NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Chemistry

RESEARCH UNIVERSITY
EXCELLENCE INITIATIVE

Department of Biomaterials and Cosmetic Chemistry

New solutions in developing cosmetic formulations and polymer materials to reduce the skin barrier

Summary of Professional Accomplishments

dr Justyna Kozlowska

Natural sciences

Discipline: Chemical sciences

Appendix No. 3

Toruń 2022

CONTENTS

1. Personal data

Name: **Justyna Kozlowska**

2. Diplomas and degrees conferred in specific areas of science

2014 **Doctor of chemical sciences**
Title: Collagen-hydroxyapatite composites for biomedical applications
Supervisor: prof. dr hab. Alina Sionkowska
Faculty of Chemistry, Nicolaus Copernicus University in Torun

2010 **Postgraduate Diploma**
Higher School of Cosmetics and Health Care in Warsaw
Postgraduate course *Knowledge about cosmetics and cosmetic chemistry*

2008 **Master of Science in Chemistry**; specialization: polymer chemistry
Title: Influence of collagen addition on the properties of polyvinylpyrrolidone
Supervisor: prof. Alina Sionkowska
Faculty of Chemistry, Nicolaus Copernicus University in Torun

2006 **Bachelor in chemistry**; specialization: polymer chemistry
Title: Gas permeability through polymer films
Supervisor: prof. Halina Kaczmarek
Faculty of Chemistry, Nicolaus Copernicus University in Torun

3. Information on employment in research institutes

2017- present **Assistant Professor**
Faculty of Chemistry, Nicolaus Copernicus University in Torun

2012 - 2017 **Assisant**
Faculty of Chemistry, Nicolaus Copernicus University in Torun

4. Description of the achievements, set out in art. 219 para 1. Point 2b of the Act

4.1. Achievement title

New solutions in developing cosmetic formulations and polymer materials to reduce the skin barrier

4.2. The cycle of scientific articles related thematically, according to art. 219 para 1. Point 2b of the Act

The scientific achievement consists of the following:

- 11 publications (H1-H11),
- 2 patents (PP1, PP2).

H1 **J. Kozlowska***, A. Kaczmarkiewicz, *Collagen matrices containing poly(vinyl alcohol) microcapsules with retinyl palmitate - structure, stability, mechanical and swelling properties*, Polymer Degradation and Stability, 2019, 161, 108-113.

IF = 4.032 (current 5.204); MSHE = 100

My contribution to the work consisted in developing the concept of all research and defining its purpose, developing the methodology for obtaining microparticles and composite materials, selecting research methods necessary to experiment, supervising the research, discussing the obtained results, and preparing the paper for printing. The research was carried out and financed as part of my managing project.

H2 **J. Kozlowska***, N. Stachowiak, W. Prus, *Stability studies of collagen-based microspheres with Calendula officinalis flower extract*, Polymer Degradation and Stability, 2019, 163, 214-219.

IF = 4.032 (current 5.204); MSHE = 100

My contribution to the work consisted in defining the purpose of the work, planning the entire experiment and managing the individual stages of the research, analyzing the results, preparing the manuscript, editing the whole paper, and corresponding

with the reviewers. The study was carried out and financed as part of my managing project.

H3 **J. Kozlowska***, N. Stachowiak, A. Sionkowska, *The preparation and characterization of composite materials by incorporating microspheres into a collagen/hydroxyethyl cellulose matrix*, Polymer Testing, 2018, 69, 350-358.
IF = 2.943 (current 4.931); MSHE = 40 (current 100);

My contribution to the publication consisted of defining the scientific problem, planning and supervising the entire research process, developing a methodology for obtaining microparticles and composite materials, discussing the results, writing and editing the manuscript, and securing research funding.

H4 **J. Kozlowska***, N. Stachowiak, A. Sionkowska, *Collagen/Gelatin/Hydroxyethyl Cellulose Composites Containing Microspheres Based on Collagen and Gelatin: Design and Evaluation*, Polymers, 2018, 10 (4), 456.
IF = 3.164 (current 4.967); MSHE = 40 (current 100);

My contribution to the work consisted of developing a prototype of the obtained material, planning all stages of labor, controlling their course, and receiving funds for their implementation. My contribution to this work includes interpreting and describing the obtained results, preparing, editing the manuscript, and discussing it with the reviewers.

H5 **J. Kozlowska***, W. Prus-Walendziak, N. Stachowiak, A. Bajek, Ł, Kazmierski, B. Tylkowski, *Modification of collagen/gelatin/hydroxyethyl cellulose-based materials by addition of herbal extract-loaded microspheres made from gellan gum and xanthan gum,* Materials (Basel), 2020, 13 (16), 3507.
IF = 3.623 (current 3.748); MSHE = 140

My contribution to the work consisted of defining the scientific problem, developing the methodology for obtaining materials, planning the course of the research, interpreting the results, preparing the manuscript, editing the entirety, and discussing with the journal editor and reviewers. The study was carried out and financed as part of my managing project.

H6 **J. Kozlowska***, K. Pauter, A. Sionkowska, *Carrageenan-based hydrogels: Effect of sorbitol and glycerin on the stability, swelling and mechanical properties*, Polymer Testing, 2018, 67, 7-11.

IF = 2.943 (current 4.931); MSHE = 40 (current 100);

My contribution to the work consisted in defining the research problem, developing the research concept, planning and supervising the various stages of the work, analyzing the results, preparing the manuscript, editing the entirety, and corresponding with the reviewers of the work. I also obtained funding for research.

H7 **J. Kozlowska***, B. Tylkowski, N. Stachowiak, W. Prus-Walendziak, *Controlling the skin barrier quality through the application of polymeric films containing microspheres with encapsulated plant extract*, Processes, 2020, 8 (5) 530.

IF = 2.847 (current 3.352); MSHE = 70

My contribution to the work consisted of presenting research hypotheses, developing the research concept, planning and supervising all stages of the work, interpreting the obtained results, writing the manuscript, and correcting the comments received in the reviews. The study was carried out and financed as part of my managing project.

H8 W. Prus-Walendziak, **J. Kozlowska***

Design of sodium alginate/gelatin-based emulsion film fused with polylactide microparticles charged with plant extract, Materials (Basel), 2021, 14 (4), 745.

IF = 3.748; MSHE = 140

My contribution to the work consisted in defining the concept of the work, planning and supervising research, and obtaining funds. I participated in analyzing the results, and I prepared and sent the manuscript for printing.

H9 W. Prus-Walendziak, **J. Kozlowska***

Lyophilized emulsions in the form of 3D porous matrices as a novel material for topical application, Materials (Basel), 2021, 14 (4), 950.

IF = 3.748; MSHE = 140

My contribution to the publication was to present research hypotheses, develop a concept and work plan, conduct research, and raise funds for research. My participation included analyzing the results, preparing the manuscript, and correspondence with the reviewers of the work.

H10 W. Prus, **J. Kozlowska***, *The influence of new polymeric microbeads in peeling products on skin condition*, Molecular Crystals and Liquid Crystals, 2018, 671, 140-147.

IF = 0.559 (current 0.672); MSHE = 15 (current 40)

My contribution to this work included developing the project concept, supervising the research, analyzing and discussing the results, preparing the article for publication, and correspondence with reviewers. The study was carried out and financed as part of my managing project.

H11 **J. Kozlowska***, W. Prus, N. Stachowiak, *Microparticles based on natural and synthetic polymers for cosmetic applications*, International Journal of Biological Macromolecules, 2019, 129, 952-956.

IF = 5.162 (current 8.025); MSHE = 100

My contribution to the work consisted of defining the research problem, developing the research concept, planning individual stages of labor and monitoring their course, and obtaining funds for research. My participation included analyzing the results, preparing the manuscript, and correspondence with the reviewers of the work.

PP1 **J. Kozlowska**, W. Prus, *A method of producing a cosmetic composition for exfoliating epidermal cells*, PL nr 239755 B1, 2022.

MSHE = 75

I am the originator of the patent. I developed the research concept and edited the assumptions and content of the patent application.

PP2 **J. Kozlowska**, W. Prus, *A cosmetic preparation for cleansing and exfoliating the epidermis and the method of its production*, PL nr 236187 B1, 2020.
MSHE = 75

I am the originator of the patent. I developed the research concept and edited the assumptions and content of the patent application.

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* corresponding author

Summary

	According to the year of publication	Current
Total IF	36.801	48.53
Total MSHE	1075	1280*

* following the Ministry of Science and Higher Education list of 1 December 2021.

4.3. The scientific purpose of the research

Introduction

The skin, the largest human organ that covers and protects the entire body, has long been the site of the application of dermatological drugs used in the local treatment of skin diseases. Currently, preparations intended for application to the skin are appearing more and more often on the market, the purpose of which is to cause a general effect or to alleviate pain and inflammation of muscles and joints. Administration of the drug by the percutaneous route has many advantages, including eliminating the possibility of a potential drug substance decomposition in the gastrointestinal tract or excluding its adverse effects on the digestive system [1]. A significant problem to this day is the penetration of many drugs and other active substances into the skin, resulting from the specific structure of the epidermis, which is entirely different from the structure of other layers of the skin. For this reason, when developing a formulation of a preparation intended for skin application, several factors should be considered, including the physicochemical properties of the active ingredient and base ingredients.

Obtaining the proper penetration of biologically active substances through the skin to induce a specific therapeutic effect remains one of the main problems in skin pharmacotherapy [2-5].

Currently used dermatological preparations are most often in the form of an emulsion or a hydrogel. In addition to active substances, they contain several ingredients mixed, e.g., in the form of a product or excipients that do not show any dynamic effect. The same is true for cosmetic products. The effectiveness of active substances contained in creams, ointments, patches, or gels applied to the skin depends on their ability to reach the appropriate areas in the deeper layers of the skin and thus on overcoming the barrier constituted by the *stratum corneum* [1,2]. Currently, there is a lot of interest in the issue of the transport of active substances through the skin. Many scientific teams research the mechanisms influencing this process to increase the effectiveness of dermatological preparations [3-5].

Recently, the scientific literature has reported a dynamic increase in publications focused on improving the form of controlled-release drugs, using micro- and nanoencapsulation techniques, mainly to overcome the inconveniences associated with using conventional medicines [6-10]. The properties of microparticles, such as biocompatibility, the ability to transport various, even unstable, active substances, and, at the same time, a controlled profile of their release in a specific place, contributed to the extension of the scope of microparticle application, especially in medicine and pharmacy [11,12]. They are used to prepare medicinal products, mainly for intramuscular, intranasal, and subcutaneous administration [10]. The synthesis and characterization of polymer microparticles (microspheres, microcapsules) is also the subject of many research works in other areas, including the food, cosmetic, textile, and agricultural industries [13-16].

Despite the dynamic development of encapsulation techniques and making massive progress in understanding the physiology of the skin, research on new solutions improving the effectiveness of penetration of biologically active ingredients through the skin is still a challenge in the formulation of dermatological and cosmetic products. Analyzing the current literature and reviewing recent scientific achievements in this subject allowed me to develop new research hypotheses and propose new solutions. I noticed shortcomings in this field, which allowed me to specify the goals of the research carried out as part of my habilitation thesis.

As a new solution to improve the penetration of active substances into the skin, I have developed a prototype of an **innovative polymer material** with a complex structure that determines the multi-stage operation. The main direction of my research was to design, obtain and characterize a **new class of materials** by **incorporating microcapsules or microspheres containing the active substance into the polymer matrix** (Fig. 1). In addition, to enhance the

effect of the penetration of active substances from microparticles into the skin, some of the matrices have been modified with the addition of the **penetration enhancers (accelerants)**.

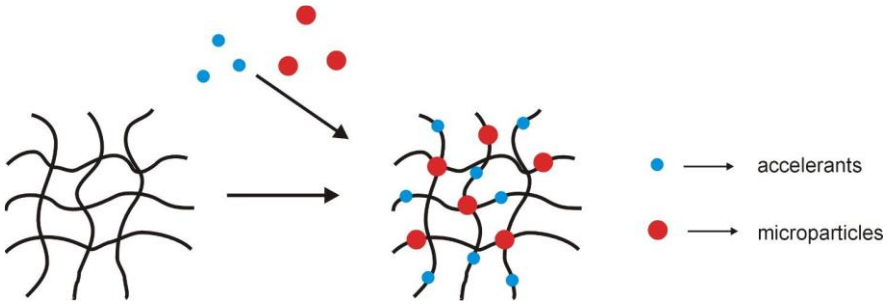

Fig. 1. Polymer matrix modified with the addition of microparticles and penetration enhancers (accelerants)

I obtained funds for implementing my original idea under the NCN Sonata competition (project no. UMO-2016/21/D/ST8/01705 *New materials containing microparticles incorporated into polymer matrix for medical, pharmaceutical, and cosmetic applications*).

Another method to reduce the skin barrier is removing dead epidermal cells (corneocytes) from their surface before applying a medicinal or cosmetic preparation. The epidermis is a dynamic tissue that constantly undergoes the process of keratinization [17]. Mechanical peels (containing abrasive ingredients) and enzymatic or chemical peels are recommended to cleanse the skin and remove corneocytes. After washing the skin with peeling, the effectiveness of the preparations applied to it is observed due to the partial removal of the *stratum corneum*, facilitating the penetration of active ingredients. Preparations intended for mechanical peeling, as abrasives, may contain elements of natural origin, such as seeds, fruit seeds, ground nut shells, and coffee beans that mechanically peel the epidermis. However, due to the difficulties in modifying the shape and hardness of the aforementioned abrasive substances, the cosmetic peelings market has been dominated by synthetic (mainly polyethylene) microspheres with a diameter of 5 μm to 1 mm, characterized by a spherical shape, smooth surface, and repeatable size. Unfortunately, due to their small size, the microgranules reaching the sewage system were not always separated from the sewage. Thus they ended up in the environment, causing a severe threat, especially to aquatic organisms [18,19]. Accordingly, Cosmetics Europe - the European trade association for the cosmetics and personal care industry - recommended in October 2015 that the use of solid plastic particles (microplastics), which are not biodegradable in the aquatic environment, should be discontinued in skin exfoliation and cleansing products [20]. The recall of synthetic microbeads is a challenge for the cosmetics industry. Much research is currently focused on finding

alternative, biodegradable abrasive components suitable for skin cleansing. To fill the gap in this subject, I used encapsulation techniques to develop new solutions in the formulation of cosmetic peels, where I proposed **microparticles based on biopolymers as abrasives**.

The aim of research carried out as part of the habilitation

Taking into account the progress in understanding the mechanisms of penetration of active substances through the epidermal barrier, as well as methods of their strengthening, as well as the need for innovative materials and preparations intended for application to the skin, showing high effectiveness, I undertook research aimed at:

- design, prepare, and evaluation of the physicochemical properties of innovative materials by incorporating polymer microparticles with an encapsulated active ingredient in the structure of a polymer, porous matrix to provide a multidirectional action increasing the effectiveness of penetration of active substances through the skin barrier;
- developing a methodology for obtaining materials to be applied to the skin in the form of a film or hydrogel, taking into account the addition of penetration (sorption) promoters modifying the properties of the *stratum corneum*;
- production of innovative emulsion materials, an alternative to traditional cosmetic emulsions;
- developing a methodology for obtaining biodegradable microparticles to use as abrasives in designing new cosmetic formulations that reduce the skin barrier by exfoliating and removing dead cells from the *stratum corneum* surface.

4.4. Presentation of the most important results

The common denominator of the entire cycle of papers included in the scientific achievement is new solutions for increasing the efficiency of transdermal penetration of active ingredients in preparations applied to the skin surface.

The prototype of a new material containing microparticles incorporated in a polymer matrix

The concept of works [**H1-H5**] assumed encapsulation processes to enclose active ingredients in polymer microparticles (microspheres or microcapsules), which were then incorporated into polymer matrices.

In cosmetic products, the most frequently encapsulated are essential oils, fragrances, vitamins, and pigments, which are usually released during the mechanical destruction of the shell when using the preparation [21]. Active substances and medicinal products intended for transdermal administration until recently were often incorporated into liposomes. However, they are used less and less due to low stability and high production costs [22]. Lipid nanoparticles have recently become popular carriers of active substances as an alternative to liposomes. However, they are also not free from disadvantages, among which the low capacity of loading the carrier with the active substance or problems with stability during storage [23].

Due to the challenges of modern dermatology and the cosmetics industry, I proposed a new solution to increase the effectiveness of preparations applied to the skin. I have developed a prototype of a material composed of a polymer matrix in which microparticles have been incorporated with the encapsulated active ingredient. I assumed that such a material would be active against the *stratum corneum*, which is the skin's most important protective barrier component. The basis for the claim that the materials obtained as a result of the incorporation of microparticles in the polymer matrix will promote transepidermal (transepidermal) transport of active substances was the method of construction of these materials, conditioning their two-stage action. In the first stage, the contact of hydrated polymer matrices with the skin, as a result of occlusion, will contribute to weakening its barrier functions. In the next step, due to the slow degradation of microparticles, the active substance will be gradually released from them, which will be able to diffuse more freely into the deeper parts of the skin.

In the initial stages of the work, to prepare the matrix of the prototype of the new material, collagen isolated from northern pike scales was used by the previously developed methodology [24]. Retinyl palmitate was used as the active ingredient, and it was encapsulated in poly (vinyl alcohol) (PVA) capsules utilizing coacervation and cross-linking methods. The primary goal was the effective entrapment of retinyl palmitate in polymer microcapsules and then incorporating them into the structure of a three-dimensional collagen-based matrix. The detailed methodology of incorporating capsules into a three-dimensional, porous collagen matrix and the results of testing the properties of the obtained material are presented in

publication **H1**. To increase the stability of the prepared materials, the collagen chain cross-linking mixture: EDC/NHS (N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride) and N-hydroxy succinimide) was used. I also decided to check how adding different amounts of microcapsules would affect the selected properties of the final materials. For this reason, the microcapsule suspension was added to the collagen suspension at a concentration of 5 mg/ml in such amounts as to obtain mixtures, in which the weight ratio of collagen (Col) to microcapsules was 1:1 (Col/PVA 1: 1) and 1:10 (Col/PVA 1:10).

The analysis of materials by scanning electron microscopy (SEM) confirmed that microcapsules with a diameter range of 20-60 μm were incorporated in porous collagen matrices. The materials obtained had a porosity of 40% (Col/PVA 1:10) and ~50% (Col/PVA 1:1). The materials were characterized by high swelling capacity (decreasing with increasing content of microcapsules in the matrix) after swelling, they were elastic and adhered to the skin very well. Sample SEM photos of the obtained material are shown in Figure 2.

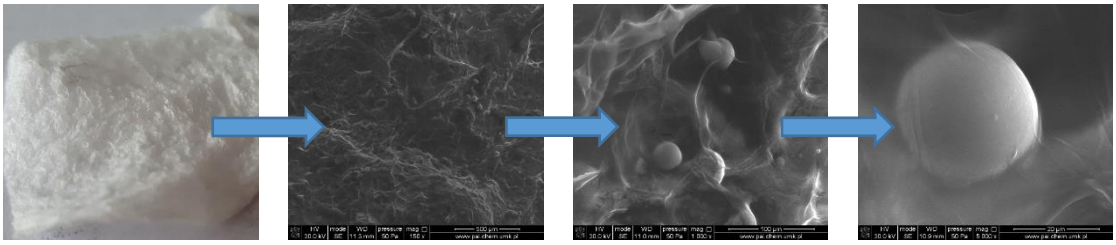

*Fig. 2. SEM photos of the collagen matrix with incorporated PVA capsules containing retinyl palmitate (Col/PVA 1:1) [**H1**]*

In the following stages of work, I decided to use collagen isolated from scales of *Esox lucius* to obtain microparticles. This protein was still the base component of the prepared matrix, which I simultaneously modified with the addition of other polymers (hydroxyethyl cellulose and gelatin), mainly to improve the mechanical parameters of the material. I also decided to use the marigold flower extract (*Calendula officinalis* flower extract) as a model active substance enclosed in microparticles. Marigold flowers contain many active substances, including carotenoids, polyphenols, coumarins, quinones, saponins, and triterpene alcohols, valued in the pharmaceutical and cosmetic industry for their antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, anti-swelling and anti-swelling properties. Therefore, marigold flower extract treats various skin injuries and diseases, such as burns, ulcers, inflammations, rashes, eczema, bruises, cuts, frostbite, and abrasions [25-27]. Selecting one active ingredient for further work allowed us to avoid additional variables. It made it possible to compare the

materials obtained, especially regarding the release profile of the active substance. The results of the work were presented in publications **H2**-**H4**.

The subject of the **H2** publication was the development of a methodology for obtaining gelatin (gel) and collagen-gelatin (col-gel) microspheres using various cross-linking techniques, as well as determining the release profile of the water-glycolic extract from marigold flowers. An important stage of the work was the assessment of the stability of the microspheres depending on the method of modification used. Gelatin or collagen-gelatin microspheres were prepared using the emulsion-cross-linking technique, and the EDC/NHS mixture and the vacuum dehydration (DHT) method were used as cross-linking agents. The obtained microspheres were immersed in the water-glycolic extract of marigold flowers for 24 hours. The comparative analysis of the results showed that the incorporation efficiency of the plant extract was more significant in the case of gelatin microspheres than in the case of collagen-gelatin microspheres, and taking into account the cross-linking technique, higher loading capacity of microspheres treated with high-temperature as a cross-linking agent was observed. However, the DHT cross-linked microspheres released the active substance much faster than chemically cross-linked microspheres.

For the following stages of the research work, I prepared the gelatin and collagen-gelatin microspheres again, which, taking into account the results of research from **H2**, this time was cross-linked only with a chemical agent - the EDC/NHS mixture (Figure 3).

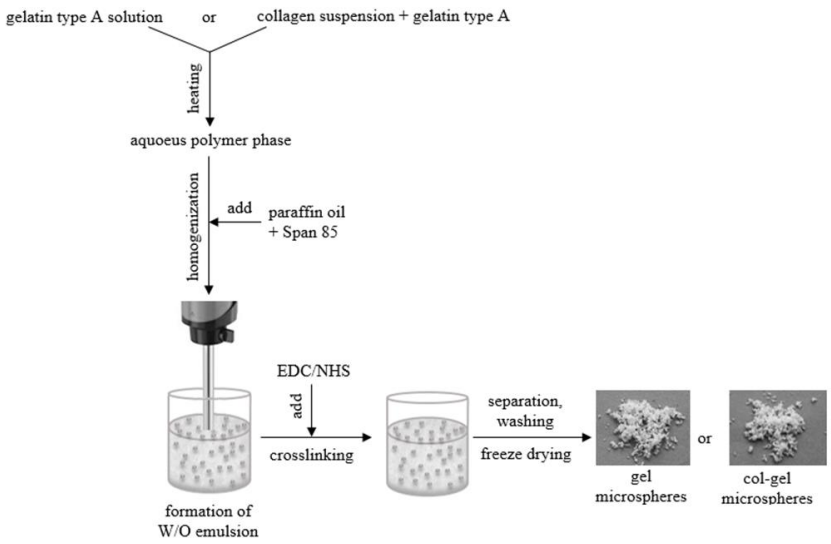

*Fig. 3. Scheme of preparation of gelatin (gel) or collagen-gelatin (col-gel) microspheres cross-linked with EDC/NHS mixture [**H3**]*

Spherical microparticles with dimensions of ~21 μm and ~26 μm, respectively, were obtained in the case of gelatin and collagen-gelatin microspheres. In the following stages of work, I prepared two types of porous matrices based on fish collagen containing the exact amounts of gelatin or collagen-gelatin microspheres:

• matrices modified with the addition of hydroxyethyl cellulose (col/hec) (**H3**);

• matrices modified with the addition of gelatin and hydroxyethyl cellulose (col/gel/hec) (**H4**).

The freeze-drying technique was used to obtain materials with a porous structure. It was observed that the obtained polymer matrices had a high swelling capacity, and after 30 minutes of incubation in PBS buffer pH 5.7, they reached the maximum degree of swelling. The matrices enriched with gelatin were characterized by higher swelling capacity. In contrast, the largest ones - the matrix consisting of collagen and gelatin with the addition of hydroxyethyl cellulose, not containing microparticles (swelling degree after 30 minutes was 3680%). In comparison, the least swellable were matrices based on collagen and hydroxyethylcellulose, containing gelatin microspheres in their structure (their swelling after 30 minutes was about 2400%). Moreover, significant differences were observed when comparing the stability of the obtained materials in phosphate buffer. The matrices consisting of collagen, hydroxyethyl cellulose, and gelatin became more stable than those without adding gelatin. After 28 days of incubation in phosphate buffer, their weight decreased by about 8.5-10%. By comparison, the degradation of the col/hec matrices after 28 days of incubation in buffer was within the range of 10-20% (depending on the type of incorporated microspheres).

The fusion of microspheres into the matrices did not significantly change the mechanical properties of the materials. On the other hand, it was observed that materials with incorporated microspheres absorbed higher amounts of marigold flower extract. The col/gel/hec matrices with the applied gelatin microspheres showed the highest efficiency of the active substance incorporation in the papers **H3** and **H4**.

Most importantly, the release profile of the extract was determined by the Folin-Ciocalteu method from marigold flowers by monitoring the amount of released phenolic compounds. The marigold flower extract incorporated in the tested matrices was released entirely after three days of incubation in an acetate buffer at pH 5.7. When comparing the release profile of the active substance, it can be seen that the extract was released faster from the col/hec matrices than from matrices modified with the addition of microspheres. About 50% of the active substance was removed from the matrices without microspheres during the 24 h

incubation of the samples in acetate buffer at 37°C. For comparison - in the case of matrices with the addition of microspheres, this value after the first day of incubation was about 25% and 37%, respectively, for the col/hec (gel) and col/hec (col-gel) matrices [**H3**].

A similar relationship was noticed when the release profile of the plant extract was assessed from col/gel/hec matrices [**H4**]. The active substance was released twice as fast from matrices without microspheres as from matrices modified by their addition. After the first day of incubation of the base matrices in the buffer, about 51.5% of the active substance was released from them. On the other hand, in the case of matrices with incorporated microspheres, this result was half lower and amounted to ~26%. This allowed for the conclusion that the structure of the matrices had a significant impact on the release profile of the extract. Along with the addition of microspheres to the matrices, a significant decrease in their porosity was observed, which in turn could slow down the diffusion of the extract. In the case of both types of matrices - col/gel and col/gel/hec- the extract release rate analysis confirmed the two-stage profile of this process. In the first stage, there was a rapid ejection of polyphenolic compounds from the samples, followed by a gradual, sustained release of the flower extract of *Calendula officinalis*. This burst may be related to the initial and rapid release of the extract adsorbed on the matrix surface. On the other hand, the second phase of extract release, characterized by a much milder course, could result from its gradual diffusion from microparticles and matrix pores.

To sum up - I obtained new, porous three-dimensional polymer materials with incorporated microspheres with *Calendula officinalis* flower extract, intended for application to the skin. The obtained results conclude that the properties of this type of material can be regulated by changing both the matrix composition and microspheres, depending on the needs and parameters of the final material.

In the next stage of the work, I decided to extend the research on the obtained matrices based on fish collagen, gelatin, and hydroxyethyl cellulose in terms of the possibility of including other microparticles containing encapsulated plant extract in their structure. For this purpose, I used gellan gum (GG) and xanthan gum (XG) to prepare the microparticles, and to obtain them, I used two techniques - extrusion and emulsification. In varying amounts, microparticles were incorporated into the matrix structure (Table 1). The test results of the obtained materials are presented in [**H5**].

*Table 1. Composition of materials in [**H5**] (COL - collagen, GEL - gelatin, HEC - hydroxyethyl cellulose, GG - gellan gum, XG - xanthan gum)*

	The weight ratio of polymers (%)			**(% w/v)**
	COL	**GEL**	**HEC**	**Microspheres***
COL/GEL/HEC	25	25	50	-
COL/GEL/HEC + 1.5% (GG)	25	25	50	1.5
COL/GEL/HEC + 3% (GG)	25	25	50	3.0
COL/GEL/HEC + 1.5% (GG + XG)	25	25	50	1.5
COL/GEL/HEC + 3% (GG +XG)	25	25	50	3.0

* microspheres were obtained by extrusion or emulsion method

The first significant difference concerned the microspheres size (Table 2). The microspheres obtained by the emulsion method were much smaller than the microspheres obtained by the extrusion technique.

*Table 2. Sizes of microspheres obtained by extrusion and extrusion techniques [**H5**] (GG - gellan gum, XG - xanthan gum)*

microspheres	**swollen (μm)**		**dry (μm)**	
	extrusion method	**emulsion method**	**extrusion method**	**emulsion method**
GG	1214 ± 31	219 ± 15	384 ± 52	5,75 ± 1,16
GG + XG	1256 ± 41	226 ± 13	448 ± 23	7,05 ± 1,23

Despite the significant differences in the sizes of the microparticles obtained, it was observed that the active substance was wholly released from all tested microspheres within four hours of incubation in an acetate buffer. However, the analysis of the release profile of the extract allowed for the conclusion that adding xanthan gum to the microspheres extended the release time of the active substance. The extract was released much faster from gellan gum-based microspheres, primarily when the emulsion technique obtained the microspheres.

After analyzing all the results, it can be seen that the final parameters of the obtained material, such as porosity, density, mechanical properties, swelling capacity, and stability, are significantly dependent on the amount of added microparticles and their size. However, the most important information was obtained by conducting *in vitro* cytotoxic tests using mouse fibroblasts (3t3). It turned out that the col/gel/hec matrices modified with a 3% addition of gellan gum microspheres obtained with the emulsion technique ensure high cell viability. Therefore, their potential practical use may be extended in the future for biomedical applications.

Modification of polymer matrices by adding penetration enhancers

To enhance the effectiveness of the penetration of active substances into the deeper layers of the skin, I also modified some of the polymer matrices with the addition of the **penetration enhancers**, i.e., components that help to reduce the barrier functions of the epidermis as a result of temporary changes in the lipid structure of the *stratum corneum* [28]. Due to their hygroscopic properties, penetration enhancers increase access to the water spaces between the lipid layers of intercellular cement, thus improving the penetration of hydrophilic substances through the skin barrier. The next part of the series of publications constituting the scientific achievement concerned the design and testing of polymer matrices with the addition of sorption promoters [**H6**-**H9**].

The **H6** publication aimed to obtain hydrogel matrices based on carrageenan containing various amounts of glycerin (an accelerant) and sorbitol and to evaluate the influence of these additives on the stability, swelling capacity, and mechanical properties of the obtained materials. There were prepared four types of hydrogel matrices containing a variable amount of glycerin and sorbitol, as well as equal amounts of carrageenan and active and auxiliary cosmetic ingredients such as hyaluronic acid, collagen, allantoin, panthenol, propylene glycol, soybean oil, grape seed oil, tocopherol, extract from goji fruit, phenoxyethanol and Polysorbate 20. The most important conclusion revealed that controlling the amount of glycerin and sorbitol could significantly influence the physicochemical parameters of the obtained hydrogel materials, especially their mechanical properties. The more sorbitol the samples contained, the stiffer they were and the lower their swelling capacity was. The model containing 20% sorbitol and 1.5% glycerin was the most optimal for cosmetic applications. The advantage of this hydrogel was its high resistance to degradation, thanks to which it can be stored in a hydrated state. In addition, the material adhered very well to the skin, which was tested by forming a hydrogel in the shape of an eye pad and its application to the skin around the eye. The hydrogel based on carrageenan, sorbitol, and glycerin presented in **H6**, enriched with the addition of active ingredients, is a product that can potentially find cosmetic or dermatological applications.

Sodium alginate and starch were other polymers used to achieve the goals of scientific achievement. These biopolymers were used to prepare materials in the form of films. Some of the films were modified with the addition of microspheres. The description of these materials preparation methods and their characteristics is the subject of publication **H7**. Films were prepared from sodium alginate and sodium alginate mixtures and starch (Fig. 4). Gelatin microspheres containing marigold flower extract were obtained per the previously developed

methodology [**H2**]. A series of films without microspheres was prepared, as well as a series with a 0.6% addition of microspheres. Glycerin was used as a sorption promoter and plasticizer, and it was added to the films in various amounts: 1.5%, 2%, and 2.5%.

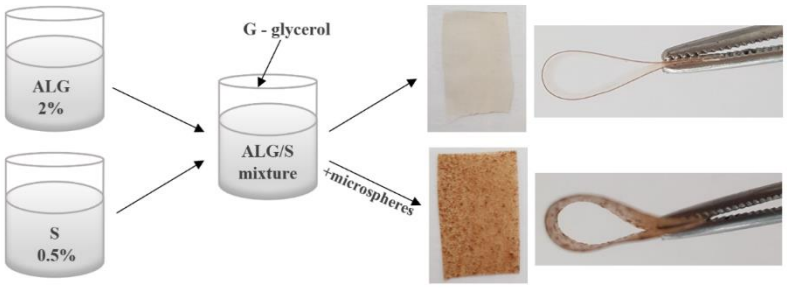

*Fig. 4. Scheme of obtaining films based on sodium alginate (ALG) and starch (S) with glycerin (G) and microspheres [**H7**]*

Most importantly, the results of the application tests of the obtained films confirmed that their modification with the addition of microspheres with marigold flower extract contributed to getting materials improving the condition of the epidermal barrier. The film based on sodium alginate and starch, containing 2.5% glycerin and microparticles with encapsulated plant extract, turned out to be the most promising for cosmetic applications.

When analyzing the release profile of the active ingredient, it was seen that it was gradually released from the materials obtained within 180 minutes of incubation in the acetate buffer. It can be seen that the addition of microspheres caused apparent changes in the mechanical parameters of the film (a reduction in the value of Youngs modulus was observed compared to the results of the film tests without the addition of microspheres). However, this did not degrade the functional properties of the films, and the swollen materials could be successfully applied to the skin.

Based on interesting results obtained during the work [**H7**], I decided to go a step further and add fatty components to the system at the film-making stage, which leads to emulsion films. The following paper [**H8**] focuses on developing a methodology for obtaining film emulsion materials and their modification by incorporating microparticles with plant extract.

Emulsions are colloidal systems consisting of two immiscible liquids where the inner phase is dispersed in the outer phase (called the continuous phase) in the form of fine drops. It is necessary to add emulsifiers to lower the surface tension between the phases. Emulsions are widely used in the cosmetics, pharmaceutical, and food industries. Classic cosmetic emulsions

- in the form of creams, lotions, or milk - have several advantages. Thanks to the base components appropriately selected for the active substance, enhancing the therapeutic effect of the preparation can be obtained. Thin emulsion films have recently gained increasing interest in the packaging and food industries. The materials I propose in the form of emulsion films with embedded microparticles containing the active ingredient are an innovative idea for cosmetic applications and dermatological.

The publication **H8** presents the developed methodology for obtaining this type of material, where sodium alginate and gelatin were used to prepare a biopolymer film, and cottonseed oil, as well as beeswax, were used as the oil phase ingredients. Glycerin was added to each film as a plasticizer and, simultaneously, a sorption promoter. Several versions of emulsion films differing in the content of individual ingredients were developed to indicate the material with the best application parameters, which was then modified with the addition of microparticles with encapsulated marigold flower extract. This material turned out to be a film in which the concentration of sodium alginate (ALG) was 2%, gelatin (GEL) 1%, glycerin (G) 1%, cottonseed oil 2.4%, beeswax 1% (in total (L) lipids was 3.4%). Span-80 (0.7%) was used as the emulsifier. This film was modified with a 0.6% addition of microspheres containing plant extract (MPs). A biodegradable polymer - polylactide (PLA) was used to create the microspheres, and microparticles with a size of about 60 μm were obtained by the emulsion method. The diagram of the process of obtaining emulsion films is shown in Figure 5.

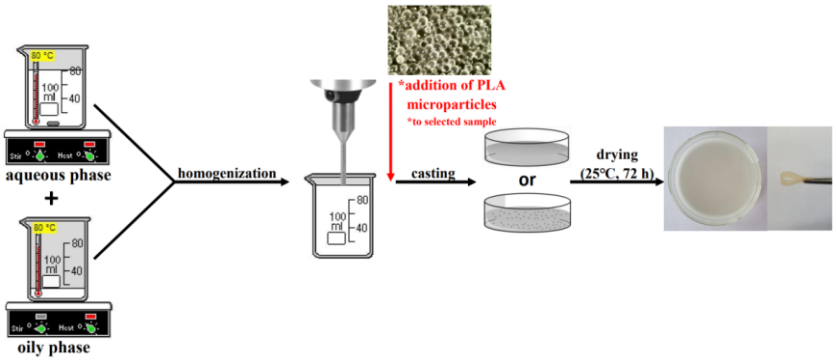

*Fig. 5. Scheme of preparation of emulsion films [**H8**]*

The obtained films were characterized in detail - the analysis included, among others, their mechanical parameters, surface properties (contact angle), transparency, moisture content, and particle size distribution of the dispersed phase. I compared the influence of the addition of fatty components on the properties of the films concerning non-emulsion films and the impact of the incorporation of microparticles in the selected film to a sample containing no

microparticles. The results confirmed that the properties of films based on sodium alginate and gelatin significantly change with the increase in glycerin. Emulsion films with a more significant addition of glycerin were more transparent and were characterized by greater flexibility, moisture, and thickness. The introduction of lipids (cottonseed oil and beeswax) also significantly changed the properties of the materials, especially the color, moisture content, and surface polarity of the films.

The important research stage assessed the cosmetic action of the obtained emulsion materials. These studies were carried out with the participation of 5 female probands, aged 24-27, under controlled conditions (temperature 20-22℃, air humidity 40-60%). The selection of probands took into account the Helsinki Declaration of 1964, the current EU legal regulations, and the Cosmetics Europe guidelines with the inclusion and exclusion criteria. The Local Bioethics Committee approved all the procedures. The volunteers selected for the study met the requirements for inclusion in the study, were acquainted with the purpose of the study and the method of its conduct, and signed a consent to participate in the study. Samples of the water-wetted emulsion films were placed on the inside of the probands forearms for 10 minutes; then they were removed from the skin, and measurements were taken at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours from removing the films from the skin. Measurements of selected skin parameters were performed, including skin hydration and the transepidermal water loss index (TEWL) compared to the control area of the skin (Fig. 6).

The research results showed the satisfactory cosmetic effectiveness of the ALG/GEL/G(1%)/L(3.4%)+MPs film, which contained 1% glycerin, 3.4% lipids, and 6% microparticles with encapsulated marigold flower extract. There was a visible increase in skin hydration and a slight and temporary decrease in its barrier properties in the absence of irritation.

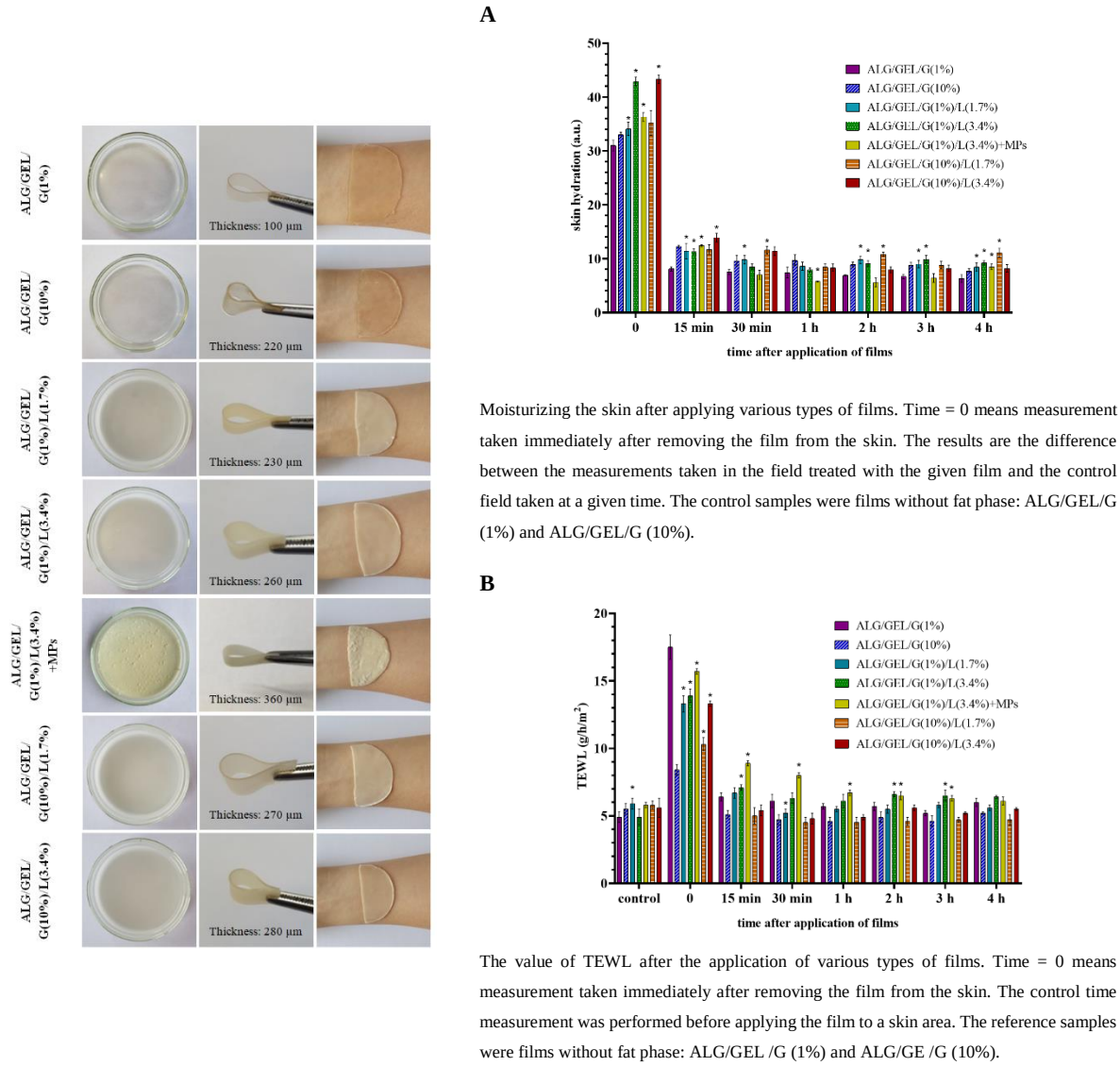

*Fig. 6. The obtained emulsion films based on sodium alginate and gelatin compared to non-emulsion films. Results of skin hydration (A) and TEWL (B) measurements [**H8**] (ALG – sodium alginate, GEL – gelatin, G – glycerin, L – lipids, MPs – microparticles)*

As I mentioned, emulsions are one of the most popular cosmetic forms due to several advantages. However, it should be emphasized that they are thermodynamically unstable systems that require a suitably selected emulsifier or a mixture of emulsifiers. Due to the high water content, proper maintenance of emulsion preparations is also necessary. Moreover, in an improperly composed practice, undesirable reactions between the components may occur, leading to a decrease in its therapeutic effect. The stability of the emulsion decreases with the time of its storage, which also limits its durability [29]. In order to meet these challenges and prevent the limitations mentioned above, I proposed an innovative solution - using the freeze-drying technique, I designed innovative, porous polymer-based emulsion materials for cosmetic

purposes. Freeze-dried emulsions combine the advantages of both emulsions and freeze-dried forms. Compared to conventional emulsions, freeze-dried emulsions have several technological advantages, such as extended shelf life, more accessible and cheaper transport, and high resistance to microbial contamination due to the very low water content. The procedure for obtaining three-dimensional matrices, as well as the results of their tests, are the subject of publication **H9**. In order to meet the assumptions, several mixtures were prepared with the composition analogous to that in the work **H8**, with a variable amount of glycerin penetration promoter, as well as with different content of fatty components (Table 3).

Table 3. Composition of matrices based on sodium alginate (ALG) and gelatin (GEL) with the addition of various amounts of glycerin (G), lipid components (L), or polylactide microparticles (PLA Mps) [H9]

	Water phase **[%]**			Oil phase **[L] [%]**			**PLA MPs**
	ALG	GEL	G	Cotton oil	Bee wax	Span80	**[%]**
ALG/GEL/G(1%)	2	1	1	-	-	-	-
ALG/GEL/G(10%)	2	1	10	-	-	-	-
ALG/GEL/G(1%)/L(1.7%)	2	1	1	1.2	0.5	0.35	-
ALG/GEL/G(1%)/L(3.4%)	2	1	1	2.4	1	0.7	-
ALG/GEL/G(1%)/L(3.4%)+MPs	2	1	1	2.4	1	0.7	6
ALG/GEL/G(10%)/L(1.7%)	2	1	10	1.2	0.5	0.35	-
ALG/GEL/G(10%)/L(3.4%)	2	1	10	2.4	1	0.7	-

To increase the obtained materials' stability, some were cross-linked by immersion in a calcium chloride solution, re-freezing, and freeze-drying. Then, it was assessed how the physicochemical properties of the matrices changed after cross-linking and re-lyophilization.

Marigold flower extract was encapsulated in polylactide microparticles, and the ALG/GEL/G(1%)/L (3.4%) matrix selected based on the analysis results was modified by including microparticles in its structure (Figure 7).

*Fig.7. Polylactide microparticles and ALG/GEL/G(1%)/L(3.4%)+MPs matrix containing 2% sodium alginate (ALG), 1% gelatin (GEL), 1% glycerin (G), 3.4% oil phase (L): 2.4% oil from cotton seeds and 1% beeswax, and 6% microparticles (MPs) [**H9**]*

There was a clear difference between the samples with 1% and 10% glycerin addition. After the first freeze-drying process, materials containing 1% glycerin were soft and had the porous structure, while matrices with 10% glycerin were sticky and flexible. However, these materials became more rigid after cross-linking and a second freeze-drying process. A porous structure with irregular macropores characterized the matrices with 1% glycerin. On the other hand, the porosity of the matrices containing the more significant addition of glycerin was much lower. The samples containing 3.4% lipids also showed lower porosity than matrices with 1.7% lipids. In addition, the obtained results confirmed that the processes of cross-linking and re-lyophilization contributed to the increase in porosity of samples containing 3.4% lipids and the control sample consisting of sodium alginate, gelatin, and 10% glycerin. The opposite effect was observed in the case of samples containing 1.7% lipids and in the case of the control sample with a 1% addition of glycerin. In turn, introducing microparticles into the matrix increased the porosity of the matrix.

The cross-linking process, variable amounts of glycerin, lipids, or the addition of microspheres significantly influenced other material properties, such as swelling capacity, stability, and mechanical properties. All dependencies are described in detail in **H9**.

We obtained valuable information by analyzing the release profile of the plant extract from a sample enriched with the addition of microparticles with encapsulated marigold flower extract. The active substance enclosed in the microparticles was released entirely within 7 hours of incubation of the sample in the acetate buffer, and the process proceeded in two steps. The first step lasted 6 hours, and during this time, approximately 51% of the plant extract was released from the matrix at a relatively constant rate. The second stage was very rapid - the remainder of the extract was removed from the material within 1 hour.

New peeling formulations containing biodegradable microparticles

One of the methods used to increase the penetration of active substances from the product applied to the skin is to partially remove the *stratum corneum* before applying the preparation to the skin. For this reason, it is recommended to use cosmetic peels. As a result of the implementation of the assumptions of the scientific achievement, to meet the requirements related to the obligation to withdraw microplastics from washable products, I offered new, alternative solutions in the formulation of cosmetic peels. As abrasives, I proposed microparticles based on biopolymers, and I prepared two types of microparticles with the

extrusion technique - microparticles based on sodium alginate [**H10**, **H11**, **PP1**] and mixtures of sodium alginate with starch [**H11**, **PP1**] (Fig. 8).

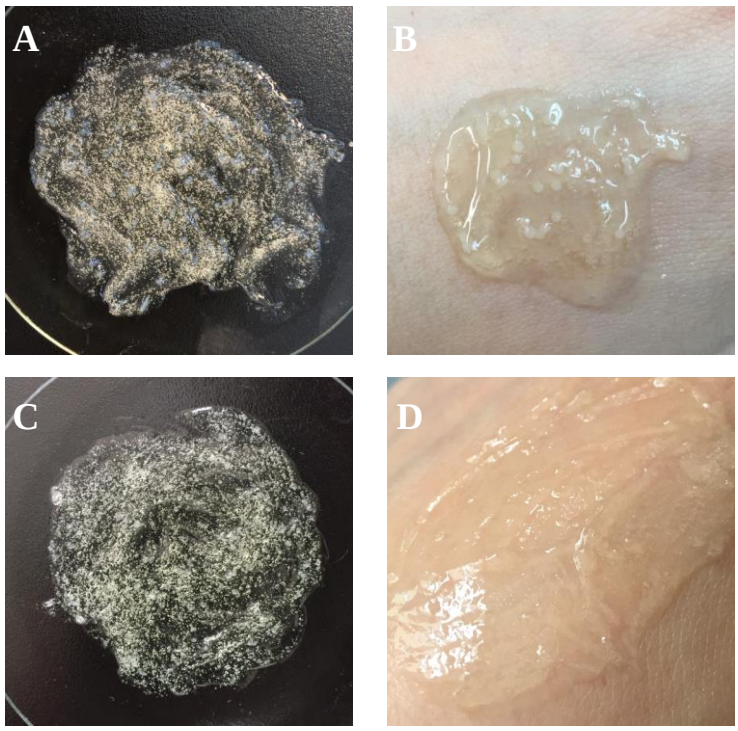

Fig. 8. A, B - peeling containing sodium alginate microparticles obtained by extrusion using the Büchi B-395 Pro encapsulator; C, D - peeling containing microparticles of sodium alginate and starch obtained by extrusion using the Büchi B-395 encapsulator [**H11**]

Regardless of their composition, all the obtained microparticles had a spherical shape, which is indicated for application in cosmetic peels. Then, the microparticles were suspended in a gel prepared according to an original recipe. The obtained preparations were assessed in terms of their cosmetic effect. The application tests confirmed the lack of adverse side effects on the skin, including irritation. In turn, the moisturizing properties of the obtained preparations were demonstrated, which is their great advantage. A temporary reduction in the epidermal barrier was observed for a few minutes after washing the skin, so the goal was achieved. However, after a short time from the application of the peeling, the TEWL index had the same values as before the application of the preparations, which confirms that the protective barrier of the epidermis was not damaged. The method of producing a cosmetic composition for the exfoliation of corneocytes containing microparticles based on sodium alginate or sodium alginate with starch has obtained patent protection [**PP1**].

An innovative idea was also to develop a formulation containing the enzyme bromelain in microparticles based on sodium alginate. A synergistic effect characterized the obtained

formulation - the preparation was intended for mechanical abrasion of corneocytes with polymer microspheres, which additionally contained an encapsulated enzyme, supporting the process of exfoliation of dead epidermal cells. This invention has also been granted patent protection [**PP2**].

4.5. Summary

The research problem that was the subject of my scientific achievement concerned the development of new preparations intended for application to the skin, the action of which will contribute to a temporary reduction in the *stratum corneum* barrier, which will result in an improvement in the therapeutic effectiveness of preparations containing active ingredients applied to the skin. To achieve the achievement goals, I used various factors and mechanisms influencing the increase in the penetration of active ingredients, such as **penetration enhancers** or the creation of **occlusions**. I also used the **encapsulation** method of selected active ingredients to increase their stability and control the release profile. In addition, I developed a methodology for obtaining biopolymer microparticles for their use as abrasives in the formulation of cosmetic **peels**, which reduced the skin's barrier by exfoliating and removing dead epidermal cells (corneocytes) from its surface.

The most significant achievements of my research can be summarized as follows:

- ✓ Development of a methodology for obtaining microparticles using natural and synthetic polymers - collagen, gelatin, sodium alginate, starch, gellan gum, polylactide - containing marigold flower extract as a model active ingredient [**H2-H5**, **H7**, **H8**, **H9**].
- ✓ Development of a methodology for obtaining microparticles based on poly (vinyl alcohol) (PVA) with encapsulated retinyl palmitate [**H1**].
- ✓ Obtaining and characterizing three-dimensional collagen-based matrices containing the resulting microparticles in the structure:
  - collagen matrices with PVA microparticles with retinyl palmitate [**H1**];
  - matrices based on collagen and hydroxyethyl cellulose with the addition of microparticles with marigold flower extract [**H3**];
  - matrices based on collagen, gelatin, and hydroxyethyl cellulose with the addition of various types of microparticles containing marigold flower extract [**H4**, **H5**].
- ✓ Preparation and characterization of polymer matrices containing penetration promoters:
  - in the form of a hydrogel based on carrageenan [**H6**];

- thin polymer films based on sodium alginate and starch with incorporated gelatin microparticles with marigold flower extract [**H7**].

✓ Obtaining new emulsion materials and their modification by adding microparticles and penetration promoters:

- preparation of emulsion films based on sodium alginate and gelatin containing polylactide microparticles with marigold flower extract [**H8**];
- preparation of porous emulsion matrices based on sodium alginate and gelatin containing polylactide microparticles with marigold flower extract [**H9**].

✓ Developing a recipe for new cosmetic peels:

- containing microparticles based on sodium alginate [**H10, PP1**];
- containing microparticles based on sodium alginate and starch as a replacement for synthetic microgranules [**H11, PP1**];
- containing microparticles of sodium alginate with an encapsulated enzyme [**PP2**].

A significant result of my research was the development of a **prototype of a material with an innovative design**, combining the advantages of microparticles (as carriers of active substances) and sorption promoters (increasing the penetration of active substances through the skin), incorporated in a matrix made of biodegradable polymers. I prepared the matrices in the form of thin **films**, **hydrogel**, or **three-dimensional porous samples**. These types of materials can become the basis for new forms of cosmetic and dermatological preparations. This prototype of innovative material for topic skin application tested with marigold extract can be modified depending on the skin's specific needs. Depending on the active/therapeutic substance encapsulated in microparticles, it can potentially be used as a controlled release preparation in skin care, treatment of dermatoses, or as a dressing material supporting the healing of wounds and burns. Further application research of this material, which I am currently working on, is recommended.

The practical use of the results of scientific achievement may, in the future, contribute to the development of technological innovations and their use in materials chemistry.

5. Presentation of significant scientific activity

Description of the scientific and research activity before obtaining the doctoral degree

I developed my interest in polymer biomaterials after defending my BA thesis in 2006 while continuing my studies at the Faculty of Chemistry at the Nicolaus Copernicus University in Toruń (2006-2008). Implementation of the assumptions of the masters thesis entitled *Influence of collagen addition on the properties of polyvinylpyrrolidone* prepared under the supervision of prof. Alina Sionkowska allowed a better understanding of the knowledge gained during the studies, especially in polymer chemistry. It also allowed applying this knowledge in practice in designing new polymer biomaterials. The main component of the materials I obtained and characterized was collagen, which I got from the rat tail tendons in laboratory conditions. At my masters thesis stage, I modified collagen materials by adding a biocompatible synthetic polymer - polyvinylpyrrolidone. The obtained materials were in the form of thin films, and their properties were modified by exposure to ultraviolet radiation. The research results, while still studying, were presented at a scientific conference (*V Polish - Ukrainian Conference: Polymers of special applications*, June 17-19, 2008, Święta Katarzyna, Poland). They were also published in 3 articles [30-32].

The acquired experience and knowledge strengthened my scientific interests and also became the motivation to start work in the field of chemistry of polymer biomaterials, as part of my PhD studies, which began in 2008 at the Faculty of Chemistry of the Nicolaus Copernicus University in Toruń, in the Biopolymer Group, at the Department of Chemistry and Photochemistry of Polymers (now the Department of Biomaterials and Cosmetics Chemistry). Research as part of the doctoral dissertation was partially carried out in cooperation with the Collegium Medicum NCU in Bydgoszcz and the Jagiellonian University in Kraków. The subject of the doctoral thesis concerned collagen-based composite materials and hydroxyapatite nanoparticles as potential biomaterials for tissue engineering [33-38]. The biocompatibility of the obtained materials was confirmed *in vitro* tests (using cell cultures) [37] and *in vivo* (animal models) [38]. These materials were characterized by high porosity, adequate resistance to degradation, high absorbency, and swelling capacity. Among the cross-linking agents tested, the most promising results were obtained using the thermal dehydration method. Moreover, the impact of the doctoral dissertation was the development of a new way of precipitation of calcium phosphate in the collagen matrix [36]. While implementing the assumptions of the doctoral thesis, I noticed the possibility of isolating collagen from another new source - the

scales of *Esox lucius* fish (northern pike). I was able to take up this challenge and implement it thanks to the Preludium grant from the National Science Center (NCN). The results of the research obtained under project No. UMO-2012/05 / N / ST8 / 02283 entitled *Fish collagen for biomedical, cosmetic and pharmaceutical applications (isolating and characterization of collagen from a new source)* were published in 4 publications [24, 39-41]. Managing the NCN project (2013-2015), as well as three grants from the Faculty of Chemistry at the Nicolaus Copernicus University from the statutory subsidy for young scientists and participants of doctoral studies (2011, 2012, 2013), taught me scientific independence.

Before obtaining the doctoral degree, I was a co-author of 11 publications in the journals awarded in the Journal Citation Reports, ten publications in the journals mentioned on the Ministry of Science and Higher Education list, and two peer-reviewed non-scored publications. The total IF ratio was 26.64, and the number of points from the Ministry of Science and Higher Education = 320. The research results were presented at international (45 presentations) and national (12 presentations) conferences in the form of 1 invited lecture, four scientific communications (3 I gave personally), and 52 posters (26 I gave personally). Some conference presentations were awarded:

• 2nd place in the competition for the best poster presented at the Regional Doctoral Seminar, October 24, 2008, Toruń, Poland (J. Kozlowska, A. Sionkowska, *The influence of polyvinylpyrrolidone on the structure of collagen*);

• First place in the competition for the best poster presented at the Biomaterials in Medicine and Veterinary Medicine conference, October 13-16, 2011, Rytro, Poland (J. Kozlowska, A. Sionkowska, *Preparation and properties of porous three-dimensional collagen scaffolds*);

• First place in the competition for the best scientific communication delivered at the 6th Copernican PhD Seminar, June 13-15, 2012, Toruń, Poland (J. Kozlowska, A. Sionkowska, *Composites of collagen and hydroxyapatite for tissue engineering*).

During my PhD studies, I received a scholarship under the project *Scholarships for PhD students 2008/2009 - ZPORR* (implemented under Measure 2.6 Regional Innovation Strategies and Knowledge Transfer of the Integrated Operational Program for Regional Development 2004-2006 and co-financed by the European Union from the European Social Fund and the state budget), as well as two scholarships under the project *Step into the future - scholarships for PhD students* (implemented under Sub-measure 8.2.2 Regional innovation strategies of the Human Capital Operational Program 2007-2013 and co-financed by the European Social Fund,

state budget and voivodship budget (3rd edition: October 2009 - July 2010 and 4th edition: October 2011 - September 2012).

I also participated in several training courses in the field of chemistry (including *Toruń School of Computational Chemistry - 1st edition: Organic Chemistry*, September 21-27, 2009, Faculty of Chemistry, NCU in Toruń; Training course in teaching and using computational chemistry methods at the Faculty of Chemistry Nicolaus Copernicus University in Toruń as part of the project *Strengthening the teaching potential of the Nicolaus Copernicus University in Toruń in the fields of mathematics and natural sciences*, September 2013).

In addition, I began to be interested in the possibilities of commercializing knowledge. I participated in several specialized training courses in this field (e.g., *Course on the commercialization of knowledge* carried out at the Marshals Office of the Kujawsko-Pomorskie Voivodeship; *Multidimensional promotion of the idea of spin-off* activity in the Kujawsko-Pomorskie Voivodeship organized by the European Center of Youth Co-operation). To pass the training on the project *Academic Entrepreneurship - the direction of tomorrow economy*, I proposed a business plan for a company that could arise due to the commercialization of my research, for which I received the leading award.

Scientific and research work was appreciated by the Rector of the Nicolaus Copernicus University in Toruń with a team distinction for achievements in science and research in 2010. In 2012, I received a prestigious award - the Minister of Science and Higher Education scholarship for outstanding achievements for 2012/2013.

On October 1, 2012, I was employed as an assistant in the Department of Chemistry and Photochemistry of Polymers, and from 2013 in the newly established Department of Chemistry of Biomaterials and Cosmetics. I defended my doctoral dissertation entitled *Collagen/ hydroxyapatite composites for biomedical applications* on October 22, 2014.

Description of scientific and research activity after obtaining the doctoral degree

Since 2017 I have worked as an assistant professor at the Department of Biomaterials and Cosmetics Chemistry. The knowledge gained so far, and my experience working with polymer materials allowed me to define new research directions after obtaining a doctoral degree. Participation in numerous international and national conferences permitted the exchange of experiences and the establishment of scientific cooperation. I managed a project financed by the National Science Center as part of the Sonata competition entitled *New*

materials containing microparticles incorporated into polymer matrix for medical, pharmaceutical, and cosmetic applications (2016-2022). Personal development in the subject of encapsulation of active ingredients resulted in receiving invitations to deliver thematic lectures. I presented two invited lectures at international conferences: *International Symposium on Encapsulation Technologies* in Tarragona (Spain) and the *7th International Caucasian Symposium on Polymers and Advanced Materials (ICSP&AM7)* in Tbilisi (Georgia). In addition, I gave two invited lectures during the meetings of the Scientific Groups: Scientific Club Materials in Medicine of the Gdańsk University of Technology and the Biocosmetology Section of the Scientific Club of Biologists of the University of Life Sciences in Lublin.

After obtaining the doctoral degree, 28 publications were published, of which I am a co-author, including 26 in journals with Impact Factor, located in the Journal Citation Reports database. Moreover, I am the author or co-author of 6 chapters in monographs and two patents. The total IF after the doctorate is 104.16. Part of the research work was carried out in cooperation with other centers, including foreign ones:

- Department of Mechanical Engineering, Universitat Rovira i Virgili, Tarragona, Spain [42];
- Department of Chemical Engineering, Universitat Rovira i Virgili, Tarragona, Spain [42, 43];
- Eurecat, Centre Tecnològic de Catalunya, Tarragona, Spain [**H5**, **H7**, 42, 43];
- Department of Chemical, Materials and Production Engineering, University of Naples Federico II; Napoli, Italy [42];
- Institute for Polymers, Composites and Biomaterials, National Research Council of Italy, Pozzuoli (NA), Italy [42];
- Procter&Gamble Services Company, Strombeek-Bever, Belgium [43];
- Institute of Physiological Chemistry and Pathobiochemistry, Münster, Germany [44];
- Department of Cellular and Structural Biology, UT Health Science Center, San Antonio, USA [44];
- Department of Dermatology, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, USA [44];
- Pathology and Laboratory Medicine Service, VA Medical Center, Birmingham, USA [44];
- Department of Dermatology, University of Münster, Münster, Germany [44];
- Department of Urology and Andrology, Faculty of Medicine; Nicolaus Copernicus University in Torun; Collegium Medicum in Bydgoszcz, Bydgoszcz [**H5**, 38, 42, 43];

- Department of Plastic Surgery, Faculty of Medicine, Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Bydgoszcz [38];
- Chair and Department of Clinical Pathomorphology, Faculty of Medicine, Nicolaus Copernicus University in Toruń; Collegium Medicum in Bydgoszcz; Bydgoszcz [38];
- Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków [37, 45].

In addition to research activities related to international cooperation, I was also involved in other research conducted by the team, and the results have been published in subsequent papers [46-48]. After obtaining my PhD, in addition to the already-mentioned NCN Sonata project, I also participated in implementing the NCN Opus project as a co-investigator [49]. Currently, I am a member of an interdisciplinary research team implementing a project entitled *Lyophilisate of glycosaminoglycans and collagen from fish skin for biomedical applications* led by Dr. Beata Kaczmarek-Szczepańska, which is financed by the Tango competition (NCBiR). In addition, I am a member of the research team implementing two grants under the Innovation Incubator UMK 4.0 projects.

In 2018 I did an internship under the Erasmus + STA program at Universitat Rovira i Virgili (Tarragona, Spain). I established a scientific cooperation that has continued to this day. The internship resulted, among others, in applying for a joint project application for Innovative Training Networks (ITN) under the Maria Skłodowska-Curie activities in H2020.

The NCU Rector has appreciated my scientific and research activity with numerous awards. Among them are six 2nd Team Awards of the Rector of the Nicolaus Copernicus University in Toruń for achievements in the field of science (2014, 2016, 2018, 2019, 2020, 2022) and one award for the 3rd degree (2015). In addition, publishing activity was honored with the NCU Rector awards (total 8) for highly scored scientific publications in journals with the assigned number of points from 140 to 200 on the Ministry of Science and Higher Education list. I received an award from the Dean of the Faculty of Chemistry for papers in highly-scored scientific journals in 2018. In addition, I received 2 Awards from the Dean of the Faculty of Chemistry of the Nicolaus Copernicus University in Toruń for obtaining funds under external grants.

In addition to publishing activities, the results of scientific research were also presented at many international and national scientific conferences in the form of scientific announcements (35) and posters (62).

Currently, I am the assistant supervisor of two PhD students. One of PhD students is pursuing doctoral studies at the Faculty of Chemistry of the Nicolaus Copernicus University in Toruń. The second is pursuing a doctorate at the Interdisciplinary Doctoral School of NCU *Academia Copernicana Interdisciplinary Doctoral School*. Research as part of the doctoral dissertation is carried out in cooperation with Lancaster University, United Kingdom (supervisor: Dr. Timothy Douglas). Some PhD students' papers are already published [50, 51].

Since 2014, I have been an ordinary member of the Polish Biomaterials Association (PSB), and since 2022 an ordinary member of the Polish Chitin Society (PTChit), the Polish Society of Cosmetologists Chemists (PTCK), and the European Society for Biomaterials (ESB).

After obtaining the doctoral degree, I reviewed over 70 publications in many scientific journals: Biomedicines (MDPI, IF= 4.757); Carbohydrate Polymers (Elsevier, IF= 10.723); Composites part B: Engineering (Elsevier, IF= 11.322); Cosmetics (MDPI); European Polymer Journal (Elsevier, IF=5.546); Fishes (MDPI, IF=3.170); Food and Bioprocess Technology (Springer, IF= 5.581); International Journal of Biological Macromolecules (Elsevier, IF= 8.025); Journal of Bioactive and Compatible Polymers (SAGE Journals, IF=2.137); Journal of Biomaterials Applications (SAGE Journals, IF=2.712); Journal of Cosmetic and Laser Therapy (Taylor & Francis, IF=1.982); Journal of Materials Research and Technology (Elsevier, IF= 6.267); Journal of Polymers and the Environment (Springer, IF= 4.705); LWT - Food Science & Technology (Elsevier, IF= 6.056); Marine Drugs (MDPI, IF=6.085); Materials (MDPI, IF= 3.748); Micromachines (MDPI, IF= 3.523); Molecules (MDPI, IF= 4.927); New Journal of Chemistry (The Royal Society of Chemistry, IF= 3.925); Pharmaceutics (MDPI, IF=6.525); Polymers (MDPI, IF= 4.967); Processes (MDPI, IF= 3.352); Reactive and Functional Polymers (Elsevier, IF= 4.966); Scientific Reports (Springer Nature, IF=4.996).

In addition, I am the editor of a special edition *Polymer-Based Nano/Microparticles* in the Polymers, MDPI [IF = 4.967; MNiSW = 100], as well as the editor of the special issue *Micro- and Nanoparticles in Biomedical and Cosmetic Applications* in Materials, MDPI [IF = 3.748; MNiSW = 140]. I am also a member of the board of reviewers of the journal Marine Drugs, MDPI [IF = 5.118; MNiSW = 100].

Another important step for my research activity was membership in the competition committee evaluating projects, which aims to support scientific initiatives of students and doctoral students, in line with the goals of the Nicolaus Copernicus University as a research university. In this competition, I was a substantive reviewer in the field of exact sciences, natural sciences, and technical (ST). In addition, I participated in the committee selecting the

best oral presentation and the best poster presented by PhD students participating in the international conference: *7th International Caucasian Symposium on Polymers and Advanced Materials (ICSP&AM7).*

I am a Toruń Center of Excellence member in the research area *Towards personalized medicine*. In January 2019, the competition *Priority research teams of the Nicolaus Copernicus University* was announced, addressed to employees of the Nicolaus Copernicus University who have demonstrated significant, documented scientific activity in the last five years, activity in the field of obtaining funds for research and human resources development and in terms of increasing the internationalization of the Nicolaus Copernicus University. This competition was part of a project implemented by Nicolaus Copernicus University in Toruń as part of the Strategy of Excellence - Research University project financed by the Ministry of Science and Higher Education. In April 2019, 16 priority teams were awarded, including the Interdisciplinary Innovation in Personalized Medicine Team (IPM Team), of which I am one of the leaders. The interdisciplinary team consists of employees and PhD students of the Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz (leader Prof. Barbara Bojko), Department of Urology and Andrology of the Faculty of Medicine of Collegium Medicum in Bydgoszcz (leader: Prof. Małgorzata Maj) and the Department of Chemistry of Biomaterials and Cosmetics at the Faculty of Chemistry of the Nicolaus Copernicus University in Toruń (leader: dr Justyna Kozlowska). Due to the NCU obtaining the status of a research university, the IPM Team has become a part of the Toruń Center of Excellence in the research area *Towards personalized medicine*, whose scientists focus on searching for new biomarkers of diseases, developing new therapeutic strategies, and regenerative medicine.

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science

Didactic activity

My didactic activity is related to the subject of cosmetic chemistry. Due to the creation at the Faculty of Chemistry of the Nicolaus Copernicus University, initially a specialization, and now the field of study - *Cosmetic chemistry* - I took an active part in creating new specialized subjects in this field and developing syllabuses. To improve my competencies, I completed post-graduate course at the Academy of Cosmetics and Health Care in Warsaw *Knowledge about cosmetics and cosmetic chemistry*. My final thesis was awarded and

published in Cosmetology Today [52]. I conduct my lectures with students of the Faculty of Chemistry of Nicolaus Copernicus University in the following subjects: *The structure of the skin and appendages* (15 h), *Recipe and form of a cosmetic* (15 h), *Cosmetic formula* (10 h), *Safety and effectiveness of cosmetics* (15 h), *Phytocosmetics* (15 h), *Marketing and PR in the cosmetics industry* (15 h), *Encapsulation methods in pharmaceutical techniques* (10 h). In addition, I have a 10 h general university lecture: *Modern trends in the cosmetics industry*. I developed the content and preparation instructions for many tasks as part of various laboratories and exercises in subjects such as *Safety and effectiveness of cosmetics, Care cosmetics, Cosmetic recipes, Cosmetic raw materials and the basics of cosmetics production*, and Phytocosmetics. I am a coordinator of several subjects (*Safety and effectiveness of cosmetics, Construction of skin and appendages, Marketing and PR in the cosmetics industry, Phytocosmetics, Encapsulation methods in pharmaceutical techniques*) and a head of laboratories: *Cosmetic formulation* and *Safety and effectiveness of cosmetics*. I also have exercises and research workshops on the *Universitas Copernicana Thoruniensis In Futuro II* project - modernization of the Nicolaus Copernicus University as part of the Universitys Integrated Program.

I am the author of 2 chapters: *Legislation* and *The structure of skin and appendages* in the academic textbook *Cosmetic chemistry - selected issues*, edited by A. Sionkowska.

To improve my qualifications, I completed a training course as part of the *Improving the Competencies of NCU teaching staff* (Operational Program Knowledge Education Development 2014-2020, NCBiR) covering issues related to Design Thinking, teaching with the use of educational games and other innovative didactic methods.

Currently, I am the assistant supervisor of two PhD students. So far, I have promoted 13 master's and 29 bachelor dissertations. I was also a reviewer of 10 MA theses and 28 BA theses. In addition, I was a mentor within the program of the Faculty of Chemistry of the Nicolaus Copernicus University, *Studies with a mentor*, and I mentored five students of Cosmetic chemistry. Mentoring support has resulted in their numerous successes, including two scholarships from the Minister of Science and Higher Education for outstanding achievements, scholarships from the President of the City of Toruń, the title of the best graduate of the Faculty of Chemistry at the Nicolaus Copernicus University or the award of the Polish Chemical Society for the best BA thesis. I encouraged students to present their research results at national and international conferences, where they won numerous awards and distinctions. I also supported them in writing their first research projects. I am pleased to be the content supervisor of 3 student grants as part of the *Initiative of Excellence - Grants4NCUstudents* competition.

In 2013/2014, 2014/2015, 2016/2017, and 2021/2022, I tutored first-year students of *cosmetic chemistry*. I was again entrusted with this function in the current academic year, 2022/2023.

In addition to active work with students of my home university, I also cooperate with international students, for whom I enable the implementation of a research internship under my supervision. The training is related to the current research interests regarding the microencapsulation methods of active ingredients in designing new materials for cosmetic and biomedical purposes. Among others, I was a student supervisor from the Université de Rennes in France during her internship at the Faculty of Chemistry of the Nicolaus Copernicus University 08/04/2019-21/06/2019 as part of the preparation for her diploma. From Polytech Nantes in France, I hosted two students for a 3-month internship under the *Erasmus* program and one student from the German Jordanian University for a 2-month internship from the same program. I was also a supervisor for a student from Universitat Rovira i Virgili (Reus, Spain) and students from NOVA School of Science and Technology (Lisbon, Portugal). They completed a 4-week internship at the Faculty of Chemistry at Nicolaus Copernicus University under the *TSSP NatSci - Toruń Students Summer Program in Natural Sciences*.

Organizational activities

Apart from research and teaching, I develop organizational skills. During my doctoral studies, I organized conferences for PhD students: the *3rd Regional Doctoral Seminar* (2009) and the *4th* and *5th Copernican Doctoral Seminar* (2010, 2011), which took place at the Faculty of Chemistry of the Nicolaus Copernicus University in Torun. The annual meetings of doctoral students from all over Poland resulted in creation of a new journal, Copernican Letters, of which I was one of the editors (vol.1 from 2010 and vol.2 from 2011).

After defending my doctoral dissertation, I am still actively participating in the *Copernican PhD Seminar* as a scientific committee member. In 2017 and 2018, the conference had international status. In addition, I was a member of the organizing committee of the *Chemistry for Beauty and Health* conference (June 8-10, 2017, Toruń), where I also reviewed the submitted abstracts of the presentation of the participants of the Young Sessions. In the next edition of the conference, which was already an international event, I performed similar organizational functions (*Chemistry for Beaty and Health*, June 13-16, 2018, Torun). In 2019, I participated in the organization of the 4th interdisciplinary conference, *NanoBioMaterials - from theory to application* (June 6-7, 2019, Toruń), during which I also chaired one session. I

also conducted one session during the international conference, the *7th International Caucasian Symposium on Polymers and Advanced Materials* (July 27-30, 2021).

I was part of the team preparing the self-assessment report in the field of cosmetic chemistry at the Faculty of Chemistry of Nicolaus Copernicus University in Toruń for PKA (2017). In the 2018/2019 academic year, I was a member of the Council of the Faculty of Chemistry at Nicolaus Copernicus University. Since 2019 I have been a member of the Discipline Council of the Faculty of Chemistry at Nicolaus Copernicus University.

In addition, I actively participate in the organization of campaigns promoting the Faculty of Chemistry of Nicolaus Copernicus University, taking part in the *open doors* or delivering popular science lectures for high and primary school students visiting our faculty. I also promoted our university in primary school in Kurzętnik, during event *Healthy skin - long life* was organized on November 16. The organizational activity was awarded the team distinction of the Rector of the Nicolaus Copernicus University in Toruń for achievements in the organizational field in 2020.

Activities popularizing science

I am also eager to take part in all kinds of events popularizing science. As a doctoral student, I actively participated in 4 editions of the *Toruń Festival of Science and Art* (2011, 2012, 2013, and 2014) and the *Toruń Scientists Night* in 2011. During these events, I co-organized and conducted workshops on formulating cosmetics.

After obtaining the title of doctor of chemical sciences, I developed the activity of disseminating and popularizing science. In addition to participating in the 16th and 20th Toruń Festival of Science and Art, I was invited several times by the Science Center: Centrum Nowoczesności Młyn Wiedzy in Toruń to appear as an expert at various thematic meetings. I participated in the following events in this place:

• the event: *I am a woman* - I acted as an expert in the field of cosmetics chemistry (interview, consultation) (17/09/2015),

• the event *I am a woman* - I also acted as an expert in the field of cosmetics chemistry (8.03.2018),

• ecological evening for adults - I gave a lecture at the invitation *Eco-revolution - the end of the plastic era* and consulted as an expert in the field of household chemicals and polymer chemistry (November 14, 2019),
• the event: *Naturally beautiful. I am a woman* - I acted as an expert in the field of cosmetic chemistry, conducted workshops with the participants of the event, and gave an invited lecture *Is chemistry harmful in cosmetics*? (March 5, 2020)
• event *I am a woman* - I gave a lecture, *What is hidden in the cream*? (03/04/2022).

The activities popularizing science also include cooperation with the DLA cosmetic company and joint recording of educational films made available on the websites of the company and the Faculty of Chemistry of Nicolaus Copernicus University.

In addition, since 2018, I have been participating in the University of Young People, an educational project organized by the Amicus Universitatis Nicolai Copernici Foundation, offering young people to expand their knowledge of chemistry. As part of the project, I conduct laboratory classes with primary or secondary school students.

7. Research plans

I am currently participating in research on cosmetic preparations intended for cancer patients. The work is carried out under the NCU 4.0 Innovation Incubator grant, one of which was establishing a university spin-off company. The assumption was successfully implemented, and since 2022, I have been a partner of NatChemLab Ltd. I educate and develop my skills in a systemic and algorithmic approach to inventiveness - I have already obtained the 1st degree of certification from the International TRIZ Association (MA TRIZ).

In the near future, I plan to expand my research interests and scientific cooperation to use encapsulation techniques in other key areas of today, such as agriculture and environmental protection.

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9. Scientific parameters of the entire publication output

Impact Factor*	**130.8**
Citations - without self-citations *	**781**
Hirsch index**	**15**
MSHE *	**2727**

* according to the year of publication

** according to the Web of Science Core Collection (27/10/2022)

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