Liposome Loaded Chitosan Hydrogels, a Promising Way to Reduce the Burst Effect in Drug Release

A comparativ analysis

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In order to analyze the effect induced by encapsulating the drug in liposomes, comparative studies were performed for control hydrogels and complex systems (the same hydrogels containing liposomes). The results showed that the use of liposomes entrapped within chitosan hydrogels allows a strong decrease of burst effect and, moreover, the mechanism of drug release is a complex Fickian diffusion, consisting of drug diffusion through the swollen hydrogel and/or water filled pores, being continuously fed by calcein loaded in liposomes.

Keywords: liposome, chitosan, drug release, Fickian diffusion

Because of its favorable properties (non-toxicity, biodegradability, mucoadhesiviness) chitosan was studied as a biomaterial and as a pharmaceutical excipient in drug formulations. These formulations may be produced in a wide variety of forms including hydrogels or hydrogel based particles. In these formulations chitosan hydrogels are able to provide local delivery of loaded therapeutic agents but their delivery can be rapid and not easily time-controllable due to, particularly, the burst effect. This leads to a loss in drug efficiency and lifetime. To overcome the consequences of burst effect, systems involving liposomes incorporated into chitosan hydrogels may appear as a promising material in tissue engineering, regenerative medicine and drug loading systems. Liposomes are spherical self-closed structures, composed of curved lipid bilayers, which enclose part of the surrounding solvent into their interior. The simplicity of production, their biocompatibility, absence of toxicity [1, 2], the size and similar composition to cell make them a revolutionary tool in nanomedicine and biomedical domains.

The goal of this study is to develop drug delivery systems containing chitosan hydrogels and liposomes able to accurately control this delivery, without any burst effect, and to model the drug release kinetics from these biomaterials.

Experimental part

Materials

According to the chemical definition, the compound named Chitosan is a natural aminopolysaccharide comprising glucosamine and N-acetyl glucosamine units. This biopolymer is widely used for the development of biomedical applications and it shows diverse biological activities towards mammalian cells, including mucoadhesion, the ability to condense and transport oligonucleotides, and adjuvant activity.

Chitosan Medium Molecular Weight (C) (weight average molar mass of 210,000 g.mol^{-1} and degree of deacetylation (DD) of 75% determined by ^{1}H NMR technique), gelatin B (G) of bovine origin, glutaraldehyde (GA) 25% aqueous solution, sodium tripolyphosphate (TPP), calcein (hydrophilic model drug) 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 PrimexChim (Bucharest, Romania).

Preparation of liposomes

Calcein-encapsulating phosphatidylcholine (PC) based Multi-Lamellar Vesicles (MLV) and Small Unilamellar Vesicles (SUV) were used for complex hydrogels formation in order to check the effect of the liposomes as an extra barrier for the calcein release. For complex systems preparation, in each hydrogel a certain volume of liposomes suspension was added before the GA addition (used as covalent crosslinking agent) and the total amount of included calcein was calculated by breaking the liposomes with the surfactant Triton X-100. Also, for the liposomes distribution studies throughout the polymeric matrix, Rhodamine B labeled liposomes were prepared. MLVs were prepared by the thin film hydration method [3] with minor modifications, using a chloroform/methanol mixture (2:1 v/v). SUVs were prepared by sonication method [4].

Preparation of polymer-liposomes-calcein systems

In order to achieve a controlled release of calcein complex systems were prepared. As a first stage, calcein was encapsulated in liposomes. The suspension of calcein loaded liposomes was then added to the polymer solution prior to the introduction of GA as covalent crosslinking

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Sample code	C/G (w/w)	GA (µL)	TPP (mg)	Na_2SO_4 (mg)	Calcein loaded (µg)
CG-S1	1.9	31.5	-	60,5	579.0
CG-S2	9	40	-	64	545.6
CG-S3	4.5	37.5	-	79	562.8
CG-S4	4.5	37.5	-	59,5	585.9
CG-T1	1.9	31.5	78	-	503.7
CG-T2	9	40	83	-	496.5
CG-T3	4.5	37.5	102	-	503.0
CG-T4	4.5	37.5	77	-	508.9

Table 1
THE HYDROGELS PREPARATION PARAMETERS

agent [5]. Subsequently, the aqueous solutions of specific amounts of GA were added under vigorous stirring and the crosslinking process continued with the introduction of ionic crosslinker (Sodium TripolyPhosphate, TPP, or sodium sulphate) [6]. Eight complex systems were obtained in duplicate using the preparation parameters presented in table 1.

Results and discussions

Reported hydrogels have been analyzed from the point of view of drug loaded liposome entrapment. The formation of polymeric networks has been proved by FT-IR spectra and the hydrogels character has been demonstrated by swelling degree measurement. The mean diameters measured for the vesicles used in this study were 1.266±0.237 mm for MLVs and 0.112±0.014 mm for SUVs. The dimensional dispersity is a single-mode and presents relatively narrow distributions [6]. From confocal microscopy observation, the liposomes are well distributed throughout the whole surface area of the hydrogels.

The release of calcein was first studied according to the nature of the ionic crosslinking agent (sodium sulphate or tripolyphosphate) [6]. For the CG-S hydrogels about 80% of the loaded drug was gradually released into the supernatant over seven days. A similar behavior was observed for CG-T hydrogels but, in this case, there was a lower efficiency of calcein release, which was about 65%. It is explained by the fact that TPP is a stronger ionic crosslinking agent, leading to a denser hydrogel matrix and, furthermore, to longer stability and lower efficiency of CG-T hydrogels compared to CG-S ones. From kinetics studies and modeling of kinetics curves we have shown that interpenetrated networks based on double-crosslinked chitosan are capable of releasing hydrophilic drugs through a multi-scale mechanism, characterized by four distinct phases, each characterized by a different kinetics model [7]. The first phase, i.e. the burst effect, can be well described by the Higuchi equation. The second phase is dominated by the swelling of the polymer matrix, and the most appropriate model for this is that of Korsmeyer and Peppas. Its temporal length is proportional with the crosslinking density of the polymer matrix. The analysis of the Korsmeyer-Peppas parameters shows that the mechanism of drug release is the complex Fickian diffusion, consisting of drug diffusion through the swollen hydrogel and/or water filled pores and that the release rate also depends on the crosslinker type. The equilibrium phase is the third one and is characterized by a constant concentration and its time length is dependent on the crosslinking density. The equilibrium phase can be analyzed through two equations, Peppas-Sahlin and Weibull. The quantitative analysis of the equation parameters shows the preponderance of the Fickian mechanism compared to the polymer matrix relaxation. The fourth phase, observed at large time scales (of days order), reveals a decrease of the drug amount, that can be attributed to interactions appearing between calcein and hydrogel fragments resulting from hydrogel degradation that bonds each other. Because the classical theory of the phenomena occurring at high time scales leads is very complex due to the high number of variables and parameters (which are hardly determinable), the nonlinear mathematical approach proved to be more appropriate [7-9].

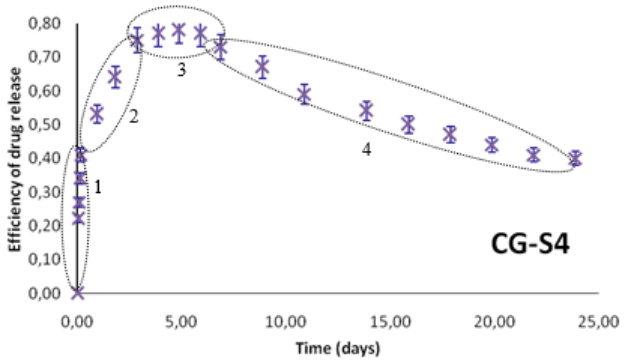

Fig. 1. Release kinetics of calcein from hydrogels based on chitosan and gelatin crosslinked with sodium sulphate

The first and the fourth phases are undesirable in the release of active agent and the inclusion of liposomes allows to decrease it. The role of the type of liposomes was studied. Systems containing MLVs release higher amounts of calcein compared to systems containing SUVs, although these liposomes are more stable in the matrix (being mainly multilamellar) and diffuse with difficulty. The likely explanation is that, although MLVs are more stable than SUVs, they transport a much higher quantity of calcein (in relation with their volume). Moreover they present a better efficiency in controlling the burst effect and the release kinetics. The release kinetics of calcein from chitosan based delivery systems with and without SUV liposomes are shown in figure 2.

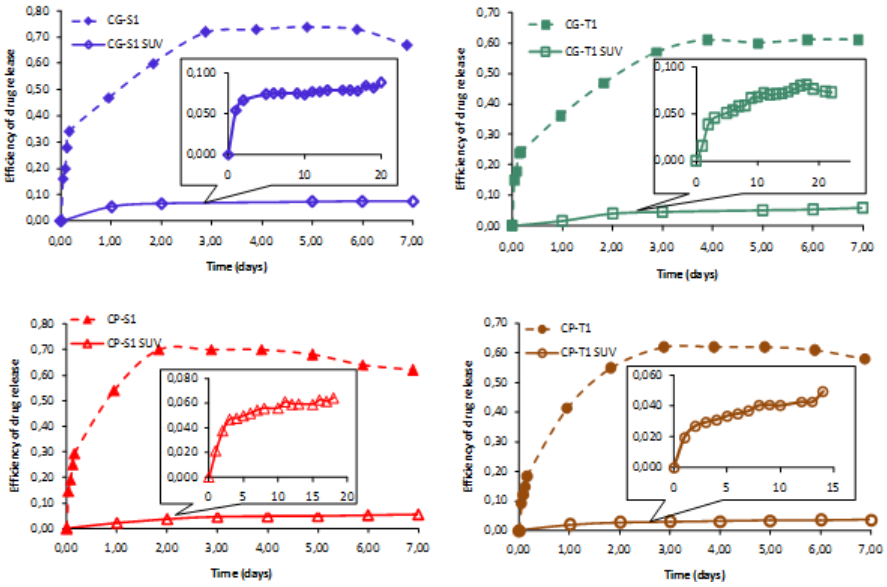

Fig. 2. Release kinetics of calcein from chitosan based hydrogels with and without liposomes

Table 2

KINETICS PARAMETERS FROM HIGUCHI AND KORSMEYER-PEPPAS MODELS

	CS/GEL (w/w)	CS/PAV (w/w)	Na_2SO_4 (g)	TPP (g)	k_H	k_{KP}	n
CG-S1	1.9	-	0.0605	-	0.72	0.50	0.32
CG-S1 SUV					**0.11**	**0.06**	**0.16**
CG-T1	1.9	-	-	0.078	0.66	0.40	0.30
CG-T1 SUV					**0.08**	**0.05**	**0.18**
CP-S1	-	9	0.0605	-	0.68	0.55	0.40
CP-S1 SUV					**0.08**	**0.04**	**0.18**
CP-T1	-	9	-	0.078	0.42	0.40	0.44
CP-T1 SUV					**0.04**	**0.03**	**0.25**

An obvious decrease of the burst effect was observed and the uniform increase of calcein release continues even at large time scales, of days order, the first and the fourth phases being eliminated. The evolutions of drug release from hydrogel systems, with and without SUV, were compared through the mathematical modeling of experimental data, the values of Higuchi constant (k_H) and Korsmeyer-Peppas coefficients (k_{KP} and n) being presented in table 2.

The Higuchi and Korsmeyer-Peppas constants, indicators of release rates during the burst effect and uniform release stages have much lower values (four to eight times smaller) compared with those without liposomes. The values of diffusional exponent n are, still, below 0.5, two times lower than for systems without liposomes, demonstrating that the mechanism of drug release is a complex Fickian diffusion, consisting of drug diffusion through the swollen hydrogel and/or water filled pores, being continuously *fed* by calcein loaded in liposomes [7].

Comments and perspectives. Currently, for a superior understanding of diffusional transport patterns, in the case of a (real) polymer chain dynamics in relationship with membrane's pores at various dimensional scales, we recommend reading a few articles in the field [10, 11]. Furthermore, regarding the diversity of the classical theoretical models for drug release, these turn out to be reducible to only one singular model in which controlled drug release systems, considered as physical systems, are analyzed through the complex dynamics of their structural units of fractal type [12, 13]. Thus, it legislates multiscale type mechanisms for which the dynamic variables are described by fractal mathematical functions dependent both of space-time coordinates and of resolution scales [14 - 24].

Conclusions

The use of liposomes entrapped within chitosan hydrogels allows a strong decrease of burst effect as well as a better control of release kinetics.

The release kinetics mechanism was modelled and their knowledge will be a very interesting tool for designing new formulations for tissue engineering, regenerative medicine and drug delivery systems.

The fact that the drug release mechanism is a complex Fickian diffusion, it was clearly demonstrated.

The experimental results are in good agreement with the theoretical models chosen. They will be improved and applied in our planned future research.

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