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Article

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Development and Investigation of Mucoadhesive Polymers Based on Chitosan for Intravesical Therapy

Intravesical drug delivery (IDD) refers to the administration of therapeutic agents directly into the urinary bladder through a catheter. Low permeability of the urinary bladder epithelium, poor retention of the therapeutic agents due to dilution and periodic urine voiding as well as frequent catheterizations (with potential risk of infections) are the major limitations of IDD used in the treatment of bladder-related disorders, such as bladder cancer. In this work, the mucoadhesive properties of polymeric materials based on chitosan, chitosan-gellan gum, and chitosan-Carbopol™ 940 containing sodium fluorescein (NaFI) were investigated for their potential application in intravesical drug delivery. The evaluation of mucoadhesive properties was carried out using an in vitro flow-through method with fluorescent detection that simulates the interaction conditions of polymers with the urinary bladder mucosa. Additionally, the release kinetics of NaFI from polymer compositions under conditions mimicking the physiological environment of the bladder was studied using a fluorescence spectrometry. The acquired data confirm the promise of using chitosan-based mucoadhesive polymers in developing systems for intravesical drug delivery, which could significantly enhance the efficacy of IDD therapy to treat urinary bladder-related disorders.

Keywords: chitosan, gellan gum, Carbopol™ 940, in situ gel, mucoadhesion, release, urinary bladder, intravesical drug delivery.

Introduction

Mucoadhesion is a critical property of materials that determines their capacity for prolonged interaction and adhesion to mucosal surfaces. This property holds a particular significance in the domain of transmucosal drug delivery, which is extensively utilized across various mucosal membranes, including ocular, nasal, rectal, and vaginal routes. Transmucosal delivery offers numerous advantages, including rapid absorption of active pharmaceutical ingredients into the systemic circulation and reduced degradation of drugs within the gastrointestinal tract, thereby presenting a promising strategy for a wide range of therapeutic applications [1–6]. In particular, mucoadhesive drug formulations can be especially beneficial for the local treatment of posterior segment eye diseases [7, 8] neurological disorders [9, 10] and urogenital diseases [11].

Intravesical drug delivery (IDD) involves the direct administration of active pharmaceutical ingredients into the bladder via a catheter through the urethra, proving to be highly effective for treating various conditions such as bladder cancer and interstitial cystitis. However, the effectiveness of this method is limited by several factors, including dilution and washout during periodic urination, which diminish the therapeutic efficacy and retention of the administered agents. Furthermore, the necessity for frequent catheterization poses potential risks of irritation, inflammatory reactions, and infections, making this procedure quite uncomfortable for patients [12–14].

To enhance the efficacy of IDD, mucoadhesive materials can be employed, which are capable of prolonging the retention time of the drug within the bladder cavity, thereby ensuring a more sustained therapeutic effect. These materials include hydrophilic polymers that are traditionally used as matrices in many formulations for transmucosal drug delivery, such as chitosan, carbopol (a weakly cross-linked polyacrylic acid), alginate, cellulose derivatives, etc. [15–17]. These macromolecules are able to interact with the glycosylaminoglycans/mucin present on the surface of mucus membranes through non-covalent interactions such as hydrogen bonding, electrostatic attractions, diffusion, and chain entanglement/interpenetration pro-

moting increased contact time with the mucosal lining [18]. The mucoadhesive properties of these polymers are mostly based on the mix of several mentioned mechanisms. Recent studies also indicate that thiolated polymers exhibit enhanced mucoadhesive properties due to the formation of disulfide bonds with mucin, further augmenting their ability to interact with mucosal surfaces [19–21]. Various strategies have also been introduced to improve the mucoadhesive properties of hydrophilic polymers by chemically modifying them with specific adhesive groups such as methacryloyl and maleimide moieties. These unsaturated functional groups are able to establish covalent bonds with cysteine residues within mucin glycoproteins through thiolene click Michael-type addition reactions to form strong mucoadhesive bonds [22–25].

The structure of the bladder wall, composed of transitional epithelium, plays a crucial role in the permeability and efficacy of drug delivery. The urothelium, consisting of multiple cellular layers, serves important protective functions and facilitates interactions with therapeutic agents, which can significantly influence treatment outcomes [26].

In this study, polymeric formulations based on chitosan with Carbopol™ 940 and gellan gum was developed. A comprehensive evaluation of their mucoadhesive properties was carried out, enabling an assessment of their potential application in intravesical drug delivery. The results of this research aim to contribute to the development of effective therapeutic strategies for the treatment of bladder diseases and to enhance the quality of life for patients.

Experimental

Materials

Chitosan (low molecular weight, Mw = 50–190 kDa), gellan gum (Phytagel™, Mw = 1000 kDa), sodium fluorescein (NaFl), urea, uric acid, and creatinine were obtained from Sigma-Aldrich (Germany); Carbopol™ 940 was sourced from Acros Organics (Belgium). All other chemicals were of analytical grade and used without further purification.

Development of Mucoadhesive Drug Formulations

The development of mucoadhesive drug formulations involved the systematic preparation of polymers, including chitosan, gellan gum, and Carbopol™ 940, mixed in 1:1 volume ratio containing sodium fluorescein (NaFl).

To prepare a 2 % chitosan solution, the polymer was dissolved in 100 mL of 0.1 M hydrochloric acid (HCl) to improve its solubility after which the solution pH was adjusted to pH 5.50 with 1 M NaOH. A 0.5 % Carbopol™ 940 solution was prepared by dispersing it in 50 mL of deionized water. A 0.5 % gellan gum solution was prepared by dissolving it in deionized water at 50–60 °C to ensure complete dissolution of the polymer, and a sodium fluorescein (NaFl) solution (concentration 0.1 mg/mL) was prepared in deionized water as a model drug compound.

Following the preparation of the polymer solutions, chitosan-based mixtures were formulated in a volumetric ratio of 50:50, combining chitosan with both gellan gum and Carbopol™ 940. This combination was found to balance optimal viscosity, mucoadhesion, and controlled release properties of the resulting formulations and to evaluate their potential applications in IDD.

Ex vivo Experimental Studies on Retention in Sheep Bladder Mucosa

Preparation of Artificial Urine Solution:

The artificial urine solution was prepared according to the methodology described in the literature [27]. Briefly, to achieve a final volume of 2000 mL, the following components were dissolved in deionized water at room temperature: urea (24.27 g), uric acid (0.34 g), creatinine (0.90 g), disodium citrate ($Na_3C_6H_5O_7 \times 2H_2O$, 2.97 g), sodium chloride (NaCl, 6.34 g), potassium chloride (KCl, 4.50 g), ammonium chloride (NH_4Cl, 1.61 g), calcium chloride dihydrate ($CaCl_2 \times 2H_2O$, 0.89 g), magnesium sulfate heptahydrate ($MgSO_4 \times 7H_2O$, 1.00 g), sodium bicarbonate ($NaHCO_3$, 0.34 g), sodium oxalate ($Na_2C_2O_4$, 0.03 g), sodium sulfate (Na_2SO_4, 2.58 g), disodium hydrogen phosphate ($NaH_2PO_4 \times H_2O$, 1.00 g), sodium phosphate dibasic (Na_2HPO_4, 0.11 g).

The prepared artificial urine solution with pH 6.20 was maintained at 37 °C throughout the experiment. This solution served as a physiological mimic to evaluate the retention and performance of the mucoadhesive formulations under conditions that closely resemble those in the urinary bladder.

Preparation of Sheep Bladder Mucosa:

The *in vitro* retention of chitosan and its polymeric mixtures with gellan gum and Carbopol™ 940 on sheep bladder mucosa was assessed using a modified experimental method described in the literature [24, 28]. Freshly collected samples of sheep bladder mucosa were obtained from the Zhetysu Abattoirs (Almaty, Kazakhstan). The collected biological tissues were transported to the laboratory in a frozen plastic container to minimize structural changes. Upon arrival, the bladder was dissected, and the obtained mucosal tissue sections were prepared for further experimental investigations.

The bladder tissues were placed in a specialized glass vessel designed to mimic the anatomical structure of the bladder, oriented with the mucosal surface facing upward. Prior to the experimental procedures, the tissues were rinsed with 3 mL of artificial urine solution (pH 6.20). All experiments were carried out in an incubator maintained at 37 °C to ensure physiological conditions.

Aliquots of 5 mL from the mucoadhesive formulations containing either chitosan, chitosan-gellan gum, or chitosan-Carbopol™ 940, all formulated with sodium fluorescein (NaFl), were aspirated and uniformly applied to the mucosal surface of the bladder and subsequently irrigated at a constant flow rate of 2.0 mL/min using a syringe pump. This setup facilitated stable distribution of the tested materials over a period of 250 min, allowing for comprehensive evaluation of their retention and mucoadhesive properties.

Fluorescent images of the bladder tissues were captured using an iPhone 13 Pro under ultraviolet lighting provided by a Winzwon UV Torch. At each time interval following rinsing with artificial urine, the obtained microscopic images were analyzed using ImageJ® software for quantitative assessment of pixel intensity. The pixel intensity of control samples (bladder mucosa without fluorescent material) was subtracted from the measured values, allowing the data to be converted into objective quantitative metrics.

The experiment parameters, including the setup of the equipment and the conditions of the study, are illustrated in Figure 1. The experimental apparatus comprised a microscope, ultraviolet (UV) light source, and bladder simulator, all securely positioned using laboratory stands. The distance between the microscope objective and the mucosal surface was standardized at 10 cm, with the UV light source also maintained at 10 cm from the mucosa. Additionally, the needle tip was positioned 10 cm away from the mucosal surface. Artificial urine was administered to the mucosal surface from a distance of 20 cm at a constant flow rate of 2.0 mL/min. To ensure statistical significance and the reliability of the results, all experiments were carried out in triplicate.

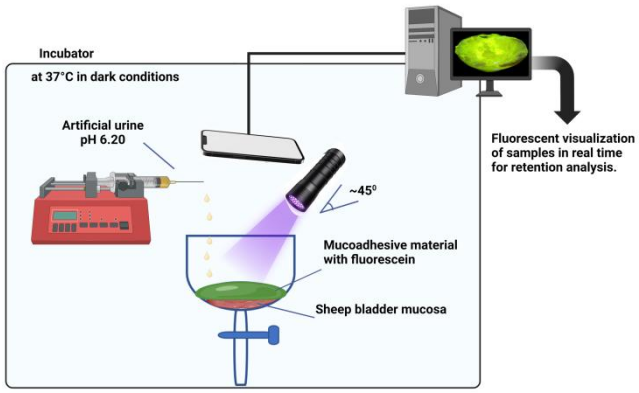

Figure 1. Experimental setup for investigating the retention of mucoadhesive formulations on the bladder mucosa

Release of the Model Drug Compound from Mucoadhesive Formulation

In vitro release of sodium fluorescein (NaFl) from polymeric materials was studied under controlled conditions using the dialysis method described in the literature [23]. Polymeric formulations containing NaFl, with a volume of 2 mL, were placed in a dialysis membrane and immersed in 30 mL of artificial urine solution (pH 6.20). The process was carried out at a temperature of 37 °C with continuous stirring at 80 rpm for 24 h.

At regular time intervals, aliquots of 5 mL were withdrawn from the dialysis solution and replaced with fresh artificial urine to maintain a constant volume within the system. The concentration of released sodium fluorescein (NaFl) was quantified using a fluorescence spectrometer (Varian Cary Eclipse fluorescence spec-

trophotometer, UK) by exciting the sodium fluorescein at $\lambda_{excitation}$ = 460 nm and detecting the emitted light $\lambda_{emission}$ = 514 nm, which corresponds to the peak emission of sodium fluorescein. The concentration of NaFl was determined based on the emitted signal at 514 nm, as it is directly related to the amount of fluorescein in the sample. Figure 2 presents the standard curve used for the quantitative analysis of the release. All release experiments were carried out with a minimum of three replicates to ensure statistical significance and the reliability of the results obtained.

Statistical Analysis

The obtained data were subjected to statistical analysis, including the calculation of means ± standard deviations. One-way analysis of variance (ANOVA) was employed, followed by Student's *t*-test and *post hoc* Bonferroni correction for multiple comparisons. All statistical calculations were performed using GraphPad Prism software (version 7.0), with $p < 0.05$ set as the threshold for statistical significance.

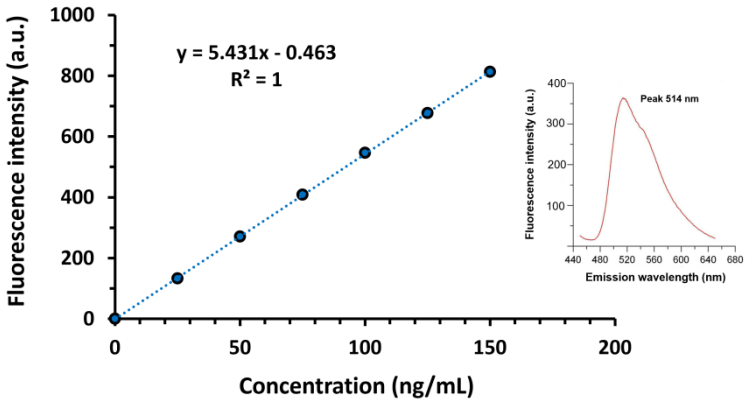

Figure 2. Standard curve used for the quantitative determination of sodium fluorescein (NaFl) released from polymeric material using a fluorescence spectrometer (Insert is an emission peak of NaFl)

Results and Discussion

Intravesical Delivery of Mucoadhesive Polymeric Materials

The mucoadhesive properties of polymeric materials based on chitosan, chitosan-gellan gum, and chitosan-Carbopol™ 940, containing sodium fluorescein (NaFl), were investigated using an updated version of in-house made *in vitro* flow-through method with fluorescent detection [28]. Sheep bladder mucosae were rinsed with 500 mL of artificial urine solution for 250 min period [24, 29]. The schematic of the experimental approach is presented in Figure 3.

In this study, gellan gum and Carbopol™ 940 were selected to enhance the mucoadhesive properties of chitosan. Chitosan is widely considered to be a "gold standard" mucoadhesive polymer due to its polyelectrolyte nature that usually exhibits superior mucoadhesive properties compared to non-ionic polymers [2, 15]. Gellan gum, a water-soluble anionic polysaccharide produced by the bacterium *Sphingomonas elodea*, undergoes a sol-to-gel transition in response to ion presence (ions present in urine), forming viscoelastic gels that enhance retention on mucosal surfaces. Carbopol™ 940, a weakly crosslinked synthetic polymer, provides a viscous gel environment, supporting drug retention on mucosal surfaces. Both polymers were chosen for their ability to further improve mucoadhesiveness of chitosan and extend drug release duration [30, 31]. This minimizes wash-out effects, thereby resulting in improved patient compliance and reduced administration frequency which is beneficial in IDD systems [2, 13].

Freshly excised samples of bladder mucosa were positioned in a specialized vessel designed to replicate the anatomical structure of the bladder and were rinsed with 3 mL of artificial urine solution. Subsequently, a fluorescent image of the mucosal surface was captured after the application of the polymeric material sample containing sodium fluorescein (NaFl), documenting the initial fluorescent image prior to rinsing. Throughout the experiment, microscopic images were analyzed at each rinsing time interval using ImageJ® software to quantitatively assess pixel intensity.

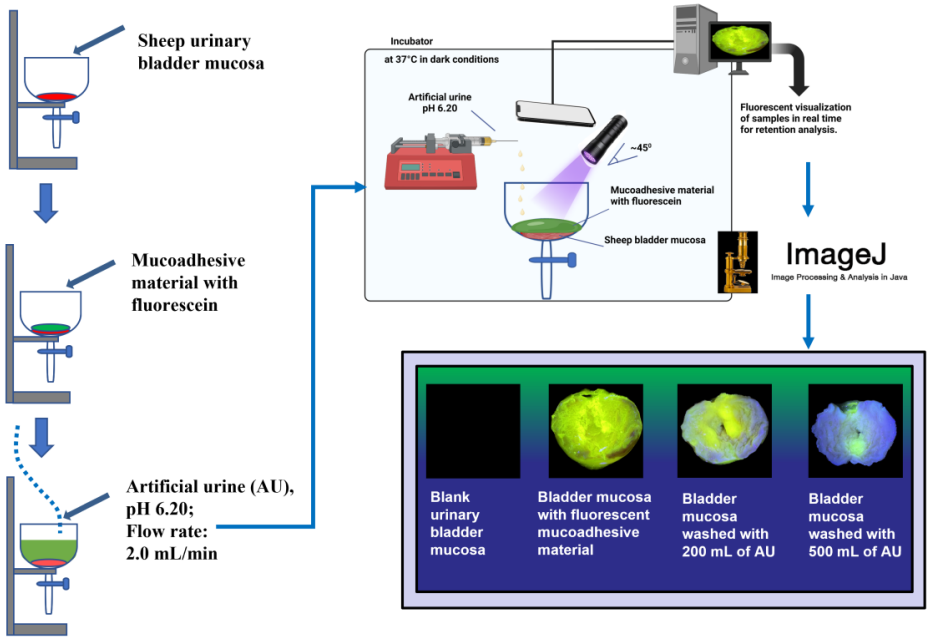

Figure 3. Stepwise process of the *in vitro* retention experiment on sheep bladder mucosa

The results of the fluorescent image analysis, illustrated in Figure 4, demonstrate the retention levels of chitosan and its mixtures with gellan gum and Carbopol™ 940 on the bladder mucosa following exposure to artificial urine. Analysis carried out using ImageJ® revealed that the retention of the chitosan-gellan gum and chitosan-Carbopol™ 940 mixtures on the bladder mucosa was statistically significantly higher compared to pure chitosan ($p < 0.05$). The enhanced mucoadhesive properties of the chitosan-gellan gum mixture can be attributed to the formation of an *in situ* gel within the saline solution. This phenomenon is further depicted in Figure 5, which presents the retention values for chitosan, chitosan-gellan gum, chitosan-Carbopol™ 940, and sodium fluorescein (NaFl) as the control.

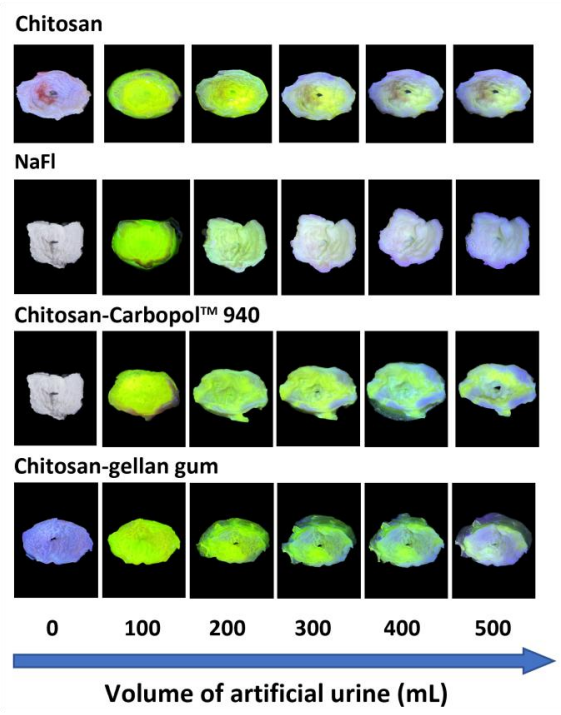

Figure 4. Fluorescent images illustrating the adhesion of polymeric materials to sheep bladder mucosa washed with artificial urine solutions of varying volumes

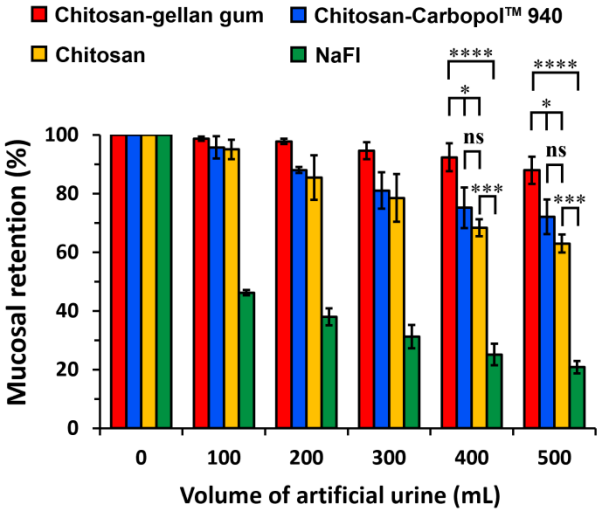

Figure 5. Retention of chitosan and its mixtures with gellan gum and Carbopol™ 940 on sheep bladder mucosa after washing with artificial urine solutions of varying volumes. Data are presented as mean ± standard deviation (n = 3). Statistically significant differences are indicated as follows: * — $p < 0.05$; *** — $p < 0.001$; *ns* — no significant differences

The adhesive properties of these polymers are primarily attributed to their ability to interact with glycosaminoglycans and mucins on mucosal surfaces through non-covalent interactions, including hydrogen bonding, electrostatic attraction, and conformational changes in the polymer chains. These interaction mechanisms are critical in determining the efficacy of mucoadhesive materials in the context of intravesical drug delivery.

Release of the Model Drug Compound

In vitro studies on the release of sodium fluorescein (NaFl) from polymeric formulations were carried out using a dialysis method in artificial urine solution at 37 °C. The cumulative release profiles are presented in Figure 6. Chitosan demonstrated rapid release of NaFl, reaching saturation levels within 4 h. In contrast, the chitosan-Carbopol™ 940 and chitosan-gellan gum mixtures provided prolonged release, achieving approximately 95 % release within 10–12 h, respectively. The extended release of NaFl from the chitosan-gellan gum polymeric mixture can be attributed to the formation of an *in situ* gel in artificial urine, which enhances the release efficiency and maintains therapeutically significant concentrations of the drug within the bladder following intravesical administration. Moreover, the sustained release of NaFl from the polymeric formulations may contribute to improved retention of the model drug compound within the bladder.

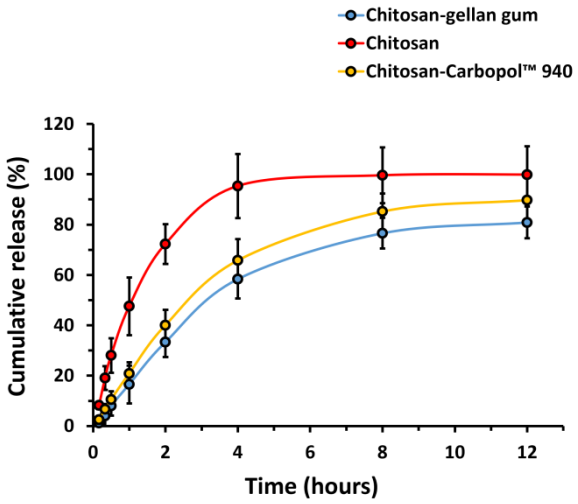

Figure 6. Cumulative release profile of sodium fluorescein from polymeric formulations. Data are presented as mean ± standard deviation (n = 3)

Conclusions

In the present study, the mucoadhesive properties of chitosan-based polymeric materials, as well as its mixtures with gellan gum and Carbopol™ 940 containing sodium fluorescein (NaFl), were analyzed. Experiments carried out using an *in vitro* flow-through method demonstrated that these polymers effectively interact with sheep bladder mucosa.

Fluorescent images confirmed that the retention of the chitosan-gellan gum and chitosan-Carbopol™ 940 mixtures on the mucosal surface significantly exceeds that of pure chitosan ($p < 0.05$). This indicates that the incorporation of gellan gum and Carbopol™ 940 enhances the mucoadhesive characteristics of chitosan, which may be attributed to the formation of an *in situ* gel in the saline solution.

The key factor that makes the polymer complexes (chitosan-gellan gum and chitosan-Carbopol™ 940) more effective than chitosan alone is the synergistic enhancement of mucoadhesive properties. While chitosan contributes to the initial adhesion through electrostatic and hydrogen bonding interactions with mucins, the addition of gellan gum and Carbopol™ 940 enhances both the physical retention (through gel formation) and the sustained release of the drug, which collectively lead to prolonged therapeutic effects in IDD.

The release profiles for sodium fluorescein (NaFl) indicated that chitosan enables a rapid release of the active compound, attaining saturation levels within 4 h. In contrast, the chitosan-Carbopol™ 940 and chitosan-gellan gum formulations demonstrated a markedly prolonged release, with nearly 100 % of the drug released within 10 h. This extended release behavior is primarily attributed to the formation of a physically entrapped gel matrix, which significantly enhances the efficiency of drug delivery while simultaneously facilitating the maintenance of therapeutically relevant concentrations of NaFl in the bladder cavity over an extended period.

The formation of this gel structure not only creates a reservoir effect that supports sustained drug release but also mitigates the risk of concentration fluctuations, optimizing the therapeutic efficacy of the treatment. Such characteristics are particularly advantageous for intravesical drug delivery systems, where the maintenance of effective drug levels is crucial for the management of bladder-related pathologies. These findings highlight the potential of chitosan-based polymeric formulations, particularly in combination with gellan gum and Carbopol™ 940, to enhance therapeutic outcomes in clinical applications targeting bladder conditions.

Consequently, the findings of this investigation substantiate that the incorporation of chitosan mixtures with gellan gum and Carbopol™ 940 markedly improves mucoadhesive properties and facilitates the controlled release of therapeutic agents. This advancement presents novel opportunities for the development of effective mucoadhesive drug delivery systems within the field of urology.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Daulet Bolatovich Kaldybekov** data curation, formal analysis, funding acquisition, resources, supervision, methodology, writing–review & editing; **Elvira Orynbasarovna Shatabayeva** data curation, formal analysis, visualization, writing–original draft; **Aziza Almaskhankyzy Polatkhan** data curation, formal analysis, investigation; **Rysgul Nurlanovna Tuleyeva** data curation, formal analysis, investigation; **Galiya Serikbayevna Irmukhametova** writing–review & editing; **Vitaliy Victorovich Khutoryanskiy** methodology, conceptualization, writing–review & editing.

Conflicts of Interest

The authors declare no conflict of interest.

References

1 Caramella, C. M., Rossi, S., Ferrari, F., Bonferoni, M. C., & Sandri, G. (2015). Mucoadhesive and thermogelling systems for vaginal drug delivery. *Advanced Drug Delivery Reviews*, *92*, 39–52. https://doi.org/10.1016/j.addr.2015.02.001

2 Khutoryanskiy, V. V. (2011). Advances in mucoadhesion and mucoadhesive polymers. *Macromolecular Bioscience*, *11*(6), 748–764. https://doi.org/10.1002/mabi.201000388

3 Neves, J., & Sarmento, B. (2014). *Mucosal Delivery of Biopharmaceuticals: Biology, Challenges and Strategies*. https://doi.org/10.1007/978-1-4614-9524-6

4 Prosperi-Porta, G., Kedzior, S., Muirhead, B., & Sheardown, H. (2016). Phenylboronic-acid-based polymeric micelles for mucoadhesive anterior segment ocular drug delivery. *Biomacromolecules*, *17*(4), 1449–1457. https://doi.org/10.1021/acs.biomac.6b00054

5 Rieger, C., Kunhardt, D., Kaufmann, A., Schendel, D., Huebner, D., Erdmann, K., Propping, S., Wirth, M. P., Schwenzer, B., Fuessel, S., & Hampel, S. (2015). Characterization of different carbon nanotubes for the development of a mucoadhesive drug delivery system for intravesical treatment of bladder cancer. *International Journal of Pharmaceutics*, *479*(2), 357–363. https://doi.org/10.1016/j.ijpharm.2015.01.017

6 Seo, Y. G., Kim, D. W., Yeo, W. H., Ramasamy, T., Oh, Y. K., Park, Y. J., Kim, J. A., Oh, D. H., Ku, S. K., Kim, J. K., Yong, C. S., Kim, J. O., & Choi, H. G. (2013). Docetaxel-loaded thermosensitive and bioadhesive nanomicelles as a rectal drug delivery system for enhanced chemotherapeutic effect. *Pharmaceutical Research*, *30*(7), 1860–1870. https://doi.org/10.1007/s11095-013-1029-0

7 Fangueiro, J. F., Veiga, F., Silva, A. M., & Souto, E. B. (2016). Ocular drug delivery — new strategies for targeting anterior and posterior segments of the eye. *Current Pharmaceutical Design*, *22*(9), 1135–1146. https://doi.org/10.2174/1381612822666151216145900

8 Hornof, M., Weyenberg, W., Ludwig, A., & Bernkop-Schnürch, A. (2003). Mucoadhesive ocular insert based on thiolated poly(acrylic acid): Development and in vivo evaluation in humans. *Journal of Controlled Release*, *89*(3), 419–428. https://doi.org/10.1016/S0168-3659(03)00135-4

9 Kapoor, M., Cloyd, J. C., & Siegel, R. A. (2016). A review of intranasal formulations for the treatment of seizure emergencies. *Journal of Controlled Release*, *237*, 147–159. https://doi.org/10.1016/j.jconrel.2016.07.001

10 Kozlovskaya, L., Abou-Kaoud, M., & Stepensky, D. (2014). Quantitative analysis of drug delivery to the brain via nasal route. *Journal of Controlled Release*, *189*, 133–140. https://doi.org/10.1016/j.jconrel.2014.06.053

11 Mugabe, C., Matsui, Y., So, A. I., Gleave, M. E., Baker, J. H. E., Minchinton, A. I., Manisali, I., Liggins, R., Brooks, D. E., & Burt, H. M. (2011). In vivo evaluation of mucoadhesive nanoparticulate docetaxel for intravesical treatment of non-muscle-invasive bladder cancer. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *17*(9), 2788–2798. https://doi.org/10.1158/1078-0432.ccr-10-2981

12 GuhaSarkar, S., & Banerjee, R. (2010). Intravesical drug delivery: Challenges, current status, opportunities and novel strategies. *Journal of Controlled Release*, *148*(2), 147–159. https://doi.org/10.1016/j.jconrel.2010.08.031

13 Kolawole, O. M., Lau, W. M., Mostafid, H., & Khutoryanskiy, V. V. (2017). Advances in intravesical drug delivery systems to treat bladder cancer. *International Journal of Pharmaceutics*, *532*(1), 105–117. https://doi.org/10.1016/J.IJPHARM.2017.08.120

14 Kolawole, O. M., & Khutoryanskiy, V. V. (2024). Potential bladder cancer therapeutic delivery systems: a recent update. *Expert Opinion on Drug Delivery*, *21*(9), 1311–1329. https://doi.org/10.1080/17425247.2024.2396958

15 Sogias, I. A., Williams, A. C., & Khutoryanskiy, V. V. (2008). Why is chitosan mucoadhesive? *Biomacromolecules*, *9*(7), 1837–1842. https://doi.org/10.1021/bm800276d

16 Hombach, J., & Bernkop-Schnürch, A. (2010). Mucoadhesive drug delivery systems. In M. Schäfer-Korting (Ed.), *Drug Delivery* (pp. 251–266). Springer. https://doi.org/10.1007/978-3-642-00477-3_9

17 Grassiri, B., Zambito, Y., & Bernkop-Schnürch, A. (2021). Strategies to prolong the residence time of drug delivery systems on ocular surface. *Advances in Colloid and Interface Science*, *288*, 102342. https://doi.org/10.1016/j.cis.2020.102342

18 Bravo-Osuna, I., Noiray, M., Briand, E., Woodward, A. M., Argüeso, P., Martínez, I. T. M., Herrero-Vanrell, R., & Ponchel, G. (2012). Interfacial interaction between transmembrane ocular mucins and adhesive polymers and dendrimers analyzed by surface plasmon resonance. *Pharmaceutical Research*, *29*(8), 2329–2340. https://doi.org/10.1007/s11095-012-0761-1

19 Bernkop-Schnürch, A., Guggi, D., & Pinter, Y. (2004). Thiolated chitosans: Development and in vitro evaluation of a mucoadhesive, permeation enhancing oral drug delivery system. *Journal of Controlled Release*, *94*(1), 177–186. https://doi.org/10.1016/j.jconrel.2003.10.005

20 Bernkop-Schnürch, A. (2005). Thiomers: A new generation of mucoadhesive polymers. *Advanced Drug Delivery Reviews*, *57*(11), 1569–1582. https://doi.org/10.1016/j.addr.2005.07.002

21 Barthelmes, J., Perera, G., Hombach, J., Dünnhaupt, S., & Bernkop-Schnürch, A. (2011). Development of a mucoadhesive nanoparticulate drug delivery system for a targeted drug release in the bladder. *International Journal of Pharmaceutics*, *416*(1), 339–345. https://doi.org/10.1016/j.ijpharm.2011.06.033

22 Kolawole, O. M., Lau, W. M., & Khutoryanskiy, V. V. (2018). Methacrylated chitosan as a polymer with enhanced mucoadhesive properties for transmucosal drug delivery. *International Journal of Pharmaceutics*, *550*(1), 123–129. https://doi.org/10.1016/j.ijpharm.2018.08.034

23 Tonglairoum, P., Brannigan, R. P., Opanasopit, P., & Khutoryanskiy, V. V. (2016). Maleimide-bearing nanogels as novel mucoadhesive materials for drug delivery. *Journal of Materials Chemistry B*, *4*(40), 6581–6587. https://doi.org/10.1039/C6TB02124G

24 Kaldybekov, D. B., Tonglairoum, P., Opanasopit, P., & Khutoryanskiy, V. V. (2018). Mucoadhesive maleimide-functionalised liposomes for drug delivery to urinary bladder. *European Journal of Pharmaceutical Sciences*, *111*, 83–90. https://doi.org/10.1016/j.ejps.2017.09.039

25 Agibayeva, L. E., Kaldybekov, D. B., Porfiryeva, N. N., Garipova, V. R., Mangazbayeva, R. A., Moustafine, R. I., Semina, I. I., Mun, G. A., Kudaibergenov, S. E., & Khutoryanskiy, V. V. (2020). Gellan gum and its methacrylated derivatives as in situ gelling mucoadhesive formulations of pilocarpine: In vitro and in vivo studies. *International Journal of Pharmaceutics*, *577*, 119093. https://doi.org/10.1016/j.ijpharm.2020.119093

26 Grasso, M. (2008). Bladder Cancer: A Major Public Health Issue. *European Urology, Supplements*, *7*(7), 510–515. https://doi.org/10.1016/j.eursup.2008.04.001

27 Chutipongtanate, S., & Thongboonkerd, V. (2010). Systematic comparisons of artificial urine formulas for in vitro cellular study. *Analytical Biochemistry*, *402*(1), 110–112. https://doi.org/10.1016/j.ab.2010.03.031

28 Phuong Ta, L., Bujna, E., Kun, S., Charalampopoulos, D., & Khutoryanskiy, V. V. (2021). Electrosprayed mucoadhesive alginate-chitosan microcapsules for gastrointestinal delivery of probiotics. *International Journal of Pharmaceutics*, *597*, 120342. https://doi.org/10.1016/j.ijpharm.2021.120342

29 Kaldybekov, D. B., Filippov, S. K., Radulescu, A., & Khutoryanskiy, V. V. (2019). Maleimide-functionalised PLGA-PEG nanoparticles as mucoadhesive carriers for intravesical drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *143*, 24–34. https://doi.org/10.1016/j.ejpb.2019.08.007

30 Vigani, B., Rossi, S., Sandri, G., Bonferoni, M. C., Caramella, C. M., & Ferrari, F. (2020). Recent Advances in the Development of In Situ Gelling Drug Delivery Systems for Non-Parenteral Administration Routes. *Pharmaceutics*, *12*(9). https://doi.org/10.3390/pharmaceutics12090859

31 Kulkarni, R., Fanse, S., & Burgess, D. J. (2023). Mucoadhesive drug delivery systems: a promising noninvasive approach to bioavailability enhancement. Part II: formulation considerations. *Expert Opinion on Drug Delivery*, *20*(3), 413–434. https://doi.org/10.1080/17425247.2023.2181332