ORIGINAL ARTICLE

IN VITRO CHARACTERIZATION OF POLYVINYL ALCOHOL/ CHITOSAN HYDROGELS AS MODIFIED RELEASE SYSTEMS FOR BISOPROLOL

ALINA-DIANA PANAINTE [1], GRAȚIELA POPA [1]*, DANIELA PAMFIL [2], ELENA BUTNARU [2], CORNELIA VASILE [2], LILIANA MITITELU TARȚĂU [3], CARMEN GAFIȚANU [1]

[1]*Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, 15 Universității Street, 700115, Iași, Romania*
[2]*"Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Vodă Alley, 700487, Iași, Romania*
[3]*Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 15 Universității Street, 700115, Iași, Romania*

**corresponding author: gratielapopa@yahoo.com*

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Abstract

The aim of this paper was the evaluation of bisoprolol fumarate (BF) incorporated into polyvinyl alcohol/chitosan (PVA/CS) hydrogels and the study of its *in vitro* kinetic release, further used in the pharmaceutical field in oral dosage forms as modified release systems. For all the hydrogels tested, the swelling degree decreased with the chitosan proportion increase. Drug release was delayed and the release mechanism of the active principle was found to depend on the matrix composition. The results obtained for these hydrogels may show interest for application in pharmaceutical and medical fields.

Rezumat

Scopul acestei lucrări a constat în înglobarea bisoprololului fumarat (BF) în hidrogeluri pe bază de alcool polivinilic/chitosan (PVA/CS) şi evaluarea cineticii sale de eliberare *in vitro*. Pentru toate hidrogelurile testate, gradul de umflare a scăzut cu creşterea proporţiei de chitosan. Eliberarea medicamentului a fost întârziată şi s-a constatat că mecanismul de eliberare al principiului activ depinde de compoziția matricei. Rezultatele obținute pot prezenta interes pentru utilizarea acestor hidrogeluri în domeniile medical şi farmaceutic.

Keywords: bisoprolol, hydrogels, controlled delivery, *in vitro* characterization

Introduction

Mixtures of natural polymers (e.g. chitosan, starch, cellulose, etc.) and synthetic polymers (polyvinyl alcohol, polyethylene, polystyrene, polypropylene, etc.) are investigated as biodegradable materials, in order to obtain hydrogels with various biomedical and pharmaceutical applications [2, 5]. Hydrogels as drug delivery systems have gained specialists' increased attention due to their versatile properties: they can be adapted for modified release, thus decreasing side effects and toxicity [4]. Biopolymer hydrogels are recommended for use as carrier systems for controlled drug release and as tissue engineering materials [14]. Drug molecules are physically trapped in the hydrogel matrix and released through diffusion mechanism [9].

Many drugs have been incorporated into polymeric structures with chitosan (CS) in order to modify their release profiles: cimetidine [16], capecitabine [1], mesalamine [7], metronidazole [17], ramipril [3]; literature also presents the encapsulation of bisoprolol fumarate. Sarath Chandran *et al*. included bisoprolol in CS-based buccal patches, in patches based on CS and hydroxypropyl methylcellulose and on CS and sodium carboxymethyl cellulose [11-13].

Polyvinyl alcohol (PVA) is a polymer that has received special attention from researchers, having a simple structure that can be easily modified through chemical reactions [9]. Lately, PVA has been used in the production of nanofibers as PVA-Chitosan copolymers. Applications of PVA in modified release systems for beta-blocking agents have not been reported in literature yet. Bisoprolol fumarate (BF) is a selective beta-blocker very used in the treatment of cardiovascular diseases, due to its higher efficiency compared to other agents, like atenolol and betaxolol [4, 6]. Its applications in cardiovascular therapy include: hyper-tension and cardiac ischemic diseases, management of certain types of cardiac arrhythmias, as an adjuvant in hyperthyroidism, cardio-protection prior and post myocardial infarction [15].

The purpose of this study was the *in vitro* characterization of PVA/CS (polyvinyl alcohol/ chitosan) hydrogels loaded with bisoprolol fumarate, to be further used in the pharmaceutical field in oral dosage forms as modified release systems.

Materials and Methods

Materials: bisoprolol fumarate (100.07% purity, Unichem Laboratories LTD, India - BF); low molecular weight chitosan (CS, Sigma, Steinheim, Germany), hydrochloric acid (Fluka, Germany); polyvinyl alcohol (PVA), (Romacril Râșnov, Romania); sodium hydroxide (Fluka, Germany); concentrated acetic acid solution (Fluka, Germany).

Equipments: analytical balance Kern ABT 100-5M; mechanical stirrer with heater NHT 691-2; dissolution test station Agilent 708-DS, equipped with a UV-VIS Carry 60 spectrophotometer.

The PVA/CS hydrogels as carrier systems had been previously prepared dried and were kindly supplied by the "Petru Poni" Macromolecular Chemistry Institute from Iași, Romania [8]. The PVA/CS hydrogel ratios were: 95/05, 90/10, 80/20, 60/40. The drying process was carried out with and without lyophilisation. The evaluation of the hydrogels involved also the swelling capacity, taking into account that this parameter can highly influence the drug release through diffusion.

The influence of chitosan concentration on the swelling capacity of hydrogels was evaluated and was expressed as the maximum swelling capacity after 48 hours. Samples of hydrogels were analytically weighed at the beginning of the experiment and immersed in 50 mL bi-distilled water at 37°C; at various time intervals they were periodically weighed. The temperature was kept constant by using a hot plate stirrer with temperature monitoring system. Prior to each weighing, the extra-water retained on the surface of the hydrogel was removed using filter paper. At the end of the test, the hydrogels were taken out of the liquid, dried at room temperature and analytically weighed. The amount of retained water was determined according to the equation:

$$Degree\ of\ swelling\ = \frac{W_t - W_i}{W_i} \times 100,$$

where: W_t = weight of water-imbibed hydrogel at various time points; W_i = initial weight of the hydrogel. The kinetic swelling parameters *n, k* (min^{-1}) in water at 37°C were determined for the PVA/CS tested hydrogels [10].

The loading capacity of PVA/CS for bisoprolol was evaluated for both types of hydrogels (with and without lyophilisation), in order to point out their eventual pharmaceutical applications. The drug was loaded by diffusion process of a 0.05% bisoprolol solution throughout the hydrogel matrix, at the same time with hydrogel swelling at 37ºC for 48 hours with continuous stirring. The loaded samples were subsequently dried in the oven for 24 hours at 40ºC. The loading capacity of bisoprolol in the tested hydro-gels was done through spectrophotometric method. The calibration curve was drawn at the maximum absorption wavelength in the UV-VIS domain for bisoprolol in water (225 nm). Bisoprolol release was tested using an Agilent 708-DS dissolution test station, equipped with a spectrophotometer, with the following protocol: Apparatus 2 (paddle), 100 rpm; dissolution medium: 100 mL phosphate buffer solution pH = 7.4. Drug release was tested over a 72 hour period, until a drug concentration plateau in the dissolution medium was reached. The bisoprolol concentration was determined spectrophotometrically method using as blank the phosphate buffer solution.

Results and Discussion

Polyvinyl alcohol/chitosan matrix hydrogels with 4 different PVA/CS ratios were previously dried (with and without lyophilisation) and tested for the swelling capacity. The swelling curves are presented in Figures 1 and 2. The hydrogels showed increased swelling capacity, the maximum swelling degree varying with the polymer matrix composition. According to the swelling curves, it can be noticed that the degree of hydrogel swelling for all samples increased rapidly over the first minutes, due to the hydrophilic properties of chitosan. In the first 50 - 55 minutes a high amount of water was retained in the hydrogel matrix; afterwards the swelling degree did not change significantly; 4 hours after immersion in the liquid, the maximum swelling degree was reached and a swelling degree plateau was achieved. However, the swelling degree of the lyophilised hydrogels was higher. Also, the increase of chitosan proportion in the hydrogel matrix (5 to 40%) was inversely correlated to the swelling degree, decreasing it. This phenomenon can be explained by the formation of new cross-linking bridges between chitosan particles and the PVA polymeric net, thus forming a hydrogel with a lower swelling degree. The kinetic swelling parameters of the tested hydrogels (Table I) were evaluated for a maximum weight ratio M_t/M_∞ of 0.6 using the de Korsmeyer-Peppas equation [10]. The *n* values indicate a Fickian swelling behaviour of PVA/CS hydrogels. The calibration curve of bisoprolol in bi-distilled water had a correlation coefficient equal to 0.99997, which confirms the accuracy of the experimental results (Figure 3). The amount of bisoprolol loaded in the hydrogels (encapsulation efficiency) was spectrophotometrically determined, making possible to deduct the remaining unloaded bisoprolol concentration. The calculation of loading capacity was done according to the calibration curve equation: Absorbance = 0.00808 x concentration + $3.6945 \cdot 10^{-4}$. The bisoprolol amounts loaded in the tested hydrogels are presented in Table II. Drug release from a carrier material is a complex process, involving drug transfer from the release system to the targeted site through various media.

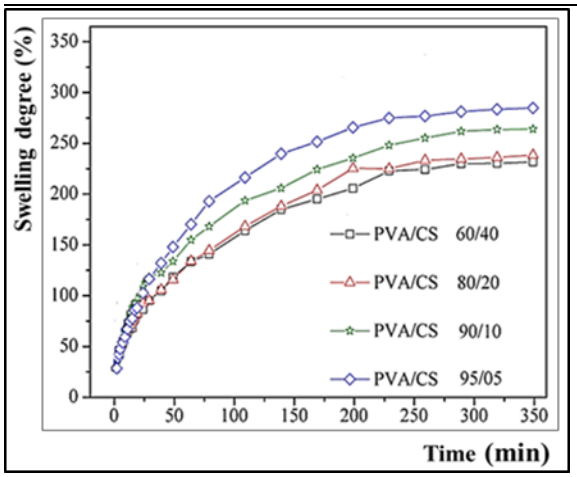


Figure 1.
Swelling behaviour of non-lyophilised hydrogels in bi-distilled water at 37°C

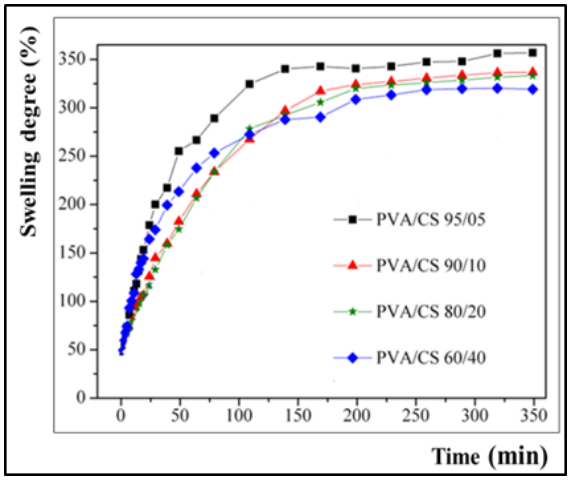


Figure 2.
Swelling behaviour of lyophilised hydrogels in bi-distilled water at 37°C

Table I
The kinetic parameters of hydrogel swelling in bi-distilled water 37°C

Sample code	Kinetic parameters			
	n		k (min^{-1})	
	WL	L	WL	L
PVA/CS 95/05	0.32	0.43	0.23	0.28
PVA/CS 90/10	0.31	0.39	0.18	0.16
PVA/CS 80/20	0.25	0.33	0.22	0.14
PVA/CS 60/40	0.23	0.22	0.27	0.21

n = diffusion coefficient; k = diffusion rate; WL = samples without lyophilisation; L = lyophilised samples

A direct correlation could be observed between the swelling degree of hydrogels and drug loading: the highest bisoprolol amount was noted for the PVA/CS 95/5 ratio hydrogels. A better diffusion of bisoprolol was noted through the lyophilised matrices, due to their higher porosity (Table II).

Table II
Loaded drug amounts in the tested hydrogels (mg)

Sample code	Loaded drug amount (mg)	
	WL	L
PVA/CS 95/5	0.38	0.92
PVA/CS 90/10	0.35	0.59
PVA/CS 80/20	0.31	0.39
PVA/CS 60/40	0.28	0.34

WL = samples without lyophilisation; L = lyophilised samples.

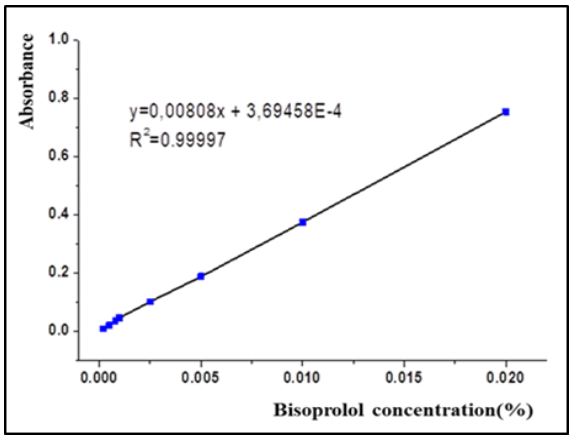


Figure 3.
Calibration curve for loading capacity testing of bisoprolol

The main drawback of hydrogels is the fast diffusion of low molecular weight drugs loaded through these matrices, due to large capillary spaces of the polymer network. Hydrogels with different PVA/CS ratios were evaluated for the release behaviour of loaded BF and the released drug concentration was calculated according to the regression equation of the calibration curve in phosphate buffer (Figure 4). Figure 5 displays the release profile curves of bisoprolol from the tested hydrogels and Table III presents the kinetics parameters fitted on the Peppas model.

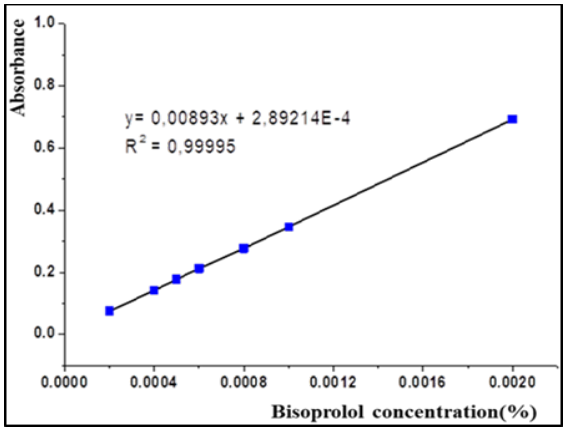
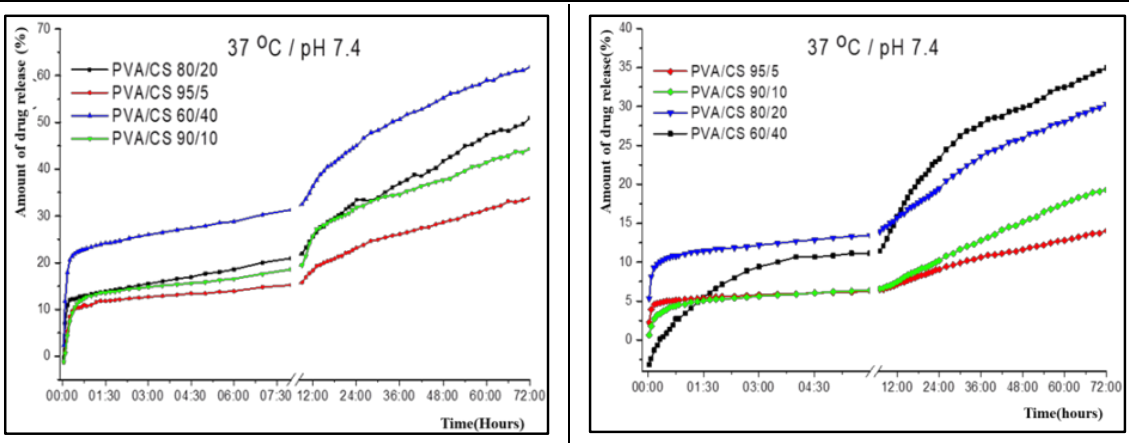


Figure 4.
Calibration curve for dissolution release testing in phosphate buffer solution pH = 7.4



A B

Figure 5.
Bisoprolol release from PVA/CS hydrogels without (A) and with lyophilisation (B)

Table III
The kinetics release parameters of BF from the PVA/CS hydrogels

Kin. parameters	n		k, min^{-n}		r^2	
Sample code	WL	L	WL	L	WL	L
PVA/CS 90/5	0.247	0.085	0.0015	0.0047	0.991	0.997
PVA/CS 90/10	0.215	0.23	0.0036	0.031	0.996	0.994
PVA/CS 80/20	0.359	0.11	0.188	0.082	0.998	0.993
PVA/CS 60/40	0.418	0.19	0.20	0.064	0.993	0.998

r^2 = regression coefficient, n = diffusion exponent, k = diffusion rate; WL = samples without lyophilisation; L = lyophilised samples.

From the kinetics point of view, bisoprolol release from hydrogel matrices showed a burst effect for all the tested hydrogels, but more intense for the hydrogels with higher chitosan proportions. The non-lyophilised hydrogels with PVA/CS ratios of 95/5, 90/10, 80/20 had a fast release of about 10% bisoprolol in the first hour, whereas for the hydrogel with PVA/CS 60/40 ratio the first hour release was 20% (Figure 5). Following this burst effect, a slightly linear continuous release could be observed, other 15% of the loaded drug being released. This second release step was followed by a progressive increase in drug release over 72 hours. After 72 hours BF was released very slowly and in very low amounts, thus 3 days afterwards a remaining drug amount could be determined in all the hydrogel samples.

The release kinetics parameters for the non-lyophilised hydrogels indicate *n* values very astray from 0.5, suggesting that the release behaviour is governed by other mechanisms apart from diffusion. The k constant shows different values for the tested matrices, being higher for the hydrogels with 80/20 and 60/40 PVA/CS ratios (Table III). The release kinetics of bisoprolol from the lyophilised hydrogels suggests a fast release of 5% drug in the first hour for the hydrogels with PVA/CS 95/5, 90/10, 60/40 ratios and of 10% drug for the PVA/CS 80/20 ratio hydrogel (Figure 5). Like in the case of non-lyophilised hydrogels, this release step is followed by a slightly linear profile, other 12% of the loaded drug being released. Afterwards, drug release from the lyophilised hydrogel matrices increases progressively over 72 hours. A flow amount of the remaining bisoprolol is released after 72 hours and with a very slow release rate. Also, the release kinetics parameters for these hydrogels, with even lower *n* and *k* values indicate that the release behaviour is governed by other mechanisms apart from diffusion. The *n* values are also astray from the 0.5 value, indicating probably one extra perturbing factor; the low *n* values could be explained by hydrogen bonds formation between bisoprolol and hydrogel polymers, due to the basic pH value of the phosphate buffer dissolution medium. A remaining amount of bisoprolol could be determined in all hydrogels after 3 days from the beginning of the dissolution test. The unreleased (un-diffused) drug was retained thoroughly in the polymer matrix pores, from which bisoprolol could be further released only *via* enzymatic degradation.

Conclusions

The present study presents the characterization of polyvinyl alcohol/chitosan hydrogels in various polymer ratios, dried by two methods (with and without lyophilisation) in order to obtain controlled release systems for bisoprolol fumarate. For all the hydrogels tested, the swelling degree decreased with the higher proportion of chitosan. The loading capacity was

higher for the hydrogels with lower chitosan content. Loading efficiency and bisoprolol release were higher for PVA/CS hydrogels dried by lyophilisation. Generally, the kinetics parameters indicate a release dependent on the hydrogel composition.

References

1. Agnihotri SA, Aminabhavi TM, Novel interpenetrating network chitosan-poly(ethylene oxide-g-acrylamide) hydrogel microspheres for the controlled release of capecitabine. *Int J Pharm.*, 2006; 324(2): 103-115.
2. Avram I, Lupaşcu FG, Confederat L, Constantin SM, Stan CI, Profire L, Chitosan microparticles loaded with antidiabetic drugs - preparation and characterization. *Farmacia*, 2017; 65(3): 443-448.
3. Chadha R, Swati B, Darpan K, Sushma G, Exploring Lecithin/Chitosan Nanoparticles of Ramipril for Improved Antihypertensive Efficacy. *J Nanopharm Drug Deliv.*, 2013; 1(2): 173-181.
4. Imre S, Ormenişan A, Tero-Vescan A, Muntean DL, Vari CE, Hplc Enantioseparation of β-blockers on ovomucoid stationary phase. J Chromatogr Sci., 2016, 54(9): 1578-1583.
5. Lin CC, Metters AT, Hydrogels in controlled release formulations: network design and mathematical modeling. *Adv Drug Deliv Rev.*, 2006; 58: 1379-1408.
6. Nuttall SL, Routledge HC, Kendall MJ, A comparison of the beta1-selectivity of three beta1-selective beta-blockers. *J Clin Pharm Ther.*, 2003; 28(3): 179-186.
7. Patel NV, Formulation and *in-vitro* evaluation stability of chitosan of mesalamine matrix tablets using chitosan for colonic drug delivery. *J Pharm Res.*, 2009; 2(8): 1319-1323.
8. Părpăriţă E, Cheaburu CN, Paţachiab SF, Vasile C, Polyvinyl alcohol/chitosan/montmorillonite nano-composites preparation by freeze/thaw cycles and characterization. *Acta Chem Iasi*, 2014; 22(2): 75-96.
9. Peppas NA, Hydrogels Clases of materials used in medicine. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, Biomaterials Science: An introduction to materials in medicine. Ed. 2. New York: Academic Press, 2004; 60-64.
10. Peppas NA, Korsmeyer RW, Dynamically swelling hydrogels in controlled release applications. Hydrogens in medicine and pharmacy. *Boca Raton: CRC Press*, 1986; 3: 109-136.
11. Sarath Chandran C, Shijith KV, Vipin KV, Augusthy AR, Chitosan based mucoadhesive buccal patches containing bisoprolol fumarate. *IJAPBC*, 2013; 2(3): 465-469.
12. Sarath Chandran C, Shijith KV, Vipin KV, Augusthy AR, Formulation and evaluation of bisoprolol fumarate buccal patches by using selected polymers. *IJPCBS*, 2013; 3(3): 854-860.
13. Singh A, Sharma PK, Garg VK, Garg G, Hydrogels: A review. *IJPSRR*, 2010; 4(2): 97-105.
14. Smith C, Fisher M, McKay G, Bisoprolol. *Pract Diab Int.*, 2008; 25(8): 324-325.
15. Struszczyk MH, Chitin and chitosan - Part II. Applications of chitosan. *Polimery*, 2002; 47(6): 396-403.
16. Vipin Bansal V, Sharma PK, Sharma N, Prakash Pal O, Malviya R, Applications of chitosan and chitosan derivatives in drug delivery. *Advan Bio Res.*, 2011; 5(1): 28-37.
17. Zupančič Š, Potrč T, Baumgartner S, Kocbek P, Kristl J. Formulation and evaluation of chitosan/polyethylene oxide nanofibers loaded with metronidazole for local infections. *Eur J Pharm Sci.*, 2016; 95: 152-160.