CHITOSAN/POLY(VINYL ALCOHOL) HYDROGELS FOR ENTRAPMENT OF DRUG LOADED LIPOSOMES

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The paper describes the modeling of drug release from chitosan/poly(vinyl alcohol) hydrogels obtained by using a double crosslinking technique. These complex hydrogel systems were able to reduce the burst effect observed in control hydrogels and to prolong the drug release from 24-48 hours to two weeks. The principle consists in drug entrapment in phosphatidylcholine based liposomes of different sizes and lamellarity along with their subsequent dispersion in hydrogels during the preparation process.
Liposomes containing calcein as a model hydrophilic drug diffuse from the prepared matrices as a function of the network characteristics and the liposome diameter. Multilamellar vesicles (MLVs) containing calcein were detected in the supernatant, due to their better stability, and were covered by multiple lipid bilayers. On the contrary, small unilamellar vesicles (SUVs) containing calcein were less stable during the diffusion through the hydrogel and released their content mostly during their movement inside the matrix. The drug release can be adjusted by modeling the release of liposomes from such hydrogels. Considering the biocompatibility and biodegradability of the components, these hydrogels could be applied in the medical field, when a long period of drug release is needed.

INTRODUCTION

The use of hydrogels, which are mainly seen as biomaterials, in different fields of technology, is determined by their unique properties, such as their intermediary behavior between both solid and liquid materials. The hydrophilic character of the polymers constituting the hydrogels determines their capacity to retain quantities of water, which can be very high at some point and, at the same time, of the substances dissolved in water. In addition, if they are biocompatible and nontoxic as the natural ones (polysaccharides, proteins), the corresponding hydrogels might be used as carriers for inclusion, transport and sustained/controlled drug release.[1-4]Their hydrated network shows a very good compatibility with proteins, living cells and fluids in living organisms. The first signals in the literature regarding the possibility to use hydrogels for biomedical purposes are associated with Wichterle and Lim,[5]and refer to materials based on poly(2-hydroxyethyl methacrylate). Eversince, many other hydrogels have been developed for biomedical and pharmaceutical applications, for the release of drugs and other bioactive compounds in particular. The presentation form varies depending on the intended use and may include films, sponges and particles. This type of material formulation revealed in time a series of disadvantages, such as reduced ability to include drugs, the capacity to load only water soluble drugs or in water/alcohol mixtures, the release of the active compound with an initial burst effect, determining the process to continue with a relatively constant speed and making it possible to control. Often, the toxic nature of the crosslinkers used must be added, especially of the covalent ones, which involves lasting and expensive purification operations. The main release

mechanism is drug diffusion through the polymeric matrix, according to the principle of Fick's Law,[6] but it is often disturbed by factors such as crosslinking density, drug interactions with the polymeric support, matrix porosity, etc. The introduction of an additional barrier in the drug release process may be a possibility to control and to regulate the release kinetics by reducing or even eliminating the burst effect. Drug encapsulation into a reservoir, whose membranes could constitute an additional barrier, may represent a solution for the adjustment and control of the kinetics. Such reservoirs can be represented by micro/nanocapsules with a much higher drug loading capacity, compared to full micro/nanospheres of the same size. A special category of such micro/nano reservoirs is constituted by liposomes, self-assembled lipid vesicles, with the advantage that they are biocompatible and can encapsulate both hydrophilic and lipophilic drugs; drug formulations based on liposomes are already on the market.[7] The main disadvantage of these vesicles stands in the poor mechanical and biochemical stability, which limits their resistance in the blood stream to only a short period of time, after which they break and suddenly release the encapsulated drug, which produces an undesired burst effect on the release kinetic curves. The inclusion of liposomes in polymeric matrices can combine the benefits of integrating the two types of carriers and represents a way of modeling the release kinetics, which is the main objective of this work. *In vitro* studies showed that the diffusion resistance of gels containing liposomes is higher than that of hydrogels and liposomes alone.[8-10] If modeling the release of small molecular weight drugs from prepared hydrogels involves the use of additional amounts of crosslinkers, the present study focuses on the liposomes behavior inside the hydrogels or during their diffusion and passage through the hydrogel matrix.

This paper is part of a series of studies and aims to model the drug release kinetics from hydrogels and to establish appropriate kinetic models corresponding to the proposed transport and release mechanism. Hydrogels used as matrices for the inclusion of hydrosoluble drugs (in this case, represented by a model compound – calcein) are obtained from a mixture of chitosan and poly(vinyl alcohol) with an interpenetrating network type. The addition of poly(vinyl alcohol) aims to control the hydrophilicity of the system in order to avoid the use of additional crosslinker (a common option to control the network properties through crosslinking density). We have chosen the double crosslinking of the polymer mixture, an option that significantly reduces the amount of covalent crosslinker (glutaraldehyde) by partially substituting it with ionic compounds (sulphate or tripolyphosphate anions) with an important reduction in the toxicity of the material used.[11] We have analyzed the influence of some factors on the kinetics of the release process (the crosslinker type, the composition of the IPN obtained, the type and the size of calcein loaded liposomes).

EXPERIMENTAL

Materials

Medium molecular weight chitosan (C), degree of deacetylation~75%, poly(vinyl alcohol)(PVA) 9.000-10.000 mol/g, 80% degree of hydrolysis, glutaraldehyde (GA) 25% aqueous solution, sodium tripolyphosphate (TPP), Sephadex G-25, calcein (fluorescent hydrophilic model drug– Figure 1) and Triton X-100 were purchased from Sigma-Aldrich (St. Luis, MO, USA). Phospholipon-90G was received as a gift sample from Phospholipid GmbH, Nattermannallee 1, D-50829,Köln. Sodium sulphate was obtained from Primex Chim (Bucharest, Romania). All other chemicals used were of analytical grade.

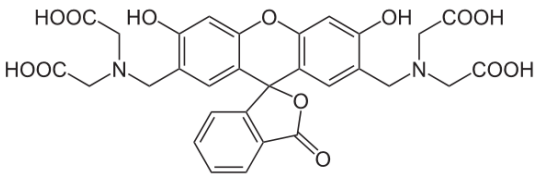


Figure 1: Chemical structure of calcein

Preparation methods

Preparation of liposomes

Large multilamellar vesicles (MLVs) and small unilamellar vesicles (SUVs) composed of phosphatidylcholine were prepared in order to encapsulate calcein, a self-quenching hydrophilic model drug currently used in liposomes technology, and to study the release behavior from such systems.

Multilamellar vesicles (MLVs) were prepared by the thin film hydration method.[12] Briefly, this involves the dissolution of 200 mg Phospholipon-90G in a chloroform/methanol mixture and then evaporating the solvent using a rotary vacuum evaporator (Heidolph Laborota 4002) to obtain a lipid film on the wall of the flask. Then, the lipid film was dried under vacuum for two hours. The hydration of the film with a drug solution and vortex shaking (calcein – 30 mg/mL) resulted in liposomes self-assembling in multilamellar liposomes containing hydrophilic drug. For liposome separation from free calcein, centrifugation was performed (3 cycles at 15.000 rpm for 40 min), using Hetich Zentrifugen Universal 320R equipment.

SUVs were prepared by the sonication method,[13] which reduced the size of the previously obtained multilamellar liposomes. A Bandelin Sonopuls GM 2200 probe type sonicator (uniform sonic waves), 20 kHz processing frequency (10 pulses of 60s duration and 30s break to prevent overheating of the sample) was used. The separation of the SUVs from MLVs and free calcein was realized by Size Exclusion Chromatography (Sephadex G-25 column, 25 cm length, 1cm diameter, eluted with PBS buffer -2.2 mL/min). Due to their instability, liposomes were used in the first hours of preparation. Subsequent diameter analysis of liposomes was performed by the laser light diffractometry technique (Shimadzu – SALD 7001); three successive measurements were performed and the standard deviation was calculated.

Preparation of hydrogels

Plain hydrogels were prepared by dissolving the polymers (2.5% final concentration) overnight in 2% acetic acid solution (v/v) at 25°C. The solutions were filtered using a reusable syringe filter holder coated with filter paper and partially crosslinked with GA. A sufficient quantity of GA was taken for analysis in order to provide the crosslinking of approximately 20% of the amino groups present in chitosan by taking into account the degree of deacetylation of the polymer. The homogenization of GA in the polymer solution was performed by stirring manually. The gels were then dried in the oven for 1 hour at 50°C and then were immersed in 10 mL of ionic crosslinker (1% concentration). Sodium sulphate and sodium tripolyphosphate were used for this purpose in a sufficient amount to provide 80% crosslinking of the remaining amine groups from the system (in the form of ammonium ion). Double crosslinking was used to reduce the amount of glutaraldehyde, known as toxic, but not below a certain limit (about 20% crosslinking of amino groups) in order to preserve the integrity of the obtained hydrogels.[14,15]The studied parameters were the C/PVA ratio (w/w), as well as the ionic crosslinking agent type, as can be remarked in Table 1.

Control hydrogels containing calcein (not encapsulated in liposomes) were prepared by mixing the calcein solution with the polymer mixture subjected to crosslinking (125 mg). In each hydrogel, 640 μg calcein (20μL stock solution – 32 mg/mL) was added prior to GA addition, which was partially released during the ionic crosslinking procedure. This amount was determined and was subtracted from the total included into hydrogels, as can be seen in Table 1.

To create an extra barrier in the drug diffusion process calcein was entrapped in liposomes of different sizes. Before GA addition, a fixed volume of liposomes (250 μL) was mixed with the polymer solution to obtain the complex systems. The total calcein content included in the hydrogels was calculated by treating a liposome suspension with the surfactant Triton X-100, capable of disrupting the liposomal membrane. The preparation followed the same protocol as for the preparation of plain hydrogels.

Table 1
Hydrogel preparation protocol

Sample code	C/PVA (w/w)	GA 25% (μL)	Ionic crosslinker (10 mL – 1%)	Calcein loaded in control hydrogels (μg)*	Calcein loaded in complex hydrogels (μg)*
CP-S1	9	32.3	Na_2SO_4	581.8	455
CP-S2	5.7	33.6	Na_2SO_4	592.1	455
CP-S3	4	35	Na_2SO_4	591.7	455
CP-S4	3	36.3	Na_2SO_4	588.4	455
CP-T1	9	32.3	TPP	571.6	507.2
CP-T2	5.7	33.6	TPP	583.2	507.2
CP-T3	4	35	TPP	585.8	507.2
CP-T4	3	36.3	TPP	580.4	507.2

*the amount of hydrogel loaded with calcein or liposome suspension was 125 mg in all cases

Characterization methods

Swelling capacity

In order to study the swelling behavior of the hydrogels prepared, the gravimetric method was used. Briefly, the protocol consisted in completely drying the samples at 100°C (three hours ensured a constant weight). Then, the samples were weighed and immersed in PBS solution (pH=7.4) for 48h. The films were then removed from the swelling medium and weighed after elimination of the excess water from their surface by gently touching with filter paper. The swelling degree was calculated using the equation:

$$Q(\%)=\frac{W_1-W_0}{W_0}\times 100 \quad (1)$$

where:W_1 –swollen sample weight; W_0 –dry sample weight.

Measurement of liposome integrity

For measuring liposome integrity, calcein self-quenching was a useful tool, since at the concentration used (calcein from the interior of the vesicles), no fluorescence appeared. Only after the liposomes' destruction with the surfactant and subsequent dilution of calcein in the surrounding media, we could determine liposome integrity and attributed it to cargo vesicles by making the difference between the total and the initial calcein. The protocol consists in with drawing samples of 1 mL from the supernatant (containing free calcein and calcein entrapped in liposomes) at regular intervals, which were then replaced by fresh PBS solution. Then, 20 μL of the liposome suspension was diluted with 4 ml buffer (pH 7.4) to measure the free calcein intensity. Afterwards, the samples were treated with Triton X-100, able to rupture the liposomal membrane and total calcein intensity was measured again. The latency parameter was calculated using the following equation:

$$\%Latency=\frac{1.1\cdot I_{AT}-I_{BT}}{1.1\cdot I_{AT}}\cdot 100 \quad (2)$$

where: I_{BT} and I_{AT} are the calcein amounts released before and after the addition of Triton X-100, respectively.

The latency was used to determine the encapsulation efficiency after the column separation. In each experiment, at least 95% of calcein was entrapped in liposomes.

Calcein release from hydrogels

The calcein release from the prepared hydrogels was spectrophotometrically studied. The hydrogels containing calcein were immersed in 20 mL of phosphate buffer saline (pH=7.4) and calcein was monitored in time, using a Perkin Elmer LS 50B fluorescence spectrometer (excitation/emission = 490/515 nm, bandwidth 5 nm). The *in vitro* conditions were the following: 60 rpm and 37±0.1 ºC settled by GFL 1092 water bath. Each hydrogel was prepared in duplicate. Then, 1 mL of supernatant was removed, analyzed and replaced with fresh PBS solution. In the case of complex systems, for liposome breakage, 1% final concentration of Triton X-100 was used.

The calcein released was calculated by using the calibrating plot presented in Figure 2 and equation (3):

$$m_{\mathrm{ci}}=C_{ci}\times V+\sum_{i=1}^{i-1}m_{ci-1}\times 100 \quad (3)$$

where: m_{ci} – total calcein released at the moment *i*, C_{ci}– calcein concentration at the moment *i* (ng/ml); V – the buffer volume where the calcein is released(20 ml); m_{ci-1}– calcein content in one mL of suspension or solution equal with concentration (C_{ci}) removed from supernatant for determination and replaced with fresh PBS buffer at previous moments of experiments (t_{i-1})(g).

The release efficiency was calculated by equation (4).

$$\boldsymbol{E_i}(\%)=\frac{\boldsymbol{m_{ci}}}{\boldsymbol{m_{ct}}}\times\mathbf{100} \quad (4)$$

where: m_{ci} is the total calcein released at moment *i* and m_{ct} is the total calcein included into hydrogels.

To determine the calcein concentration in the samples that were periodically drawn from the release medium for analysis, the calibration curve was performed (R^2 =0.999), where: *y* =intensity and x= concentration (ng/mL).

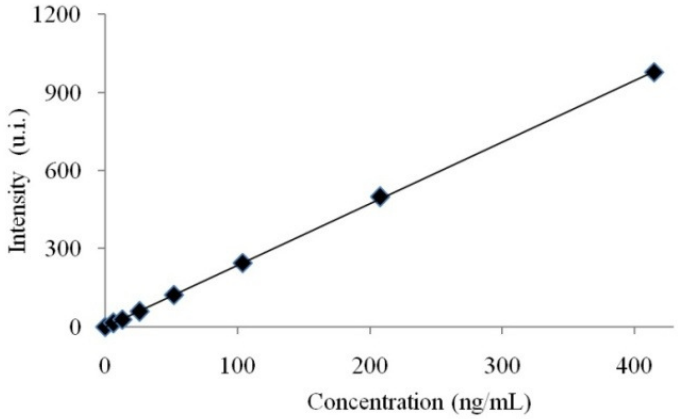


Figure 2: Calibration curve of calcein in PBS (pH=7.4)

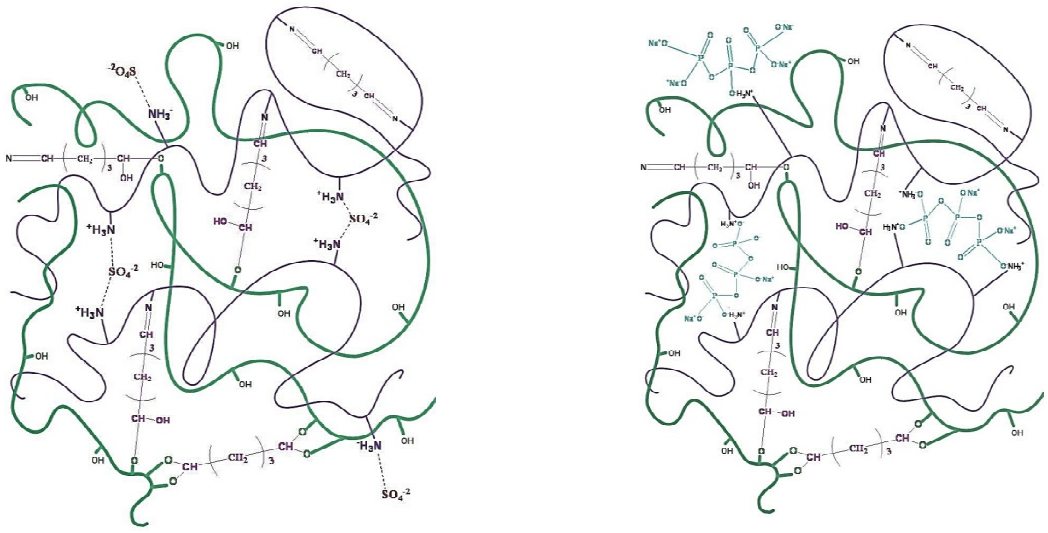
RESULTS AND DISCUSSION

Small molecular weight drugs usually diffuse fast from polymeric hydrogels. In order to reduce the release rate, it is necessary to increase the amount of crosslinker, which improves the density of the network, although high precision is difficult to accomplish due to the large mesh size of the hydrogel. Moreover, raising the amount of crosslinker may be dangerous to the human body, especially when covalent crosslinkers are involved–glutaraldehyde[16] or formaldehyde.[17] Hydrogel systems with low levels of covalent crosslinker and well controlled drug release can be obtained by encapsulation of the drug in liposomes and subsequent dispersion of liposomes into hydrogels. Therefore, a composite system with at least two control points of drug release can be obtained: first, the hydrogel itself can be reasonably well tuned in terms of swelling capacity and mesh size, by controlling the type and amount of the crosslinker (hydrogel swelling has implications in the diffusion of liposomes from such matrices); secondly, the great variability in liposome composition and size represents a second release mechanism that can be adjusted, depending on the desired release capacity.

In this study, two hydrogels were prepared with a minimum amount of GA and two types of accepted biological ionic crosslinkers. The main difference between them is the functionality (theoretically, TPP binds 5 protonated amino groups, and sodium sulphate only 2), leading to the formation of different matrices in terms of the crosslinking density. Moreover, the ratio between the polymers varied in order to check the implications of PVA in terms of the release behavior. This polymer is capable of being crosslinked by glutaraldehyde, despite the fact that it does not contain amino groups as substituent on the backbone. However, it may participate to the crosslinking process, with hydroxyl groups that are capable of forming either semiacetal links or acetal cycles with glutaraldehyde. In the second release point, liposomes of different sizes were used in order to check the implications of liposome diameter in the diffusion process. A major challenge was to retain the integrity of the liposomes during the hydrogel preparation process in the presence of the crosslinking agents. To completely characterize the release mechanism, it was necessary to study if the calcein was released in a free form or encapsulated in liposomes.

The network that was formed under these conditions is the interpenetrating/interconnected type, the bridges between macromolecules being assured, on one side, by glutaraldehyde and, on the other side, by ionic crosslinker molecules (sulphate or tripolyphosphate anions). Figure 3 shows the schematic structure of such a hydrogel.

The main diameters measured for the vesicles used in this study are 1.26±0.237 μm for MLVs and 0.12±0.014 μm for SUVs. The size distribution of liposomes can be visualized in Figure 4.



A B

Figure 3: Schematic representation of the structure of a double crosslinked hydrogel based on chitosan and poly(vinyl alcohol) – A – with Na_2SO_4 and GA; B – with TPP and GA

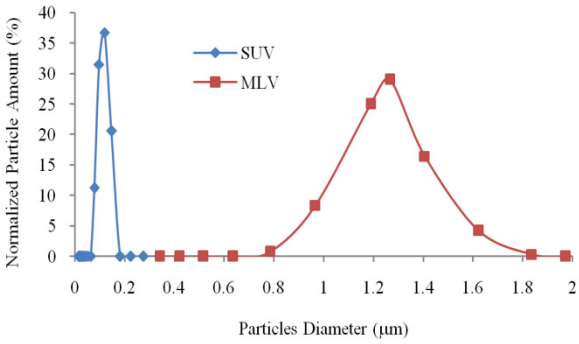


Figure 4: Liposome distribution curve determined by the laser light diffractometry technique

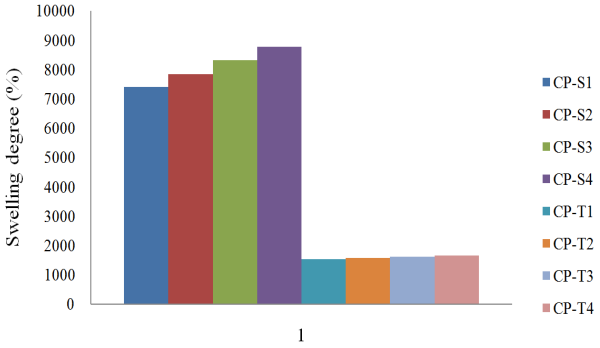


Figure 5:Maximum swelling degree after 48h in PBS (pH=7.4)

Swelling capacity of hydrogels

Hydrogels based on C/PVA were characterized according to the capacity to swell in phosphate buffer pH=7.4, since this characteristic significantly affects the release through the diffusion process of the drugs included in such hydrogels. The swelling capacity of the hydrogels prepared, assessed through the maximum degree of swelling reached after 48 hours depends on the amount of the crosslinking agent used and the PVA content. In the case of hydrogels crosslinked with Na_2SO_4, the degree of swelling exceeds 7000%, reaching up to 9000% in the case of samples with less chitosan in composition. Increasing the amount of chitosan leads to denser networks and, therefore, reduces the maximum degree of swelling (CP-S1 to CP-S4).

In the case of the hydrogels obtained by crosslinking with TPP, the maximum degree of swelling was lower than the one obtained by crosslinking with the sulphate anion, as a result of a higher crosslinking density (CP-T series), but does not vary according to the parameters of the preparation process. It should be noted that in this case the determined maximum degree of swelling exceeds 1500% (Figure 5).

Release of calcein from control hydrogels

The release profile of small molecular weight drugs from polymeric hydrogels usually presents a burst release unsuitable for many bioengineering and drug delivery applications. Aside from the therapeutic situations when an attack dose is needed,[18] in most pathologies, when a long term treatment is needed, the drug released during the burst effect has to be minimum. This is an interesting research topic especially in the case of low molecular weight drugs administered for long periods of time. In the present study, we intended to reduce the burst release or even eliminate it by introducing a supplementary barrier in the drug release from polymeric hydrogels – namely, the liposomes. In Figure 6, the kinetics of calcein release (cumulative calcein release) in PBS (pH=7.4) from control hydrogels crosslinked with GA and Na_2SO_4/TPP is presented. In general, the burst effect is intense in the first 6 hours and in less than 24 hours at least 50% of the calcein loaded is released in the supernatant (Figure 6). Compared to other hydrogels based on polysaccharides (based on CMC, for example[19]), calcein release is nevertheless slower. Its inclusion into the hydrogel during its preparation process is mostly based on the formation of ionic bonds between its carboxylate ions and ammonium cations formed by quaternization of amino groups from chitosan in acidic medium. In the weak base medium in which the release of calcein was studied, although the ionic interactions between the two types of ions disappear, hydrogen bonds between the carboxyl and amino groups of calcein may occur which delays its diffusion through the polymer network (compared to the hydrogels based on CMC).

The matrix preparation parameters do not determine a different release behavior of calcein. This can be explained by the small size of this molecule, which diffuses more easily from the prepared hydrogels with a higher mesh size. After 48h, the release profile reaches a steady state, the films ionically crosslinked with sulphate anions showing a release efficiency between 57-67% and the films prepared by TPP crosslinking between 73 and 80%.

From the presented data regarding the evolution of the swelling process, it seems that although TPP achieves a higher crosslinking density and the calcein amount included in hydrogels is identical to those used in the case of

sulphate anions crosslinked hydrogels, the calcein release efficiency is higher in this set of hydrogels. This fact leads to the idea that besides diffusion, there are other mechanisms involved in calcein release from such hydrogels. The explanation that we propose is the following: the higher degree of crosslinking when using TPP is determined by its higher functionality, but it is possible that not all anions per TPP molecule participate in the crosslinking process. In mildly basic medium, when calcein emerges from the interaction with ammonium cations, to which it was tied during the crosslinking process, electrostatic rejection occurs between calcein and TPP anions, which causes faster calcein diffusion (and in greater amounts) through the hydrogel.

Release of calcein from hydrogels containing MLVs

As mentioned above, the inclusion of liposomes loaded with calcein in the prepared hydrogels should have a delay effect of the model compound release and should reduce the amount of calcein released during the burst period. Figure 7 presents the kinetics of calcein release efficiency from hydrogels entrapping MLV liposomes. The release efficiency was estimated on the basis of the total amount of calcein released into the supernatant, which can be found both in a free state and in liposomes, which have not been disintegrated during the diffusion within the matrix. Indeed, in Figure 7A, we can note the absence of the burst effect of the hydrogels ionically crosslinked with sodium sulphate, due to the gradual release of liposomes loaded with calcein from this polymeric network. Their diffusion is mainly controlled by the hydrogel swelling and matrix mesh size, which is appropriate for liposome retention. The ≈60% release efficiency presented by control hydrogels after 24-48h is greatly reduced when calcein is entrapped in MLV liposomes (reaching ≈20% after 20 days in the case of sodium sulphate crosslinking and much more attenuated in the case of TPP crosslinking). In the case of complex systems, the viability duration is prolonged (three weeks for sodium sulphate crosslinking).

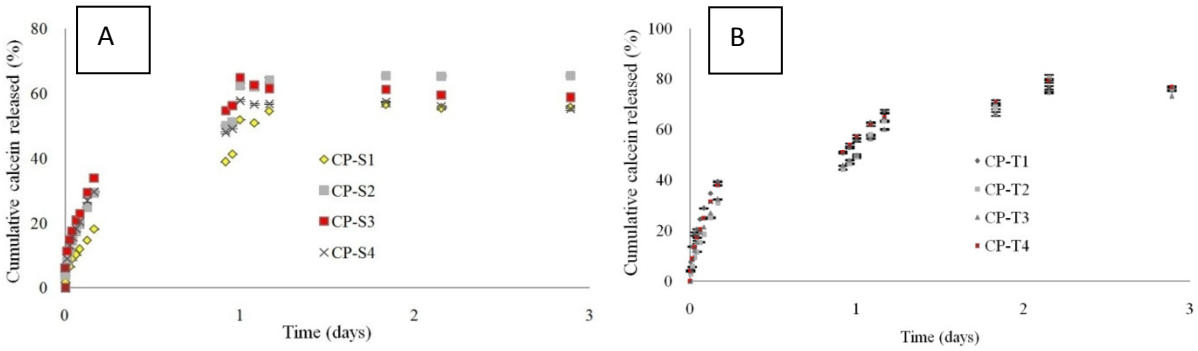


Figure 6: Kinetics of calcein release efficiency at pH=7.4 from control hydrogels crosslinked with GA/Na_2SO_4 (A) and GA/TPP (B)

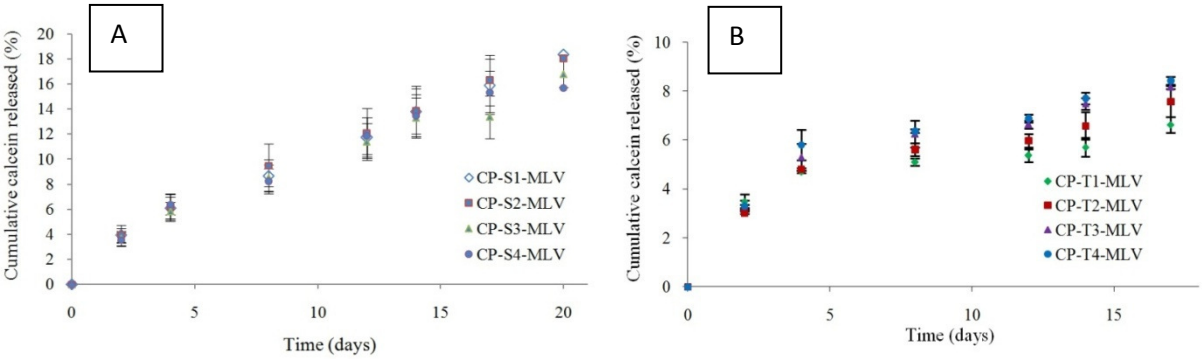


Figure 7: Kinetics of calcein release efficiency in PBS(pH=7.4) from hydrogels crosslinked with GA/Na_2SO_4 (A) and GA/TPP (B) entrapping MLVs after Triton X-100 treatment

Also, the hydrogels prepared by TPP crosslinking presented the ability to further reduce the diffusion of calcein included in the MLV liposomes. In this case, due to the superior

crosslinking density achieved by TPP (the functionality of the phosphate ion is 2 and that of TPP is 5), these hydrogels present a reduced mesh size, which delays the free diffusion of liposomes. The release efficiency in this case is reduced compared to the previously prepared hydrogels crosslinked with sodium sulphate.

Release of calcein from hydrogels containing SUVs

In order to reduce the burst effect the calcein was also entrapped in SUV liposomes, which were then dispersed into hydrogels. Due to the greater sizes of the liposomes, compared to calcein molecule (Figures 1 and 4) the drug release is significantly reduced, as can be remarked in Figure 8, presenting the kinetics of calcein release efficiency at pH=7.4 from different hydrogels entrapping SUVs. An interesting observation is that calcein concentration is constantly increasing during the examination period. Moreover, the burst release noticed is not as pronounced as in the case of the control hydrogels. Although it was expected that small unilamellar liposomes would present higher calcein release efficiency, due to the greater diffusion capacity than that of multilamellar liposomes, this was not observed. The explanation may lie in the high encapsulation capacity of MLVs compared with SUVs: the volume of an MLV liposome is about 1000 times larger than that of an SUV liposome, determining a higher encapsulation efficiency. When such a liposome destabilizes during the diffusion through the hydrogel network, the amount of calcein released is obviously much higher than in the case of breakage of an SUV. The release rate and encapsulation efficiency are two main parameters that must be taken into account when designing such composite systems. Although small liposomes have a lower loading capacity, compared to large multilamellar liposomes, their easier diffusion through the matrix leads to lower drug release efficiency.

Measurement of liposome integrity

In the case of the systems obtained, different behavior was observed regarding the integrity of liposomes in the supernatant. Regarding large liposomes, they show superior stability compared to the SUVs, due to multiple layers that cover the liposome core. As shown in Figure 9, the treatment of the liposome suspension extracted from the release medium – PBS (pH 7.4) with Triton X-100 causes the release of an amount of calcein carried by liposomes. The determination of this amount of active ingredient sequestered in liposomes is important in terms of the potential uses of these matrices, such as for controlled drug delivery systems, because the natural extracellular body fluids contain amphiphiles that may have a similar action to that of surfactant Triton X-100.[20]

In the case of the systems crosslinked with sodium sulphate, the latency reached values in the range 25-35%. The value of this parameter is increasing throughout the release kinetics study. When hydrogels are obtained by crosslinking with TPP, resulting in denser networks, the diffusion of liposomes is more difficult. A large number of them are broken before going out of the matrix due to the resistance opposed by the network and therefore the latency shows lower values, compared with the process of sodium sulphate crosslinking. In the case of complex systems containing SUV liposomes, the latency values are near zero. This is due to the instability of small liposomes that break during the diffusion through the matrix (data not shown).

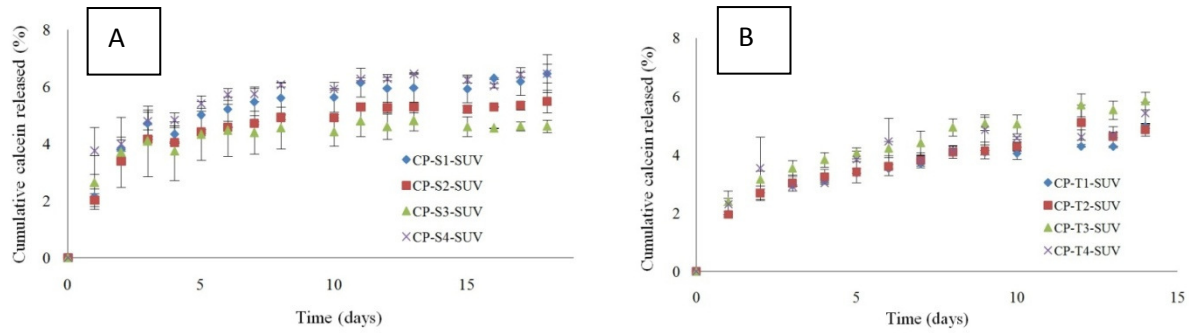


Figure 8: Kinetics of calcein release efficiency at pH=7.4 from hydrogels crosslinked with GA/Na_2SO_4 (A) and GA/TPP (B) entrapping SUVs after Triton X-100 treatment

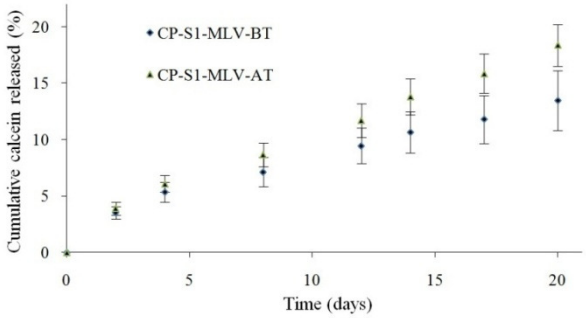


Figure 9:Kinetics of calcein release efficiency before (BT) and after addition of Triton X-100 (AT) for a randomly selected sample

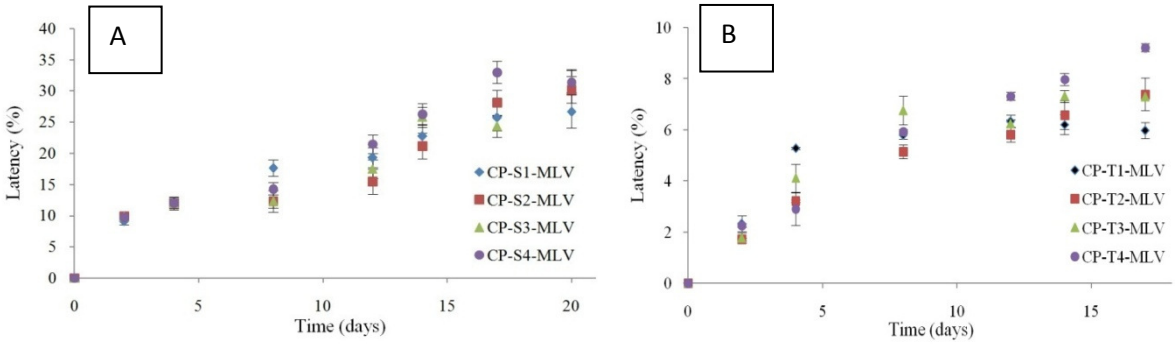


Figure 10:Calcein latency for hydrogels crosslinked with GA/Na_2SO_4 (A) and GA/TPP (B) entrapping MLVs

Mechanism of calcein release from complex systems

The calcein encapsulating liposomes, which are located close to the hydrogels surface and have the appropriate size to escape from the mesh network (after swelling occurs), are first released from the hydrogels; then, another calcein amount is released outside the hydrogels after the liposomes disintegrate. While the diffusion of the buffer solution in the hydrogel network determines its swelling, liposomes, during diffusion, begin migrating slowly from the internal layers to the surface of the hydrogel and outside. A part of them (the most bulky) get broken, because of the rigidity or high density of the network, and calcein is released and diffuses soon out of the hydrogel. The others that reach the film surface gradually disintegrate (or in the presence of Triton X-100), releasing in their turn calcein. Concurrently, the free calcein from the hydrogel (resulted from the liposomes that collapse during the crosslinking process or during the diffusion of PBS) can interact strongly with hydrogel constituent polymers, as has been stated in the discussion about calcein release from control hydrogels. This explains why calcein release from such system is generally slower, confirming our starting hypothesis. In these circumstances, it is obvious that the release kinetics will be influenced on the one hand by the polymer matrix characteristics (composition, crosslinking density or nature of the crosslinking agent) and, on the other hand, by the liposomes characteristics: size and stability.

CONCLUSION

Polymeric hydrogels have been widely used in drug delivery applications, due to their great properties. A drawback of such systems is the fast diffusion of drugs, as we demonstrated in this study using calcein as model hydrophilic drug. Therefore, due to the intense burst effect, these hydrogels could be used only for short term treatment. Moreover, the drug amount released during the burst period may lead to toxic levels, could be metabolized and excreted without being used by the body, which is a financial and therapeutic drawback. The greatest challenge is to create a support able to release the drug in a well-controlled manner over a prolonged period of time (at least two weeks). Therefore, the release characteristics of the hydrogels must be adapted to these requirements and this can be possible through the use of liposomes, which affords a composite system with new properties. Liposomes are very diverse in terms of diameter,

nature of the lipids that form the membrane, lamellarity, electric charge, mechanical or other properties. In the present study, we used two types of conventional liposomes (composed of phosphatidylcholine), which differ in size and lamellarity, in order to characterize the behavior regarding the encapsulation and release of active ingredients included in such vesicles. The liposomes were dispersed in hydrogels with different crosslinking characteristics and the results showed that they kept their integrity during both hydrogel preparation and the release process testing. Remaining intact, liposomes block the rapid diffusion of calcein from the hydrogels. Multilamellar liposomes (larger in diameter than SUV) diffuse more slowly than the SUVs, but their higher encapsulation capacity makes the amount of calcein released to be close to that released by the SUVs. In terms of drug release from these matrices, it may be established that TPP reduces the diffusion capacity of liposomes loaded with drugs, due to the density of the network, which is higher than in the case of crosslinking with sodium sulphate. This is noted especially in the case of large multilamellar liposomes, when the mesh network obtained restricts the diffusion of these liposomes and hence the diffusion of the drug molecules they carry. The composition of hydrogels, mainly the amount of chitosan, influences to a lesser extent both the degree of swelling of hydrogels and the calcein release efficiency. Both characteristics vary slightly from one sample to another, but in a logical way. Another important conclusion that can be drawn from the results of this study is the fact that the burst effect decreases with increasing liposome diameter.

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