Sofia University “St. Kliment Ohridski”

Faculty of Chemistry and Pharmacy

Department of Pharmaceutical and Applied Chemistry

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ST. KLIMENT OHRIDSKI

Denitsa Valerieva Nikolova

“Smart Polymer Materials for Modified Release of Timolol Maleate in the Eyes”

ABSTRACT

of a dissertation submitted for acquiring the educational and science degree “Doctor”

Scientific field: 4.2 Chemical Sciences

Polymers

Thesis supervisors: Assoc. Prof. Elena Vasileva, PhD
Assoc. Prof. Lachezar Christov, PhD

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The dissertation contains 118 pages, of which 11 pages with references and 3 pages with appendices. The work includes 20 schemes, 24 figures and 24 tables. The bibliographic reference covers 163 literary sources. **The numbering of the sections, figures, tables and literary sources in the abstract does not match those in the dissertation.**

The dissertation work has been discussed and directed for defense by the Departmental Council of the Department of Pharmaceutical and Applied Organic Chemistry of the Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski", held on 30.01.2023.

The PhD student is enrolled in regular doctoral studies at the Department of Pharmaceutical and Applied Organic Chemistry with order No. RD 20 - 242 of the Rector of SU "St. Kliment Ohridski" from 28.01.2019

The research on the dissertation work was carried out in the Laboratory of Structure and Properties of Polymers at the Department of Pharmaceutical and Applied Organic Chemistry of the Faculty of Chemistry and Pharmacy at SU "St. Kliment Ohridski".

The public defense of the dissertation work will be held on **08.06.2023 at 2:00 p.m**. in the Meeting Hall of the Faculty of Chemistry and Pharmacy at SU "St. Kliment Ohridski", Blvd. "J. Boucher' No. 1.

Defense materials are available to those interested in the office of the Faculty of Chemistry and Pharmacy at SU "St. Kliment Ohridski", Blvd. "J. Boucher" No. 1, room 107 and on the FHF website.

Table of Contents

Abbreviations:

ζ – potential – dzeta potential

DLC – dynamic light scattering

VP – 2-vinyl pirrolidone

EE – drug entrapment efficiency

DLC – drug loading capacity

poly(SBM-co-VP) – copolymer hydrogel of sulfobetain methacrylate and vinyl pirrolidone

PSBM – poly(sulfobetaine methacrylate)

PSBM Lin – linear nanoparticles of poly(sulfobetaine methacrylate)

PSBM NP – crosslinked nanoparticles of poly(sulfobetaine methacrylate)

ESR – equilibrium swelling ratio

SBM – sulfobetaine methacrylate

TM – Timolol maleate

PBS – phosphate buffer saline solution

I. Introduction

Glaucoma is one of the most common eye diseases associated with increased intraocular pressure. The most common medication prescribed to patients suffering from this disease is the non-selective beta blocker timolol maleate. It works by blocking the beta receptors in the ciliary body, leading to reducing the secretion of intraocular fluid, thereby lowering intraocular pressure. The drug is most often administered in the form of an eye drops, which, however, are quickly washed out of the tear fluid. Thus, the drug does not have enough time to contact the eye surface, its concentration in the eye rapidly decreases and as a result, the bioavailability of timolol maleate is lower than 5%. This requires more frequent application of the eye drops, which makes the treatment more expensive. Therefore, the development of a suitable drug carrier for drugs administered in the eye, and in particular the most common such timolol maleate, represents a challenge in pharmaceutical industry.

Two of the possible solutions to these problems are: (i) the development of nano/micro carriers for timolol maleate that would increase the contact time between the medication and the ocular surface and (ii) the development of drug delivering soft contact lenses for eyes that would not allow reducing the concentration of the drug substance and washing it out by the tears.

Due to its properties, the zwitterionic polymer poly(sulfobetaine methacrylate) stands out to be used for the devotement of such carriers for timolol maleate. Poly(sulfobetaine methacrylate):

- exhibits intelligent behaviour, i.e. responds to external stimuli of the environment, such as a change in temperature or salt concentration;
- has biomimetic properties that determine the so-called "stealth" effect, which reduces the body's immune response;

These properties of poly(sulfobetaine methacrylate) form the basis of this dissertation. The obtained results demonstrate the potential of poly(sulfobetaine methacrylate) to be used as a drug carrier for timolol maleate in two different forms.

II. Aim and tasks

The aim of this thesis is to demonstrate the potential of smart polymeric materials based on the zwitterionic polymer poly(sulfobetaine methacrylate) as drug delivery systems for timolol maleate, in the form of cross-linked and linear nanoparticles, with potential ophthalmic application, or copolymer hydrogels of poly (sulfobetaine methacrylate-co-vinyl pyrrolidone), with potential application as soft contact lenses for eyes. The choice of the specific polymers was made in order to give the obtained materials appropriate properties that help prolong the contact between the medicinal substance and the ocular surface, and hence increase the medicinal bioavailability.

The tasks that were set to achieve the aim are the following:

- Synthesis of linear and cross-linked nanoparticles of poly(sulfobetaine methacrylate)
- Characterization of the obtained particles
- Loading of polyzwitterionic particles with timolol maleate and investigating their potential as drug delivery systems for ocular administration
- Synthesis of hydrogels of poly(sulfobetaine methacrylate-co-vinyl pyrrolidone) copolymers with different compositions
- Characterization of the properties of the thus obtained copolymers
- Loading of the copolymer hydrogels with timolol maleate
- Investigating the properties of the obtained hydrogels as potential materials for drug-delivery soft contact lenses.

III. Results and discussion

III.1. Linear and crosslinked nanoparticles of poly(sulfobetaine methacrylate)(PSBM)

III.1.1 Hydrodynamic diameter of nanoparticles linear (PSB Lin) and crosslinked (PSB NP) PSBM

Particle size is a critical parameter in eye drops (suspensions) that ensures optimal biological activity, physical stability and bioavailability of the administered medication and plays a major role in patient comfort. Dynamic laser light scattering (DLS) was used to estimate the size of the nanoparticles obtained from linear and cross-linked PSBM. Since PSBM is known to be a thermosensitive polymer, it was expected that the particle size would depend on temperature. The temperature dependence of the hydrodynamic diameter of PSBM lin and PSBM NP is presented in Figure 1. At room temperature, both linear poly(sulfobetaine methacrylate) particles (PSBM lin) and cross-linked poly(sulfobetaine methacrylate) particles (PSBM NP) have hydrodynamic diameters of 2.5 – 3.5 μm. As the temperature increases, their hydrodynamic diameter decreases and passes into the nanometric scale.

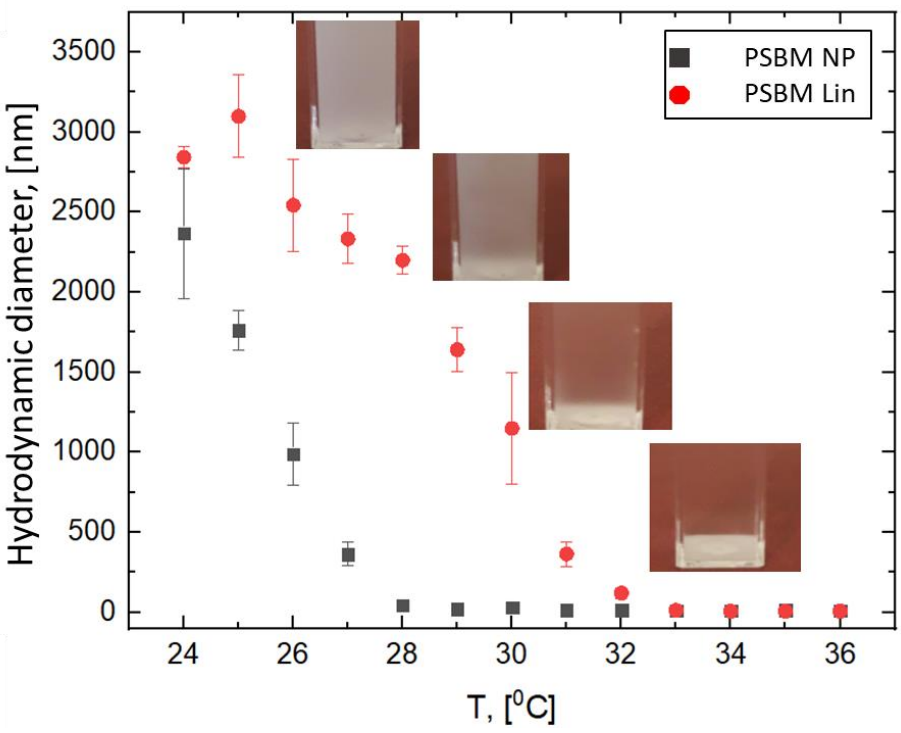
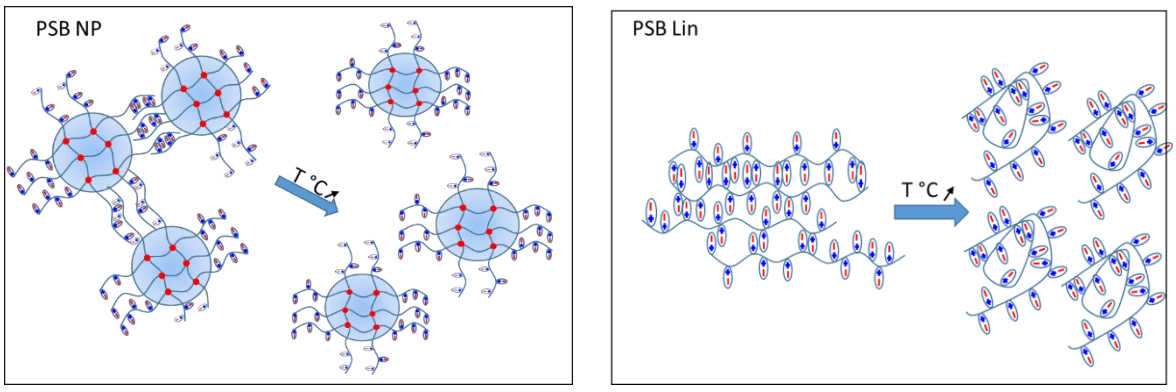


Figure 1. Temperature dependence of the hydrodynamic diameter of PSB NP and PSB Lin. The photos are taken for PSB Lin solution but the same transition from turbid to transparent solution upon temperature increase is observed also for PSB NP.

In the temperature range of 33 to 36 °C, PSBM NPs have a hydrodynamic diameter of ~11 nm, while the hydrodynamic diameter for PSBM lin is ~7 nm. The nanosize of both types

of PSBM particles is achieved in the temperature range corresponding to the surface temperature of the human eye. According to some authors, the temperature of the cornea of a person at rest is 32.17 °C with the eyelid open and 34.43 °C when the eyelid is closed [1]. Other authors determine a minimum temperature of the cornea of 33.82 ± 1.10 °C and a maximum of 35.41 ± 0.73 °C, i.e. mean eye surface temperature 34.51 ± 0.82 °C according to this study [2]. Therefore, both types of PSBM particles are expected to be nanosized within the temperature range of the intended ocular application. It is known that the size of the particles in suspensions for eye application should be below 10 µm in order not to cause irritation in the eye and not to create discomfort for the patient [3]. The results in Figure 1 show that the sizes of both types of PSBM particles remain well below 10 µm, even at room temperature. Thus, the application of the particles would not cause eye irritation when instilling such a suspension.

The temperature dependence of the hydrodynamic diameter of both types of PSBM particles shows a decrease in their size with increasing temperature (Figure 1). This behavior can be explained by the self-association that takes place between neighboring PSBM macromolecules/particles. Intermolecular/interparticle associates are obtained due to the formation of physical bonds through dipole–dipole interactions characteristic of zwitterionic polymers (Scheme 1). As the temperature increases, these physical bonds are broken, the associations between PSBM particles/macromolecules are destroyed and the hydrodynamic diameter decreases (Scheme 1).



(a) (b)

Scheme 1. Disintegration of the inter-macromolecular/inter-particle associates formed via dipole–dipole interactions at room temperature between (a) PSB linear macromolecules (PSB Lin) and (b) PSB nanoparticles (PSB NP).

Further evidence of the disruption of these associations is the change in solution turbidity with increasing temperature (Figure 1). At room temperature, the solution is milky white, while as the temperature increases, it gradually clears, and at a temperature of 36 °C, it

is transparent. When comparing the hydrodynamic diameters of PSBM NP and PSBM lin in the temperature range from 33 to 36 °C, a difference in their size is observed, which can be explained by the cross-linking effect - for PSBM NP, larger particles are obtained due to their network structure, in which several PSBM macromolecules are chemically linked by a cross-linking agent. At room temperature, however, PSBM lin particles have a larger size. The reason is that the linear PSBM has a more spread out structure of its macromolecules compared to the cross-linked PSBM, due to the restraining action of the cross-linking cross-links in the latter. Self-association between more extended linear PSBM molecules leads to the formation of larger associates, which break at higher temperatures, since they probably also include a larger number of macromolecules. Thus, DLS measurements provide indirect confirmation of the successful cross-linking of PSBM NPs.

The possibility that the decrease in the hydrodynamic diameter of PSBM particles was due to sedimentation was tested. The results of this experiment confirm that the disruption of dipole-dipole intermolecular/interparticle interactions is the cause of hydrodynamic diameter reduction, not sedimentation. The results are detailed in the thesis: Section V.1.2 Figure 8, from the thesis.

III.1.2 Zeta (ζ) potential of PSBM linear and crosslinked nanoparticles

The above-described changes in the conformation of PSBM macromolecules caused by increasing temperature are expected to affect the zeta potential of PSBM particles. The zeta potential of the particles, in turn, determines the behavior of these particles as carriers of medicinal substances for ocular application - it is known that the ocular surface is negatively charged, and tears have a pH of ~7.4. Thus, drug carriers with a positive charge or those that are neutral can provide a longer residence time in the eye, and hence a greater amount of drug substance released from them can pass through the cornea.

Therefore, the temperature dependence of the ζ-potential of the two types of PSBM particles was also investigated. The results presented in Figure 2 show that at room temperature both types of particles are negatively charged, but as the temperature increases, their ζ-potential increases and approaches zero. These results support the hypothesis of self-association of PSBM at room temperature and disruption of these associates upon increasing temperature. At room temperature, due to the formed associates between neighboring macromolecules (in the case of PSBM lin) and parts of chains (for PSBM NP) (Scheme 1), the positively charged groups of the zwitterionic structure "hide" inside the associates, leaving the negatively charged groups on the surface. Therefore, at temperatures close to room temperature PSBM NP and

PSBM lin have a negative ζ-potential. This explanation is consistent with other similar studies on PSBM-based materials. For example, Dong et al. observed a negative ζ-potential, at room temperature, of a polydisperse colloidal system consisting of particles with a polyzwitterionic shell and a hydrophobic core. In their study, variation of the ζ-potential from -43 mV to -19.8 mV was observed, depending on the composition of the investigated particles [4]. Li et al. investigated the effect of pH on the ζ-potential of PSBM and found negative ζ-potential values at pH values ~ 6, which corresponds to the pH used in the present research [5]. However, no other studies were found in the literature to reveal the temperature dependence of the ζ-potential of PSBM.

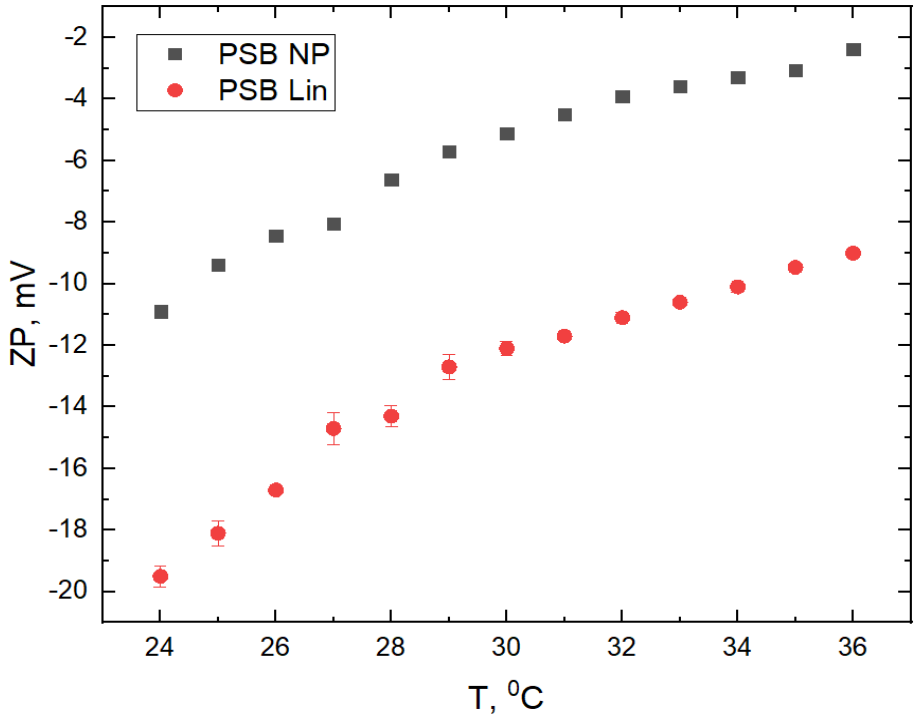


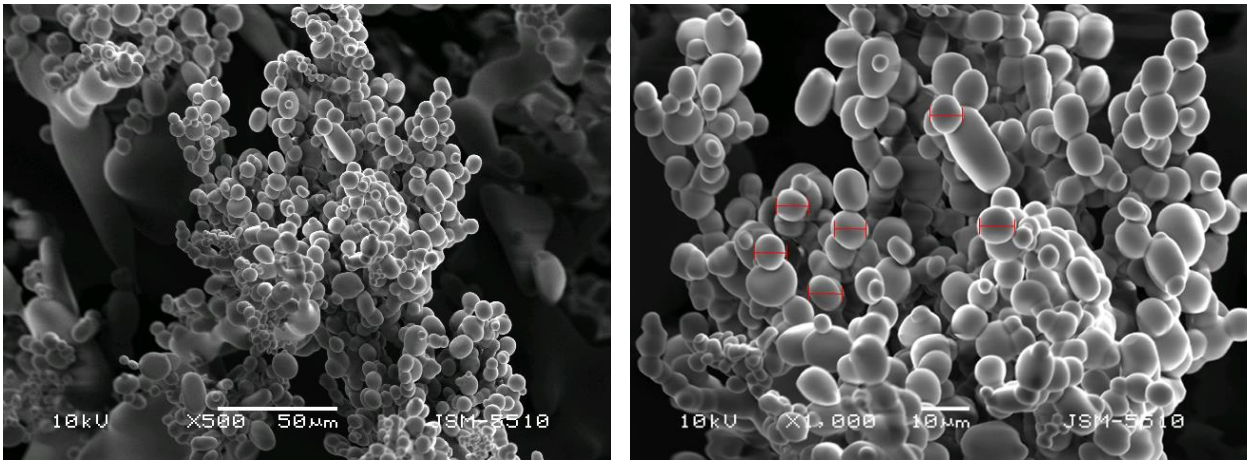
Figure 2. Temperature dependence of the ζ potential of PSB NP and PSB Lin.

When the temperature increases, the PSBM associates are destroyed, the intermolecular dipole-dipole interactions decrease, and this leads to the restoration of the electroneutrality of the particles by forming intramolecular interactions, by analogy with the internal salts that, for example, amino acids form when they are in their zwitterionic form.

If we compare the ζ-potentials of PSBM NP and PSBM Lin, the cross-linked particles are less charged, i.e. have a higher ζ-potential. This is due to the more compact structure of PSBM NP due to cross-linking, compared to the more spread out structure of PSBM Lin (Scheme 1). Thus, the temperature dependence of the ζ-potential is another proof of the effective cross-linking in PSBM NPs.

III.1.3 Morphology of the crosslinked PSBM nanoparticles

The morphology of PSBM NPs observed by scanning electron microscopy (SEM) is presented in Figure 3. The particles of cross-linked PSBM are oval in shape, and the images clearly show adhesion between the individual particles. This observation is consistent with the assumption of self-association of PSBM NPs (Scheme 1a).



(a) (b)

Figure 3. SEM images of PSB NP at (a) 500× and (b) 1000× magnification.

The average particle size of PSBM NPs was determined by making a size distribution of 100 particles using SEM images. The resulting size distribution is presented in Figure 4. A relatively broad distribution was observed, with the mean diameter determined to be ~ 6 µm, which is a comparable value to the result for the hydrodynamic diameter obtained with DLS at room temperature (~ 2.5 µm). The two values can be compared because the SEM images were taken on lyophilized PSBM NP particles that were stored at room temperature before freezing.

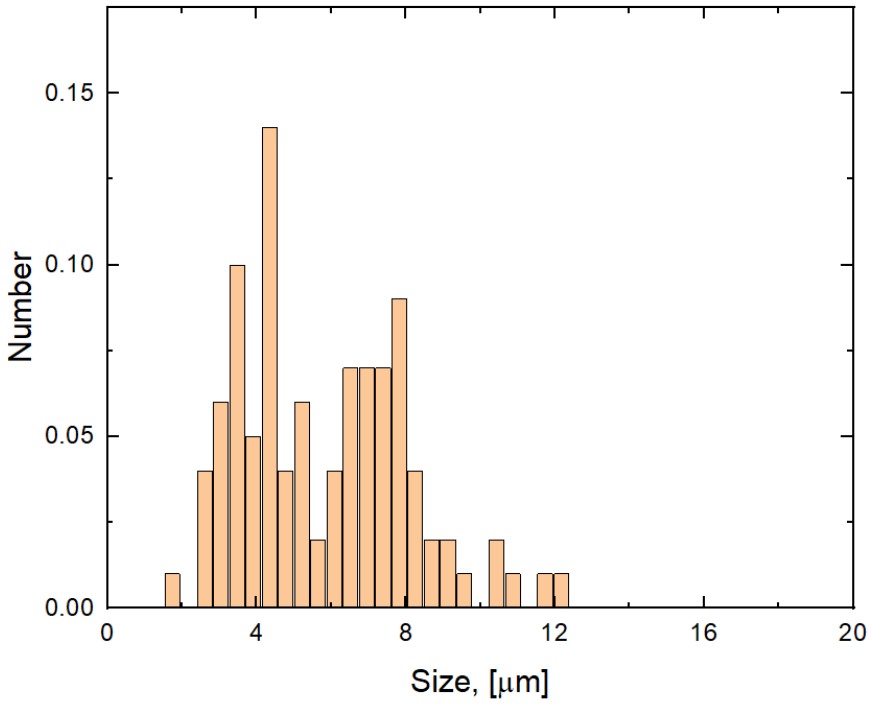


Figure 4. Size distribution of 100 PSBM NP particles based on SEM images

It should be noted that an attempt was made to study the morphology of PSBM Lin, but they were unstable under the electron beam and the attempt was unsuccessful.

III.1.4. Calibration curve for Timolol maleate (TM) at 298 nm wavelength

Using standard solutions of the drug with concentrations: 0.5 μg/ml, 1 μg/ml, 1.5 μg/ml, 2 μg/ml, 2.5 μg/ml, 3 μg/ml, 3.5 μg/ ml, 4 μg/ml, 4.5 μg/ml and 5 μg/ml, a calibration curve for TM was obtained. The absorbance of the standard solutions measured using a UV spectrophotometer at a wavelength of 298 nm were used to plot their dependence on TM concentration (Figure 5).

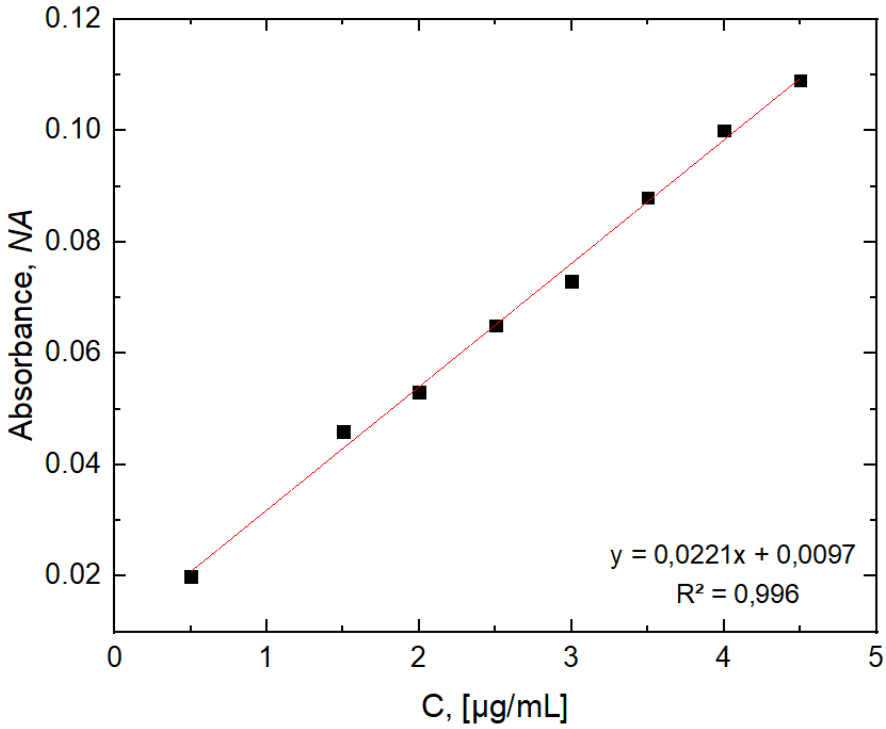


Figure 5. Calibration curve for TM, obtained at 298 nm wavelength

According to the obtained linear equation, the quantities of the unloaded medicinal substance TM, remaining in the supernatant after centrifugation of the solution in which the loading of the PSBM nanoparticles was carried out, are calculated, and from there the amount of TM loaded in the respective sample is determined.

III.1.5 Drug entrapment efficiency (EE) and drug loading capacity (DLC) of PSBM nanoparticles with Timolol maleat

The entrapment efficiency as well as TM loading capacity in PSBM Lin and PSBM NP particles are presented in Table 1. The results show that PSBM Lin exhibits both higher EE and higher TM loading capacity.

Table 1. TM entrapment efficiency and loading capacity in PSBM particles

Sample	EE [%]	DLC [%]
PSBM NP	23 ± 3.5	2.5 ± 0.33
PSBM Lin	31 ± 1.9	3.2 ± 1.7

On the one hand, this is due to the cross-linked structure of PSBM NP, which determines a more difficult entry of TM into the particles during loading. On the other hand,

the lower ζ-potential possessed by PSBM Lin results in more negative charges on the particle surface that can interact with the positively charged TM. These two factors lead to an improvement in both TM loading efficiency and an increase in the capacity of linear PSBM particles to load TM.

III.1.6 TM release profiles from PSBM particles

The release profiles of TM from the two types of PSBM particles are presented in Figure 6. The experiment was performed in an environment that mimics the conditions of the surface of the human eye - a physiological phosphate buffer with pH= 7.4 and a temperature of 36 ± 0.5 °C.

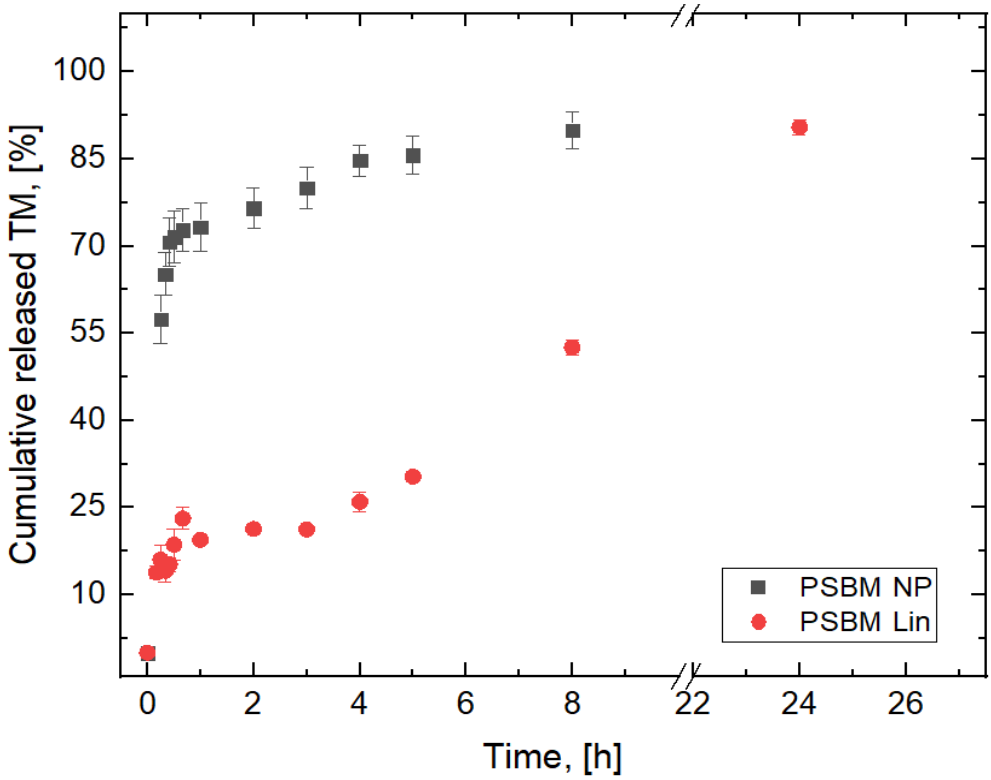


Figure 6. TM release profiles in ocular-like conditions from PSBM NP and PSBM Lin

In the release profile of TM from PSBM NP, an initial "burst" effect of release is observed, and in the first 10 minutes about 50% of the medicinal substance is released. The release then slows down and follows an almost linear profile. Within 9 hours, about 90% of TM is released from the carrier. The observed "burst" effect in PSBM cross-linked particles is probably related to the accumulation of more TM on the surface of the particles, which drug substance is then easily and suddenly released when the environmental conditions change, i.e. in mimicking the eye application. For PSBM Lin particles, only 20% of TM is released in the

first 10 minutes, followed by another 30% within 9 hours. In 24 hours, about 90% of the TM loaded in the PSBM line is released. This delay is again related to the structure of the linear PSBM particles – they have a more spread out conformation and to release the drug contained in them they probably have to begin to disintegrate, a process that requires a longer time. Considering the potential ocular application of TM-loaded PSBM particles, we can conclude that PSBM NPs show a more suitable release profile because PSBM Lin do not release the entire amount of Timolol maleate loaded in them, even within 24 h. Thus, in order to achieve the desired therapeutic effect and to release the entire amount of TM loaded in them, the particles of linear PSBM should stay in the eye for more than 24 hours. Retention of particles on the surface of the eye for a long time could damage the epithelial tissue of the cornea. Therefore, there are mechanisms for washing away particles attached to the surface of the eye. The tear film washes away such particles that fall into the nasolacrimal duct, and from there are expelled from the eye by blinking. These mechanisms make it unlikely that PSBM Lin remain on the surface of the eye long enough to release enough LV to maintain the required therapeutic dose. On the other hand, PSBM NP releases an initial dose of 50% of the included Timolol maleate, thanks to the "burst" effect, and then the rest of the drug is released in the form of a maintenance dose until the 9th hour.

III.1.7 Kinetic study of drug release profiles of TM from PSBM particles

The four main kinetic models were applied to the obtained TM release profiles from the two types of PSBM particles (Figure 6), and the results are presented in Table 2. The first-order kinetic model best described the TM release profiles, since at this model observed the highest correlation coefficient. First-order kinetics for the release profile means that the release rate of TM is proportional to its concentration, i.e. the greater the amount of loaded TM, the faster its release [6]. A more detailed assessment of TM release profiles from both types of PSBM particles can be made by applying the Korsmeier-Peppas model. The diffusion coefficients (n) for both PSBM carriers are less than 0.45, which indicates a Quasi-Fickian diffusion mechanism of release of TM from the PSBM particles, i.e. the TM release mechanism is mainly controlled by drug diffusion, as it is much faster than the time required for the relaxation of the polymer chains.

Table 2. Kinetic models, describes the drug release profiles of TM from PSB NP and PSB Lin

Model/Sample	**PSBM NP**	**PSBM Lin**

Zero order	K_0, *[μg/(ml.h)]*	3.3446	3.2234
	R_0^2	0.775	0.963
First order	K_1, $[h^{-1}]$	-0.0706	-0.0394
	R_1^2	0.9201	0.974
Higuchi model	K_H	11.27	16.122
	R_H^2	0.868	0.913
Korsmeyer – Peppas	K_{KP}	73.0299	21.365
	n	0.1016	0.3271
	R_{KP}^2	0.891	0.827

A possible explanation for this fact is that the diffusion of TM is mainly influenced by the functionality of the polymer carriers, for example, by the electrostatic interactions between the positively charged molecules of the drug substance and the negative charges in the PSBM chains, which can delay or accelerate the release of LV, depending from the conditions of the external environment, which affect the conformation of the zwitterionic molecules, and hence the available charges. Thus, TM diffusion is the controlling parameter for its release from both types of PSBM particles [7].

III.2. Copolymer hydrogels

III.2.1 Equilibrium swelling ratio (ESR) of hydrogels poly(SBM-co-VP) and PSBM homopolymer hydrogel

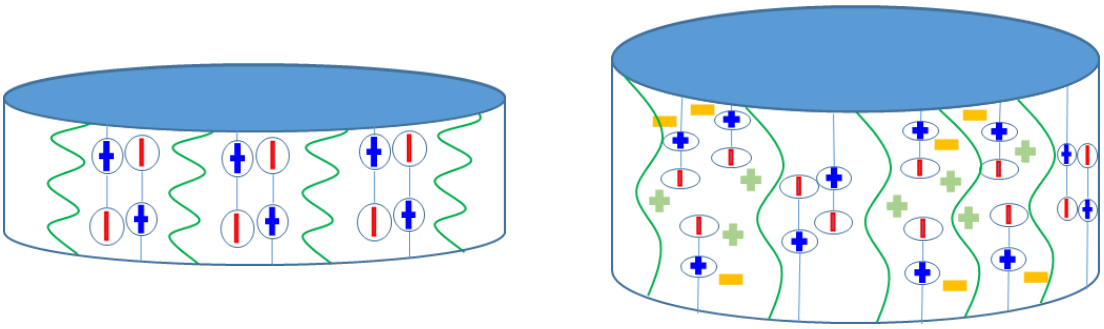
The equilibrium swelling ratio in distilled water of the three copolymer hydrogels as well as the ESR of PSBM hydrogel are presented in Table 3. The data shows that as the molar content of vinyl pyrrolidone (VP) units in the copolymer hydrogel decreases, the ESR decreases.

Table 3. Equilibrium swelling ratio of the hydrogels in distilled water and room temperature

Sample	ESR
SV 1-2	2.55 ± 0.02
SV 1-1	2.2 ± 0.03
SV 2-1	1.98 ± 0.02

PSBM	1.72 ± 0.04

This trend could be explained by two distinct features of the monomeric units, namely: (i) VP is more hydrophilic as compared to SBM and (ii) SBM is able to form a physical network via dipole-dipole zwitterionic clusters (Scheme 2), which reduces its ESR. Thus, both factors define the observed decrease in ESR of the pVP-co-pSB hydrogels in distilled water, as the VP content decreases.



PSBM in H_2O, 25 °C

PSBM in PBS*, 37 °C

*PBS – phosphate buffer saline solution

Scheme 2. Dipole-dipole zwitterionic clusters acting as junctions of the PSBM physical network.

It could be summarized that the proper choice of the copolymer's components as well as the molar ratio between them allows for control on the swelling ability of the newly developed copolymeric hydrogels.

III.2.2 Elastic Modulus of poly(SBM-co-VP)

Elastic moduli of poly(SBM-co-VP) hydrogels at their ESR in distilled water were determined following the Hertz contact theory (Table 4). The results demonstrate a slight increase in the elastic moduli as the SBM content in the copolymeric hydrogels increases. This could be explained by the increase in the number of the dipole-dipole clusters (Scheme 2), which enhances the effect of the PSBM physical network on the properties of the copolymeric networks. This result is in line with the ESR values presented in Table 1, where the same effect of the PSBM physical networks on the swelling ability of the copolymeric networks is clearly observed.

Table 4. Elastic moduli of poly(SBM-co-VP) and PSBM at their ESR in distilled water at room temperature

Sample	Elastic moduli [MPa]
SV 1-2	8.82 ± 0.04
SV 1-1	8.78 ± 0.09
SV 2-1	9.07 ± 0.05
PSBM	9.22 ± 0.04

This result correspond well with the ESR value presented in Table 3, where it is clear that there is an increase in the dense of the physical network with an increase in the content of SBM monomer units and therefore a decrease in the swelling ability of the copolymers.

III.2.3 Swelling kinetics of poly(SBM-co-VP) networks

To elucidate the behaviour of the copolymer hydrogels under conditions similar to the conditions of *in vitro* release of TM, the swelling kinetics of poly(SBM-c-VP) hydrogels was investigated, in a medium of phosphate buffer saline solution (PBS) and a temperature of 37 ° C (Figure 7). For comparison, the swelling kinetics of a PSBM network was also investigated under these conditions. The PSBM hydrogel swelled the most under these conditions, compared to the copolymeric hydrogels (Figure 7), although it had the lowest equilibrium swelling ratio in distilled water (Table 3). This can be explained by the existing physical network in PSBM formed by the dipole-dipole clusters (Scheme 2). These clusters are stable in distilled water at room temperature, but break down (i) on increasing the temperature, in the case of 37 °C, the same process of dipole–dipole cluster breakup being behind the behaviour of the upper critical solution temperature, observed for PSBM [8] and (ii) when adding a low molecular weight salt, in this case, the PBS used in the experiment was prepared from a mixture of inorganic salts. For comparison, the ESR data presented in Table 3 were obtained for swelling in distilled water. Thus, two of the conditions used in the swelling kinetics experiment disrupt the physical network created between the monomer units of SBM and therefore the hydrogel containing the highest amount of SBM swells significantly more both compared to the copolymeric hydrogels and in compared to its swelling in distilled water (Scheme 2).

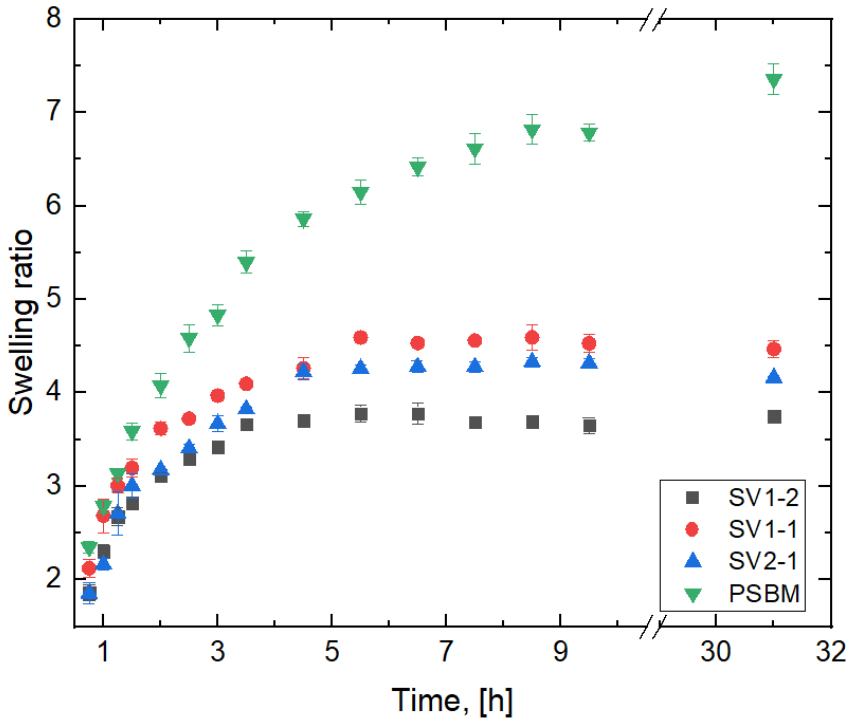


Figure 7. Swelling kinetics of poly(SBM-co-VP) and PSBM hydrogels in PBS at 37°C

The copolymer hydrogels have a smaller initial slope of the curves describing their swelling kinetics, as well as lower values of the degree of swelling, compared to the PSBM network. Similar to the pure PSBM networks, the swelling degrees of the copolymer hydrogels in PBS at 37 °C (Figure 7) were higher than their ability to swell in distilled water (Table 3). The reason is again the disruption of the physical network defined by the presence of sulfobetaine monomer units, although in the copolymers the number of dipole-dipole clusters is less compared to that in the PSBM hydrogel. Therefore, the least swelling can be expected for the copolymer hydrogel with the lowest content of SBM monomer units, namely SV 1-2. Since in this hydrogel the amount of SBM monomer units is the least, the increase in temperature and salt concentration really have the least effect on its swelling.

It is interesting to note that the two other copolymer hydrogels, namely SV 1-1 and SV 2-1, have very close time-dependent profiles of their swelling degrees. This means that the two factors described above, namely the different hydrophilicity of the two monomer units, as well as the physical network formed by SBM, have comparable effects. However, since these effects "oppose" each other, neither is superior to the other in these samples.

III.2.4 Calibration curve of TM at 294 nm wavelength

In an analogous way, described in point *III.1.4*, a calibration curve for TM at a wavelength of 294 nm was made (Figure 8).

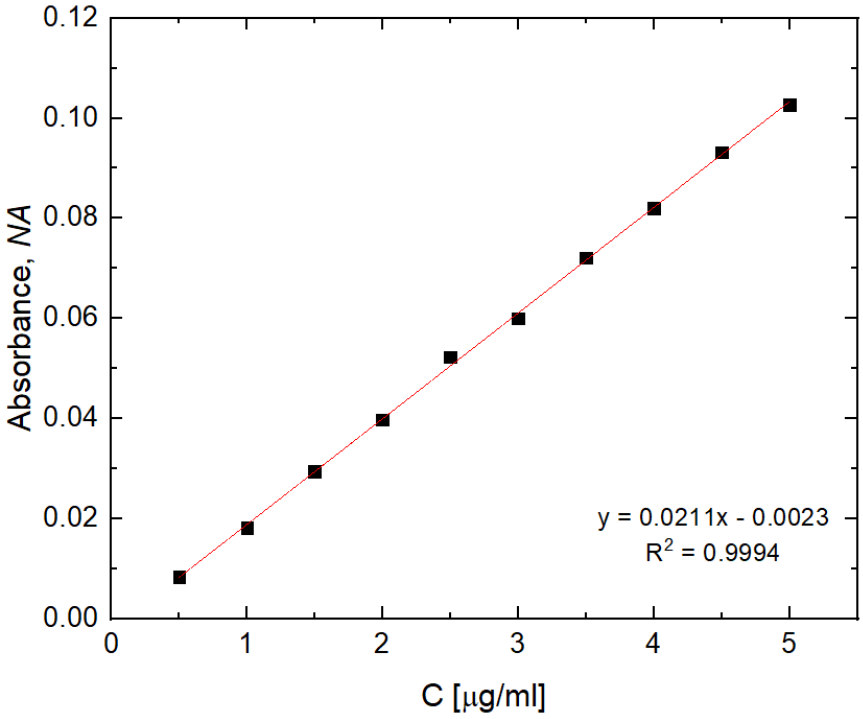
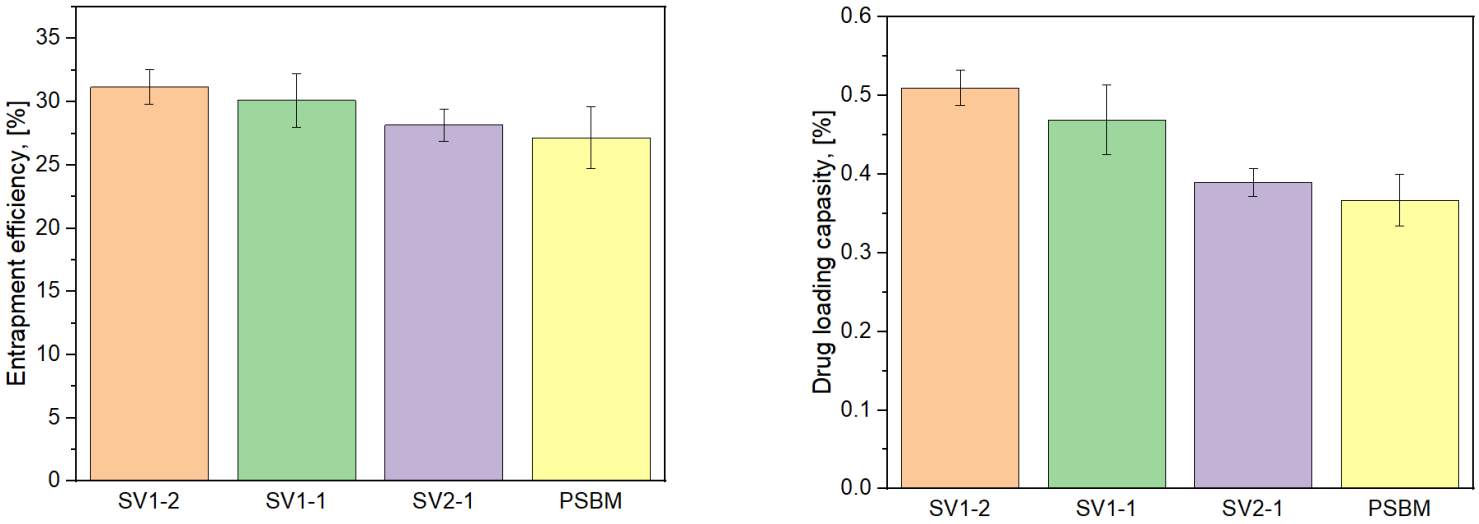


Figure 8. Calibration curve of timolol maleate at 294 nm

III.2.5 Drug entrapment efficiency (EE) and drug loading capacity (DLC) of TM in copolymers poly(SBM-co-VP) hydrogels

Figure 9 (a) and (b) present the results obtained for the loading efficiency and loading capacity of TM in the hydrogels, respectively. The results do not show a clear dependence of the loading efficiency on the copolymer composition – all three copolymer hydrogels have an EE of ~30%, with the PSBM hydrogel having the lowest EE.



(a) (b)

Figure 9. (a) Drug entrapment efficiency and (b) drug loading capacity of TM in copolymeric hydrogels. For comparison, the data for PSBM are also presented

The dependence of DLC of the composition of hydrogels, Figure 9b, can be explained either by (i) interactions between the monomer units of VP and TM, e.g. formation of hydrogen bonds, and/or (ii) by the greater swelling capacity of the rich VP copolymer networks, which improves the diffusion of TM in them, i.e. pure diffusion loading of TM into the polymer hydrogels takes place. The latter assumption correlates well with the results obtained for ESR, presented in Table 3, which demonstrate the higher ability of the VP-rich monomer unit copolymer networks to swell more in distilled water (TM loading is performed in distilled water). Thus, the dependence of DLC of the copolymer composition can be explained, on the one hand, by the increased swelling ability of the copolymer network containing more VP monomer units. This allows more TM molecules to enter the networks, thus increasing the DLC. On the other hand, the formation of a physical network with the participation of SBM monomer units in the copolymer determines a higher degree of crosslinking of the SBM-rich copolymer networks and thus prevents the diffusion of TM in them.

Additional experiments were perfpormed to examine to what extent the concentration of the TM solution used to load the copolymer networks affects the respective EE and DLC. The results show that it is possible to achieve a higher TM loading efficiency by increasing the concentration of the drug substance in the starting solution in which the polymer networks are loaded. In the literature sources that were studied before planning the experiments, the amounts used to load TM in different carriers are very small, for example 8 – 19 µg/ml [9] or a series of

concentrations: 100 µg/ml, 200 µg/ml, 500 µg/ml and 1000 µg/ml [10]. The latter paper shows that although the amount of drug loaded increases, its release profiles do not change. This means that the dose of released TM itself can also be controlled by the initial concentration of TM in the loading solution, but this would not change its release profile.

The ophthalmic solution of timolol maleate most often prescribed to patients with glaucoma has a concentration of 0.125%, and it should be applied 2 times a day. This provides a daily TM dose of approximately 125µg. As already mentioned above, the bioavailability of TM provided by eye drops is less than 5%, usually about 1–2%, which means that the daily intake of TM is about 2.5 µg [11]. On the other hand, it is estimated that about 50% of the drug substance delivered through soft contact lenses reaches the cornea of the eye [12]. With these considerations in mind, we performed simple mathematical calculations of the amount of TM that the poly(CBM-co-VP) copolymer hydrogels would deliver to the eye if applied as a TM-delivery system for timolol maleate in the form of soft contact lenses. These calculations showed that the TM drug loaded in the copolymer hydrogels was about 300 µg, and the average value of 30% was taken as the loading efficiency. This makes a dose of 210 µg TM, of which 105 µg would reach the stratum corneum, at an average of 70% cumulative drug release. This convincingly illustrates the potential of the obtained novel poly(SBM-co-VP) hydrogels as drug delivery systems for TM applied in the form of contact lenses for eyes.

III.2.6 TM release profiles from copolymeric hydrogels of poli(SBM-co-VP)

The TM release profiles from the copolymer hydrogels are presented in Figure 10. All samples exhibited a “burst” effect, as within the first 30 min more than 40% of the loaded TM was released. After the initial explosive release, however, it slows down and exhibits a clear dependence on the composition of the copolymers. The higher the SBM content, the slower the release of TM is (Figure 10 (a)). The time required to achieve full drug release also depends on the copolymer composition: the two samples with the highest SBM content, namely SV 2-1 and PSBM, reach full TM release in 48 h (Figure 10 b).

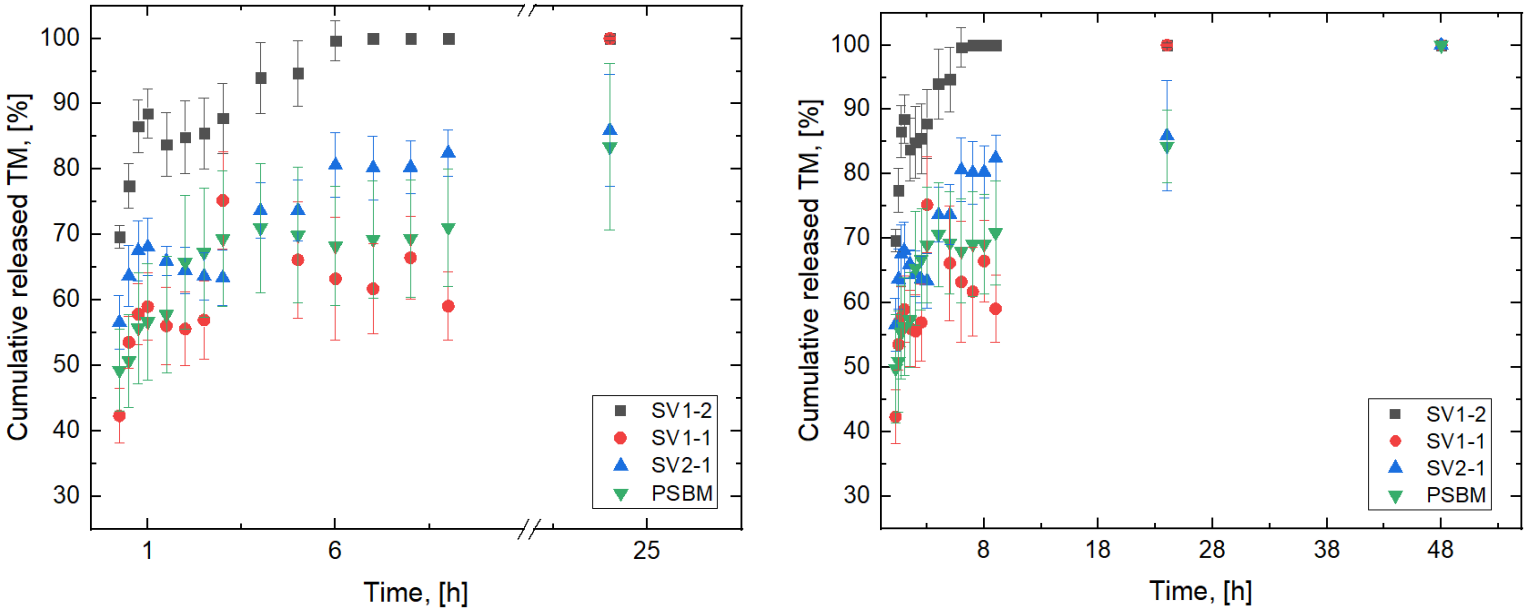


Figure 10. TM release profiles from copolymer hydrogels for period of (a) 24 hours and (b) 48 hours

On the other hand, the sample with the highest VP content, SV 1-2, released the drug substance the fastest (in ~6 hours). This is not entirely expected, as the swelling kinetics curve of this sample, under conditions resembling TM release conditions, shows the slowest swelling, as well as the lowest swelling ratio, of the three copolymer hydrogels (Figure 7). Therefore, if TM release depends only on its diffusion, defined by the swelling ability of the polymer network under the conditions used for the TM release experiment, SV 1-2 should be the slowest TM-releasing hydrogel. This result can be due, on the one hand, to drug-polymer interaction, but on the other hand, to the disintegration of the physical network due to the monomer units of SBM. This decay occurs concurrently with the swelling and drug release process, also influencing the TM release profile. In support of this explanation is the fact that the two copolymer networks where the monomer units of SBM are 1:1 or predominate compared to those of VP slow down the release of TM compared to the one in which the monomer units of VP predominate. The copolymer hydrogel SV 1-1, where the ratio between the monomer units SBM and VP is 1:1, releases TM much more slowly after the initial "burst" effect - about 60% of TM is released in the first 9 hours and 100% of TM is released for 24 hours (Figure 10a).

The copolymer hydrogel with the highest SBM content, SV 2-1, released TM faster than SV1-1 but slower than SV 1-2 during the first 9 h, probably precisely due to the fact that it took time to destroyed the physical network of SBM. After 9 h, however, SV2-1 released

TM much more slowly and 100% of TM was released from this hydrogel after 48 h (Figure 10 (b)). The release profile of TM from the SV 2-1 copolymer hydrogel closely resembles the drug release from the PSBM network (Figure 9), with both hydrogels releasing the entire loaded amount of TM in 48 h (Figure 9(b)). The amount of TM released by them during the first 9 hours was also comparable (Figure 9).

The obtained TM release profiles from the copolymer hydrogels (Figure 10) can be related to the amount of SBM monomer units in them, since they can form a physical network that would further hinder TM release. This physical network is not static, as it gradually breaks down under the influence of temperature and salt concentration (part of the experimental conditions of the TM release experiment). It takes some time for the zwitterionic physical network to be destroyed and the copolymer hydrogel to release more and more TM, which extends the drug release time up to 48 hours, in cases where the content of SBM monomer units is the highest. Thus, Figure 10 well illustrates the influence of sulfobetaine monomer units on TM release profiles.

III.2.7 Kinetic study of drug release profiles of TM from copolymer hydrogels

The release profiles of TM from the copolymer hydrogels were investigated by applying the basic mathematical models describing the kinetics of drug release from carriers (Table 5). None of the four used models perfectly described the TM release profiles presented on Figure 10. The Higuchi diffusion model obtained the highest correlation coefficients for the four samples studied, which is somewhat expected since the Higuchi diffusion model was developed namely for hydrogel drug delivery systems.

The Korsmeyer-Peppas model also shows high correlation coefficients, especially for samples SV2-1 and PSBM, but it cannot be applied for sample SV1-2, where the "burst" effect of TM release is too high (almost 70% of the drug is released in the first 15 minutes). The diffusional exponent (n) for the studied hydrogels are less than 0.45, which indicates a Quasi-Fickian diffusion of TM upon its release from the copolymer gels. This means that the time for diffusion of TM out of the hydrogels is much faster than the time required for relaxation of the macromolecular chains in the hydrogel [7].

Table 5. Kinetic models, describeing drug release profiles of TM from poly(SBM-co-VP) and PSBM

Model/Sample		SV1-2	SV1-1	SV2-1	PSBM
Zero order	K_0 $[\mu g/(ml.h)]$	1.023	1.826	1.163	1.227
	R_0^2	0.775	0.739	0.619	0.644
First order	K_1 $[h^{-1}]$	0.005	0.011	0.0069	0.008
	R_1^2	0.408	0.662	0.584	0.574
Higuchi model	K_H	6.721	9.268	8.974	7.321
	R_H^2	0.678	0.691	0.812	0.829
Korsmeyer-Peppas	K_{KP}	-	54.475	70.958	56.079
	n	-	0.104	0.162	0.099
	R_{KP}^2	-	0.551	0.998	0.913

This conclusion is consistent with the observed influence of the content of SBM monomer units on TM release. The formed zwitterionic physical network, due to the presence of SBM in the carriers, “hardens” the copolymer matrix, which leads to the retention of the drug substance inside the hydrogel. The more "stiffened" the polymer network, the greater will be the difference between the TM diffusion rates and the relaxation of the polymer chains. As the in vitro release conditions (temperature and PBS) begin to disrupt the sulfobetaine clusters, the drug is gradually released and begins to leave the hydrogel, thus the content of monomer units of the SBM strongly affects the TM release profile.

III.2.8 Light transmittance of TM-loaded hydrogels of poly(SBM-co-VP) and PSBM

The application of poly(SBM-co-VP) hydrogels as drug delivery contact lenses requires that they have to be transparent and not blur the vision of patients. Therefore, the light transmission through the TM-loaded copolymer hydrogels was measured (Figure 11).

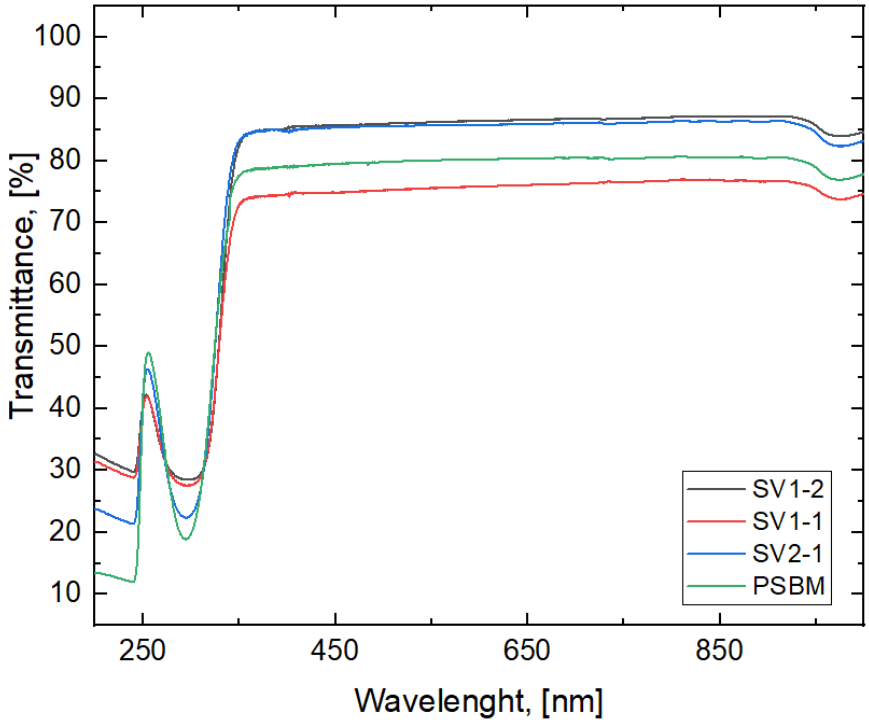


Figure 11. Transmittance spectrum of TM-loaded hydrogels of poly(SBM-co-VP) and PSBM

The absorption of TM loaded into the respective hydrogels was clearly observed with an absorption maximum at 294 nm. The results presented in Figure 11 show that in the visible region of the spectrum, the SV1-1 hydrogel has the lowest transparency, around 70%. The PSBM hydrogel loaded with TM has a transparency of about 75%, while the hydrogels of SV1-2 and SV2-1 have higher light transmittance, which is approximately 85%. A transparency of 85% is sufficient to provide clear vision through the materials when they are used as eye contact lenses. Gulsen et al. al determined that the transmittance of conventional soft contact lenses made from PHEMA was 87% [13]. Another study by Zhu et al. [14] showed that a transmittance higher than 67% does not affect the optical properties of the materials, i.e. they can be used to make contact lenses. Copolymer hydrogels SV1-2 and SV2-1 have the highest transparency, although the other two hydrogels would also be good potential materials for soft contact lenses.

The permeability of the polymer hydrogels was also determined after 5 hours of TM release, i.e. after some of the loaded TM leaves the hydrogel to test whether the drug release process will affect the transparency of the copolymer hydrogels. The results presented in Figure 12 show that the permeability of all poly(SBM-co-VP) copolymer hydrogels increased after 5 h of TM release, most likely due to the decrease in drug content and not to processes occurring

in the networks. It should be remembered here that the experiment tracking the drug release starts from a swollen state of the hydrogels, so they do not significantly change their volume and thickness, which could cause discomfort to the patient.

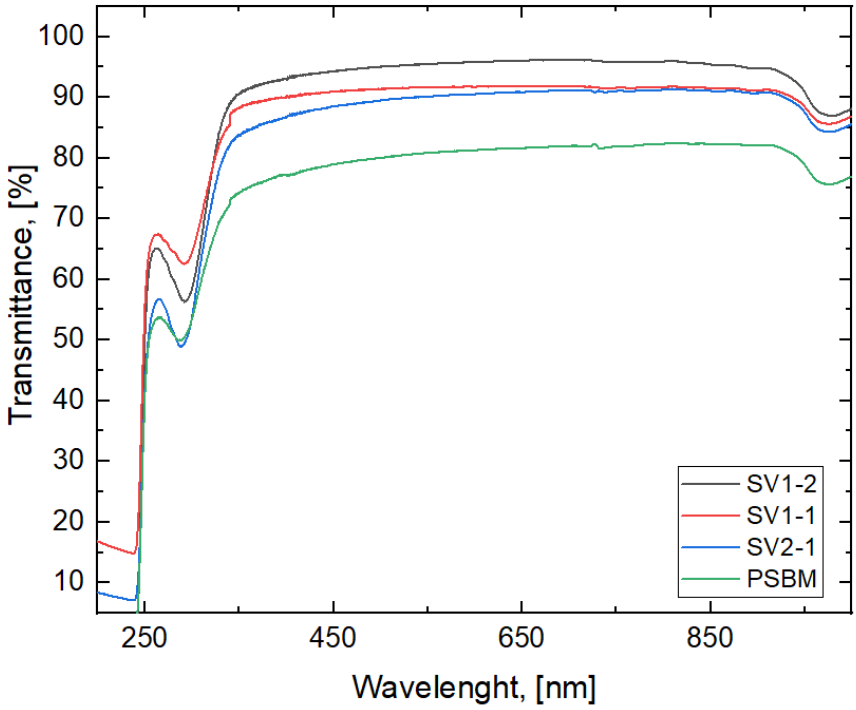
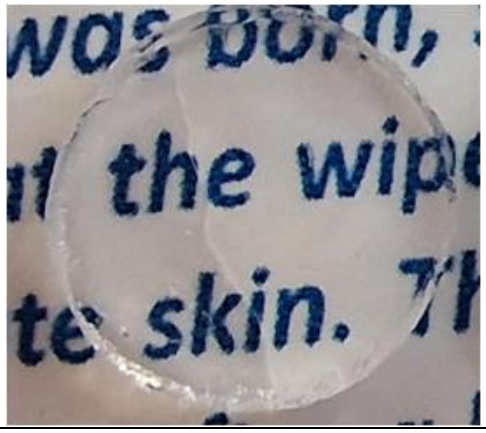


Figure 12. Transmittance spectrum of TM-loaded hydrogels after 5 h of drug releasing in PBS at 37 °C

The sample that after 5 hours of TM release had the highest drug substance content (PSBM) (Figure 10) showed the lowest permeability (Figure 12), although it had the highest degree of swelling when determining the kinetics of swelling (Figure 7), i.e. it should also have the highest degree of swelling in this experiment. On the other hand, the hydrogel that after 5 h had released ~95% of the loaded drug had the highest transparency, namely SV1-2 had 95% permeability (Figure 12). Thus, the results in Figure 12 show that the permeability of the copolymer hydrogels increased after release of TM for all copolymer hydrogels, but did not change for the PSBM hydrogel. Thus, it can be expected that the release of TM will not degrade either the light transmission of the hydrogels or the wearing comfort – after 5 hours of TM release time, no detectable change in the thickness of the hydrogels is observed.

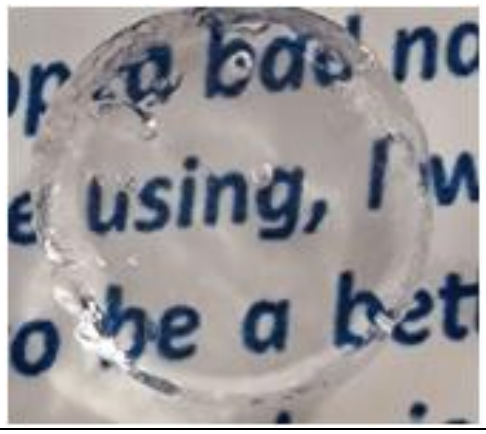
Further proof of the transparency of the copolymer hydrogels are the photographic images presented in Figure 13. These images clearly show that the hydrogels are completely transparent when loaded with TM, i.e. before his release begins.

(a)



(b)

(c)



(d)

Figure 13. Photographic images of TM-loaded hydrogels of (a) SV1-2, (b) SV1-1, (c) SV2-1, and (d) PSBM

Additional analyzes of the obtained data (Figure 13) show that all hydrogels do not transmit a large part of the waves in the UV-B region. Their transmittance in this region of the UV spectrum is between 19% and 28%, depending on their composition, meaning they block between 72% and 81% of UV-B radiation (Table 6).

Table 6. Optical transmittance of poly(SBM-co-VP) and PSBM hydrogels, loaded with TM, determined for the different parts of the UV and visible spectrum

Sample/ Transmittance	**UV-B (280–315nm)**	**UV-A (316–380nm)**	**SBR* (381–460nm)**	**LBR** (461–500nm)**
SV1-2	28.55%	81.43%	85.61%	85.84%
SV1-1	27.53%	72.12%	74.78%	75.06%
SV2-1	22.48%	82.58%	85.16%	85.5%
PSBM	19.16%	77.12%	79.22%	79.70%

*shortwave blue region
**longwave blue region

Recent studies have outlined the relation between the UV-B radiation present in sunlight, and the formation of natural lens cortical opacities. Moreover, as UV-B rays have shorter wavelength, but higher energy level as compared to UV-A, they do not penetrate much in the tissues. Thus, they hit the corneal epithelium, and are known to accelerate the loss of corneal epithelial cells and enhance the related photo keratitis [15]. That is why soft contact lenses are required to also have UV protection, which is usually imparted by adding special additives. TM-loaded poly(SB-co-VP) and PSBM hydrogels exhibit an inherent UV-B blocking ability, which is an additional advantage for their application as soft contact lenses.

IV. Conclusions

- For the first time, PSBM homopolymer particles were investigated as TM drug delivery systems for ocular application. Two types of PSBM particles were successfully synthesized - from linear and from chemically cross-linked PSBM. For the first time, the temperature dependence of the hydrodynamic size of PSBM particles, as well as the temperature dependence of their ζ-potential, were determined. These dependencies are explained by the association of PSBM particles, which depends on temperature. Linear PSBM (PSBM Lin) particles have a smaller hydrodynamic diameter and lower ζ-potential, compared to cross-linked PSBM (PSBM NP) particles, in the temperature range characteristic of the surface of the human eye. The two types of particles show comparable TM loading efficiency - about 30% for PSBM Lin and 23% for PSBM NP. The structure of the PSBM particles was shown to influence the release profile of TM: the cross-linked particles provided a linear release of TM between the first and ninth hours, as well as a release of 90% of the loaded Timolol maleate within the first 9 hours, while linear poly(sulfobetaine methacrylate) particles, despite having a higher drug loading efficiency, released only 50% of the incorporated drug within the first 9 hours. Thus, the release profile of TM from PSBM NPs is considered to be more suitable for ocular application.
- Copolymer hydrogels of poly(sulfobetine methacrylate-co-vinyl pyrrolidone), cross-linked with poly(ethylene glycol) diacrylate, with different compositions were successfully synthesized for the first time. Copolymer hydrogel composition was shown to influence TM loading capacity—copolymers rich in VP monomer units showed higher drug loading capacity. The composition of the copolymer was also found to affect the TM release profiles: a higher content of monomeric units of SBM determined a slower release of TM, which was explained by the presence of an additional physical network due to dipole-dipole clusters, formed between the zwitterionic side groups of SBM. Thus, the results of the dissertation show that copolymer networks of poly(SBM-co-VP) have a very good potential as drug-delivery systems for timolol maleate in the form of soft contact lense, and the composition of the copolymers can controlled the TM loading capacity and its release profile. The newly synthesized copolymer hydrogels have good optical properties: they exhibit high transmittance of visible light while at the same time blocking harmful UV-B rays.

In conclusion, both types of newly developed polymeric carriers were shown to prolong the release of TM while simultaneously providing the required therapeutic dose.

V. Dissertation contributions

- Homopolymer nanoparticles based on poly(sulfobetaine methacrylate) crosslinked with poly(ethylene glycol diacrylate) were synthesized for the first time
- For the first time, the temperature dependence of the hydrodynamic size and ζ - potential of linear and cross-linked PSBM nanoparticles were determined
- For the first time, homopolymeric linear and cross-linked PSBM nanoparticles were used as timolol maleate carriers in the eye
- Copolymer hydrogels of sulfobetaine methacrylate and vinyl pyrrolidone crosslinked with poly(ethylene glycol diacrylate) were synthesized for the first time
- For the first time, these hydrogels were considered as potential drug-delivering soft contact lenses for timolol maleate eyes

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VII. List of publications:

VII.1 Publication on the dissertation topic

1. Nikolova, D., Ruseva, K., Tzachev, C., Christov, L., & Vassileva, E. (2022). Novel Poly(Sulfobetaine methacrylate) based carriers as potential ocular drug delivery systems for timolol maleate. *Polymer International*. https://doi.org/10.1002/pi.6368 (Q1, IF=3.213)

2. Nikolva, D., Tzachev, C., Christov, L., Vassileva, E., (2023) Poly(Sulfobetaine Methacrylate-co-Vinyl Pyrrolidone) Hydrogels as Potential Contact Lenses Delivery Systems for Timolol Maleate. *Gels.* https://doi.org/10.3390/gels9020114 (Q1, IF=4.432)

VII.2 Publication outside the dissertation topic

1. Simeonov, M., Gussiyska, A., Mironova, J., Nikolova, D., Apostolov, A., Sezanova, K., Dyulgerova, E., & Vassileva, E. (2019) Novel hybrid chitosan/calcium phosphates microgels for remineralization of demineralized enamel – A model study. *European Polymer Journal*. https://doi.org/10.1016/j.eurpolymj.2019.07.005 (Q1, IF=5.546)

2. Nikolova, D., Simeonov, M., Tzachev, C., Apostolov, A., Christov, L., & Vassileva, E. (2021). Polyelectrolyte complexes of chitosan and sodium alginate as a drug delivery system for diclofenac sodium. *Polymer International*. https://doi.org/10.1002/pi.6273 (Q1, IF =3.213)