DESIGN AND CHARACTERIZATION OF CHITOSAN AND CHITOSAN/PVA MICROBEADS FOR GALLIC ACID DELIVERY

Zuzanna Pawlak-Likus[1]*, Patrycja Domalik-Pyzik[2]

[1] AGH University of Krakow,
Faculty of Electrical Engineering, Automatics, Computer Science and Biomedical Engineering,
Department of Biocybernetics and Biomedical Engineering,
al. A. Mickiewicza 30, 30-059 Krakow, Poland
[2] AGH University of Krakow,
Faculty of Materials Science and Ceramics,
Department of Biomaterials and Composites,
al. A. Mickiewicza 30, 30-059 Krakow, Poland
*e-mail: zpawlak@agh.edu.pl

Abstract

Tissue engineering enables the development of tissues and organs that closely replicate physiological dimensions and functions. This field aims to address challenges related to organ transplantation, regenerative medicine, and the treatment of damaged tissues by designing biomaterials that can support cellular growth and tissue repair. One of the most important aspects of tissue engineering is the development of advanced delivery systems for drugs and active substances, which play a critical role in promoting regeneration. Controlled release, stability, and compatibility with the engineered environment are crucial parameters for these systems, as they influence the effectiveness and safety of therapeutic applications. In this study, microbeads for active compounds delivery were designed using two materials: a chitosan-polyvinyl alcohol (9:1 CS:PVA) polymer blend and pure chitosan modified with a polyphenolic compound, gallic acid. The physicochemical properties of the obtained microspheres, such as swelling ratio, microstructure, wettability, and active compound release, were analysed. The 9:1 CS:PVA+GA composite demonstrated the most promising characteristics as an active substance carrier, particularly due to its favourable release profile. These results suggest that this material could be an effective drug delivery system that offers controlled and sustained release of therapeutic agents. Further research, especially investigating the biological properties of these materials, is needed to fully confirm their suitability for practical applications in drug delivery and tissue engineering.

Keywords: *tissue engineering, compounds delivery systems, microcapsules, polymer blends, chitosan*

[Engineering of Biomaterials 172 (2024) 10]

doi:10.34821/eng.biomat.172.2024.10

Submitted: 2024-10-08, Accepted: 2024-11-19, Published: 2024-11-21

Introduction

Medicine and pharmacy are increasingly focusing on the development of drug delivery systems (DDS), which enable the controlled release of active substances, enhancing therapeutic efficacy and minimizing side effects. Conventional drugs, such as tablets or syrups, are rejected from the body too quickly, which leads to difficulties in maintaining drug levels within the therapeutic window [1]. The short duration of conventional drug levels may be a problem in reaching the desired therapeutic effect. Drug concentration should always be between the minimum effective concentration and the toxic concentration, and to obey the limitations offered by the drugs, the investigation of DDS has started. It aimed to maintain a constant drug level at a specifically targeted point for a specific time [1,2].

Various strategies have been developed for delivering drugs using DDS, including nanocarriers, membranes, liposomes, polymeric systems, and hydrogels - the latter being particularly promising [3-5]. Hydrogel properties, such as high water capacity, can closely mimic native tissues, which is the main reason for their excellent biocompatibility. Additionally, their biodegradability, achievable through enzymatic, environmental or hydrolytic mechanisms, makes them highly desirable for DDS applications [6,7]. Their porosity and stiffness can be tunable, though their primary limitation is low tensile strength [5,7]. A key advantage of hydrogels is their ability to encapsulate cells, as well as micro- and macromolecules within their polymeric mesh [5,8]. There are multiple ways to functionalise hydrogels, one of which is the formation of an interpenetrating polymer network (IPN), where two or more polymers are entangled with an already cross-linked polymer structure [6,8,9]. This approach makes hydrogel physical properties more tunable and enhances drug loading capacity compared to conventional hydrogels [7]. Another strategy involves adding micro- and nanoparticles, which strengthen the mechanical properties of hydrogels and allow for extended and more controlled drug release profiles [7,8]. Another approach is polymer blending, what is achieved by physical mixing of two or more polymers [10]. With this method, it is possible to improve and customize polymer properties [11]. Polymer blending is attractive, particularly due to its cost-effective and not complicated process [10].

To increase the biocompatibility of DDS, the use of natural substances is a logical and effective approach. During the preparation of matrices for active substances, two primary groups of natural materials are emphasized: polysaccharides and proteins [12]. Among polysaccharides, particular attention is given to chitosan (CS) and hyaluronic acid (HA) [12]. The first one is reported to possess great biocompatibility, bioactivity, and non-toxic nature [12,13]. Chitosan is an excellent natural biopolymer for delivering plant-based compounds, including polyphenols such as curcumin or quercetin [14]. Its properties enhance the mucoadhesion, solubility, dissolution rate, and targeted delivery of these bioactive substances, making it a highly effective carrier [14]. Natural polyphenols exhibit diverse biological activities, including antioxidant, anticancer, antibacterial, antiviral, anti-inflammatory, and immune-regulatory properties, while also protecting cells from UV-induced damage and oxidative stress [14].

Gallic acid is a phenolic acid that is a very common compound in many plants [15]. It can be found in plants such as grapes, tea leaves, sumac, and gallnuts [16]. It can exist as a free molecule, but also as a chemical component in tannic acid [16,17]. Gallic acid is a well-known ingredient of Chinese gall, present in many prescribed Chinese herbs [18].

Besides being easy to find, it possesses also a lot of other advantages. It was proven that gallic acid has antibacterial, anti-inflammatory, anti-carcinogenic, antioxidant, and antifungal properties [16,19-22]. What is more, it can be consumed up to 5000 mg/kg body mass without any toxic effects [20]. It has selective cytotoxicity for cancer cells, while being neutral for normal cells [20]. Gallic acid can suppress tumour angiogenesis, induce apoptosis in different cancer cells, or inhibit cancer cell growth [17,19,20].

This study focused on the preparation and characterization of microcapsules composed of chitosan and chitosan/poly(vinyl alcohol) blend, both with and without the addition of gallic acid. The microcapsules were tested to determine their structure and properties. The microstructure was examined using a digital microscope, while spectrographic analysis provided insights into the material structure. Absorption studies were conducted to determine shrinkage and the capacity of the microcapsules to absorb active substances. Additionally, foils were prepared to evaluate the hydrophilicity of the materials through wettability testing. Finally, the release profile of the active compound was measured.

Materials and Methods

First, a 1.5% chitosan solution (CS, Sigma-Aldrich, high molecular weight) was prepared by dissolving chitosan powder in a 1% acetic acid (POCH, 99.5%) aqueous solution. Next, a 10% poly(vinyl alcohol) (PVA, POCH, molecular weight 72,000) solution was prepared by dissolving PVA powder in distilled water, which was stirred on a magnetic stirrer at 80°C for 24 h to ensure complete dissolution. The chitosan solution was then mixed with the PVA solution in a 9:1 CS:PVA volume ratio. Gallic acid (GA, Sigma-Aldrich) was added to the CS and CS:PVA systems to achieve a final concentration of 20 wt% relative to the dry polymer mass. This process resulted in the formation of four systems: CS, CS:PVA, CS+GA, and CS:PVA+GA.

Microcapsules preparation

A 5% tripolyphosphate solution (TPP, Acros Organics), supplemented with 1 ml of vanillin solution (Stanlab) and 1 ml of SPAN 80 (Sigma-Aldrich), was used as the cross-linking solution. To prepare the microcapsules, the previously prepared polymer solutions were loaded into a syringe with a 0.7 mm diameter needle, which had been filled to ensure a straight tip. Each solution was carefully dripped from the syringe, positioned on a stand, into the cross-linking solution, which was continuously stirred on a magnetic stirrer. The distance between the needle tip and the cross-linking solution was maintained at 6 cm. The mixture was left on the magnetic stirrer for 2 days to allow complete cross-linking of the capsules. Afterward, the microcapsules were collected by pouring the solution through a sieve. The microbeads were then washed with isopropanol (Avantor Performance), acetone (Avantor Performance, 96%), ethanol (Stanlab), and distilled water, using a laboratory centrifuge set to 500 rpm for 5 min. After washing, the microcapsules were placed in Petri dishes and dried in an incubator at 70°C for 24 h.

Microstructure characterization

The microstructure was tested using a digital microscope (Keyence VHX-900F). Photos of the microcapsules before and after drying were taken at various magnifications. Additionally, the diameters of each microbead set were measured and the average diameter together with the standard deviation were calculated.

Structure analysis

The attenuated total reflection (ATR) Fourier transform infrared spectroscopy (FTIR) method was chosen to analyse the structure of samples. The spectra were recorded with the Bruker Tensor 27 equipment at ambient conditions, with a resolution of 4 cm^{-1}, 32 scans, in the range 4000-600 cm^{-1}. The dried beads were pressed to the diamond crystal and 32 scans were performed to obtain samples spectra with a resolution of 4cm^{-1}, in the range 4000-600 cm^{-1}. The obtained graphs were analysed in Spectragryph software.

Swelling ratio

Before and after drying, 10 microcapsules from each set were weighed on an analytical scale (Sartorius). By comparing the weight of the dried beads with the mass in the hydrated state, it was possible to calculate their swelling. Three independent mass measurements were obtained for each material, from which the average was calculated. The formula used to calculate the swelling ratio (SR) is as follows:

$$\text{Swelling Ratio (SR)} = \frac{(M_{wet} - M_{dry})}{M_{dry}} \cdot 100\%$$

where M_{wet} is a mass of microcapsules before drying, and M_{dry} is a mass of dried microcapsules.

The percentage decrease in the diameter of the microcapsules after drying was calculated to determine their shrinkage. The formula used to calculate the percentage reduction in a microcapsule diameter was as follows:

$$P = 100 - X$$

where:

$$X = \frac{D_{dry} \cdot 100}{D_{wet}}$$

where D_{dry} is the diameter of dried microcapsules, and D_{wet} is the diameter of microcapsules before drying.

Wettability

To assess the wettability of the obtained materials, three foils were prepared from solutions of 9:1 CS:PVA + GA, CS + GA, and CS. The solutions were stirred on a magnetic stirrer at 70°C and then poured onto Teflon trays. The trays were then transferred to a hot plate set at 70°C. After 2 days, the trays were removed and three dried foils were obtained.

Water contact angle (WCA) measurements were performed on the three foils using a goniometer (Kruss DSA25E) at 20°C, employing the sessile drop method. For each material, 10 measurements were taken on both the top and bottom surfaces. The results from both sides were found to be very similar, so only measurements from one side of each foil were included in the analysis.

Bioactive compound release

To evaluate the release of the active substance, 5 mg of microbeads from each type were weighed and placed in separate tubes, each containing 5 mL of phosphate-buffered saline (PBS, PAN Biotech). The samples were incubated at 37°C. After 30 min, 1 mL of the solution was sampled from each tube and then replaced with fresh PBS. This process was repeated at 1 h, 2 h, 24 h, and 1 week. The amount of gallic acid released was quantified spectrophotometrically by measuring the absorbance at 260 nm. The measurements were made in triplicate.

Results and Discussion

Microstructure

The analysis of the microstructure of microbeads is very important, as morphology and functionality directly influence their performance in drug delivery systems. Particle size, surface smoothness, and uniformity affect key parameters of the release systems, i.e. active compound release rate, stability, and interaction with the surrounding environment. Qualitative and quantitative analyses of the microbeads were done using the Keyence digital microscope and appropriate software.

FIG. 1 and 2 present the loaded and unloaded hydrogel microspheres in a hydrated state. Notable differences are present in both size and colour, with the microspheres containing gallic acid being visibly larger. This increase in size can likely be attributed to the interaction between chitosan and gallic acid, which may promote swelling or changes in polymer behaviour during preparation. While the apparent surface roughness of the microspheres is generally comparable, the chitosan-only microspheres appear slightly smoother than their gallic acid-loaded counterparts. This difference in surface texture could also be linked to the presence of gallic acid, which may alter the polymer's surface properties.

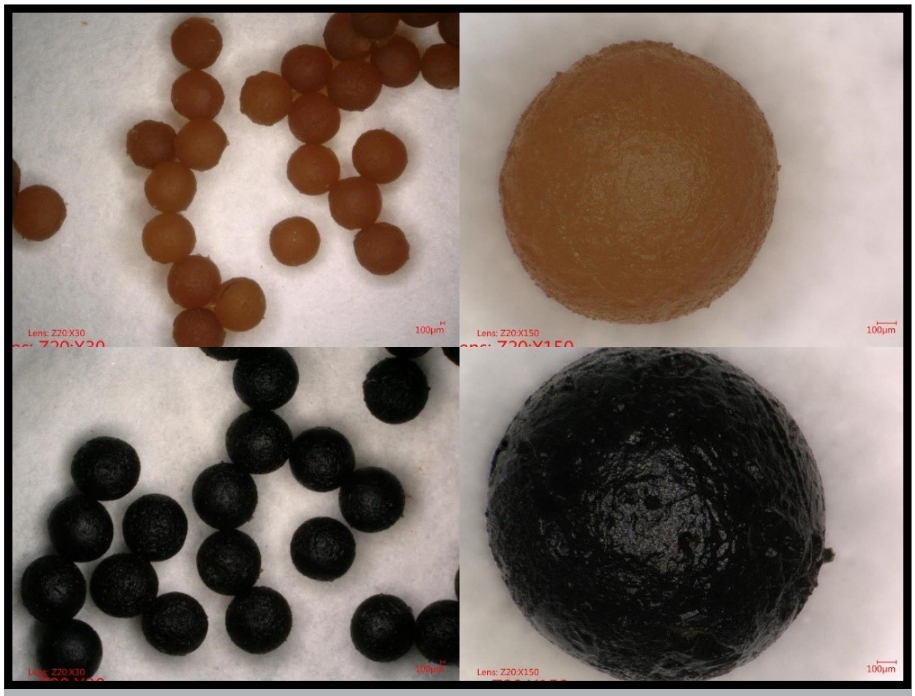

FIG. 1. Microcapsules before drying, at the top CS (from left: zoom 30x; zoom 150x); at the bottom CS+GA (from the left: zoom 30x; zoom 150x).

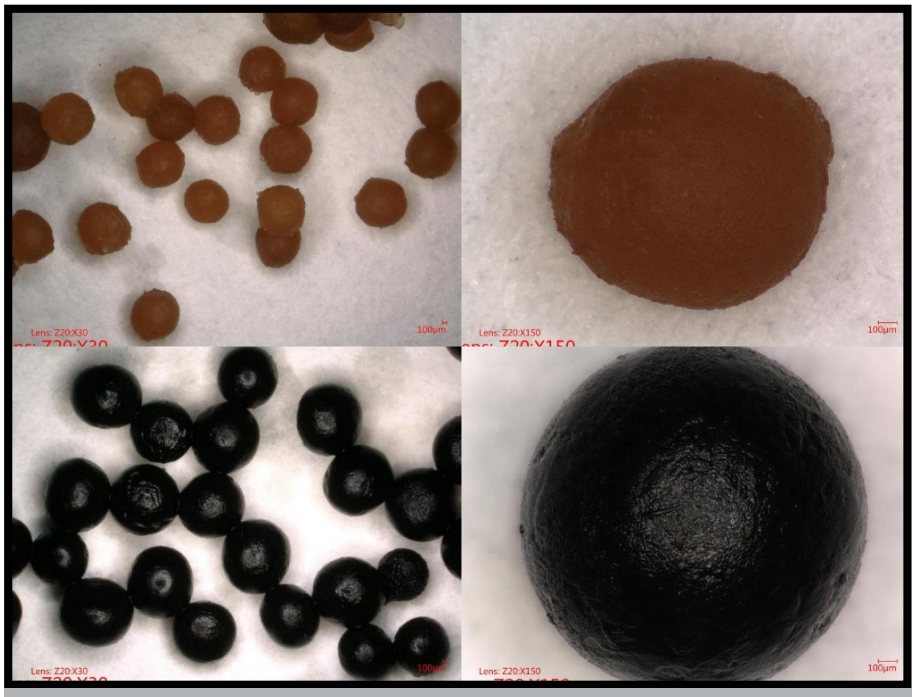

FIG. 2. Microcapsules before drying, at the top 9:1 CS:PVA (from left: zoom 30x; zoom 150x); at the bottom 9:1 CS:PVA+GA (from the left: zoom 30x; zoom 150x).

The average diameters of both wet and dry microbeads for each material are presented in FIG. 3. The measurements confirmed the initial microscopic observations that gallic acid-modified microbeads are larger in a wet state than unmodified materials (1823 µm and 1856 µm for CS+GA and CS:PVA+GA, respectively, versus 1411 µm and 1286 µm for CS and CS:PVA, respectively). This suggests that gallic acid may promote greater swelling or water retention. The differences were less evident in the dry state with average diameters for all sample types in the range of 565-690, but still the GA beads were slightly larger than the unmodified ones.

FTIR analysis

FTIR-ATR analysis was performed to assess specific chemical interactions, such as those between polymer matrix and gallic acid, which can influence the swelling behaviour, stability, and drug release profiles of the resulting microbeads. FIG. 4 presents the overlaid absorbance spectra of the 9:1 CS:PVA and 9:1 CS:PVA+GA microbeads. It shows a mild peak between 3500-3200 cm^{-1}, typical for hydroxyl and amino groups [23]. The interactions of gallic acid and chitosan can be particularly evidenced by the broadening and increase of intensity of this peak due to the formation of hydrogen bonds between the hydroxyl groups of gallic acid and the amine groups of chitosan. The two prominent peaks in the 3000-2800 cm^{-1} range are indicative of symmetric and asymmetric C-H bonds [23,24]. The peak around 1800 cm^{-1} is likely associated with C=O stretching vibrations, followed by two additional peaks that may correspond to the presence of the amide I and amide II groups [23]. The three smaller peaks in the 1500-1100 cm^{-1} range suggest the presence of C-H bending and C-N stretching vibrations [23]. Finally, the peaks between 1200-900 cm^{-1} are likely related to C-O stretching vibrations [23].

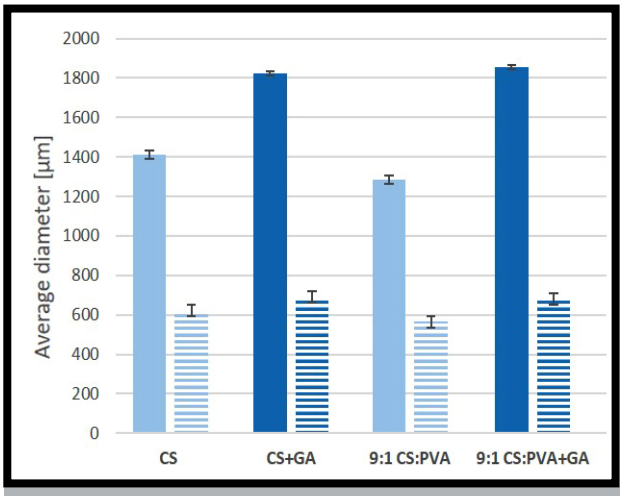

FIG. 3. Average diameter values for microbeads in a hydrated state (solid) and dried (striped) for each material. Results are shown with standard deviation poles.

As observed in the previous spectra, the broad peak in the 3500-3200 cm^{-1} region in FIG. 5 is likely attributed to O-H stretching vibrations [23]. No notable differences after the introduction of gallic acid are visible in this area. A less intense band in the 3000-2800 cm^{-1} range corresponds to symmetric and asymmetric C-H bonds [23,24]. Further peaks in the 1800-1500 cm^{-1} range suggest the presence of C-H wagging and bending vibrations. Two prominent peaks between 1150-1000 cm^{-1} may correspond to C-O stretching vibrations. The spectrum also reveals a peak near 800 cm^{-1}, which could be attributed to C-H bond rocking vibrations [23,24].

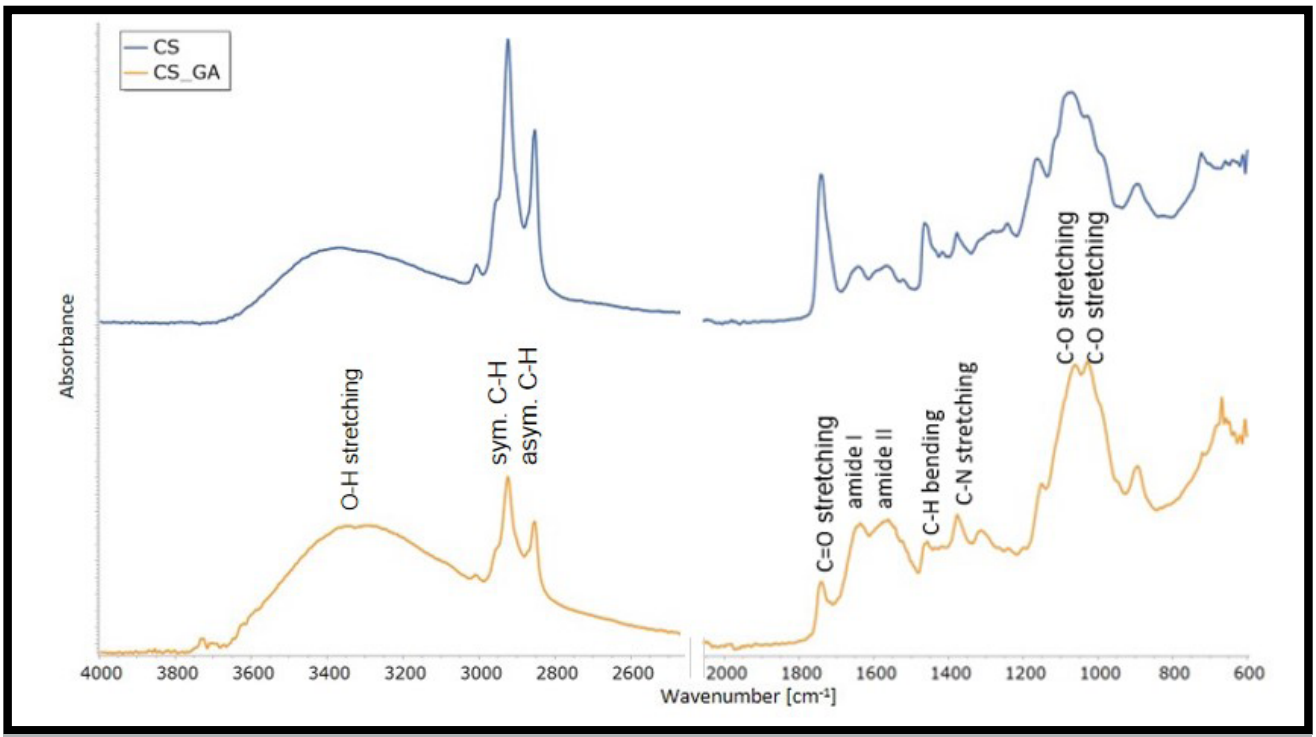

FIG. 4. FTIR-ATR spectra of CS (blue line) and CS+GA (yellow line).

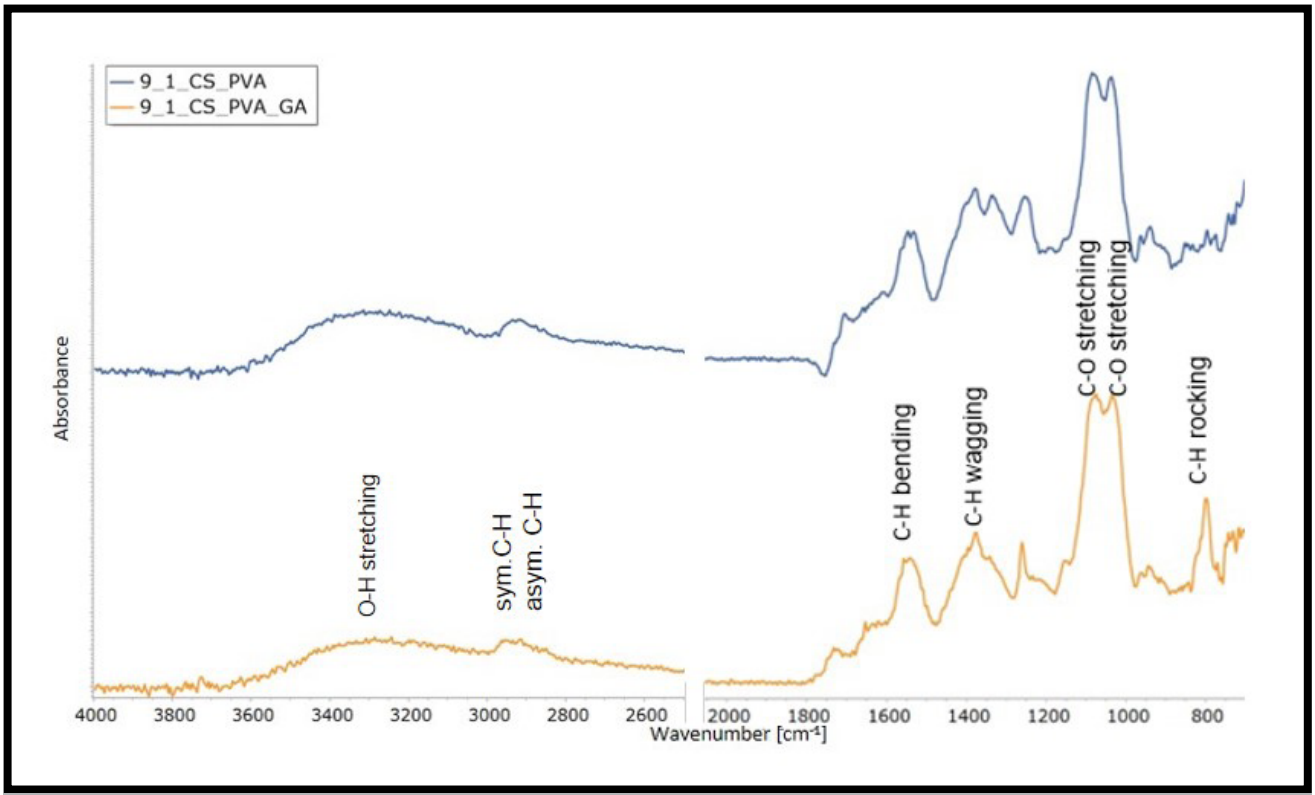

FIG. 5. FTIR-ATR spectra of 9:1 CS:PVA (blue line) and 9:1 CS:PVA+GA (yellow line).

Swelling ratio

Tests were conducted to determine the swelling ratio and shrinkage properties of microcapsules, which are crucial parameters for systems designed to deliver active compounds. These factors influence the capacity of the microbeads to absorb substances and the extent to which their size changes upon drying. FIG. 6 presents the absorption percentage for each material. The influence of gallic acid is evident, as the formulations containing the additive demonstrated higher absorption. The most visible difference was observed in the 9:1 CS:PVA polymer blend, where gallic acid increased absorption by nearly 5%, showing its beneficial effect on the system's performance.

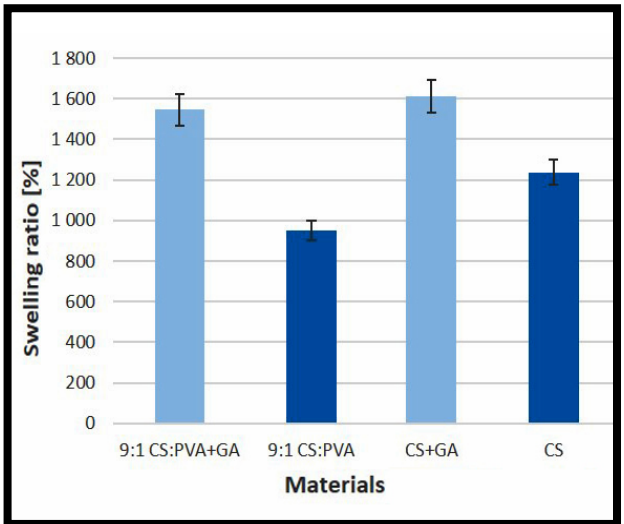

FIG. 6. The swelling ratio of the microbeads.

FIG. 7 shows the percentage decrease in the diameters of the microbeads as a result of drying. Samples containing gallic acid demonstrated noticeably higher shrinkage compared to those without the additive. This can be attributed to the interaction between gallic acid and the polymer matrix, which may lead to a more pronounced swelling behaviour in the wet state, followed by a greater contraction upon drying.

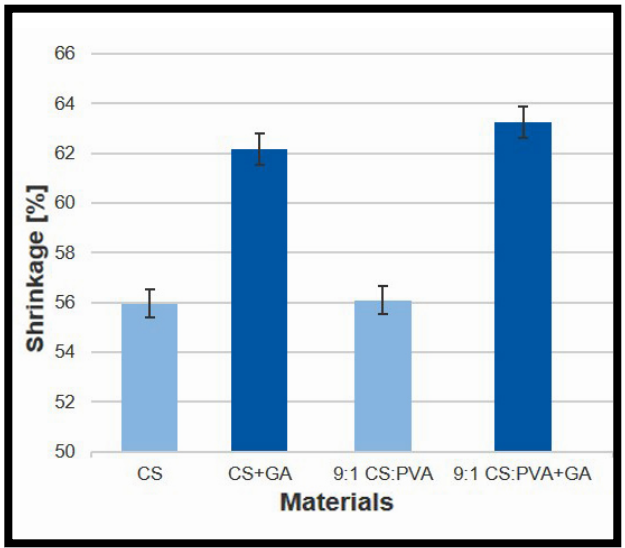

FIG. 7. Graph showing the percentage decrease in microbeads diameter as a result of drying.

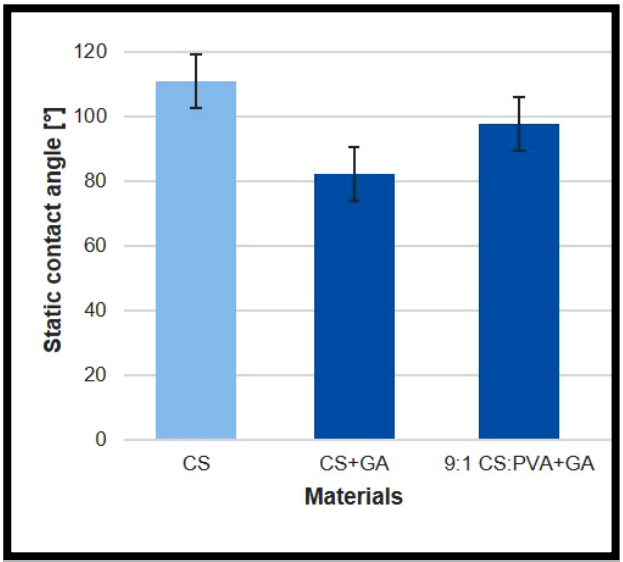

FIG. 8. Averages of static contact angle measurements along with standard deviation poles for individual films and a representative picture of a droplet on each material.

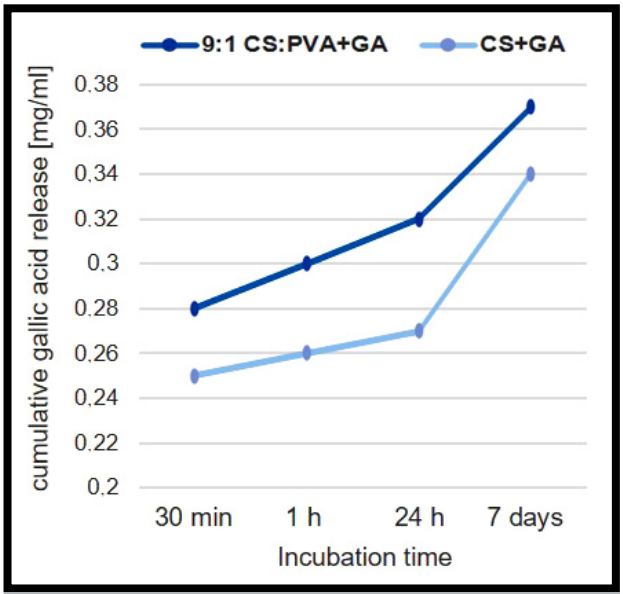

FIG. 9. Graph of the dependence of gallic acid release on incubation time for 9:1 CS:PVA+GA and CS+GA.

Wettability

The wettability of a hydrogel directly influences its swelling behaviour, drug release rate, and overall performance in biological systems. A hydrophilic surface, indicated by a lower contact angle, enhances the hydrogel's ability to absorb water, which is essential for its swelling and the controlled release of encapsulated drugs. On the other hand, a hydrophobic surface could limit water absorption and, consequently, drug release. FIG. 8 presents the contact angle measurement results, demonstrating a significant impact of gallic acid addition on the wettability of the materials. The WCA of CS+GA sample was nearly 30° lower than in the case of the reference CS sample. This means that the addition of gallic acid increased the surface hydrophilicity of the material. This can be attributed to the chemical structure of gallic acid, which contains three hydroxyl groups (-OH) [16]. These groups enhance its ability to interact with water molecules, likely through hydrogen bonding, thereby improving the water affinity of the modified material. The system based on the blended polymer matrix (CS:PVA+GA) shifted towards higher WCA values, showing a more hydrophobic character.

Bioactive compound release

Spectrophotometric analysis is an effective method for quantifying the release of gallic acid from a hydrogel matrix. The absorbance measurements were done at 260 nm in a 7-day perspective. To ensure that the observed changes show gallic acid release, control samples without the active substance were also tested, but, as expected, no absorbance was detected. The cumulative gallic release graph (FIG. 9) clearly indicates that the bioactive compound is being released from the polymer matrix over time. The 9:1 CS:PVA+GA system exhibited a slightly higher release profile compared to the CS+GA sample. This effect may result from weaker interactions between gallic acid and the blended polymer matrix. Pronounced interactions between gallic acid and chitosan matrix were confirmed in the FTIR analysis and were not that evident in the case of CS:PVA system.

Conclusion

In conclusion, this study successfully developed and characterised hydrogel microbeads designed for gallic acid delivery, using two material systems: a 9:1 chitosan-polyvinyl alcohol (CS:PVA) polymer blend and pure chitosan (CS). The microstructure of the microbeads was evaluated by digital microscopy, and their swelling ratio and shrinking ability were determined. Wettability was assessed using foils cast from the respective solutions, and Fourier-transform infrared spectroscopy (FTIR) with attenuated total reflection (ATR) provided insight into their structural properties. The addition of gallic acid positively influenced microcapsules' ability to uptake water and swelling capacity, which can be assigned to its hydroxyl groups enhancing hydrogen bonding interactions. Finally, the kinetics of gallic acid release from the microcapsules were analysed. Among the materials tested, the 9:1 CS:PVA+GA composite demonstrated the most promising characteristics as an active substance carrier, particularly due to its favourable release profile. However, further research, especially investigating the biological properties of these materials, is needed to fully confirm their suitability for practical applications in drug delivery and tissue engineering.

Acknowledgement

This study was supported by the subsidy (No 16.16.160.557) for the AGH University of Krakow.

References

[1] S. Adepu, S. Ramakrishna: Controlled drug delivery systems: Current status and future directions. Molecules 26(19) (2021), doi:10.3390/molecules26195905.
[2] F. Sabbagh, B.S. Kim: Recent advances in polymeric transdermal drug delivery systems. J. Control. Release 341 (2022) 132-146, doi:10.1016/j.jconrel.2021.11.025.
[3] S. Bandopadhyay, S. Manchanda, A. Chandra, J. Ali, P.K. Deb: Overview of different carrier systems for advanced drug delivery. Elsevier Inc., 2019.
[4] M. Vigata, C. Meinert, D.W. Hutmacher, N. Bock: Hydrogels as drug delivery systems: A review of current characterization and evaluation techniques. Pharmaceutics 12(12) (2020) 1-45, doi:10.3390/pharmaceutics12121188.
[5] J. Li, D.J. Mooney: Designing hydrogels for controlled drug delivery. Nat. Rev. Mater. 1(12) (2016) 1-18, doi:10.1038/natrevmats.2016.71.
[6] Z. Zou et al.: A sodium alginate-based sustained-release IPN hydrogel and its applications. RSC Adv. 10(65) (2020) 39722-39730, doi:10.1039/d0ra04316h.
[7] T.R. Hoare, D.S. Kohane: Hydrogels in drug delivery: Progress and challenges. Polymer (Guildf) 49(8) (2008) 1993-2007, doi:10.1016/j.polymer.2008.01.027.
[8] S. Jacob, A.B. Nair, J. Shah, N. Sreeharsha, S. Gupta, P. Shinu: Emerging role of hydrogels in drug delivery systems, tissue engineering and wound management. Pharmaceutics 13(3) (2021), doi:10.3390/pharmaceutics13030357.
[9] W. Wei et al.: Synthesis and characterization of a multi-sensitive polysaccharide hydrogel for drug delivery. Carbohydr. Polym. 177 (2017) 275-283, doi:10.1016/j.carbpol.2017.08.133.
[10] M. Kaur et al.: Chitosan-Based Polymer Blends for Drug Delivery Systems. Polymers (Basel) 15(9) (2023), doi:10.3390/polym15092028.
[11] N.N. Nyamweya: Applications of polymer blends in drug delivery. Futur. J. Pharm. Sci. 7(1) (2021), doi:10.1186/s43094-020-00167-2.
[12] X. Tong, W. Pan, T. Su, M. Zhang, W. Dong, X. Qi: Recent advances in natural polymer-based drug delivery systems. React. Funct. Polym. 148 (2020) 104501, doi:10.1016/j.reactfunctpolym.2020.104501.
[13] T. Kean, M. Thanou: Biodegradation, biodistribution and toxicity of chitosan. Adv. Drug Deliv. Rev. 62(1) (2010) 3-11, doi:10.1016/j.addr.2009.09.004.
[14] H.S. Rahman et al.: Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. Int. J. Nanomedicine 15 (2020) 2439-2483, doi:10.2147/IJN.S227805.
[15] N. Kahkeshani et al.: Pharmacological effects of gallic acid in health and disease: A mechanistic review. Iran. J. Basic Med. Sci. 22(3) (2019) 225-237, doi:10.22038/ijbms.2019.32806.7897.
[16] J. Zhao, I.A. Khan, F.R. Fronczek: Gallic acid, Acta Crystallogr. Sect. E Struct. Reports Online 67(2) (2011), doi:10.1107/S1600536811000262.
[17] Y. Lu et al.: Gallic acid suppresses cell viability, proliferation, invasion and angiogenesis in human glioma cells. Eur. J. Pharmacol. 641(2-3) (2010) 102-107, doi:10.1016/j.ejphar.2010.05.043.
[18] B. Zhao, M. Hu: Gallic acid reduces cell viability, proliferation, invasion and angiogenesis in human cervical cancer cells. Oncol. Lett. 6(6) (2013) 1749-1755, doi:10.3892/ol.2013.1632.
[19] N. Oršolić, M. Kunštić, M. Kukolj, D. Odeh, D. Ančić: Natural Phenolic Acid, Product of the Honey Bee, for the Control of Oxidative Stress, Peritoneal Angiogenesis, and Tumor Growth in Mice. Molecules 25(23) (2020) 5583, doi:10.3390/molecules25235583.
[20] S. Choubey, S. Goyal, L.R. Varughese, V. Kumar, A.K. Sharma, V. Beniwal: Probing Gallic Acid for Its Broad Spectrum Applications. Mini-Reviews Med. Chem. 18(15) (2018) 1283-1293, doi:10.2174/1389557518666180330114010.
[21] B.H. Kroes, A.J.J. Van Den Berg, H.C. Quarles Van Ufford, H. Van Dijk, R.P. Labadie: Anti-inflammatory activity of gallic acid. Planta Med. 58(6) (1992) 499-504, doi:10.1055/s-2006-961535.
[22] H.M. Chen et al.: Gallic acid, a major component of Toona sinensis leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. Cancer Lett. 286(2) (2009) 161-171, doi:10.1016/j.canlet.2009.05.040.
[23] A.A. Menazea, A.M. Ismail, N.S. Awwad, H.A. Ibrahium: Physical characterization and antibacterial activity of PVA/Chitosan matrix doped by selenium nanoparticles prepared via one-pot laser ablation route. J. Mater. Res. Technol. 9(5) (2020) 9598-9606, doi:10.1016/j.jmrt.2020.06.077.
[24] K.V. Hiatsevich, K.S. Hileuskaya, V.V. Nikalaichuk, A.I. Ladutska, O.R. Akhmedov, N.N. Abrekova, L. You, P. Shao, M.M. Odonchimeg: Chitosan-Gallic Acid Conjugate with Enhanced Functional Properties and Synergistic Wound Healing Effect (preprint) (2024), 1-20, doi:10.21203/rs.3.rs-4982795/v1.