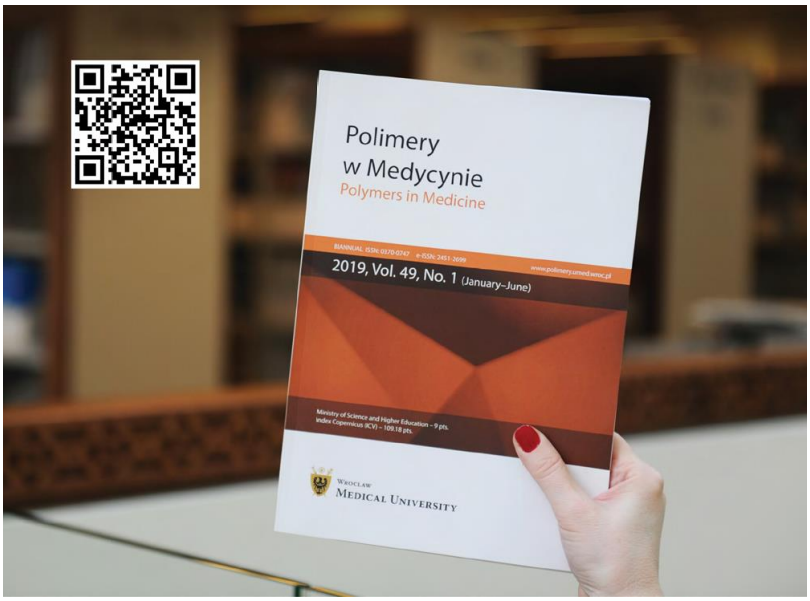

Wroclaw Medical University
Auditory Hall of the Faculty of Pharmacy
Borowska 211 st., Wrocław
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Editors:
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Witold Musiał

Wroclaw Medical University, Department of Physical Chemistry and Biophysics
211A Borowska St., 50-556 Wrocław

Wrocław 2025

Dear Colleagues and Friends,

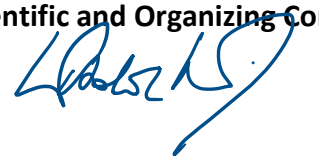
We kindly invite you to participate in the third edition of the scientific conference "Physical Chemistry and Biophysics for Pharmacy 2025". The aim of the conference is to give researchers, PhD candidates and undergraduates, an opportunity to discuss the latest important research developments in physical chemistry and biophysics in medical and pharmaceutical applications. The topics of the Conference include the structure and dynamics of macromolecules and biomacromolecules, intermolecular interactions, experimental and theoretical methods in physicochemical and pharmaceutical research of future drugs. The context of the Conference concerns the studies and development of new drugs and their carriers, and the design of new medical devices and appliances, appropriate methods of synthesis, analysis and application.

Welcome to Wroclaw!

On behalf of the Scientific Committee and the Organizing Committee:

Prof. Witold Musiał, PhD, DSc

Chairman of Scientific and Organizing Committee



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About the Department of Physical Chemistry and Biophysics

Pharmaceutical Faculty of Wroclaw Medical University

The Department of Physical Chemistry and Biophysics is active in the field of physicochemical and biophysical aspects of the development, production and analysis of innovative medicinal products, including polymer drug carriers. The research methods used by our scientists cover preparative methods: surfactant free precipitation polymerization, other radical polymerization methods, liposomes preparation, classic pharmaceutical preparation and layer by layer techniques. The analytical methods include spectral studies (NMR, LC-MS, FTIR, XRPD), HPLC chromatography and its variants, pharmacopoeial methods, studies with biopharmaceutical models, measurements based on Langmuir π-A isotherms, electrochemical methods, other physicochemical methods as well as mathematical modeling in kinetic studies. Researchers are co-authors of patents and many publications in peer-reviewed scientific journals, and cooperate with other research units of domestic and foreign universities and with the pharmaceutical industry.

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The Overall Conference Program:

No.	**4th of December 2025**	hrs
1.	Opening	10.00 – 10.20
2.	Lectures of the 1st session and discussion - *Surface tension in physical methods for drug development*	10.20 – 11.40
3.	Coffee break	11.40 – 11.55
4.	Lectures of the 2nd session and discussion - *Drug development and pharmaceutical technology*	11.55 – 13.10
5.	Coffee break 2	13.10 – 13.25
6.	Lecture of the 3rd session and discussion - *Conjugates, proteins and similarities – targeting cells and tissues*	13.25 – 14.45
7.	Lunch break	14.45 – 15.30
8.	Lectures of the 4th session and discussion - *Kinetics and models in pharmaceutical sciences*	15.30 – 16.25
11.	Summary of the first day of conference	16.25 – 16.30
12.	Poster session on billboards	10.00 – 16.30
	5th of December 2025	
13.	Opening	09.00 – 09.10
14.	Lectures of the 5th session and discussion - *Drug carriers and active pharmaceutical ingredients: new particles, modifications and natural sources*	09.10 – 10.45
15.	Coffee break	10.45 – 11.05
16.	Lectures of the 6th session and discussion - *Carriers for active pharmaceutical ingredients – between basic science and applications*	11.05 – 12.35
17.	Coffee break 2	12.35 – 12.50
18.	Lecture of the 7th session and discussion - *Analytics and interactions in pharmaceutical systems*	12.50 – 14.00
19.	Lunch break	14.00 – 14.50
20.	Lectures of the 8th session and discussion - *Pharmaceutical sciences and industry*	14.50 – 16.00
21.	Summary of the conference	16.00 – 16.10
22.	Poster session on billboards	09.00 – 16.10

The Scientific Program, 4th of December:

Opening 10.00 – 10.20	*Witold Musiał (Wroclaw Medical University)*	
Session 1	**Surface tension in physical methods for drug development**	
10.20 – 10.50	OP1	**Toward understanding the impact of hydrophobic surfaces on insulin agglomeration: Therapeutic implications and challenges** *Sourav Bhattacharjee (University College Dublin, Ireland)*
10.50 – 11.10	OP2	**Molecular insights into surfactant–protein interactions: From physicochemical characterization to biomedical potential** *Żaneta Czyżnikowska (Wroclaw Medical University, Poland)*
11.10 – 11.35	OP3	**Engineering Metal Oxide Nanoparticles for Glucose Sensing Application** *Mahabubur Rahman Chowdhury (Cape Peninsula University of Technology, South Africa)*
11.35 – 11.40	**Discussion**	
Coffee break 11.40 – 11.55		
Session 2	**Drug development and pharmaceutical technology**	
11.55 – 12.15	OP4	**Studies on the interaction of ophthalmic drop packaging polymers with the self-emulsifying carrier of oil drops** *Marzena Jamrógiewicz (Medical University of Gdansk, Poland)*
12.15 – 12.40	OP5	**Industrial-scale fluid-bed granulation and PAT** *Jyrki Heinämäki* (*University of Tartu, Estonia*)
12.40 – 13.05	OP6	**Cyclodextrins: Small ring – Big impact** *Tamas Sohajda (CarboHyde Budapest, Hungary)*
13.05 – 13.10	**Discussion**	
Coffee break 13.10 – 13.25		
Session 3	**Conjugates, proteins and similars – targeting cells and tissues**	
13.25 – 13.50	OP7	**Design and Application of Hydrophilic Linkers in Next-Generation High-Payload Antibody–Drug Conjugates** *Gianfranco Pasut (University of Padova, Italy)*
13.50 – 14.05	OP8	**Design of PLGA–mupirocin conjugate scaffolds with tunable degradation and antimicrobial activity for wound healing applications** *Tomasz Urbaniak* (*Wroclaw Medical University, Poland)*

14.05 – 14.25	OP9	**Properties of Doped Resin-Modified Glass Ionomer Cement and Fluoride Release from Dental Biomaterials** *Paweł Piszko (Wroclaw University of Science and Technology, Poland)*
14.25 – 14.40	OP10	**Effect of the peptide C10-KRIWQRIKDF-NH_2 in the aqueous subphase on the compression and stability of phosphatidylinositol monolayers in the temperature range of 25–35°C** *Iwona Golonka (Wroclaw Medical University, Poland)*
14.40 – 14.45	**Discussion**	
Lunch break 14.45 – 15.30		
Session 4	**Kinetics and models in pharmaceutical sciences**	
15.30 – 15.50	OP11	**Computational Biophysics and Clinical Pharmacology: Machine Learning Approaches for Predictive Modeling of Patient Data** *Andrzej Czyrski (Poznan University of Medical Sciences, Poland)*
15.50 – 16.10	OP12	**Pharmacokinetic Approaches in Preclinical Evaluation of Drug Candidates** *Małgorzata Szafarz (Jagiellonian University Medical College, Poland)*
16.10 – 16.25	OP13	**The Response Surface Methodology** *Remigiusz Zapolski (Wroclaw Medical University, Poland)*
16.25 – 16.30	**Discussion and summary of the conference** *Witold Musiał*	
Poster session 10.00 – 16.30	**The posters will be available for viewing on boards during the conference.**	

The Scientific Program, 5th of December:

Opening 09.00 – 09.10	*Witold Musiał*	
Session 5	**Drug carriers and active pharmaceutical ingredients: new particles, modifications and natural sources**	
09.10 – 09.30	OP14	**Selected examples of physico-chemical methods applied for development of drug carriers** *Witold Musiał (Wroclaw Medical University, Poland)*
09.30 – 09.55	OP15	**When Molecules Join Hands: The Journey of Cocrystals from Mechanochemistry to Therapeutic Application** *Beatrice Perissutti (University of Trieste, Italy)*
09.55 – 10.20	OP16	**Harnessing liposome technology to unlock the potential of natural products in therapy** *Carla Caddeo (University of Cagliari, Italy)*
10.20 – 10.40	OP17	**Physical modifications of poorly soluble drugs as a way to create supersaturated systems: The case of bosentan hydrate** *Anna Krupa (Jagiellonian University, Medical College, Poland)*
10.40 – 10.45	**Discussion**	
Coffee break 10.45 – 11.05		
Session 6	**Carriers for active pharmaceutical ingredients – between basic science and applications**	
11.05 – 11.30	OP18	**Chemico-physical and biological properties of monoclonal antibodies, under real-life light doses: a mechanistic approach** *Giorgia Miolo (University of Padova, Italy)*
11.30 – 11.55	OP19	**Dermal delivery of clotrimazole using microneedle technologies** *Bozena Michniak-Kohn (Rutgers-The State University of New Jersey, USA)*
11.55 – 12.15	OP20	**Where biophysics meet pharmacy - parametrization of mechanistic model for drug release from PLGA-based biodegradable implants** *Sebastian Polak (Jagiellonian University Medical College, Poland)*
12.15 – 12.30	OP21	**The impact of formulation composition on the bioavailability of the active substance in probiotic emulsions for the topical treatment of dermatological conditions** *Monika Gasztych (Wroclaw Medical University, Poland)*
12.30 – 12.35	**Discussion**	

Coffee break 12.35 – 12.50		
Session 7	**Analytics and interactions in pharmaceutical systems**	
12.50 – 13.15	OP22	**Physicochemical and biological properties of multi-target Michael acceptors based on cinnamanilides** *Josef Jampilek (Palacky University Olomouc, Czech Republic;* *Comenius University Bratislava, Slovakia)*
13.15 – 13.35	OP23	**Analytical challenges and clinical relevance of vitamin D epimers** *Marta Karaźniewicz – Łada (Poznan University of Medical Sciences, Poland)*
13.35 – 13.55	OP24	**You cannot step into the same river twice – imaging of hidden processes in hydrophilic matrix tablets** *Przemysław Dorożyński (Medical University of Warsaw, Jagiellonian University Medical College, Poland)*
13.55 – 14.00	**Discussion**	
Lunch break 14.00 – 14.50		
Session 8	**Pharmaceutical sciences and industry**	
14.50 – 15.10	OP25	**Physicochemical methods for the study of the interaction between exogenous factors and endogenous antioxidants proteins (EAPs)** *Wojciech Rogóż (Medical University of Silesia in Katowice, Poland)*
15.10 – 15.25	OP26	**Impact of PEG-type crosslinker molecular size on the structural and thermal characteristics of thermosensitive polymers** *Agnieszka Gola (Wroclaw Medical University, Poland)*
15.25 – 15.40	OP27	**The effect of hydrothermal and chemical modifications of potato starch on its carrier properties for selected polyphenols from chokeberry fruit** *Justyna Kobryń (Wroclaw Medical University, Poland)*
15.40 – 15.55	OP28	**Kinetics of prednisolone sodium phosphate release from sodium hyaluronate based hydrogels doped with synthetic polymers** *Dorota Wójcik-Pastuszka (Wroclaw Medical University, Poland)*
15.55 – 16.10	**Discussion and summary of the conference** *Witold Musiał*	
Poster session 09.00 – 16.10	**The posters will be available for viewing on boards during the conference.**	

Poster Session

P1 - **The role of fatty acids in albumin glycoxidation – a spectroscopic analysis**

Karolina Kołacz, Agnieszka Szkudlarek*, Małgorzata Maciążek-Jurczyk

Department of Physical Pharmacy, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, 40-055 Katowice, Poland

P2 - **Physicochemical assessment of interaction between a new phenothiazine derivative and major serum carrier proteins – preliminary study**

Aleksandra Owczarzy[1]*, Wojciech Rogóż[1], Karolina Kulig[1], Andrzej Zięba[2], Małgorzata Maciążek-Jurczyk[1]

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P3 - **Analysis of cross-linked and uncross-linked human serum albumin nanocarriers**

Karolina Kulig[1]*, Magdalena Ziąbka[2], Wojciech Rogóż[1], Aleksandra Owczarzy[1], Małgorzata Maciążek-Jurczyk[1]

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P4 **- Comparison of the colorimetric tests for detecting reduced thiol groups in peptides, proteins, and their mixtures – *in silico* analysis**

Wojciech Rogóż*, Aleksandra Owczarzy, Karolina Kulig, Małgorzata Maciążek-Jurczyk*

Department of Physical Pharmacy, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Katowice 40-055, Poland

P5 - **UPLC-MS/MS method for quantification of rivaroxaban and its M1 metabolite in plasma samples**

Kornel Pawlak[1,2]*, Marta Karaźniewicz-Łada[1]

[1]Poznan University of Medical Sciences, Department of Physical Pharmacy and Pharmacokinetics, 3 Rokietnicka Street, 60-806 Poznan, Poland

[2]Doctoral School, Poznan University of Medical Sciences, 60-812 Poznan, Poland

P6 - **Selected properties of thermo- and pH- sensitive pAMPSA as a potential drug carrier.**

Magdalena Bruchal*, Agnieszka Gola, Witold Musiał

Wroclaw Medical University, Department of Physical Chemistry and Biophysics, Pharmaceutical Faculty Borowska 211, 50-556 Wroclaw, Poland

P7 - **Drug Transport Systems in Modern Therapeutics: Natural and Synthetic Carriers in Pharmaceutical Sciences**

Laura Jonderko*, Anna Choromańska

Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211A, 50-556 Wroclaw, Poland

P8 - **Hydrogels with Tetracyclines for the Treatment of Acne: The Latest Achievements, Difficulties, and Directions for Development**

Agnieszka Kostrzębska[1]*, Adam Junka[2], Witold Musiał[1]

[1]Department of Physical Chemistry and Biophysics, Wroclaw Medical University, Borowska 211A, 50-556 Wroclaw, Poland, [2]Platform for Unique Models Application P.U.M.A., Department of Pharmaceutical Microbiology and Parasitology, Wroclaw Medical University, Borowska 211, 50-556 Wroclaw

P9 - **New Horizons in Local Periodontal Therapy: A review of advances in polymeric mucoadhesive dressings with phytocompounds (2020–2024)**

A.Dołowacka-Jóźwiak[1]*, K. Małolepsza-Jarmołowska [1], A. Gawin-Mikołajewicz [1], D. Bursy [1], D. Muciek[1], A. Krawczyk[1], M. Gasztych[2], R. Dudek-Wicher [3], I. Gabryś [4], D. Figurski [5], A. Alaimo[6], D. Haznar-Garbacz[1]

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P10 - **Natural compounds and pharmaceutical forms in the treatment of psoriasis – a review based on current reports**

Aleksandra Chojecka[1]*, Katarzyna Ptak[1], B. Baylan[2] , D. Bursy [1], M. Gasztych[3], R. Dudek-Wicher [4], A. Alaimo[5], K. Małolepsza-Jarmołowska[1], D. Haznar-Garbacz[1], A.Dołowacka-Jóźwiak[1]

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P11 - **Dendrimer-Based Drug Delivery Systems: with Protein Corona Structure – in vitro study**

Julita Kulbacka[1]*, Barbara Jachimska[2], Magdalena Szota[2], Urszula Szwedowicz[1], Nina Rembiałkowska[1], Anna Szewczyk[1], Anna Janicka-Kłos[3]

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P12 - **Safety and Efficacy of Irreversible Electroporation (IRE) Combined with Chemotherapeutics and Calcium Ions in Pancreatic Cancer**

Julita Kulbacka[1]*, Agnieszka Gajewska-Naryniecka[1], Julia Rudno-Rudzińska[2], Vitalij Novickij[3,4], Katarzyna Bieżuńska-Kusiak[1], Wojciech Kielan[2]

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P13 - **Is ion exchange chromatography (IEX) suitable for albumin conjugates separation?**

Bogusława Konopska[1]*, Krzysztof Gołąb[2]

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P14 - **Biotic elicitors as physicochemical drivers of isoquinoline alkaloid network dynamics in *Chelidonium majus***

Marcel Białas[1SSC]*, Anna Sroka-Bartnicka[2,3], Mikołaj Krysa[3], Malwina Brożyna[4], Adam Junka[4], Magdalena Dziągwa-Becker[5], Adam Matkowski[6,7], Weronika Kozłowska[1], Sylwia Zielińska[1]

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P15 - **Natural triterpenoid surfactants and bioactive polyphenols from in vitro cultures of Gypsophila elegans and Agrostemma githago**

Michał Dziwak[1]*, Monika Bielecka[1], Marta Stafiniak[1], Łukasz Pecio[2], Solomija Pecio[2], Reneta Gevrenova[3], Izabela Nawrot-Hadzik[1], Alexander Weng[4], Fabian Bülow[4], Adam Matkowski [1,5], Weronika Kozłowska[6]

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P16 - **Evaluation of Celugel potential as a semi-solid polymer-base in the design of ophthalmic antibiotic in pharmacy practice: preliminary studies and physicochemical characterization**

D. Bursy[1]*, D. Haznar - Garbacz[1] , B. Karolewicz[1] , K. Gach[1], A. Alaimo[2], A. Dołowacka-Jóźwiak[1]

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P17 **- Structure and Complexation Properties of EDTA-Crosslinked Polycyclodextrins for Advanced Polyelectrolyte Systems**

Zuzanna Podgórniak[1]*, Tomasz Urbaniak[1], Michał Kulus[2], Witold Musiał[1]

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P18 **- sST2 as a Diagnostic and Prognostic Biomarker in Patients with Thrombotic Microangiopathy after Allogeneic Hematopoietic Stem Cell Transplantation**

Aleksandra Musz[1]*, Tomasz Jarmoliński[2], Iwona Bil-Lula[3]

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P19 **- Enhancement of the antimicrobial activity of plant-derived terpenes by the incorporation of cocamidopropyl betaine**

Aleksandra Woytoń*, Gabriela Skoczek, Adam Junka

Division of Translational Technologies, "PUMA", Platform for Unique Model Applications, Faculty of Pharmacy, Wroclaw Medical University, Wrocław, Poland

P20 **- Application of Point-of-Care EPR Spectroscopy in Oxidative Stress Diagnostics and Pharmacotherapy Monitoring**

Arkadiusz Bujas[1],[2]*, Julita Kulbacka[2], Anna Choromańska[2], Nina Rembiałowska[2], Wojciech Szlasa[2],[3]

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P21 - **Cyclodextrin-Based Polymer Carriers for Acyclovir Adsorption and Controlled Release**

Prajzner Maja*, Podgórniak Zuzanna; Urbaniak Tomasz; Musiał Witold

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P22 - **Study of the interaction of sulfasalazine with human albumin protein depending on the environment**

Anna Janicka-Kłos[1]*, Laura Karpińska[2]

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*presenting Author

ABSTRACTS
LECTURES

OP1

Toward understanding the impact of hydrophobic surfaces on insulin agglomeration: Therapeutic implications and challenges

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Introduction: Due to a soaring patient load of diabetes, the therapeutic relevance of insulin is established beyond doubt. Unfortunately, insulin interacts with hydrophobic surfaces, including polymers, and agglomerates (1,2). Such agglomeration risk alleviating both its bioavailability and bioactivity. This study investigated the impact of functionalized polystyrene surfaces on insulin agglomeration with the help of confocal laser scanning microscopy (CLSM) and fluorescence lifetime imaging microscopy (FLIM).
Materials and Methods: Recombinant human insulin labeled with fluorescein isothiocyanate (FITC) was dissolved in a pH 3 solution (0.125 mg/ml, 0.25 mg/mL, and 0.5 mg/mL) and mixed with fluorescent amine- and acid-terminated polystyrene microparticles (1 µm) for t = 2 h, 4 h, 24 h, 48 h, and 72 h at 37°C, followed by inspection by both CLSM and FLIM for FITC-labeled insulin (λex=490 nm; λem=498–530 nm), amine-terminated (λex=560 nm; λem=570–650 nm), and acid-terminated (λex=625 nm; λem=640–720 nm) microparticles. The FITC was used as a *molecular pH-meter*, given its fluorescence lifetime varies with the microenvironment pH: lower lifetimes are noted at acidic pH and *vice versa*.
Results: Both the amine- and acid-terminated microparticles agglomerated FITC-insulin (amine-terminated>acid-terminated) on an overall concentration- and time-dependent manner. Interestingly, the amine-terminated microparticles caused a higher fluctuation of FITC's fluorescence lifetime in the insulin agglomerates, while such lifetime fluctuations extended beyond the particulates into the agglomerate matrices. On the contrary, such lifetime variation in FITC-insulin agglomerates were subtler in acid-terminated microparticles, and the FITC lifetime fluctuations stayed limited around particulate surfaces.
Discussion: Functionalized hydrophobic polymeric surfaces are able to exert biochemical changes in insulin identifiable with advanced microscopy. FLIM data showed that such fluctuation of FITC lifetimes extended beyond the particulate through electronic interactions, while highlighting the influence of pH on such interactions.
Conclusions: An uncontrolled, unmapped, and unpredictable agglomeration of insulin while in contact with hydrophobic polymers, with alterations in its molecular properties, carries a risk of bioactivity attenuation, and the causation of additional side effects., with particular relevance in diabetes management, where a firm control over dosing and bioavailability is of paramount importance. Further investigations are needed to understand the mechanisms driving such surface-induced insulin agglomerations.

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OP2

Molecular insights into surfactant–protein interactions:
From physicochemical characterization to biomedical potential

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Introduction: Surfactants are a broad class of amphiphilic compounds that can self-organize and recognize molecules at biological interfaces. Their interactions with proteins are crucial for understanding the relationships between structure and function relevant to drug delivery, antimicrobial activity, and toxicity. This study compares the physicochemical and biological properties of synthetic and microbial biosurfactants.
Materials and methods: Studies involved fluorescence and dichroism spectroscopy, surface tension measurements, dynamic light scattering and isothermal titration calorimetry. Computational approaches such as density functional theory, molecular docking and molecular dynamics were employed to elucidate interaction mechanism and predict binding affinities.
Results: Both classes of compounds formed stable complexes with proteins via hydrophobic, electrostatic and hydrogen bonding interactions. Isothermal titration calorimetry confirmed spontaneous binding with moderate to high affinity, depending on the length of the hydrophobic chain and the distribution charge. Molecular modeling revealed that cationic surfactants induced partial rearrangements in surface residues of proteins, whereas biosurfactants stabilized the secondary structure of protein through favorable interfacial hydrogen bonding. Biological measurements demonstrated antibacterial and anticancer activity, as well as inhibition of microbial adhesion and biofilm formation on catheter surface.
Discussion: The combination of spectroscopic and computational methods enabled a detailed understanding of surfactant-protein recognition. The results highlight fundamental differences between natural and synthetic surfactants emphasizing the selectivity and biocompatibility of biosurfactants and their potential for pharmaceutical and biomedical use.
Conclusions: The revealed interaction mechanism support the rational use of biosurfactant in designing antimicrobial and anticancer agents.

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OP3

Engineering Metal Oxide Nanoparticles for Glucose Sensing Application

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Fourth-generation glucose sensors rely on the direct electrocatalytic oxidation of glucose on non-enzymatic surfaces, providing enhanced durability and chemical stability under physiologically complex conditions. Transition-metal oxide nanoparticles—particularly those of cobalt, copper, and nickel—remain central to these developments due to their accessible redox states, catalytic selectivity, and compatibility with cost-effective, solution-based fabrication routes [1-3].

A persistent challenge in these sensors arises from the powder-based electrode assembly, where the incorporation of binder and random particle packing introduces contact resistance and non-uniform catalytic accessibility. Solution-processed direct deposition methods offer a route to mitigate these limitations. Micro-plotting of precursor inks followed by controlled thermal conversion (typically 300–350 °C) enables the formation of binder-free, conformal metal-oxide films that maintain intimate electronic contact with conductive substrates. Such routes also allow systematic tuning of crystalline phase, defect concentration, porosity, and nanoscale texturing, as reflected in structural and electrochemical characterisations using XRD, SEM, and voltametric techniques.

Engineered films produced through these approaches exhibit enlarged electroactive surface areas, improved adsorption capability, and accelerated charge-transfer kinetics. These attributes contribute to high sensitivities, short response times (<3 s), and stable operation during glucose oxidation. When deposited on flexible or biodegradable substrates, these nanostructured oxides further support the development of environmentally responsible and transient sensing platforms.

Overall, rational control over the structure and interface properties of metal-oxide nanoparticles—enabled by scalable solution deposition—positions these materials as strong candidates for robust, fourth-generation glucose sensors designed for emerging diagnostic and continuous monitoring technologies.

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OP4

Studies on the interaction of ophthalmic drop packaging polymers with the self-emulsifying carrier of oil drops

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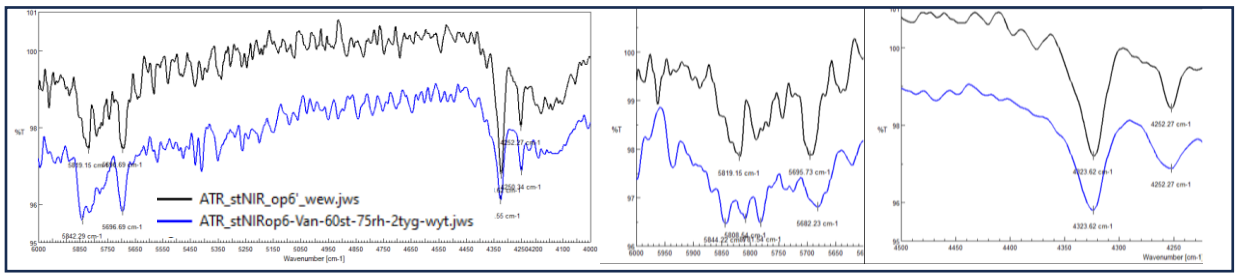
Introduction: Low-density polyethylene (LDPE) is one of the most commonly used polymeric materials in pharmaceutical packaging, including ophthalmic preparations. In pharmaceutical applications, LDPE can interact with drugs through adsorption, absorption, ingredient migration, photodegradation, and gas permeability, potentially affecting formulation stability. The introduction of new medicinal products requires comprehensive documentation in the CTD format, submitted to the Office for Registration of Medicinal Products (URPL) within the Quality Module (Module 3). Therefore, physicochemical and stress testing of packaging materials is essential.
The research is funded by the Medical Research Agency - grant 2024/ABM/03/KPO/ KPOD.07.07-IW.07-0046/24-00.

Materials and methods: The developed innovative and patented [1] pharmaceutical composition is a liquid, self-emulsifying oil suspension (SEO) consisting of Miglyol 812, Tween 20 (5%), and sodium citrate (2%) [2], containing water-unstable antibiotics (5%) – vancomycin hydrochloride and cefuroxime sodium.
Stress tests were conducted on six eye drop packages by immersing 5 mm × 5 mm fragments in the suspensions and carriers and storing them at 40–60°C / 75% RH. The tests were performed using ATR-FTIR and NIR-ATR methods, as well as DSC, XRD, and a texture meter. The mechanical strength of the eye drop package fragments was evaluated using a TA.XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell.

Results: Stress testing of the SEO packaging revealed subtle interactions between the SEO matrix and the packaging material. The APIs were not detected on the packaging surface after FTIR/NIR and DSC analyses. The matrix was shown not to degrade. The new, unused bottles exhibited higher crush resistance (compression force and compression work) compared to bottles stored for six months with oil or with the SEO system.



Discussion: Three IR bands characteristic of oil from the SEO were identified in the package spectra (1744, 1159, and 1109 cm^{-1} Three IR bands characteristic of the SEO oil were identified in the package spectra (1744, 1159, and 1109 cm^{-1}), demonstrating that oil adsorbs onto the package surface after stress testing. DSC also revealed differences in the α-relaxation temperatures and melting enthalpies of the packages after stress exposure.

Conclusions: The usefulness of IR, DSC, XRD, and texture analysis methods for studying the interaction between SEO and packaging materials was positively demonstrated. The most important result is that the active compounds suspended in SEO were not detected and did not interact with or affect the stability of the containers. Oil–packaging and oil–surfactant interactions were minor and consistent. No incompatibilities were observed.

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OP5

Industrial-scale fluid-bed granulation and PAT

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The pharmaceutical manufacturing involves often multi-variable unit-operations where several interrelated phenomena take place simultaneously. These elements together could impair the manufacturing process performance and final quality of medicinal products. Therefore, a science-based approach and building quality into medicinal products through design and understanding of manufacturing processes, are crucial.

Granulation is a major unit-operation in the manufacture of oral solid dosage forms, such as tablets. Today, one of the most common method for wet granulation in the pharmaceutical industry is a batch fluid-bed granulation. In fluid-bed processing, the granules are produced in a single piece of equipment (chamber) by spraying the binder solution onto a fluidized powder bed. After spraying, the granules are dried and discharged from the unit. The major advantage of fluid-bed granulation over other wet granulation methods is a rapid dry powder blending, wet massing, agglomeration, and drying within one unit.

In the present short lecture, recent developments and future perspectives in the batch fluid-bed granulation will be highlighted. The lecture will provide the description of a fluidization theory, and will discuss the critical process variables associated with a batch fluid-bed granulation. Process analytical technology (PAT) approach will be made to present the significance of process understanding and continuous process improvement in wet granulation. The process induced transformations (PITs) associated to the batch fluid-bed granulation are well known but difficult to predict and often difficult to control. Recent process imaging and spectroscopic techniques will be introduced as new PAT tools. Such techniques enable real time and continuous monitoring of a granulation process, and for the determination of possible PITs.

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OP6

Cyclodextrins: Small ring – Big impact

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How Will Cyclodextrins Shape Pharmaceutical Innovation?
Cyclodextrins (CDs) have evolved from academic curiosities into essential tools of modern drug development. Their ability to form inclusion complexes enhances solubility, stability, and bioavailability of active pharmaceutical ingredients, making them key enablers of safer and more effective medicines.
This presentation will showcase case studies illustrating how CD-based technologies have progressed from research to clinical and commercial success. Examples will highlight their roles in novel applications like stabilizing biologics, and enabling advanced delivery systems and as active ingredients.
Emphasis will be placed on regulatory strategy, and collaboration between academia and industry—factors that determine successful translation. The presentation will also touch on emerging CD derivatives expanding therapeutic opportunities in precision medicine and rare diseases.

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OP7

Design and Application of Hydrophilic Linkers in Next-Generation High-Payload Antibody–Drug Conjugates

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Antibody–drug conjugates (ADCs) represent a highly successful technology for the targeted delivery of cancer therapeutics. Based on the covalent coupling of a potent cytotoxic drug to a tumour-specific monoclonal antibody, ADCs have demonstrated significant clinical efficacy, with 13 ADCs currently approved and over 100 under clinical investigation.

An ADC specifically targets a tumour-associated antigen, typically a cell surface receptor, and delivers a highly potent drug (EC_{50} < 0.01–0.1 nM) directly to cancer cells. Upon antigen binding, the ADC–antigen complex is internalized and trafficked to lysosomes, where the drug is released through enzymatic, hydrolytic, or reductive cleavage.

Several biological and chemical factors can dramatically influence ADC efficacy, including the rate of endocytosis and lysosomal trafficking, and the extent of FcRn-mediated recycling of the antibody. In addition, the potency and mechanism of action of the payload, the linker chemistry, and the stability and release kinetics of the drug–antibody linkage are critical determinants of therapeutic performance.

ADCs employ two main classes of linkers: cleavable and non-cleavable. The most widely used cleavable linker is the cathepsin-sensitive dipeptide Val-Cit, often coupled with a self-immolative spacer such as *p*-aminobenzyl carbamate. In contrast, non-cleavable linkers rely on lysosomal proteolysis of the antibody backbone to release the active drug.

Recently, Pasut's group reported the design of a hydrophilic, non-cleavable PEGylated linker for the preparation of ADCs with high drug loadings. These ADCs exhibited enhanced physical stability and reduced plasma clearance in mice compared to *Kadcyla*. The linker featured a "pendant" structure with two monodisperse PEG_{12} arms extending from the central scaffold. Further optimization led to the inclusion of cleavable peptide sequences such as Phe–Gly or Val–Ala–Gly, enabling controlled drug release. Moreover, these linkers can be engineered for site-selective conjugation, either through amine (lysine) or thiol (cysteine) functional groups.

In conclusion, drug–linker optimization remains a key determinant of ADC performance. A deeper understanding of linker chemistry and design principles will support the development of next-generation ADCs with improved stability, pharmacokinetics, and therapeutic index.

OP8

Design of PLGA–mupirocin conjugate scaffolds with tunable degradation and antimicrobial activity for wound healing applications

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Chronic wounds remain a major clinical challenge, often complicated by bacterial infection and delayed healing. To address these issues, we developed biodegradable scaffolds based on poly(lactide-co-glycolide) (PLGA) covalently conjugated with mupirocin, designed to couple controlled degradation with long-term local antibacterial activity. The PLGA–mupirocin conjugates were synthesized via ring-opening polymerization and processed into porous scaffolds by solvent casting/salt leaching and electrospinning. Low-molecular-weight formulations obtained by solvent casting exhibited gradual degradation over time, while electrospun mats maintained structural integrity, supporting tunable resorption behavior. Although no measurable mupirocin release was detected by HPLC, both scaffold types displayed clear antibacterial activity against Staphylococcus aureus, suggesting that bioactive mupirocin moieties remain accessible at the material surface. The results demonstrate that covalent conjugation of mupirocin to PLGA enables stable, long-lasting antimicrobial functionality combined with controllable degradation, offering a promising strategy for the design of advanced wound healing materials.

This work was supported by the National Science Centre (NCN), Poland, under the SONATA programme (grant 2023/51/D/NZ7/00797).

OP9

Properties of Doped Resin-Modified Glass Ionomer Cement and Fluoride Release from Dental Biomaterials

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Introduction: Glass ionomers are used extensively in dentistry as provisional restorations, liners or bases, as well as for pit and fissure sealing. It is essential that this type of material exhibits favorable physicochemical and biological properties. The aim of the study was to alter resin-modified glass ionomer (RMGIC) by means of the incorporation of copper (RMGIC+Cu), with a view to evaluating its properties in terms of potential beneficial clinical applications.

Materials and methods: The antimicrobial activity of the samples was evaluated against the *C. albicans*, *S. mutans*, and *L. rhamnosus* strains. The antiviral properties of the analyzed materials were evaluated against two viruses (Herpes simplex virus type 1 and human Adenovirus 5). The materials were subjected to a cytoxicity assessment, for which the Balb/3T3 fibroblast cell line was employed. Furthermore, fluoride release up to 168 hours was measured in various incubation solutions. In addition, structural evaluation was conducted on the samples using ATR-FTIR and FT-Raman spectroscopies.

Results: The copper-modified biomaterial exhibited a diminished capacity for biofilm formation in relation to the tested strains. Multifactorial ANOVA analysis indicated that F^- release was influenced the strongest by the pH (η^2=0.93), and subsequently by combination of Cu presence and pH (η^2=0.62) and presence of copper itself (η^2=0.53). No antiviral activity against herpes simplex 1 was observed. However, both materials demonstrated the antiviral capacity against human adenovirus 5. Non-modified material exhibited no evidence of cellular toxicity, while the modified biomaterial induced minor alterations in cellular morphology.

Discussion: The reported research conducted on glass ionomer materials has focused on their antibacterial properties, with particular emphasis on their efficacy against oral bacteria. However, their antiviral activity is not vastly reported in literature. The results of the study indicate that the samples has moderate antiviral potential against human adenovirus 5, although this result was somewhat unexpected.

Conclusions: The doping of glass ionomer with copper may represent an intriguing modification, with the potential to enhance the antimicrobial properties of the biomaterial. However, further evaluation is required with respect to long-term toxicity of copper addition before additional in vivo studies are to be conducted.

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OP10

Effect of the tryptophan-to-phenylalanine substitution in modified peptides C_{10}-KRIWQRIKDF-NH_2 and C_{10}-KRIFQRIKDF-NH_2 on phosphatidylinositol monolayers studied by the Langmuir method (25–35°C)

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Introduction: *Cutibacterium acnes* is a lipophilic, anaerobic Gram-positive bacterium and a key component of the skin microbiome, contributing to its homeostasis. Its cell envelope is rich in lipids, including triacylglycerols and phosphatidylinositol, which play crucial roles in membrane organization, signal transduction, and interactions with ion channels[1]. Aromatic residues such as tryptophan and phenylalanine in peptides facilitate amphipathic helix formation, enhancing membrane interactions and antimicrobial activity. Modifications such as terminal amidation or cyclization further improve stability, selectivity, and resistance to proteolytic degradation[2]. The C_{10}-modified peptides used in this study are derivatives of the human antimicrobial peptide LL-37, designed to enhance membrane interactions and antimicrobial efficacy. These derivatives retain key structural features of LL-37 while incorporating hydrophobic modifications to increase their stability and surface activity[3].

Method: In this study, the effects of two C_{10}-modified peptides, C_{10}-KRIWQRIKDF-NH_2 and C_{10}-KRIFQRIKDF-NH_2, differing by a tryptophan-to-phenylalanine substitution, on PI monolayers were investigated using the Langmuir method. The study analyzed compression π–A isotherms and hysteresis to evaluate changes in surface pressure as a function of molecular area at 25, 30, and 35 °C.

Result: At 25 °C and 35 °C, a shift in the compression isotherms toward smaller areas per molecule was observed for both compounds, while at 30 °C the isotherms shifted toward larger areas per molecule. For C_{10}-KRIWQRIKDF-NH_2, maximum compressibility coefficients were 45.17, 52.98, and 42.33 mN/m, while for C_{10}-KRIFQRIKDF-NH_2, the values were 41.47, 51.10 and 46.90 mN/m, indicating a condensed liquid state in both systems. Hysteresis curves showed that decompression did not retrace the compression path, suggesting structural reorganization of the monolayers. The reversibility coefficient of isothermal compression increased with rising temperature, whereas the surface pressure at which monolayer collapse occurred decreased with increasing temperature for both compounds.

Discussion: The shifts in isotherms and the decrease in collapse pressure with increasing temperature indicate that the lipid monolayer becomes more fluid and susceptible to reorganization under the influence of peptides. The observed hysteresis suggests that hydrophobic modifications and aromatic residues promote peptide entry and permanent mechanical changes in the model membrane, which is consistent with current models of antibacterial peptide action[4]. The tryptophan-to-phenylalanine substitution in peptide two increases the molecular area, indicating a more relaxed and flexible monolayer.

Conclusions: The results suggest that the modified peptides C_{10}-KRIWQRIKDF-NH_2 and C_{10}-KRIFQRIKDF-NH_2 affect the physicochemical properties of the PI monolayer, which may serve as a foundation for future studies focused on the rational design of novel antimicrobial agents targeting *Cutibacterium acnes*.

Key words: Langmuir monolayer, compression isotherm, cationic peptides.

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OP11

Computational Biophysics and Clinical Pharmacology: Machine Learning Approaches for Predictive Modeling of Patient Data

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Introduction: Machine learning (ML) techniques offer advanced analytical capabilities in medical research, enabling more precise identification of critical factors influencing patient outcomes [1,2]. Two retrospective analyses applying ML methods were applied to analyze risk factors related to urinary tract infections (UTIs) and arterial blood gas (ABG) parameters in different patient populations.
Materials and Methods: The first study analyzed data from 76 patients at a Nursing and Treatment Facility, focusing on risk factors for UTIs including dapagliflozin administration, diabetes, sex, kidney failure, and urinary catheter use. Logistic regression, artificial neural networks (ANN), and decision tree were applied. The second study involved a retrospective evaluation of 71 pediatric patients with 479 ABG measurements from the Department of Pediatric Anesthesiology and Intensive Care. Regression ANNs with multiple activation functions and LASSO regression were used to identify significant variables affecting pH, pO_2, pCO_2, and lactate concentration.
Results: In the UTI study, dapagliflozin administration emerged as the predominant risk factor across ML models, with secondary contributions from sex (female), urinary catheter use, diabetes, and kidney failure. In the ABG study, ANN models achieved correlation coefficients above 0.92, identifying chloride concentration, pO_2/FiO_2, shunt fraction (Fshunt), and pH as key variables related to pH, pO_2, pCO_2, and lactate levels, respectively. LASSO regression confirmed different but complementary significant factors, underscoring ML's robustness.
Discussion: These findings demonstrate the potential of ML approaches in clinical settings to identify and prioritize impactful variables from complex biomedical data. The UTI study highlights the need for cautious use of flozins in catheterized patients, while the ABG study supports ML-driven analysis of acid-base balance in pediatric intensive care. Integration of ML with traditional statistics enhances clinical decision-making and patient management. This leads to the individualization of therapy and improvement in treatment safety[3].

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OP12

Pharmacokinetic Approaches in the Preclinical Evaluation of Drug Candidates

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Preclinical pharmacokinetic studies are essential for understanding how a drug candidate is absorbed, distributed, metabolized, and eliminated (ADME) before entering clinical trials. These investigations combine in vitro, ex vivo, and in vivo approaches. One of the main goals is to characterize metabolism, identify potential drug-drug interactions, and predict safety issues. Several complementary methods are used to achieve this.

Microsomal assays are a cornerstone of early metabolism research. Liver microsomes, rich in cytochrome P450 enzymes, enable rapid screening of metabolic stability and enzyme-specific pathways. However, microsomes mainly support phase I reactions and lack conjugation processes.

Isolated hepatocytes provide a more complete metabolic system, including phase II reactions such as glucuronidation and sulfation. However, they lack the complexity of intact organs: there is no blood flow, no biliary system, and limited ability to mimic physiological gradients. As a result, hepatocyte cultures cannot accurately predict processes like biliary excretion or perfusion-dependent clearance.

Perfused organ systems, such as isolated perfused liver, maintain vascular architecture and biliary excretion, allowing dynamic studies of clearance and metabolite distribution under near-physiological conditions. Unlike microsomes or hepatocytes, perfused organs maintain intact vascular and biliary networks, enabling assessment of processes such as biliary excretion and first-pass metabolism.

Metabolite identification relies heavily on advanced analytical tools. Mass spectrometry, often coupled with liquid chromatography (LC-MS/MS), is the gold standard for detecting and characterizing metabolites. High-resolution MS enables precise determination of molecular formulas, while tandem MS provides structural information.

To complement these in vitro approaches, in vivo studies in laboratory animals are essential for understanding systemic drug disposition. They provide ADME data under physiological conditions. They also reveal species-specific differences, critical for dose scaling and predicting human pharmacokinetics. Despite their importance, in vivo experiments are resource-intensive and raise ethical concerns, so they are guided by the 3Rs principle (Replacement, Reduction, Refinement) and strict regulatory frameworks (ICH, FDA, EMA).

By integrating in vitro, ex vivo, and in vivo methods with powerful analytical techniques like mass spectrometry, researchers build a comprehensive pharmacokinetic profile of a drug candidates that ensures safety and regulatory compliance before clinical development.

OP13

The Response Surface Methodology

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Response surface methodology (RSM) was developed by Box et al. in the 1950s and allows for modeling the relationships between multiple variables to determine parameters describing the optimal response of the studied system of variables. To describe the studied system and model the experimental conditions or their optimization, linear or polynomial functions and elements of statistical analysis are used, taking into account the correlation of variables, analysis of variance, the influence of independent variables on the dependent variable in the form of the Pareto effect, and regression parameters for the selected model. When using RSM, the following steps are distinguished: (i) selection of independent variables with main effects on the system through screening and definition of the experimental area; (ii) selecting the experimental design and conducting experiments according to the selected experimental matrix; (iii) mathematical and statistical processing of the obtained experimental data by fitting a polynomial function; (iv) assessing model consistency; (v) verification of the necessity and possibility of performing translation towards the optimal area and finally (vi) obtaining optimal values for each tested variable. The presentation will present the applications of RSM in the field of pharmaceutical sciences in the context of physicochemical research taking into account aspects of the description of dispersion systems based on experimentally determined parameters of the Langmuir surface pressure isotherms for non-ionic surfactants - based on own research; in the context of the processes of separation of bioactive substances from raw materials of natural origin and in the context of clinical pharmacology as a tool used in the process of optimizing pharmacotherapy.

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OP14

Selected examples of physico-chemical methods applied for development of drug carriers

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Classical physical chemistry arose from research at the intersection of physics and chemistry. By assigning mass and velocity, and therefore energy and momentum, to molecules, it was possible to harness the unruly and chaotic motion of molecules in the form of equations of the kinetic theory of gases. From this point on, chemical transformations can be conceptualized within the laws of physics. This allows for quantitative prediction of phenomena accompanying the synthesis, degradation, and production of drugs. Our research unit conducts research on new medicinal products. These studies are conducted either at the preformulation stage or during the development of an attractive and economically viable drug carrier, utilizing fundamental principles of physicochemistry. We do not shy away from basic research that allows us to predict the course of various processes at a more general level, as exemplified by conductometric studies on the synthesis of thermosensitive drug carriers and studies on the kinetics of drug release using electrochemical methods. Consequently, we have successfully developed a number of thermosensitive polymers, investigated the effect of antibacterial peptides on surface tension in monolayers, and obtained prototypes of antibacterial drugs for the treatment of acne, electrospun nanofibers, and a number of hydrophilic gels and carriers intended for use in joint diseases and other conditions.

OP15

When Molecules Join Hands: The Journey of Cocrystals from Mechanochemistry to Therapeutic Application

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This seminar will explore the use of mechanochemical activation as a highly sustainable approach for the preparation of multicomponent pharmaceutical solids. Mechanochemistry—driven solely by mechanical energy and, when required, by catalytic (micromolar) quantities of liquid additives—enables fine control over solid-state transformations, providing access to either well-defined crystalline cocrystals or homogeneous co-amorphous phases under appropriately selected processing conditions. The presentation will address the key factors influencing phase outcome during ball-milling, including intermolecular recognition events, the impact of liquid-assisted grinding regimes, and the kinetics governing amorphization versus nucleation. Particular focus will be given to the experimental strategies and processing parameters that allowed the isolation of a ternary cocrystal composed of three anthelmintic molecules, highlighting the role of stoichiometric design and process optimization in stabilizing this unprecedented solid form.

OP16

Harnessing liposome technology to unlock the potential of natural products in therapy

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Introduction: Natural products are a rich source of biologically active compounds and an example of molecular diversity. Natural products have been the source of many drugs, including antibiotics and anti-cancer drugs.[1] The use of plants for the cure of numerous diseases is in practice for a long time. Tremendous research has been carried out on natural products for the search and development of therapeutic agents beneficial for human health. However, natural compounds often suffer from poor solubility, rapid degradation, and low bioavailability. Incorporation within liposomes, phospholipid-based nanoparticles, can help overcome these limitations. Liposomes can carry both hydrophilic and hydrophobic compounds, increasing their solubility; protect from metabolic processes, ensuring activity until target is reached; provide protection, increasing shelf-life and stability; be customized to target cells/tissues, thereby increasing drug concentration in the desired area and reducing side effects on healthy tissue; help navigate bio-barriers and facilitate cell internalization.

Materials and methods: Phospholipid-based vesicles were developed for the delivery of plant/food-derived extracts. The vesicles were produced by a facile, solvent-free method. Key physico-chemical and technological features (i.e. size, charge, morphology, entrapment efficiency, stability in storage and in biological milieus, drug release) were studied. The cytotoxicity and bioactivity of the nanoformulations were investigated in appropriate cell lines or animal models to assess a possible enhancement of the extracts' efficacy.

Results: Nanosized vesicles were produced, typically characterized by good long-term stability, high entrapment efficiency, cytocompatibility and enhanced bioactivity.

Discussion: Phospholipid-based vesicles were demonstrated to be an efficient platform for the delivery of natural products thanks to their versatility and customization as a function of the extract and the therapeutic purpose. Most interestingly, the extracts are delivered in an aqueous formulation that makes them more feasible for application in the medical field.

Conclusions: Natural products offer an opportunity to discover new effective compounds. Vesicle-based formulations may respond to the need of high quality, safe and effective alternative treatment options for a range of disorders. While promising, the integration of liposomal delivery with natural products faces hurdles, including issues with scaling up production, ensuring the stability of the formulation over time, and navigating regulatory approval processes.

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OP17

Physical modifications of poorly soluble drugs as a way to create supersaturated systems: The case of bosentan hydrate

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Introduction

Bosentan hydrate is an endothelin receptor antagonist approved for the therapy of pulmonary arterial hypertension and systemic sclerosis (1). Since the endothelin pathway can play an important role in the pathophysiology of various diseases, recent clinical trials investigate the effectiveness of bosentan in the oral treatment of digital ulcers or unresectable pancreatic cancer. However, poor solubility of the drug limits its oral bioavailability, and in consequence, our aim was to develop enabling formulations that could make these new oral therapies more efficacious.

Materials and methods

The thermal treatment was used to obtain amorphous forms of bosentan (2). Their stability was studied using DSC and XRD. Then high energy ball milling and nano spray drying were applied to create amorphous solid dispersions of the drug in copovidone (3). Apart from physical methods of solid state analysis, dissolution studies were carried out to analyze the performance of bosentan amorphous.

Results

Bosentan vitrified easily and its two amorphous forms were described for the first time (2). The amorphous bosentan anhydrous (Tg ca. 80 °C) did not crystallize while heating. The dissolution rate and supersaturation level reached depended on the manufacturing method, but herein, the drug crystallized and precipitation was observed. In order to prevent it, high energy ball milling and nano spray drying were used to prepare amorphous solid dispersions (ASDs) of bosentan in copovidone. The polymer showed antiplasticizing effect and accelerated amorphization of bosentan (3). The drug was dispersed in copovidone at the molecular level. Depending on the ASD manufacturing method, the powder microstructure differed, which determined bosentan release rate and the supersaturation level. Bosentan was released slower from nano spray dried polymeric matrices as compared to ASDs prepared by mechanical activation, but the supersaturation lasted longer.

Conclusions

Bosentan is an easy glass former for which two amorphous forms exist. Its ASDs in copovidone can be prepared by mechanical activation or nano spray drying. The manufacturing method determines the drug performance, but a long-lasting supersaturation with maximum concentrations from four to more than ten times higher than those recorded when bosentan is vitrified alone can be reached.

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OP18

Chemico-physical and biological properties of monoclonal antibodies, under real-life light doses: a mechanistic approach.

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Keywords: Bevacizumab; Durvalumab; Photostability; Real-life

INTRODUCTION

Monoclonal antibodies (mAbs) have emerged as a prominent class of protein therapeutics for cancer treatment. However, their protein nature renders them susceptible to various stressors during manufacturing, transportation, storage, handling, and administration [1]. Notably, light exposure can induce chemico-physical changes, particularly when amino acids are located in the complementarity-determining regions (CDRs), potentially compromising efficacy and safety [2,3].

AIMS

This study aimed to evaluate the chemico-physical stability and biological activity of two mAbs, Bevacizumab (Avastin) and Durvalumab (Imfinzi) under light doses mimicking real-life exposure and the mechanism upon which the modifications took place.

RESULTS

Exposure to sunlight doses like those experienced in real-life settings did not alter the conformation of the diluted mAbs. However, light-induced aggregation was observed, with Bevacizumab exhibiting a higher extent of aggregation than Durvalumab. Durvalumab was also evaluated upon induced stress either by direct UVA/UVB exposure or by photosensitization via blue-light excitation of ruthenium(II) tris-bipyridyl dication ($Rubpy^{2+}$)[4].
Liquid chromatography-mass spectrometry (LC-MS) analysis revealed low levels of oxidative damage and deamidation. Chemico-physical modifications affected the target recognition ability of both mAbs (VEGF and PD-L1, respectively). Notably, no significant immunogenic potential was observed in dendritic cells derived from differentiated monocytes.

CONCLUSIONS

The chemico-physical changes induced by real-life light exposure did not significantly impact the overall protein structure of the tested mAbs. Minimal chemical modifications were detected in the CDRs, resulting in a marginal decrease in in vitro target recognition. While aggregation did not induce immunogenicity, it contributed to the decrease in biological activity.
Photosensitized modifications of Durvalumab showed to be predominantly mediated by singlet oxygen.

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OP19

Dermal delivery of clotrimazole using microneedle technologies

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Introduction and objective: Delivery of drugs to the lower skin layers as well as transdermally is often hindered by the thin, uppermost, dead skin layer termed the stratum corneum. One approach to overcome this penetration barrier is to use microneedles. This versatile technological platform allows for delivery of the most challenging compounds for example, large molecular weight drugs such as insulin, vaccines, genetic materials, as well as cells. A review of microneedle materials used, manufacturing techniques, applications, state of the art will be discussed in addition to a specific example of study using novel 3D printed microneedles for the delivery of clotrimazole.

Methodology: The microneedle arrays, designed using AUTODESK FUSION 360 software, possessed three distinct shapes: a cone, a double overlapping cone and a pyramid with a hexagonal base. Needle height was 2000 μm, the inter-needle spacing was varied using arrays of 17 or 37 individual needles. Arrays were fabricated using an LCD-based 3D printer (ANYCUBIC Photon Mono 5). Following characterization of the needles the optimized systems were selected and coated with two types of hydrogels containing clotrimazole using the thin-film dip-coating method. Drug release studies were conducted in vitro, as well as antifungal activity was assessed together with acute toxicity testing.

Results and discussion: The 17 microneedle arrays had improved penetration efficiency following both Parafilm and agarose gel insertion assays. Mechanical testing showed that each needle geometry responded differently to a force of 100N for 30 secs. Some needles bent, some fractured while others maintained their structural integrity. Two coatings for the microneedles consisted of 1% w/v clotrimazole-based hydrogels: one with clotrimazole dissolved in 96% ethanol and the other as suspended drug. The release profiles of the drug clotrimazole from the two coating types (clotrimazole-ethanol or clotrimazole-suspension gel) showed that the two coatings did not significantly influence the total drug released over 12 hours. Controls were placebo- ethanol and placebo suspension. For the microneedles coated with clotrimazole-ethanol gel, total drug release was 151.71 ± 14.73 μg, 156.87 ± 31.24 μg, and 140.64 ± 19.26 μg for cone, double overlapping cone and pyramid respectively. For suspension gel respective values were 179.80 ± 12.55 μg, 186.29 ± 38.33 μg, and 156.01 ± 23.29 μg. Antifungal properties of both hydrogels were tested using *Candida albicans* clinical origin and *C. Albicans* ATCC 10231 reference strain after 24 and 48 hours of incubation. Irrespective of the needle shape, the ethanol-coated needles exhibited larger inhibition zones. In addition, all the microneedles showed no acute toxicity in the *Aliivibrio fischeri* bioluminescence test.

Conclusions: Microneedles provide a painless alternative to traditional injections and patches by overcoming the skin barrier properties of the stratum corneum. This approach enables delivery of drugs to lower skin layers for treating skin diseases or if required, transdermally to treat systemic diseases. Also, challenging drugs (insulin, oligonucleotides) have now been successfully delivered in therapeutic doses in microneedle devices. More research and testing still needs to be performed to enable more microneedle products to be available for patient use.

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OP20

Where biophysics meet pharmacy - parametrization of mechanistic model for drug release from PLGA-based biodegradable implants

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Lecture summary:
Mechanistic mathematical models are designed to mimic reality and thus help to either describe it or predict its fate in changing conditions. Polymers based long acting injectables are increasingly often used, gaining interest from formulation scientists and clinicians. Their characteristics, including combination of complex processes driving drug release, long time of release, etc. make them suitable for model-based analysis. After PLGA-based long acting injectables injection water and interstitial fluid influx into the porous structure of the implant, non-catalytic and autocatalytic hydrolysis of PLGA, dissolution of small oligomers and monomers (and thus substantial mass loss of the polymer and pore network growth), liberation, dissolution, diffusion and permeation of the dissolved drug occur at the same time. The modelling is done before or in parallel with in vitro and in vivo experiments. Yet to run the mathematical model one needs to parametrize it, so considering its mechanistic nature basic, physical phenomena needs to be characterized and quantified, and here basic sciences with physical chemistry and biophysics come to help. Surface tension of the receptor fluid and contact angle or hydrophobicity of the implant helps to understand surface wetting and thus water influx, mercury intrusion or gas adsorption experiments can help to assess initial average pore size inside the implant, API solubility and diffusivity in the surrounding fluid allow to understand its dissolution and distribution, to name just a few. Further connection of the simulated in vitro release with the physiologically based pharmacokinetic (PBPK), model where disposition of API has been characterized, allows for in vitro – in vivo extrapolation and virtual scenarios testing including formulation optimization.

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OP21

The impact of formulation composition on the bioavailability of the active substance in probiotic emulsions for the topical treatment of dermatological conditions

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INTRODUCTION

The study aimed to investigate how different emulsion compositions affect the survival of the probiotic strain in a two-phase system and how stable the produced formulation is over time. In this case, the active substance was a bacterium from the *Lactobacillus genus*, which is one type of bacterium referred to as a probiotic. Probiotics have attracted increasing interest in recent years as an alternative treatment for skin diseases.

EXPERIMENTAL

Eight different biphasic systems were designed for the study. Each emulsion was prepared in two versions. A preservative was added to one of these versions. Each preparation was tested for pH value and stability using FTIR infrared spectroscopy. The type of emulsion obtained was determined for each formulation by applying three test methods. The viability of the *Lactobacillus rhamnosus* bacteria placed in each produced emulsion was determined by microbiological testing. *Lactobacillus spp.* exhibit a broad tolerance range with regard to growth and development conditions. Studies show that placing them in environments with an ambient pH below 6 leads to increased proliferation of *Lactobacillus rhamnosus* bacteria.

RESULTS AND DISCUSSION

The study therefore began by determining the pH of the prepared samples. By comparing this with the preferred pH range of the bacteria, it was assumed that the most suitable environment for *Lactobacillus rhamnosus* would be an emulsion with a pH value of 4.65. Additionally, the lower pH value of the emulsion coincides with that of human skin, i.e. equal to or less than 5; however, this value is lower than the recommended range of 4–5 for skin preparations. The prepared emulsions appeared stable, as confirmed by FTIR examination. Spectral variability was observed to correlate with the addition of *Lactobacillus rhamnosus* to the emulsion. The relative stability of the spectra of the analysed preparations was also observed over the period in which the test was performed. The surfactant-stabilised biphasic systems produced appear to provide an ideal environment for bacterial growth and development. This was confirmed by conducting a microbiological test. All of the produced formulations were subjected to this test. It was also noted that the preservative had no inhibitory effect on the growth of *Lactobacillus rhamnosus*, a finding that was confirmed in a separate study.

CONCLUSION

Each of the produced emulsions was an O/W emulsion. This is an advantageous characteristic, as microorganisms require a large amount of water to grow properly. In addition, O/W emulsions are easier to apply and remove, and they do not allow the active substance to penetrate the skin.

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OP22

Physicochemical and biological properties of multi-target Michael acceptors based on cinnamanilides

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The increasing microbial burden, the development of antimicrobial resistance (AMR) and the declining immunity of the population represent a serious threat to human health worldwide. In addition to the increased risk of patient mortality, AMR represents longer hospitalization and increased healthcare costs. To overcome this undesirable situation, it is necessary to design new entities with new/innovative mechanisms of action. A valuable approach currently being pursued is the design of so-called multi-target agents, also known as multi-target directed ligands (MTDLs) or "promiscuous drugs." These are single chemical entities designed to interact simultaneously or sequentially with two or more biological targets that are key to the mechanism or progression of a disease. This strategy contrasts with the traditional "one drug, one target" paradigm of drug discovery. Multi-target compounds can be designed using so-called Michael acceptors, which are molecules bearing an α,β-unsaturated carbonyl system that is highly reactive towards nucleophilic (i.e., electron-rich substrates, e.g., NH or SH) biological targets. Natural compounds with multiple activities are currently of particular interest for drug development, and among these compounds, cinnamic acid and its derivatives have a long history of use for various purposes. These compounds based on a ring-substituted (*E*)-prop-1-en-1-ylbenzene scaffold have led to highly effective anti-infective agents. Both ends of the molecule are substituted with lipophilic and electron-withdrawing substituents, so that the entire molecule can be considered a Michael acceptor. Moreover, these substituents have advantageous properties from the point of view of ADME properties. This brief presentation deals with the rational design of ring-substituted cinnamanilides with significant activity against bacterial and protozoal pathogens. Chemoprotemic studies and docking provided partial insight into the putative mechanisms of action of the most potent compounds.

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OP23

Analytical challenges and clinical relevance of vitamin D epimers

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Introduction: Vitamin D metabolism involves several hydroxylation steps, resulting in formation of 25-hydroxyvitamin D (25(OH)D) and the biologically active 1,25-dihydroxyvitamin D (1,25$(OH)_2$D). These metabolites undergo further epimerization into 3-epi-25(OH)D and 3-epi-1,25$(OH)_2$D [1]. The minor structural change does not affect the molecular mass; therefore, standard analytical techniques cannot distinguish them from the non-epimeric forms [2]. We developed a UPLC–MS/MS method for the analysis of 25$(OH)D_2$, 25$(OH)D_3$, and their C3-epimers in plasma. The method was applied to determine C3-epimer concentrations in patients with cardiovascular diseases.

Materials and methods: The chromatographic separation of the analytes: 25$(OH)D_3$, 25$(OH)D_2$, 3-epi-25$(OH)D_3$, and 3-epi-25$(OH)D_2$, and the internal standard d6-25$(OH)D_3$, was performed using UPLC column Kinetex® F5. The mobile phase was a 0.1% formic acid solution in methanol and water (70:30, v/v). The detection was performed in the triple quadrupole mass spectrometer with the positive electrospray ionization mode. Validation of the method was carried out based on EMA guidelines. Concentrations of the vitamin D epimers and their non-epimeric forms were determined in plasma samples collected from patients with cardiovascular diseases and healthy volunteers.

Results: Calibration curves for the analytes were linear in the range of 1- 100 ng/ml. For each analyte, the method was accurate and precise. The extraction efficiency after the liquid-liquid extraction with hexane was in the range of 70 – 97%. The matrix factors were evaluated in order to analyze the effect of plasma components on the ionization of analytes. There were no significant differences in concentrations of 25$(OH)D_3$, 25$(OH)D_2$ and their epimers between the patients and the control group. However, the percentage of 3-epi-25$(OH)D_3$ was significantly higher in the patients.

Discussion: The chromatographic separation of 3-epi-25$(OH)D_2$ and 3-epi-25$(OH)D_3$ from their non-epimeric forms prevented overestimation of 25(OH)D. In control group, 9% of subjects were misclassified if vitamin D status was determined based on total 25(OH)D concentrations (sum of 25$(OH)D_3$ and its epimeric form).

Conclusions: The separation of 3-epi-25(OH)D is critical to prevent overestimation of 25(OH)D and misclassification of subjects with vitamin D deficiency. The percentage of C3-epi-25(OH)D, rather than its absolute level, may serve as a potential biomarker reflecting its pathological increase in cardiovascular disease.

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OP24

You cannot step into the same river twice – imaging of hidden processes in hydrophilic matrix tablets

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Hydrophilic matrix tablets are among the most commonly used formulations for prolonged drug release. Their performance arises from dynamic, interdependent processes of swelling, hydration, dissolution, and erosion, all occurring within a confined space and evolving over time in ways that are difficult to capture. Understanding the role of water, polymer mobility, and structural reorganization of the matrix is essential for elucidating drug-release mechanisms; however, conventional analytical methods tend to interfere with the delicate hydrogel layers and may disturb the natural course of these processes.

In the presented research, a set of non-invasive imaging techniques was developed and applied, including magnetic resonance imaging (MRI), low-field NMR relaxometry, and X-ray microtomography (µCT). This multimodal approach enabled simultaneous monitoring of water distribution and mobility within the matrix structure as well as material changes associated with swelling and erosion.

In subsequent stages, MRI and relaxometry methods were advanced to allow precise correlation of MR signals with the quantitative water content in specific regions of the matrix. The developed segmentation procedure and localized analysis enabled determination of water content using the Karl Fischer method and assessment of water–polymer interactions via DSC. Combining these data with MRI sequences—MSME (multi-slice multi-echo) and UTE (ultra-short echo time)—provided a comprehensive description of the spatial and temporal changes occurring during hydration of sodium alginate tablets, revealing the sequential formation of hydrogel layers and the complex dynamics of water mobility.

The application of multimodal imaging techniques thus allowed a more complete characterization of the processes occurring during hydration of polymer matrices, including polymer mobilization, swelling behavior, and progressive erosion. Integrating results from multiple methods provided access to information previously inaccessible to classical analytical approaches and yielded a coherent picture of the spatiotemporal evolution of polymer matrix hydration.

OP25

Physicochemical methods for the study of the interaction between exogenous factors and endogenous antioxidants proteins (EAPs)

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Introduction: The important aspects of modern pharmacotherapy are both to eliminate the cause of the disease and to support the patient's quick recovery. It can be achieved by accelerating the restoration of the redox balance. Exogenous factors in the human body, for example drugs, can modify the antiradical activity of endogenous antioxidants proteins (EAPs). This impact can be analyzed using physicochemical methods, such as spectroscopy or calorimetry.

Materials and methods: The aim of this lecture is to present the possible applications of selected physicochemical methods in studying the interaction between exogenous modulators and EAPs.

Results and discussion: The analysis of the modulating effects of exogenous factors on EAPs can be divided into two steps. In the first one, it is necessary to compare the antiradical potential of tested ligands, EAPs and ligand-protein mixtures. To achieve that, spectroscopic colorimetric tests may be used (e.g. DPPH, ABTS and FRAP assays). Based on the results, it is possible to confirm (or rule out) the influence of the included ligands on the tested proteins` antioxidant activity. The type of the interaction between the proteins and the ligands (e.g. additive, synergistic, antagonistic) may also be predicted.

In the second step, it is necessary to identify the cause of the phenomenon observed in the first step. In fact, it is highly probable that changes in the proteins' antioxidant potential result from their conformational changes. This part of the study may involve: confirming the formation of a stable protein–ligand complex (using e.g. UV-Vis spectrophotometry; spectrofluorimetry, SFM; isothermal titration calorimetry, ITC), identifying the proteins` binding site(s) for the tested ligands (using e.g. SFM or ITC), determining the thermodynamic profile of the binding reaction (using e.g. ITC) and assessing the effect of ligand binding on the proteins` secondary and tertiary structure (using e.g. far-UV and near-UV circular dichroism (CD) spectroscopy, respectively).

A summary of the results obtained in both steps may ensure comprehensive information on the tested ligands and their potential role as modulators of EAPs activity.

Conclusions: The application of physicochemical methods into the studies of modulating the EAPs` activity may be useful for both pharmaceutical science and industry.

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OP26

Impact of PEG-type crosslinker molecular size on the structural and thermal characteristics of thermosensitive polymers.

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Introduction: Stimulus-responsive polymers, or 'smart' polymers, exhibit a nonlinear and reversible changes in their physicochemical properties when exposed to specific internal or external stimuli, undergoing characteristic phase transitions. Thermosensitive polymers are considered to be extremely promising cutting-edge tool in targeted and controlled drug delivery. [1,2] An important advantage is the possibility of precise modulation of their physicochemical properties already at the synthesis stage, which allows them to be adapted to the desired application parameters. The aim of this study was to synthesize NVCL-based thermosensitive polymers, cross-linked with PEGDMA of varying chain lengths, and to evaluate how the applied cross-linking agents influence the phase transition temperature and selected physicochemical properties that are key to their potential application in drug delivery systems.

Materials and methods: PNVCL derivatives were synthesized via surfactant-free precipitation polymerization (SFPP) at 70 °C using 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AMPA) as the initiator. Polymerization progress was monitored through conductivity measurements, and polymer formation was confirmed by ATR-FTIR and ^{1}H NMR spectroscopy. Hydrodynamic diameters (HD) and polydispersity indices (PDI) of aqueous dispersions (18–45 °C) were determined by dynamic light scattering (DLS). Zeta potential (ZP) was measured based on electrophoretic mobility. Particle morphology and surface structure were assessed using transmission (TEM) and scanning (SEM) electron microscopy. Thermal properties were analysed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) to determine phase transition temperatures and thermal stability.

Results: LCST was observed between 34 and 35 °C. At 18 °C, HD ranged from 28 to 75 nm. The polymer particles were spherical, amorphous, and highly polydisperse, and their ZP remained positive throughout the 18–45 °C range.

Discussion and Conclusions:

Synthesised polymers showed expected thermosensitive behaviour and good thermal stability. Conductivity measurements during cooling of the postreaction mixture proved effective for estimating the phase transition temperature. The results indicate that the chain length of PEGDMA affects the LCST, particle size, size distribution, ZP, and the stability of the polymer dispersions. Importantly, the relationship between cross-linker chain length and physicochemical parameters is non-linear. highlighting the need for careful optimization in designing thermosensitive systems for drug delivery

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OP27

The effect of hydrothermal and chemical modifications of potato starch on its carrier properties for selected polyphenols from chokeberry fruit

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Introduction: In pharmacy, native starch is used, among other applications, for the preparation of suspensions, starch capsules, and tablets, as it can serve as an auxiliary filling, binding, disintegrating, and lubricating agent [1]. Starch modification leads to changes in its functional properties. These alterations can be achieved through physical, chemical, enzymatic methods, or their combinations. Starch annealing is a hydrothermal process that inhibits the gelatinization of starch in the presence of water [2]. Chemical modification introduces additional functional groups that may significantly alter starch characteristics, whereas enzymatic modification involves the hydrolysis of starch by specific enzymes. The aim of this study was to obtain an economically attractive and thermally stable modified starch suitable for use as a drug carrier capable of encapsulating selected polyphenols derived from chokeberry fruit.
Materials and methods: Native potato starch was subjected to annealing, a physical hydrothermal modification, as well as to chemical etherification with chloro-hydroxypropyltrimethylammonium chloride (CHPTMA) and esterification with citric acid (CA), applied in various combinations. The starch samples were soaked in a chokeberry ethanol extract and subsequently vacuum-dried. The release kinetics of chokeberry polyphenols—chlorogenic acid (CHA), rutin (RUT), and cyanidin arabinoside (CyA)—from hydrogels was investigated. Polyacrylic hydrogels doped with the respective starches were prepared. The following parameters were determined: polyphenol concentrations during the release process using high-performance liquid chromatography (HPLC); gelatinization temperatures and enthalpy by differential scanning calorimetry (DSC); crystallinity by X-ray powder diffraction (XRPD); and functional groups by Fourier-transform infrared spectroscopy (FTIR).
Results: The annealing process of starch resulted in a substantial increase in its gelatinization temperature, a slight decrease in the enthalpy of this transition, and a reduction in crystallinity. FTIR analyses confirmed the presence of an amide group in the cationic starch. The release intensity of CHA, RUT, and CyA varied considerably and depended both on the type of starch modification and on the specific polyphenols involved.
Discussion: Annealing of potato starch was shown to increase its thermal stability, reduce its crystallinity, and decrease the amount of released polyphenols. The chemical modifications primarily affected the intramolecular interactions within the starch, as well as the intermolecular interactions among the starch, polyphenols, and the hydrogel. The agglomerative properties of the starch subjected to dual chemical modification were confirmed by scanning electron microscopy (SEM) images.
Conclusions: The studies confirmed the influence of both hydrothermal and chemical modification of starch on its carrier properties.

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OP28

Kinetics of prednisolone sodium phosphate release from sodium hyaluronate based hydrogels doped with synthetic polymers

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Introduction: Sodium hyaluronate (HA) is a natural polymer that occurs in most connective tissues, with its highest concentrations found in synovial fluid, the vitreous humor of the eye, the umbilical cord, and the hen's comb. HA plays an important role in the biomechanics of the normal knee joint, being partially responsible for its lubrication and viscoelasticity. The concentration and molecular weight of HA decrease with the progression of osteoarthritis and aging [1]. HA is widely used as a biomaterial for various biomedical purposes, including in the pharmaceutical industry as a carrier for active pharmaceutical ingredients [2].
The aim of this study was to develop pharmaceutical compositions based on HA doped with synthetic polymers (polyacrylic acid, ammonium acryloyldimethyltaurate copolymer, a mixture of polyvinyl acetate and polyvinylpyrrolidone, or polyethylene glycol) and containing prednisolone sodium phosphate (PSP) as an anti-inflammatory drug substance, and to determine the kinetics of PSP release from the obtained hydrogel formulations.
Materials and methods: Hydrogels were prepared by combining the appropriate polymers with a PSP solution and homogenizing the mixtures. Drug release studies were performed using a paddle-over-disk apparatus. The concentration of the released drug in the acceptor medium was determined spectrophotometrically. Kinetic analysis was performed according to zero-, first-, and second-order kinetics, as well as the Higuchi and Korsmeyer–Peppas models [3], [4].
Results: No significant differences were observed between the PSP release profiles from the obtained formulations. All applied kinetic models, except the zero-order model, described the release process very well, achieving correlation coefficient (R^2) values ranging from 0.98 ± 0.01 to 0.99 ± 0.01. The *n* parameter calculated based on the Korsmeyer–Peppas equation ranged from 0.58 ± 0.01 to 0.60 ± 0.01. This indicates that PSP transport from the hydrogel matrix to the acceptor medium does not follow Fick's law and involves a combination of diffusion and polymer chain relaxation. [3].
Discussion: The obtained results indicate that all tested hydrogel formulations exhibited similar release kinetics of PSP, suggesting that the addition of the studied synthetic polymers did not significantly modify the diffusion–relaxation mechanism. Although the current compositions did not extend PSP release, the results confirm that HA-based matrices provide a reproducible release profile suitable for further optimization. Future research should focus on modifying the polymer network density or introducing other synthetic polymers with higher viscosity or stronger intermolecular interactions to achieve a more sustained release profile.
Conclusions: Doping HA-based hydrogel compositions with synthetic polymers did not significantly affect the kinetics of PSP release from the obtained hydrogels. The next stage of research should involve the incorporation of other synthetic polymers that could slow down PSP release and enable a sustained drug release effect.

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ABSTRACTS
POSTERS

P1

The role of fatty acids in albumin glycoxidation – a spectroscopic analysis

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Introduction: The processes of glycation and oxidation occurring in living organisms exert a significant influence on the properties of plasma proteins. These processes are closely interrelated and mutually intensify their adverse effects [1]. The intensity of glycoxidative modifications may be affected by dietary components, including fatty acids (FAs).

Materials and methods: Due to the negative consequences of the glycoxidation process on the human body, an in vitro model of glycated and oxidized human serum albumin (HSA) was developed. Subsequently, using spectrofluorimetry and UV-Vis spectroscopy, the effect of FAs was examined on (i) the formation of advanced glycation end products (AGEs), (ii) changes in the tertiary structure of HSA, and (iii) the content of free thiol groups (-SH).

Results and discussion: The analysis of AGEs fluorescence spectra (emission, synchronous, and excitation) has demonstrated that the presence of FAs promotes their formation, thereby intensifying the glycoxidative modifications of albumin. At the same time, studies of changes in the microenvironment of the main fluorophore of HSA, tryptophan (Trp214), indicated that FAs partially inhibit glycoxidation within subdomain IIA, where this residue is located. The evaluation of the Red Edge Excitation Shift (REES) effect and the spectral parameter A showed that FAs increase the mobility of Trp214 and facilitate its contact with the polar solvent, which reflects the loosening of the local conformation of the macromolecule. On the other hand, the determination of free sulfhydryl groups revealed a significant decrease in the content of SH originating from cysteine 34 (Cys34), which indicates the intensification of oxidative damage to albumin and the reduction of its antioxidant potential in the presence of FAs.

Conclusions: The effect of fatty acids on albumin glycoxidation is complex: on the one hand, FAs facilitate the formation of AGEs, while on the other, in subdomain IIA, they may inhibit local modifications of Trp214. Although the presented results originate from an in vitro model, they suggest the need for caution and moderation in consuming products rich in fatty acids, as well as prudence in their supplementation.

Keywords: glycoxidation, human serum albumin, fatty acids, spectrofluorimetry, UV-Vis spectroscopy

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P2

Physicochemical assessment of interaction between a new phenothiazine derivative and major serum carrier proteins – preliminary study

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Introduction: Cancer diseases are a major burden to individuals, societies, and healthcare systems worldwide. In the field of modern oncology, developing effective therapeutic strategies remains a significant challenge. The efficacy of conventional cancer therapies is frequently limited by their inadequacies and the presence of numerous side effects, as well as multidrug resistance (MDR). Although biological therapy is currently gaining significance, its excessive cost suggests that it is still necessary to investigate novel substances with anticancer properties. 10-(2-(N-Piperidinyl)ethul)oxy-5-methyl-12(H)-quino[3,4-b][1,4]benzothiazinum chloride (AZMM7) exhibits antiproliferative activity in vitro against C-32, SNB-19, and MDA-MB-231 cancer cell lines. The IC50 values were 1.2±0.7 $\mu g \cdot mL^{-1}$, 1.2±0.7 $\mu g \cdot mL^{-1}$, and 3.0±0.9 $\mu g \cdot mL^{-1}$ for C-32, SNB-19, and MDA-MB-231, respectively. The reference system was cisplatin. Due to the significant harmfulness of anticancer drugs, it is necessary to determine the toxicity and therapeutic dose of the tested compound.

Materials and methods: All measurements were conducted using spectrofluorimeter (JASCO FP-6500) and spectrophotometer UV-VIS (JASCO V-730) based on the following experimental dataset: AZMM7 stock solution concentration was $3 \cdot 10^{-3}$ $mol \cdot L^{-1}$ while HSA and AGP concentrations were $3 \cdot 10^{-6}$ $mol \cdot L^{-1}$. Ligand:model carrier proteins molar ratio was from 0:1 to 6:1. Based on the Stern-Volmer method, the Stern-Volmer constant (K_{S-V}) and the bimolecular quenching constant rate (k_q) were calculated. In turn, based on the Klotz method, the association constants (K_a) and the number of binding site classes (n) were obtained. In addition, high-affinity AZMM7 binding sites for both HSA and AGP molecules were identified using binding site markers. The effect of AZMM7 on the tertiary structure of the main model carrier proteins was also determined.

Results and discussion: Based on the order of magnitude of K_{S-V} (10^4 $[L \cdot mol^{-1}]$) and k_q ($10^{12}$$[L \cdot mol^{-1} \cdot s^{-1}]$) it can be concluded that AZMM7 forms static complexes with both HSA and AGP. However, AZMM7 shows a stronger affinity to AGP than to HSA (order of magnitude of K_a for AGP was 10^5 $[L \cdot mol^{-1}]$ and 10^4 $[L \cdot mol^{-1}]$, respectively), at both excitation wavelengths. AZMM7 interacts with AGP molecule at the QR binding site (at the bottom of the β barrel), while with HSA molecule at both Sudlow site I (subdomain IIA) and II (subdomain IIIA) but slightly stronger with subdomain IIIA. In addition AZMM7 affects the tertiary structure of the studied proteins.

Conclusions: Spectroscopic techniques are a useful techniques for the analysis of the interaction between a newly synthesized substance (AZMM7) and major serum carrier proteins (HSA and AGP).

Key words: phenothiazine derivatives, major serum carrier proteins, spectroscopic techniques

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P3

Analysis of cross-linked and uncross-linked human serum albumin nanocarriers

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Introduction: Retinal is the aldehyde formulation of vitamin A. It is a stable derivative of vitamin A used in cosmeceuticals, however, its efficacy in the skin treatment is limited [1]. In order to improve the effectiveness of retinoids and overcome the problems associated with their topical application and irritant effect, attempts are being made to apply nanotechnology as retinoid carriers [2,3].
Materials and methods: Human serum albumin (HSA) nanoparticles were synthetized using the desolvation method with ethanol as an antisolvent and glutaraldehyde as cross-linking factor as well as without glutaraldehyde. The solutions of all-trans retinal (ATR) and HSA were mixed at 1:10 (ATR:HAS) mass ratio. UV-vis spectroscopy (JASCO V-760, Hachioji, Tokyo, Japan) was used to record absorption spectra in terms of encapsulation efficiency and drug release. Albumin nanoparticles without ATR were prepared as a control. The size and morphology observation was conducted using an ultra-high-resolution analytical microscope, the Apreo 2S (Thermo Fisher Scientific, Waltham, MA, USA). ATR release study was conducted with the use of sample and separate method for 24 hours. Circular dichroism spectra of HSA nanoparticles (reference groups and drug-encapsulated groups), native HSA and HSA in the presence of ATR were recorded using a Jasco J-1500 spectropolarimeter (Hachioji, Tokyo, Japan) in the wavelength range from 170 to 280 nm.
Results: Encapsulation efficiency of ATR for HSA nanoparticles was 81.29 ± 5.15 % and for uncross-linked HSA nanocarriers was 82.54 ± 1.93 %. Nanoparticles with encapsulated ATR have a radius of 116 ± 0.019 nm compared to nanoparticles in the absence of ATR, which size was about 127.68 ± 27.33 nm. The radius of the uncross-linked nanocarriers could not be read for a sufficient number of samples. Cross-linked nanoparticles were characterized by the release of larger amounts of ATR in a shorter period of time. In the case of circular dichroism spectroscopy, no significant differences were observed in the secondary structure of HSA after binding it to ATR and after forming conjugates. However, the nanoparticle preparation process degraded the secondary structure of the protein.
Discussion: Desolvation method is a great method to encapsulate water insoluble substances. The results confirmed its applicability to encapsulate ATR. Most of albumin nanoparticles are characterized by high encapsulation efficiency values due to the numerous binding sites on the albumin molecule. As was conducted in our previous study, nanoparticles preparation process affects the secondary structure of albumin. The size of the nanoparticles and their release pattern are similar to those obtained in our other studies. We have not yet obtained any uncross-linked nanocarriers.
Conclusions: Both albumin nanoparticles and uncross-linked nanocarriers can serve as carriers for ATR. The results indicate that crosslinked nanoparticles show more promising results in physicochemical studies, which provides a basis for planning further analyses.
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P4

Comparison of the colorimetric tests for detecting reduced thiol groups in peptides, proteins, and their mixtures – *in silico* analysis

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Introduction: The biological functions of the organic compounds, such as antioxidant activity, are significantly influenced by the presence of reduced thiol groups (RTGs; -SH). The availability of RTGs is one of the key factors involved in maintaining the redox balance of blood serum. Some serum proteins and peptides, such as human serum albumin and glutathione, due to RTGs, have high free radical-trapping properties.
Materials and methods: The aim of this study was to analyze data based on PubMed and Google Scholar databases to assess the usefulness of spectroscopic tests for detecting reduced thiol groups in peptides, proteins, and their mixtures.
Results and discussion: Colorimetric tests offer high accessibility, procedural simplicity, cost-effectiveness and repeatability of results. Despite their advantages, these methods have also some disadvantages, such as limited selectivity, strong correlation between results and environmental conditions (e.g pH, temperature), dependence on absorption laws (e.g the Beer-Lambert law) and their limitations, as well as difficulties in testing complex systems, such as blood serum. Spectrophotometric determination of RTGs is possible after derivatization, using some chromogenic reagent, such as N,N-dimethyl-p-phenylenediamine (DMPD), 5,5`-dithiobisnitrobenzoic acid (DTNB; Ellman's reagent) or 1-benzyl-2-chloropyridinium bromide (BCBP). These assays require different reaction conditions, such as pH (5.25, 7-8 and 9, respectively) or additional chemical compounds (e.g Fe^{3+} ions in the DMPD assay). Their reactions with the –SH group (stoichiometric ratio 1:1) results in the formation of the colored products, which exhibit absorption maxima in the range 480-510 nm (DMPD assay, depending on the tested compound's structure), at 316 nm (BCBR assay), as well as 412 nm (DTNB assay). The BCBP reagent is very difficult to obtain (or practically unavailable) on the Polish market; in contrast, both the DNTB and DMDP reagents are commercially available. The DNTB assay has also a long history of use, also in studies with plasma proteins. It simplifies the discussion of the obtained results with the other authors' works.
Conclusions: Based on the collected data, it was concluded that DNTB offers the highest utility and relevance for detecting reduced thiol groups in peptides, proteins and their mixtures.
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P5

UPLC-MS/MS method for quantification of rivaroxaban and its M1 metabolite in plasma samples

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Introduction
Rivaroxaban (RIV) is a direct oral anticoagulant (DOAC) and selective inhibitor of Factor Xa. Metabolite M1 ((S)-2-((4-(5-((5-Chlorothiophene-2-carboxamido)methyl)-2-oxooxazolidin-3-yl)phenyl)(2-hydroxyethyl)amino)-2-oxoacetic acid) was identified as the main metabolite of rivaroxaban (about 3% of total plasma radioactivity, 30% of the dose eliminated) (1,2). Therapeutic drug monitoring (TDM) was suggested due to increased risk of uncontrolled bleeding and necessity to adjust dose (e.g. in patients with renal impairment) (3). So far, no methods have been published that allow the simultaneous determination of RIV and M1. This study aimed to develop and utilize UPLC-MS/MS method for the determination of RIV and its metabolite in plasma samples collected from patients.
Materials and methods
Chromatographic separation of RIV, M1, and rivaroxaban d-4 (internal standard, IS) was achieved on Zorbax Eclipse Plus C18 column (2.1 × 100 mm; 3.5 µm) under isocratic conditions (mobile phase consisting of 0.2% formic acid in water and acetonitrile 50:50 v/v). Plasma samples for analysis were prepared using protein precipitation with acetonitrile. The method was applied for analysis of samples collected from 77 patients treated with 20 mg of rivaroxaban.
Results
The analysis time was 4 minutes. The method was linear for both RIV and M1 within the concentration range 2-500 ng/ml and 50-125 ng/ml, respectively. Intra- and inter-day precision (RSD%) were below 15% and accuracy (RE%) was below 15%. Recovery was 66.36 ± 5.78 % and 11.79 ± 1.75 % for RIV and M1, respectively. We analyzed samples from 77 patients. The RIV and M1 concentrations ranged 21.69-612.48 ng/ml and 30.36-106.93 ng/ml, respectively.
Discussion
The short run time and simple sample preparation make the method more convenient and efficient compared to time-consuming liquid-liquid extraction or solid-phase extraction methods used previously for analysis of RIV. Moreover, it is first such method for concurrent quantification of RIV and its metabolite M1 in human plasma. The LLOQ of 2ng/ml is sufficient to quantify RIV in patients treated with the drug. However, in most patients the M1 concentrations were below lower limit of quantification (LLOQ) and we observed M1 only in 5 samples. Hence further research is needed to improve recovery and LLOQ of the developed method.
Conclusion
Proposed method enables simultaneous quantification of both RIV and M1 compounds, which might provide additional tool for TDM, increasing efficacy and safety of the rivaroxaban therapy.
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P6

Selected properties of thermo- and pH- sensitive pAMPSA as a potential drug carrier.

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Introduction: Temperature- and pH-responsive polymers have been of interest for many years as potential carriers for therapeutic substances. According to their unique properties, they are capable of releasing drugs in a controlled manner from the site of administration in response to environmental changes. One of the compounds associated with this group is 2-acrylamido-2-methylpropanesulfonic acid (AMPS). Due to the presence of both hydrophilic and hydrophobic groups in its structure, the compound exhibits a characteristic phase transition. [1,2] The aim of this study was to synthesise a copolymer of AMPSA modified with a comonomer—polyethylene glycol (PEG200)—and subsequently evaluate its physicochemical properties by measuring hydrodynamic diameter (HD) and zeta potential (ZP). PEG200 was incorporated due to its excellent biocompatibility and its ability to enhance nanoparticle stability under physiological conditions.
Materials and methods: The synthesis was carried out using AMPSA as the monomer, polyethylene glycol (Mn = 200) as the comonomer, and 2,2'-azobis(2-methylpropionamidine) dihydrochloride as the initiator. The reaction proceeded via free-radical polymerization in an aqueous medium without a surfactant at 70 °C for 6.5 hours under continuous stirring. After completion of the synthesis, the product was purified by dialysis in distilled water under constant stirring at room temperature. The HD was measured using dynamic light scattering (DLS), while the ZP was determined based on electrophoretic mobility (EM).
Conclusions: As a result of the synthesis, thermo- and pH-responsive polymers with nanoscale particle sizes were successfully synthesised.
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P7

Drug Transport Systems in Modern Therapeutics: Natural and Synthetic Carriers in Pharmaceutical Sciences

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Introduction: Efficient drug delivery remains a major challenge in pharmaceutical sciences. Transport systems determine therapeutic efficacy, biodistribution, toxicity, and dosing regimens. Both natural and synthetic carriers are increasingly explored to overcome biological barriers and enhance the targeted delivery of active compounds. Exosomes—endogenous extracellular vesicles—have emerged as promising natural nanocarriers due to their biocompatibility, stability in circulation, and inherent tropism toward recipient cells. In parallel, synthetic delivery systems such as liposomes, polymeric nanoparticles, dendrimers, and inorganic nanocarriers allow precise engineering of physicochemical properties and controlled drug release profiles.

Materials and methods: This review summarizes and compares current literature on natural and synthetic drug transport systems used in medicine and pharmacy. Key aspects analyzed include structure, loading strategies, circulation time, targeting mechanisms, and safety profiles. Special attention is given to exosome-mediated delivery in oncology and regenerative medicine, and to FDA-approved synthetic nanocarriers used clinically.

Results: Natural carriers such as exosomes demonstrate superior cellular uptake and immune tolerance, enabling efficient delivery of RNA therapeutics, small molecules, and proteins. Their ability to cross biological barriers, including the blood–brain barrier, offers unique therapeutic opportunities. Synthetic systems provide advantages in large-scale manufacturing, predictable pharmacokinetics, and customizable functionalization. Liposomal formulations (e.g., doxorubicin, amphotericin B) and polymer-based nanomedicines already demonstrate clinical success, whereas hybrid technologies aim to combine the benefits of both classes.

Discussion: Although exosomes offer exceptional biological performance, limitations include low isolation yield, batch variability, and regulatory challenges. Synthetic systems, while easier to standardize, often face issues of toxicity, rapid clearance, and limited targeting specificity. Understanding the complementary strengths of both groups is essential for designing next-generation therapeutics.

Conclusions: Natural and synthetic drug transporters represent two converging domains driving innovation in pharmaceutical sciences. Integrating biological selectivity with synthetic tunability may lead to more effective, safer, and personalized therapies.

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P8

Hydrogels with Tetracyclines for the Treatment of Acne: The Latest Achievements, Difficulties, and Directions for Development

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Acne vulgaris is one of the most common dermatosis. The increasing bacterial resistance, microbiota disorders, and limited drug penetration justify the need for new, stable topical preparations. Research focuses on tetracyclines with antibacterial and anti-inflammatory properties and their combination with azeloglycine. The last component is known for its sebum-regulating and lightening effects on discoloration and scars. Tetracycline and chlortetracycline, although traditionally used orally for acne, also exhibit potential in topical therapy.

The hydrogels were based on AMPD-neutralized acrylic polymer, which has an activity against sebum components due to its ability to saponify free fatty acids, what may facilitate the cleansing of hair follicles [1]. The hydrogels provided significantly greater stability to tetracycline, when compared to aqueous solutions of the antibiotic [2]. The use of azeloglycine broadened the effect of the formulation: it limited the degradation of tetracycline in an alkaline environment, reduced the viscosity of the gels, and increased activity against model sebum. Preparations with azeloglycine also reduced *Staphylococcus aureus* biofilm more strongly in a model based on artificial sebum [3]. Ethanol up to 25% did not impair stability, while 50% increased antibiotic degradation [4]. Azeloglycine enhanced the stability of tetracycline in alkaline pH without affecting its stability at neutral and slightly acidic pH. Chlortetracycline was significantly less stable. The formulations containing tetracyclines exhibited activity against *S. aureus* and *C. acnes*. Azeloglycine enhanced anti-biofilm activity against *S. aureus*, while ethanol increased anti-biofilm activity against *C. acnes* [3,4]. Both additives modulated the physicochemical properties of hydrogels simultaneously.

The developed hydrogels combine stable tetracyclines, azeloglycine, and AMPD with cleansing properties, exhibiting activity against bacterial biofilms and demonstrating a favorable safety profile. These results suggest that such formulations could be a valuable addition to topical therapies requiring antibacterial, anti-inflammatory, and sebum-regulating properties.

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P9

New Horizons in Local Periodontal Therapy: A review of advances in polymeric mucoadhesive dressings with phytocompounds (2020–2024)

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INTRODUCTION

Periodontal diseases and inflammatory conditions of the oral mucosa are significant clinical problems. The efficacy of traditional topical therapies, such as rinses and gels, is often limited by the rapid removal of drugs by saliva and their poor retention at the target site. Many commercially available products either fail to provide adequate drug exposure or suffer from poor patient compliance. Innovative mucoadhesive drug delivery systems (MDS) have the potential to increase the amount of time a drug remains on the mucosal surface, thereby overcoming the limitations of conventional formulations. In recent years, there has been a clear trend towards replacing classic antiseptics (e.g. chlorhexidine) with natural substances (phytocompounds) that offer anti-inflammatory, antioxidant and antibacterial activities, and have a high safety profile.

AIM AND REVIEW METHODOLOGY

The aim of this study is to critically review progress in designing and formulating dental mucoadhesive dressings since 2020. The analysis focuses on the strategic transition from dressings containing synthetic drugs to new-generation systems utilising phytocompounds. A literature review was conducted using the PubMed, Scopus and MDPI databases, analysing formulations based on polymers (e.g. PVA, PVP, cellulose derivatives and chitosan) and their potential as carriers for natural compounds.
Literature review and discussion

The analysis of literature from 2020 to 2024 indicates the intensive development of biopolymer-based dressings, which are used as matrices for drug creation and transport [1]. However, the key innovation is the strategic shift from synthetic drugs to phytocompounds. These studies focus on the multifaceted action of polyphenols, such as curcumin, epigallocatechin gallate (EGCG) from green tea and quercetin, in inhibiting biofilm formation and modulating the inflammatory response [2].

The authors' own research directly aligns with this leading research trend. The first stage involved optimising multi-polymer films (cellulose derivatives, PVA and PVP) as a platform for the controlled release of a complex Reynoutria japonica extract rich in polyphenols, including resveratrol [3]. The appropriate selection of the polymer matrix was shown to have a crucial impact on mucoadhesive properties and the release profile. The next step was to design and characterise a mucoadhesive, PVA-based film aimed at precisely and sustainably releasing pure resveratrol [4]. This research confirmed that material engineering enables the development of effective phytocompound carriers adapted to the dynamic environment of the oral cavity.

CONCLUSIONS

The development of stable and effective mucoadhesive dressings containing phytocompounds is a promising strategy for periodontal therapy. Optimised formulations [3, 4] have demonstrated significant potential for application to the oral mucosa, exhibiting desirable physicochemical properties and release profiles. Combining the advantages of controlled delivery with the therapeutic potential of natural compounds, these systems set a new direction for research. However, further studies are necessary to verify their in vivo biological properties and evaluate their clinical efficacy.

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P10

Natural compounds and pharmaceutical forms in the treatment of psoriasis – a review based on current reports

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INTRODUCTION - Psoriasis is a chronic, inflammatory skin disease that affects millions of people worldwide. Although not directly life-threatening, the symptoms of the disease significantly reduce patients' quality of life due to the often extensive, visible skin lesions, which can lead to social isolation. Traditional topical treatments, based on ointments, creams, and gels, still pose challenges related to both the limitations of drug application and the optimal conditions for active ingredient penetration. Current pharmacotherapy relies primarily on glucocorticosteroids, whose long-term use is associated with numerous adverse effects, including epidermal thinning and even skin atrophy. The purpose of this review is to summarize known natural active substances and plant extracts conventionally used in the treatment of psoriasis and their potential use in modern dermatological formulations. The articleabstarct presents common, traditional chemical forms of medicinal preparations, such as ointments, creams, and gels, with their common limitations, as well as modern carriers of active substances, such as foaming emulsions, which increase the bioavailability and comfort of use. These entirely plant-based medicinal products offer safe, multifaceted, and potent therapeutic effects, representing a promising alternative or complement to conventional topical use. Further research is required to optimize the compositions and technological conditions of production and confirm the clinical benefits of herbal preparations.

AIM AND REVIEW METHODOLOGY - The growing interest in finding alternative forms of psoriasis therapy is fostering the development of new formulations using plant substances, which stems from their favorable safety profile and multifaceted action. Plant-based preparations constitute a valuable complement to psoriasis therapy, supporting the effects of traditional medications and alleviating the symptoms of the disease. Natural compounds exhibit the greatest therapeutic potential, possessing anti-inflammatory properties, regulating skin cell proliferation, and rebuilding the lipid barrier. One of the most modern vehicles with high pharmaceutical potential is therapeutic foams, which offer a more convenient and comfortable method of applying active ingredients. Foaming formulations offer many more benefits compared to traditional drug forms such as ointments, creams, or gels.

EXPERIMENTAL SECTION - This review was based on the latest literature from leading scientific databases, focusing on natural compounds used in topical psoriasis therapy and their use in modern dermatological preparations. The analysis included reports describing the activity of plant extracts such as aloe vera (Aloe vera), turmeric (Curcuma longa), mahonia (Mahonia aquifolium), indigo (Indigo naturalis), and the natural compound resveratrol,, and ……. .reynoutria sachalinensis, with particular emphasis on their anti-inflammatory, antioxidant, and barrier-supporting effects. Publications comparing traditional preparations with newer delivery systems, including therapeutic foams, were also included. The author's master's thesis, which developed and evaluated a plant-based foam formulation, was also used in parallel. Experimental work included the evaluation of its physicochemical properties and selected analytical parameters in order to determine its stability, application quality and suitability as a carrier of natural active substances.

CONCLUSIONS - Full validation requires advanced translational research to optimise the composition and determine the precise technological production parameters. This will confirm the clinical bioavailability and therapeutic efficacy of these preparations, particularly in terms of their interaction with the skin barrier at cellular and molecular levels

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P11

Dendrimer-Based Drug Delivery Systems: with Protein Corona Structure – in vitro study

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Introduction: Nanocarriers, such as Dendrimer-based Drug Delivery Systems (DDS), are platforms designed to enhance drug efficacy and minimize side effects by enabling targeted delivery [1]. The purpose of our work was to investigate the effectiveness of PAMAM decorated with BSA and loaded with 5-FU as an active substance in chemotherapeutic applications.
Materials and methods: In our study, positively charged poly(amidoamine) dendrimers (PAMAMs) were selected for analysis as potential carriers for the anticancer drug 5-fluorouracil (5FU), a drug primarily used in the treatment of colorectal cancer. For the study, the following cell lines were used: HT-26, LoVoDX, H69AR, SKOV-3, A375, HaCaT. The interaction of the G4 5 FU complex with the GPAMM protein was assessed by ITC, while selective cytotoxicity and mechanism were evaluated by MTT viability assay, which was previously described in [2], and immunofluorescent staining for caspase 3, 8, and 8 OHdG in cancer cell lines and HaCaT keratinocytes. The final concentration of 5-FU conjugated with G4PAMAM with BSA and dendrimers or unconjugated was 1, 2, 4, and 8 μM.
Results and Discussion: Immunofluorescence shows that G4 5 FU strongly increases caspase 3 activation and 8 OHdG signal in cancer cells, with the largest response in LoVoDX and H69AR, and only minimal changes in HaCaT, indicating selective induction of apoptosis and oxidative DNA damage. ITC supports stable complex formation with near 1:1 stoichiometry and micromolar affinity, and the viability data align with this, showing a marked drop in tumor cell survival. At the same time, normal keratinocytes remain largely unaffected, suggesting that G4 complexation potentiates 5-FU activity.
Conclusions: Complexing 5-FU with the G4 dendrimer significantly enhances anticancer efficacy while limiting toxicity to normal cells, making this system a strong candidate for further preclinical development. The study successfully demonstrated that the complex-formation efficiency and the maximum drug-binding capacity in a dendrimer G4PAMAM can be controlled by the physicochemical environment (pH) and by exploiting the specific, varied active sites within the nanocarrier structure.

Keywords: corona protein, dendrimers, 5-fluorouracil, colon cancer, ovarian cancer, lung cancer

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P12

Safety and Efficacy of Irreversible Electroporation (IRE) Combined with Chemotherapeutics and Calcium Ions in Pancreatic Cancer

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Introduction: Pancreatic cancer remains a major cause of cancer mortality, with fewer than 30 percent of patients eligible for curative resection. Systemic chemotherapy is essential but limited by toxicity and poor penetration into the desmoplastic, hypovascular tumor. Irreversible electroporation is a non-thermal ablation that induces tumor cell death and can be paired with drugs, such as electrochemotherapy, or with calcium ions, as in calcium electroporation. Calcium electroporation triggers lethal Ca^{2+} influx that amplifies ATP depletion after electroporation and may increase selectivity without systemic toxicity [1][2].

Materials and methods: In vitro validation was performed using two human pancreatic adenocarcinoma cell lines, BxPC-3 and HAPAF II. Cells were exposed to nanosecond and microsecond pulsed electric fields spanning clinically relevant and exploratory regimes. Membrane permeabilization was quantified by Yo-Pro-1 uptake, and MTT was used to assess metabolic viability at 48 and 72 hours. Calcium electroporation was modeled by adding CaCl2 to a final concentration of 2.5 mM during pulsing, then comparing PEF alone versus PEF plus Ca^{2+}.

Clinical safety and feasibility were assessed in a three-patient case with locally advanced unresectable pancreatic adenocarcinoma treated after induction chemotherapy. The interventions included IRE alone, IRE with intratumoral calcium administration, and IRE combined with systemic chemotherapy, as reported in the protocol. Adverse events and early outcomes were evaluated using standard peri-procedural monitoring and follow-up imaging.

Results and Discussion: In vitro, nsPEF produced strong membrane permeabilization in both models, with the highest field regime yielding near complete YO PRO 1 positivity, comparable to microsecond electroporation. Calcium dramatically increased cytotoxicity. In HAPAF II, PEF alone reduced viability modestly, while PEF plus 2.5 mM Ca2 plus decreased viability to roughly 15 to 55 percent, depending on pulse regime, at 48 to 72 hours. In BxPC 3, which was largely resistant to PEF alone, calcium electroporation reduced viability to single digits or low tens of percent across effective regimes, indicating strong synergy and cell line-specific sensitization. Clinically, overall survival was 9 months in the first case and at least 21 months in the second, with reported improvement in quality of life. The third case, treated with IRE plus chemotherapy, was feasible and did not show unexpected toxicity. Broader registry data indicate that adding IRE to standard chemotherapy does not increase severe adverse events and may reduce CTCAE grade 3 or higher events compared with chemotherapy alone

Conclusions: Our approach is a promising method for chemoresistant or unresectable disease, but requires prospective randomized confirmation, optimization of calcium timing and dose, and continued pharmacovigilance with ECG and electrolyte monitoring.

Keywords: pancreatic cancer, irreversible electroporation, calcium ions

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P13

Is ion exchange chromatography (IEX) suitable for albumin conjugates separation?

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Introduction: Albumin, a natural protein of the plasma, is widely used for the synthesis of drug conjugates to improve solubility, pharmacokinetic properties, and bioavailability of bioactive compounds. Here, we report a synthesis of chicken egg white cystatin (CWC) conjugate with bovine serum albumin (BSA) to study the anti-papain activity of such conjugate. The study aimed to develop a method for purifying the obtained CWC-BSA conjugate from unreacted albumin, taking advantage of charge differences between the conjugate and free BSA.
Materials and methods: SM(PEG)4 crosslinker (Thermo Scientific) was used for the conjugation of commercial BSA with CWC isolated from *Gallus gallus domesticus* eggs by affinity chromatography [1]. The control SDS-PAGE electrophoresis (10% gel, reducing condition) revealed a mixture of conjugate (approx. 80kDa) and free BSA (67 kDa). Isoelectric focusing was used for the precise determination of the isoelectric point of the SM(PEG)4 conjugate. It was shown that the conjugate pI (4.4) was different from that of the albumin (4.7). This finding encouraged us to proceed with chromatofocusing on the PBE94 column to separate the conjugate from free albumin. The ion exchange column equilibrated with 25 mM imidazole/HCl buffer, pH 7.4 was used in the procedure. A sample of crude conjugate was applied onto the column and eluted at 0.7 ml/min with Polybuffer74/HCl, pH=4.0. Collected fractions were monitored by absorbance measurement at λ=280 nm and, after being dialysed against PBS, assessed for protein concentration and anti-papain activity.
Results: Five fractions containing protein were separated between 250 ml and 360 ml of eluent volume, characterized by varying protein concentration and different anti-papain activity. The fractions eluted at pH 5.03-4.97 were not active against papain and yielded about 30% of the total protein quantity. The three fractions collected at pH 4.9-4.72 inhibited the papain with a specific activity of 2,5-3 IU/mg of protein.
Discussion: Ion exchange chromatography (IEX) is suitable for separating albumin conjugates as long as the conjugation alters the overall charge of the albumin molecule. The separated proteins need to be further processed by a desalting method to remove co-eluted ampholytes.
Conclusions: IEX on PBE94 can separate unconjugated albumin from its conjugates successfully.

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P14

Biotic elicitors as physicochemical drivers of isoquinoline alkaloid network dynamics in *Chelidonium majus*

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Introduction: *Chelidonium majus* L. (Papaveraceae) synthesizes a broad spectrum of isoquinoline alkaloids (IQAs), a family of heteroaromatic, π-conjugated metabolites whose electronic structures underlie both their characteristic yellow-to-red latex chromaticity and their diverse biological activities. Many IQAs function as DNA-binding and intercalating agents, engaging in π–π stacking and electrostatic interactions that can perturb nucleic-acid topology and transcriptional dynamics, while also exhibiting antiviral, antiparasitic, and antimicrobial properties.

Materials and methods: The physicochemical and metabolic responses of *Ch. majus* to biotic elicitors were examined using immobilized in vitro cultures and hydroponic seedlings. Plant cells were embedded in a non-purified bacterial nanocellulose (BNC) matrix derived from 3-day *Komagataeibacter xylinus* cultures. Microbial elicitors—*Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*—were applied to either surface of BNC discs. In hydroponics, seedlings were treated with lyophilized *P. aeruginosa* suspensions (1, 10, 50 mL/L) and methyl jasmonate (50 mL/L). Metabolic responses were analyzed using LC-MS/MS and MALDI mass spectrometry imaging (MALDI-MSI).

Results: Biotic elicitors influenced IQAs composition both released from *Ch. majus* cells cultured on cellulose matrices, as well as in hydroponics-cultivated plants. MALDI-MSI mapping revealed that BNC platforms exposed to *S. aureus* accumulated higher levels of coptisine, sanguinarine, berberine, chelerythrine, chelidonine, and allocryptopine compared to those treated with *C. albicans*, *P. aeruginosa*, or control scaffolds. In hydroponic cultures, the 10 mL/L dose of *P. aeruginosa* most effectively enhanced the biosynthesis of protoberberine and benzophenanthridine alkaloids.

Discussion: Despite commercial-scale production of selected IQAs (e.g., berberine, coptisine) in plant tissue cultures, a significant portion of the IQA chemical space, especially within Papaveraceae, remains insufficiently characterized. In our experiments, biotic elicitors differentially modulated IQAs profiles, with BNC matrices stimulated by *S. aureus* showing the strongest accumulation of key protoberberine and benzophenanthridine compounds. Consistently, the phytochemical profile of hydroponic cultivated plants was influenced by 10 mL/L of *P. aeruginosa*, highlighting elicitor-specific metabolic responses.

Conclusions: Further work is required to elucidate the mechanistic basis of strain-specific elicitation, potentially involving redox signaling, membrane perturbation, or metabolite–receptor interactions, and to determine optimal microbial concentrations and exposure durations for both *in vitro* cultures and hydroponically grown plants.

Acknowledgments

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P15

Natural triterpenoid surfactants and bioactive polyphenols from in vitro cultures of Gypsophila elegans and Agrostemma githago

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Introduction: Plants belonging to the Caryophyllaceae are known to contain diverse triterpenoid saponins and flavone-C-glycosides. The former are based on pentacyclic hydroxylated aglycons such as gypsogenin and gypsogenic, oleanolic and quillaic acids and are typically present in all plant organs. These constituents are considered major bioactive principle in numerous medicinal plants of this family. On the other hand, flavonoids, such as orientin, vitexin and their analogs are concentrated in aerial parts but the regulation of their biosynthesis is less studied, despite their high pharmacological potential.

Material and Methods: For studying the phytochemical profiles under in vitro conditions we used two species, Agrostemma githago L. and Gypsophila elegans M.Bieb. known to contain acetylated saponins able to permeabilize cell membranes. The callus, cell suspensions, shoots and roots were cultured in glass containers for solid media and scaled up from 300 mL culture flasks to temporary immersion systems (RITA and Plantform). The phytochemical profile was obtained using HPLC and LC-MS.

Results and Discussion: The presence of orientin and isoorientin as well as several saponins in various in vitro culture types was confirmed. The influence of media composition and culture system was significant and indicates a need of further studies to understand the key factors influencing the quantity and diversity of both classes of specialized metabolites.

Conclusion: The high biomass growth in temporary immersion systems provides a potential biotechnological source of bioactive flavone-C-glycosides and specifically acting acetylated quillaic acid bidesmosides for potential use as natural biomembrane permeabilization enhancers and biosurfactants.

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P16

Evaluation of Celugel potential as a semi-solid polymer-base in the design of ophthalmic antibiotic in pharmacy practice: preliminary studies and physicochemical characterization

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INTRODUCTION

The searching the viscosity-bases in ophthalmic application for improving the bioavailability of drug substances, which has a direct impact on the effectiveness of pharmacotherapy for treating infections. Studies on fluoroquinolones and aminoglycosides, among others, confirm that a higher viscosity in the formulation significantly prolongs the retention time on the corneal surface, resulting in higher concentrations of the drug in the aqueous humour. Due to the limited availability of viscometric polymers in pharmaceutical practice, adapting ready-made bases is becoming the preferred method for preparing targeted drugs. This study aimed to develop a technique for preparing eye drops in a pharmacy using the Celugel® vehicle. The research focused on optimising the physicochemical parameters in accordance with the Pharmacopoeia requirements for ophthalmic drugs. Celugel® is a ready-made hydrogel vehicle based on hydroxyethylcellulose and stabilised with glycerol and a preservative system containing sorbic acid and potassium sorbate.

EXPERIMENTAL

The amount of water for injection required to achieve system isotonicity was determined to be 2.7 g per 1 g of hydrogel, as a result of the conducted tests. Model solutions consisting of hydrogel vehicle, water for injection and a 1% chlorhexidine digluconate solution were prepared. The physicochemical analysis included osmolality measurements (cryoscopic method), pH measurements (potentiometric method) and kinematic viscosity measurements at 35 °C. The microbiological and physical purity of the solutions was assessed by examining their turbidity and colour (in accordance with the requirements of Pharmacopoeia XIII, monographs 2.2.1 and 2.2.2). The stability of the preparation was verified after autoclaving at 121°C for 20 minutes. Furthermore, the chemical and physical compatibility of the diluted hydrogel with pharmacopoeial buffering and isotonic solutions was tested.

RESULTS AND DISCUSSION

After the addition of the preservative (chlorhexidine digluconate at a concentration of 0.04%), this value increased to 284 ± 2 mOsm/L. The osmotic pressure of the prepared base solution was 279 ± 2 mOsm/L. The pH of the solutions ranged from 6.3 to 6.5 and their dynamic viscosity was 21–25 mPa·s. All of the tested solutions were clear and colourless, showing no turbidity or colouration compared to the reference standards. The steam sterilisation process did not affect the appearance (colour and clarity) or pH of the solutions. However, significant changes were observed in the other parameters. Osmotic pressure increased to 307 ± 2 mOsm/L for the preservative-free solution and to 297 ± 2 mOsm/L for the solution with the preservative. Concurrently, the dynamic viscosity of the solutions decreased, amounting to 13–15 mPa·s (preservative-free) and 12–13 mPa·s (with preservative) after sterilisation. Compatibility studies revealed that the viscosity-enhanced solution is compatible with all pharmacopoeial isotonising and buffering solutions when mixed at a ratio of 1:1.

CONCLUSION

The described method of preparing an auxiliary solution using hydrogel provides a safe way to prepare eye drops with increased viscosity, ensuring iso-osmoticity and iso-hydricity under pharmacy formulation conditions. This method ensures the preparation of a stable solution that is compatible with the most commonly used excipients. The use of Celugel® as an ingredient in prescription eye drops makes it possible to induce prolonged precorneal residence time, enhance bioavailability improve lubrication and mucoadhesion and may cause better patient compliance.

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P17

Structure and Complexation Properties of EDTA-Crosslinked Polycyclodextrins for Advanced Polyelectrolyte Systems

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Introduction: Cyclodextrins are widely used supramolecular hosts with high biocompatibility and the ability to form inclusion complexes[1]. Polymerized cyclodextrins (PCDs) further expand these capabilities, especially when functional crosslinkers such as ethylenediaminetetraacetic acid (EDTA) provide additional polyionic and chelating properties. These combined features make PCDs attractive candidates for smart drug delivery systems and advanced polyelectrolyte-based materials[2]. In this work, we report the synthesis of water-soluble EDTA-PCDs and evaluate their structural and functional characteristics relevant to biomedical applications, including their integration into complex systems assembled using the layer-by-layer method.
Materials and methods: β-Cyclodextrin (β-CD) and EDTA dianhydride (EDTADA) were reacted in anhydrous DMSO with triethylamine, using β-CD:EDTADA molar ratios ranging from 1:6 to 1:15. Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy was performed to confirm the crosslinking process and verify the structure of the synthesized products. The morphology and shape of the obtained PCDs were visualized by transmission electron microscopy (TEM). Before the analysis, the Formvar support film was hydrophilized and coated with PLL to enable binding of the negatively charged PCDs. Conductometric titration was used to quantify the unreacted carboxyl groups in the PCDs. Interactions between the PCDs and the model polycation, poly-L-lysine (PLL), were assessed using dynamic light scattering (DLS) by monitoring titration-dependent changes in hydrodynamic diameter and surface charge.
Results and discussion: In the ^{1}H NMR spectra, all PCDs presented singlets at 3.80 and 3.58 ppm, characteristic of EDTA methylene protons, confirming incorporation of the crosslinker during polymerization. Additionally, signals from residual triethylamine salt were detected. The resulting TEM images showed uniformly adsorbed, dark, nearly spherical PCD particles, with the 1:12 ratio sample displaying the most distinct morphology. Conductometric titration demonstrated that lower β-CD:EDTADA ratios required more titrant, indicating a higher content of free carboxyl groups in PCDs synthesized with increased amounts of crosslinker. Interaction with PLL led to immediate turbidity, zeta potential inversion, and pronounced increases in hydrodynamic diameter, confirming formation of polyelectrolyte complexes.
Conclusions: EDTA-crosslinked PCDs exhibit well-defined nanoscale morphology, tunable carboxyl group content, and strong interactions with polycations, highlighting their potential for use in advanced polyelectrolyte assemblies for biomedical applications.

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P18

sST2 as a Diagnostic and Prognostic Biomarker in Patients with Thrombotic Microangiopathy after Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Soluble interleukin-1 receptor–like 1 (sST2), a decoy receptor neutralizing IL-33, plays a key role in immunoregulation and tissue remodeling. Elevated sST2 levels have been linked to inflammatory activation, cardiomyocyte injury, and fibrotic processes. Recent evidence suggests its potential involvement in the pathophysiology of thrombotic microangiopathy (TMA). TMA comprises a group of syndromes characterized by thrombocytopenia, non-immune hemolytic anemia, and microvascular thrombosis leading to multiorgan damage, most commonly acute kidney injury. The aim of this study was to assess the diagnostic and prognostic value of sST2 in patients with TMA following allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Materials and methods: Plasma sST2 concentrations were measured in 14 pediatric allo-HSCT recipients with confirmed TMA and in 14 matched controls (7 females and 7 males in each group; age range: 0.8–17 years). Samples were collected prior to conditioning, on the day of transplantation, and on days 7, 14, 21, 28, 56, and 100 post-HSCT. After centrifugation, specimens were stored at –80°C. sST2 levels were determined using an enzyme immunoassay (EIA).

Results: sST2 concentrations were significantly higher in the TMA group compared with controls (median 3.940 [1.640–15.53] vs. 2.570 [1.325–4.440] ng/mL; $p = 0.0043$). No associations were observed between sST2 levels and age or sex. Patients who died due to TMA exhibited markedly elevated sST2 concentrations compared with survivors (17.68 [4.454–40.68] vs. 2.186 [1.503–7.470] ng/mL; $p = 0.029$). A moderate negative correlation was identified between sST2 levels and time to TMA onset post-transplant ($r = –0.55$; $p = 0.037$). A trend toward higher sST2 values was observed in patients with concomitant infections ($p = 0.08$), though this requires confirmation in a larger cohort. Receiver operating characteristic analysis demonstrated excellent discriminatory capacity of sST2 for TMA diagnosis (AUC = 0.982; $p < 0.001$).

Conclusions: These findings indicate that sST2 may serve as a valuable diagnostic biomarker in allo-HSCT–related TMA and holds prognostic relevance. Elevated sST2 concentrations correlate with earlier TMA onset and poorer clinical outcomes, supporting its potential utility in risk stratification and monitoring of post-transplant complications.

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P19

Enhancement of the antimicrobial activity of plant-derived terpenes by the incorporation of cocamidopropyl betaine

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Introduction: Due to growing resistance of microbes to antibiotics, plant-derived terpenes (PDT) contained in essential oils are promising alternative to treat bacterial, biofilm-related infections of chronic wounds or the oral cavity[1]. Combining PDT with appropriate surfactants may increase their distribution in infection site, at the same time maintaining low cytotoxicity towards patients' tissues. Such an approach may support the development of safe, effective topical therapies capable of more evenly covering infected tissues and better targeting biofilm structures.

Materials and methods: The antimicrobial properties of PDT (1%, 2% from thyme combined with surfactants phenoxyethanol (PE) (0.1%) or cocamidopropyl betaine (CAPB) (0.1%, 0.35% or 0.7%) were analyzed by means of modified disc diffusion method (MDDM), combined with image quantitative processing using ImageJ® software. The *Staphylococcus aureus* ATCC 6538 was used as a relevant model microorganism.

Results: The addition of 0.1% PE to 1% or 2% PDT did not increase microbial growth inhibition zones compared to PDT alone. The 0.1%, 0.35% and 0.7% concentrations of CAPB showed intrinsic antimicrobial activity themselves. Application of 0.7% CAPB combined with 2% PDT resulted in microbial growth inhibition zone of ~ 100% bigger than the one being result of application of 2% PDT alone.

Discussion: PE failed to enhance the antimicrobial effect of PDT. In contrast, CAPB showed intrinsic antimicrobial activity and markedly strengthened the overall effect of PDT. The observed enhancement most likely results from improved spreading of the formulation across the agar surface combined with the direct bactericidal properties of CAPB. Because CAPB is a surfactant that may also affect eukaryotic cells, further studies should include cytotoxicity assessment to establish safe concentration ranges for topical use.

Conclusions: CAPB effectively enhances the effect of PDT

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P20

Application of Point-of-Care EPR Spectroscopy in Oxidative Stress Diagnostics and Pharmacotherapy Monitoring

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Introduction:
Oxidative stress and mitochondrial dysfunction are fundamental mechanisms in the pathogenesis of metabolic diseases and aging [1-3]. While Electron Paramagnetic Resonance (EPR) spectroscopy is considered the "gold standard" for detecting Reactive Oxygen Species (ROS), its clinical application has been limited by the size and complexity of stationary spectrometers. This study presents the application of a miniaturized, Point-of-Care EPR system (VitaScreen) for real-time assessment of cellular redox status and monitoring the efficacy of antioxidant pharmacotherapy.
Materials and methods:
The study utilized the VitaScreen X-band EPR spectrometer (Noxygen) combined with a cyclic hydroxylamine spin probe (CMH) to quantify ROS generation in capillary blood samples. The parameter measured was Cellular Metabolic Activity (CMA), expressed in nM/s. An extended protocol (eCMA) was employed using specific metabolic modulators (including Antimycin A and L-NIO) to differentiate between mitochondrial, enzymatic (NOX, XO), and endothelial ROS sources. The impact of high-dose Vitamin C infusion (25 g i.v.) on mitochondrial function was analyzed in a case study format.
Results:
The miniaturized EPR system successfully quantified basal ROS generation and mitochondrial responsiveness in clinical settings. The study revealed significant inter-individual variability in response to Vitamin C administration. In some subjects, the infusion significantly reduced mitochondrial-dependent ROS generation (antioxidant effect), while in others, particularly those undergoing hypoxic training, it induced a pro-oxidative response (“hermetic” effect). Furthermore, basal CMA values showed a strong correlation with the expression of longevity-associated biomarkers, including SIRT1, mTOR, and AMPK.
Discussion:
The ability to distinguish between physiological oxidative eustress and pathological distress is crucial for personalized medicine [1-3]. The presented EPR method allows for the evaluation of the biological effects of pharmaceuticals on mitochondrial function, which often differs from their predicted chemical properties. This approach addresses the "ROS paradox" in antioxidant therapy.
Conclusions:
Point-of-Care EPR spectroscopy is a viable and robust biophysical tool for pharmacy and clinical practice. It enables precise monitoring of redox-modulating therapies, allowing for the personalization of supplementation and drug dosage based on the patient's individual metabolic profile rather than statistical averages.
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P21

Cyclodextrin-Based Polymer Carriers for Acyclovir Adsorption and Controlled Release

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Introduction: The aim of this study was to synthesize polymer systems containing β-cyclodextrin (β-CD) capable of adsorbing and releasing acyclovir (ACV), a poorly water-soluble antiviral drug. β-CD possesses a hydrophobic internal cavity and hydrophilic outer surface, making it suitable for interacting with hydrophobic regions of ACV. Citric acid was used as a cross-linker, while PEG400 acted as a modifier influencing the physicochemical properties of the resulting material. It was hypothesized that ACV would associate with β-CD-based polymers, enabling its temporary retention and subsequent release.

Materials and methods:
β-CD, citric acid, PEG400, catalyst and water were mixed and evaporated, followed by vacuum drying. The crude material was purified with water and ethanol and then dried.
For adsorption studies, 100 mg of polymer was incubated in 10 ml of ACV solution (1 mg/ml) and shaken at 100 rpm. Samples were withdrawn at set intervals, filtered, and analyzed.
ACV concentration was quantified using RP-HPLC with an aqueous 0.1% triethylamine mobile phase (pH 2.5). Measurements were performed at 25°C on an RP-8 column.

Results: Based on the results obtained for changes in ACV concentration in the solution taken for analysis at specific time intervals, it can be assumed that API adsorption by the synthesized systems has occurred.
A sharp decrease in ACV concentration in the first 20 minutes of the test - rapid saturation of active sites on the surface of the polymer carrier. In the intervals: 30-60 min, 90-2880 min, the ACV concentration is relatively stable - the carrier has become saturated and equilibrium has been reached. Increase in ACV concentration in the 90-2880 min interval - possible hydrolysis of the polymer carrier with the release of some ACV molecules into the solution could occur.

Discussion: The synthesized β-CD polymer successfully interacted with ACV, showing both adsorption and controlled release behavior. The observed trends support the hypothesis that β-CD-based polymer networks can transiently host hydrophobic drug molecules, making them promising candidates for delivery systems.

Conclusions: A simple synthetic route allowed the preparation of β-cyclodextrin–citric acid–PEG400 polymer carriers. Their ability to adsorb and subsequently release acyclovir was confirmed using RP-HPLC. These findings highlight the potential of modified β-CD polymers as functional materials for controlled drug delivery applications.

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Study of the interaction of sulfasalazine with human albumin protein depending on the environment

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Introduction: Sulfasalazine (SZA) is a commonly used anti-inflammatory and immunomodulatory drug. It was initially used to treat rheumatoid arthritis and is now also used to treat inflammatory bowel disease [1]. In order to work effectively, it must be transported within the body to the appropriate places. It is known that the type and strength of drug-protein binding are influenced by environmental factors such as temperature, pH, and the presence of other species in the solution. These can significantly affect both the protein conformation and the ionization state of the drug molecule, leading to changes in the nature of interactions [2]. Analysis of these relationships allows for a better understanding not only of the complexation mechanisms but also of the potential interactions of sulfasalazine with other active substances that compete for the same binding sites in albumin (HSA).

Materials and methods: Studies of the interactions between sulfasalazine and human albumin depending on environmental conditions such as pH and temperature were carried out using physicochemical and spectroscopic methods such as isothermal titration calorimetry, UV-VIS and circular dichroism spectroscopy.

Results: The thermograms recorded under all conditions showed an exothermic profile. The highest affinity is observed at pH 6.6 and 25°C, where the association constant for the first binding site is $K_{a1} = 2.92\times10^{6}M^{-1}$. At pH 7.4, T = 25°C the association constant for the first site is significantly lower ($K_{a1} = 7.64\times10^{3}M^{-1}$). In contrast in pH 6.6 and a temperature of 37°C the enthalpy of the first binding is negative (ΔH_1 = -299.1 kJ/mol). Positive value is observed for the second binding site (ΔH_2 = 32.6 kJ/mol). At the same time, very high entropy values, particularly ΔS_1 = 86.7 J/mol·K is observed. UV-VIS spectra were recorded in the wavelength range of 220–380 nm for samples containing a constant concentration of HSA and a variable molar ratio of HSA to SZA – from unbound albumin to a 1:4 HSA:SZA ratio at pH 6.6 and 7.4. The analysis revealed a number of changes in the characteristics of the absorption and CD spectra, indicating concentration-dependent interactions.

Discussion: The obtained data clearly indicate that the interaction of SZA/HSA occurs in an energetically favorable manner and it is strongly dependent on environmental conditions. Under slightly acidic conditions at 25°C, the strongest high-affinity bond is observed, while an increase in temperature to 37°C resulted in an increased enthalpy contribution to the total energy balance of the interactions. Entropic changes observed in all systems further support the hypothesis of the dominance of hydrophobic mechanisms and the formation of numerous noncovalent bonds that stabilize the formed complexes. Comparison of ITC results with pH and temperature indicates that the most favorable conditions for sulfasalazine/HSA complexation occur at pH 6.6, which may be important in the context of inflammatory sites and pathologically altered microenvironments. Analysis of the CD spectra shapes clearly indicates that sulfasalazine disrupts the α-helical structure of albumin, and this effect increases with increasing molar ratio.

Conclusions: The results of isothermal titration calorimetry, UV-VIS and circular dichroism spectroscopy confirm the interactions between sulfasalazine and human albumin. The thermodynamic parameter values indicate favorable, exothermic ligand-protein binding, consistent with literature data describing interactions of HSA with structurally similar drugs, such as salicylates and sulfonamides. UV-VIS and CD spectroscopy revealed absorption bands typical of albumin, whose shifts and intensity changes following the addition of sulfasalazine indicated complex formation and reorganization of the aromatic residue environment. Circular dichroism spectra revealed disruptions of the α-helical structure and an increase in the β-sheet fraction, similar to those reported in the interactions of albumin with ibuprofen, naproxen, and ketoprofen.

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