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Microparticles Based on Hydrophobically Modified Chitosan as Drug Carriers

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ABSTRACT: In this work, a hydrophobically modified (HM) chitosan derivative was prepared by covalent linkage of C_{12} groups to the chitosan backbone. HM-chitosan microparticles were prepared according to an emulsification-solvent evaporation method and naltrexone (NTX) was used as a model drug. For comparison, unmodified chitosan and poly lactic-*co*-glycolic acid (PLGA) microparticles were also tested as carriers for NTX. HM-chitosan formed viscous semi-dilute solutions, suggesting a high level of chain entanglements and hydrophobic associations. HM-chitosan microparticles generally showed higher production yield and encapsulation efficiency, as compared with chitosan and PLGA. The burst release shown by chitosan microparticles was significantly reduced when using the HM-chitosan derivative. An enhanced control of drug release was observed over at least 50 days. PLGA particles demonstrated inferior controlled release properties as compared to HM-chitosan subsequent to the initial release stage. These results revealed the potential of hydrophobic modification of chitosan as a means to improve the stability and sustained delivery properties of the polymer. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40055.

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INTRODUCTION

Chitosan is a natural cationic polysaccharide derived from the deacetylation of the naturally occurring chitin. It is a linear copolymer composed of β-(1,4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-d-glycopyranose.[1] This homopolymer is soluble in water at low pH, due to the protonation of the free amine groups.[2] In addition, the excellent biocompatibility and biodegradability properties of chitosan make this a particularly appealing material for pharmaceutical and biomedical applications. Its degradation products, amino sugars, are also nontoxic and completely absorbed by the human body.[1,2]

Chitosan has been extensively used in drug delivery systems, such as nanoparticles and microparticles,[3–6] where the controlled delivery of encapsulated or entrapped active agents has been demonstrated. In addition, chitosan is recognized for its mucoadhesiveness to epithelial tissues.[1]

The presence of free amine groups additionally allows the chemical cross-linking of the matrix with agents such as glutaraldehyde,[2,5,7,8] which contributes to increase the stability and strength of the network. However, the use of such cross-linkers has been questioned due to toxicity concerns.[5] Another approach has been to physically cross-link the polymer with ionic molecules such as the anionic tripolyphosphate (TPP).[9,10] In spite of the lower toxicity of the ionic cross-linkers, the cross-linking density of the network can change significantly *in vivo* in response to pH changes. Physically cross-linked chitosan networks may also lack mechanical stability and dissolve rapidly, which seriously compromises their use in long-term applications.[10]

Hydrophobically modified chitosans can show excellent biocompatibility and biodegradability *in vivo*, and form high stability systems.[11,12] The presence of hydrophobic moieties makes these polymers very appealing as drug delivery systems for poorly water-soluble drugs.[11–13] It has been shown that hydrophobically modified chitosans can self-assemble into nanoparticles with a hydrophobic core, improving the encapsulation efficiency and release profile of hydrophobic drugs.[11–14]

Le Tien et al.[15] also reported that N-acetylated chitosans with long chain side chains (C_8-C_{16}) showed improved mechanical properties and drug release features when used in tablets, which was attributed to the establishment of hydrophobic interactions between side chains. Furthermore, the authors suggested that as

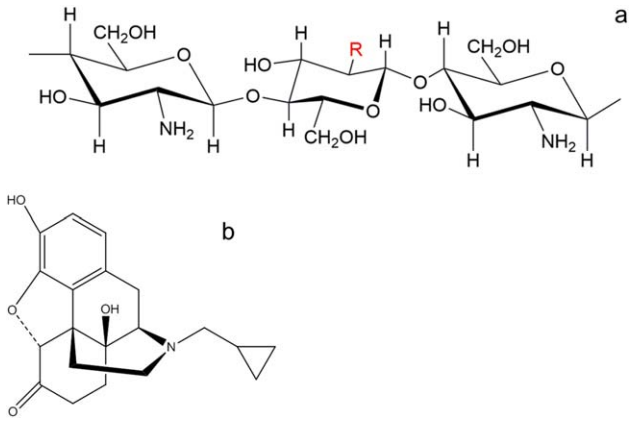

Figure 1. (a) Molecular structure of chitosan and HM-chitosan. Chitosan: R= NH_2 or R= NH-CO-CH_3; HM-chitosan: R=NH_2 or R=NH-CO-CH_3 or R= N=CH-$C_{11}H_{23}$. (b) Structure of naltrexone (NTX). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the length of the side chains increased, the swelling degree of the matrices decreased, causing the drug release to depend mostly on diffusion.[15]

Water-soluble hydrophobically modified chitosans are therefore an innovative field with great potential in drug delivery. In this work, a hydrophobically modified chitosan (HM-chitosan) was produced by chemical modification of the amine groups on the polymer chains with a C_{12} aldehyde [Figure 1(a)]. The potential of this modified polymer in controlled drug delivery was investigated by formulating microparticles containing the model drug naltrexone (NTX). Throughout the study, HM-chitosan particles were compared with counterparts based on an unmodified chitosan and on poly lactic-*co*-glycolic acid (PLGA), allowing estimation of the effect of increasing the hydrophobicity of chitosan, and comparison with an established hydrophobic polymer. The expectation was that the hydrophobic modification would improve the stability and prolong the release rate compared with unmodified chitosan, while maintaining the possibility for an easy production process employing aqueous solvents, unlike the production of traditional PLGA particles.

The work described here is part of a broader project in which the ultimate aim is to develop a two-component formulation involving drug-loaded microparticles and a thermoresponsive suspension medium that upon injection forms a soft implant *in situ*. Through this combination, it is anticipated that the control of drug release is highly improved, as shown previously for analogous formulations.[16] In this context, the purpose of this work was to produce microparticles with high stability and a high loading capacity, capable of delivering the drug over an extended period of time. The background for aiming at particles in the micrometer size range was to ensure the physical retention of the particles in the gel network, while also anticipating a more favorable loading capacity and drug release rate. Even though the work presented in this article is strictly related with the development of the microparticulate system, studies on low toxicity thermosensitive implant gel systems have also been carried out by our group.[17–19]

Our findings suggest that the hydrophobic modification of chitosan can be an effective means to improve the drug retention capacity of the microparticles, contributing to achieve a long-term drug release.

EXPERIMENTAL

Materials

The hydrophobically-modified chitosan (HM-chitosan) was prepared by modification of a chitosan sample (Pronova Biopolymers, Norway) by reaction of the amine groups on the polymer chains with a C_{12} aldehyde [Figure 1(a)]. The degree of C_{12}-aldehyde substitution in HM-chitosan was 5% with respect to free amine groups. Details on the modification can be found elsewhere.[20]

The unmodified chitosan used in this study had a degree of deacetylation of 75–85% and a molecular weight of 300−500 kDa (information provided by the manufacturer, Sigma-Aldrich, USA). PLGA (RESOMER® RG 502), ester terminated, (lactide:-glycolide (LA/GA) 50:50), M_w 70−17,000 was obtained from Aldrich (USA). Naltrexone, USP Reference Standard (Rockville, MD) was used as a model drug [Figure 1(b)].

Preparation of Microspheres

As production parameters are known to affect particle properties important for stability, loading capacity and drug release, chitosan, HM-chitosan and PLGA microspheres were prepared by an emulsification-solvent evaporation method, under varying conditions.

On the basis of a preliminary experimental screening design studying several parameters for production of blank microparticles, NTX-loaded microparticles were prepared using the conditions indicated in Table I. Chitosan and HM-chitosan were dissolved in 3.3–10 mL of 1% (v/v) acetic acid, depending on concentration. The drug was dissolved together with the polymer (50% drug:polymer mass ratio). This solution was added dropwise to the continuous phase comprising of 50 g liquid paraffin and 0.7 wt % sorbitan monooleate as emulsifier. The addition of the dispersed phase to the continuous phase was performed through a needle (0.5 mm internal diameter), while the continuous phase was kept under constant stirring (750 rpm, Eurostar power control-visc stirrer, IKA-WERKE, Germany). The stirrer was connected to a Neslab RTE-101 thermostat (Neslab Instruments, USA), allowing temperature control of the process. Stirring continued for a minimum of 5 h in order to ensure complete evaporation of the aqueous solvent. At the end, the microspheres were extensively washed with petroleum ether and recovered by vacuum filtration through a 0.8-μm pore size membrane filter (MF-Millipore Membrane, mixed cellulose esters, Millipore, USA).

When producing PLGA microparticles, the polymer was dissolved in dichloromethane. The polymer solution was added as described before to the continuous phase, which in this case consisted of an aqueous polyvinyl alcohol (PVA) solution (0.5 wt %). In the final step, the PLGA particles were thoroughly washed with water and vacuum filtered as described previously.

Each sample was shortly named according to the polymer used (C for chitosan, HM for HM-chitosan and P for PLGA),

Table I. Formulation Conditions of Naltrexone-Loaded Microspheres and Encapsulation Properties [Encapsulation Efficiency (EE) and Loading Capacity (LC)]

	Polymer	Polymer concentration (wt %)	Evaporation temperature (°C)	EE (%)	LC (%)
C-L30	Chitosan	0.5	30	45.9 ± 1.5	15.3 ± 0.5
C-L60	Chitosan	0.5	60	44.1 ± 3.6	14.7 ± 1.2
C-H30	Chitosan	1.5	30	44.5 ± 2.3	14.8 ± 0.8
C-H60	Chitosan	1.5	60	42.1 ± 1.9	14.0 ± 0.6
HM-L30	HM-chitosan	0.5	30	48.7 ± 1.9	16.2 ± 0.7
HM-L60	HM-chitosan	0.5	60	64.6 ± 2.6	21.5 ± 0.9
HM-H30	HM-chitosan	1.5	30	54.4 ± 0.2	18.1 ± 0.1
HM-H60	HM-chitosan	1.5	60	86.5 ± 4.2	28.8 ± 1.4
P-L5	PLGA	0.5	5	49.1 ± 3.5	16.4 ± 1.2
P-L25	PLGA	0.5	25	20.8 ± 2.0	6.9 ± 0.7
P-H5	PLGA	1.5	5	51.0 ± 3.2	17.0 ± 1.1
P-H25	PLGA	1.5	25	52.2 ± 6.5	15.4 ± 2.7

Results shown as average ± standard deviation, $n = 3$.

polymer concentration (L for low = 0.5 wt % and H for high = 1.5 wt %) and preparation temperature (indicated by the last number, i.e., 5, 25, 30, or 60°C).

The yield of production of NTX-loaded microparticles was estimated as follows:

$$\text{Yield}\,(\%) = \frac{\text{weight of produced particles after drying}}{\text{weight of naltrexone and polymer initially used}} \times 100$$

Drug Content: Encapsulation Efficiency and Loading Capacity

In order to determine the drug content present in chitosan and HM-chitosan microspheres, an accurately weighed amount of microspheres (3–5 mg) was firstly suspended in ethanol, in order to facilitate the dispersion of aggregates. The microspheres were subsequently dissolved in 0.1 N hydrochloric acid (pH 1) by vigorously stirring the samples at room temperature for 4 days. Samples were filtered through a 0.22-μm syringe filter (Millex, Millipore, Ireland). Naltrexone was released from PLGA microspheres by dissolving the polymer in dichloromethane. The amount of drug released was quantified by measuring the absorbance of the solution at 282 nm, using a Spectronic Helios Gamma spectrophotometer (Thermo Fisher, UK).

The encapsulation efficiency (EE) and loading capacity (LC) of the microspheres were calculated as follows:

$$\text{EE}\,(\%) = \frac{\text{measured amount of naltrexone in microspheres}}{\text{total amount of naltrexone added during manufacturing}} \times 100$$

$$\text{LC}\,(\%) = \frac{\text{measured amount of naltrexone in microspheres}}{\text{weight of microspheres}} \times 100$$

Size and Morphological Features of NTX-Loaded Microspheres

Size and size distribution were evaluated by suspending a small amount of microspheres in ethanol, so that particle concentration was lower than the instrument's threshold for individual detection of the particles. Samples were stirred for a few minutes in order to ensure homogenization and dispersion of aggregates. The analysis was performed using an Accusizer 780 (Optical Particle Sizer, PSS NICOMP, Santa Barbara, CA).

The morphological features of the microspheres were evaluated by scanning electron microscopy (SEM). Samples were sprinkled on carbon taps mounted on aluminum stubs followed by sputtercoating with 5 nm platinum in a Cressington 308UHR Coating System. Samples were imaged in a Hitachi S-4800 FE SEM at 1.0 kV and a working distance of 2.5 mm.

Rheology Measurements

Viscosity measurements were performed in a Paar-Physica MCR 301 rheometer (Physica Messtechnic GmbH, Germany) using a cone-and-plate geometry, with a cone angle of 1o and a diameter of 75 mm. Temperature control was ensured by a temperature unit (Peltier plate). Chitosan and HM-chitosan samples were dissolved in 1% (v/v) acetic acid at a concentration of 0.5, 1.0, or 1.5 wt %. The samples were introduced onto the plate and a thin layer of low viscosity silicone was added to the free surface of the sample to prevent evaporation during the measurements.

***In Vitro* Release Experiments**

The *in vitro* release experiments were carried out in phosphate buffered saline (PBS), pH 7.4, prepared according to the European Pharmacopoeia[21] and adding 0.02% sodium azide, to prevent bacterial growth during the experiment. NTX-loaded microspheres were accurately weighed (10 mg) and transferred to a dialysis tube (Spectra/Por MWCO 50,000, Spectrum Laboratories, USA). 400 μL of PBS were carefully added to suspend the particles. The dialysis tube was placed in a stoppered vial containing 25 mL of PBS solution, ensuring sink conditions. The vials were further positioned inside a water bath connected to a thermostat, keeping the temperature at 37°C. Samples were stirred throughout the experiment by a 15-position magnetic

stir plate (RO15 power, IKA, Germany), placed beneath the water bath (position 2, ~100 rpm).

At specific time intervals, the absorbance of the buffer solution was measured at 282 nm, using a UV Ultrospec II spectrophotometer (LKB Biochrom, UK).

RESULTS AND DISCUSSION

Production of NTX-Loaded Microspheres and Drug Encapsulation Efficiency

A preliminary experimental design on blank samples (absence of drug) revealed that the size of chitosan and PLGA microparticles was not significantly affected by moderate changes in the parameters of the production process of the microparticles (data not shown). However, the production of HM-chitosan microparticles was found to be significantly affected by polymer concentration and to some extent evaporation temperature. In this case, larger particles were in general formed when using higher polymer concentrations (1.5 wt%) and evaporation temperature (60°C) (the lower limits being the respective values of 0.5 wt% and 30°C). These results were very likely related with important viscosity changes in the polymer solutions, which prompted us to carry out rheological experiments under different conditions of concentration and temperature (results discussed below in section: "Viscosity measurements of chitosan and HM-chitosan solutions").

The main results from the production of NTX-loaded microparticles will be discussed in the following paragraphs.

The yield of the NTX-loaded particles was generally higher than 85% for chitosan- and HM-chitosan microspheres, but significantly lower for PLGA-based particles (51–66%). The reason for the discrepancy is the likely formation of a high number of PLGA particles smaller than 0.8 μm that is filtered out after the emulsification-solvent evaporation process. Other studies have similarly shown that low viscosity drug/polymer solutions (such as those produced here for the PLGA-dispersed phase), usually result in the formation of small particles.[22] It should be emphasized, however, that it was not the aim of this work to produce nanoparticles, but rather particles in the micrometer size range with a high loading capacity that could be used for long-term drug delivery applications. For this reason, by using 0.8 μm pore size filters, the presence of nanoparticles was largely prevented.

In contrast, samples prepared from chitosan and HM-chitosan were shown to produce viscous phases. Droplets formed by these polymer solutions were therefore more difficult to disrupt during the emulsification process, forming larger droplets that ultimately produced larger particles. These particles were easily recovered after filtration, resulting in higher yields.

The presence of a high drug to polymer ratio typically results in low encapsulation efficiencies.[23,24] As a high drug to polymer ratio (1:2 weight ratio) was used in this work in order to produce particles with high drug content, this probably accounts for the low entrapment efficiencies observed for many formulations, even though higher values were found in some cases (Table I).

When chitosan was used as the polymeric matrix, the estimated values of EE and LC (Table I) do not seem to be significantly influenced by the emulsification conditions (EE 42–46% and LC 14–15%). Interestingly, the encapsulation of naltrexone was more efficient in the presence of HM-chitosan, with the values of EE varying between formulations but being as high as 87% in the best case (HM-H60). The overall higher encapsulation efficiency in the presence of HM-chitosan is probably related to the higher hydrophobicity of the polymer due to the presence of the C_{12} groups. This means that in addition to the formation of hydrogen bonds between the hydroxyl and amine groups of the polymer and the hydroxyl and ketone groups of the drug, it is also likely that the C_{12} groups of the polymer form hydrophobic interactions with the aryl ring and cycloalkyl rings of the drug. The high affinity between the molecules explains the enhanced presence of naltrexone within the HM-chitosan microparticles. Another important fact is that HM-chitosan is less soluble in water than the unmodified analogue. In fact, it has been shown that the lower solubility of the polymer in the dispersed phase causes it to precipitate faster, thereby contributing to increase the encapsulation efficiency.[25]

The highest drug loading was found for the HM-H60 formulation, where a high polymer concentration was used, and the solvent was evaporated at a high temperature. In this respect, it is generally accepted that high polymer concentrations also contribute to a higher encapsulation efficiency.[25] In addition, 1.5 wt % HM-chitosan solutions showed to be very viscous (see rheology data in section below), which could be an indication of a high degree of chain entanglements and hydrophobic associations—acting as physical connection points of the network—which can further account for the retention of the drug.

It is interesting to note that the HM-H60 sample was prepared at a high temperature (60°C). The differences between HM-H30 (prepared at 30°C) and HM-H60 (prepared at 60°C) are probably due to a fast solvent evaporation for the latter, inducing rapid particle solidification and limiting the diffusion of the drug to the continuous phase. The fact that large microspheres were produced under these conditions may also explain the high EE, as observed before for other polymeric particles.[26] A detailed interpretation of the size distribution of the NTX-loaded particles will be given later.

The EE of PLGA particles was mostly found in the range 21–52%. The exceptionally low EE and LC values found for P-L25 (21 and 7%, respectively) are consistent with the low polymer concentration (0.5 wt %, and thus a very low viscosity) of the dispersed phase in this formulation. In addition, it has been shown that a fast evaporation can induce pore formation in PLGA microspheres,[27] enabling the drug to diffuse out to the aqueous phase during the washing procedure, which further explains the low encapsulation of NTX.

Morphological Characterization of NTX-Loaded Microparticles

The microparticles produced in this work were generally spherical, as shown by SEM (Figure 2), even though different sizes and degrees of aggregation were obtained in different formulations.

The micrographs showed that chitosan-based microparticles did not differ significantly in terms of size distribution and surface

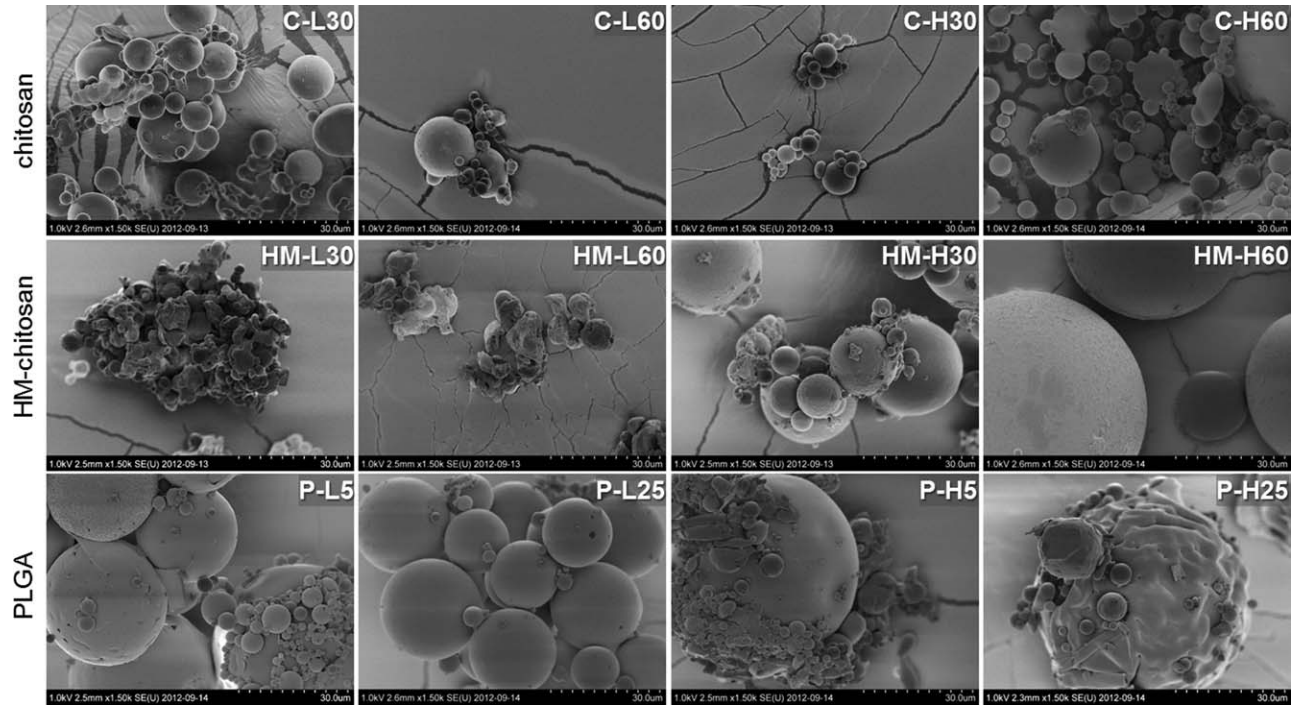

Figure 2. SEM micrographs of naltrexone-loaded microparticles prepared from chitosan, HM-chitosan and PLGA. The preparation conditions of all samples are described in Table I. Scale bar = 30 μm.

porosity. The detailed observation of samples at different magnifications showed the occasional presence of merged and/or irregularly shaped particles in C-L30 and C-L60, suggesting that the low viscosity of the polymer solution (0.5 wt %) facilitates the coalescence between droplets during the emulsification. In contrast, C-H30 and C-H60 formulations, prepared at a higher polymer concentration (1.5 wt %), formed stable droplets that resulted in well-defined microspheres.

HM-chitosan produced very different samples depending on the formulation conditions. Samples prepared at the lowest polymer concentration (HM-L30 and HM-L60) also produced irregularly shaped particles that occurred in aggregates. Conversely, particles prepared at a polymer concentration of 1.5 wt % (HM-H30 and HM-H60) were shown to be spherical and well-defined, but significantly larger when prepared at higher temperatures (HM-H60). This observation is probably related with the fast solvent evaporation and particle solidification, as mentioned above.

PLGA microparticles were largely polydisperse, with a high presence of small particles (<3 μm). Regardless of the process conditions, PLGA microparticles had a porous surface, as shown by micrographs taken at higher magnifications (data not shown). P-L25, particularly, showed the occasional presence of very large pores (up to 1 μm). P-H5 and P-H25 samples also contained a number of rod-like particles, which may be related to the elongation or coalescence of droplets at high concentrations, in a similar way to the observations made by Li et al.[28]

In all samples, SEM observations at higher magnifications showed the occasional presence of small particles (~0.5–1 μm) at the surface of larger particles.

Size and Size Distribution of Naltrexone-Loaded Particles

Figure 3 shows the size distribution of two formulations for each polymer and illustrates the influence of the drug loading on the size and size distribution of the microspheres.

It is noticeable that, in most cases, similar size distribution profiles are observed both in the presence and absence of NTX, even though the size is shifted to slightly higher values when the drug is present. Moreover, the amount of small particles tends to decrease, while the number of large particles increases. This relationship between drug loading and particle size has been reported before.[29] The increase in particle size in the presence of NTX may also be an indication of an increase in the viscosity of the dispersed phase, which in turn can suggest that polymer-drug interactions take place within the droplets. These interactions are probably favored by high polymer concentrations and temperature, as shown by the prominent size increase observed for C-H60 (chitosan) and HM-H60 (HM-chitosan) formulations, in the presence of NTX.

The size distribution of NTX-loaded microspheres prepared according to all formulations is depicted in Figure 4.

First of all, it is noticeable that particles smaller than 50 μm were produced in most cases. Small particles (0.5–2.5 μm) were often observed in high amounts (inset plots in Figure 4) and it is likely that even smaller microspheres were produced during the emulsification, but were mostly eliminated during the washing and filtration process. The presence of small particles in all samples is consistent with the SEM observations that showed the occasional presence of small particles attached to the surface of larger particles. This attachment justifies the presence of small particles in the sample, in spite of the filters used to

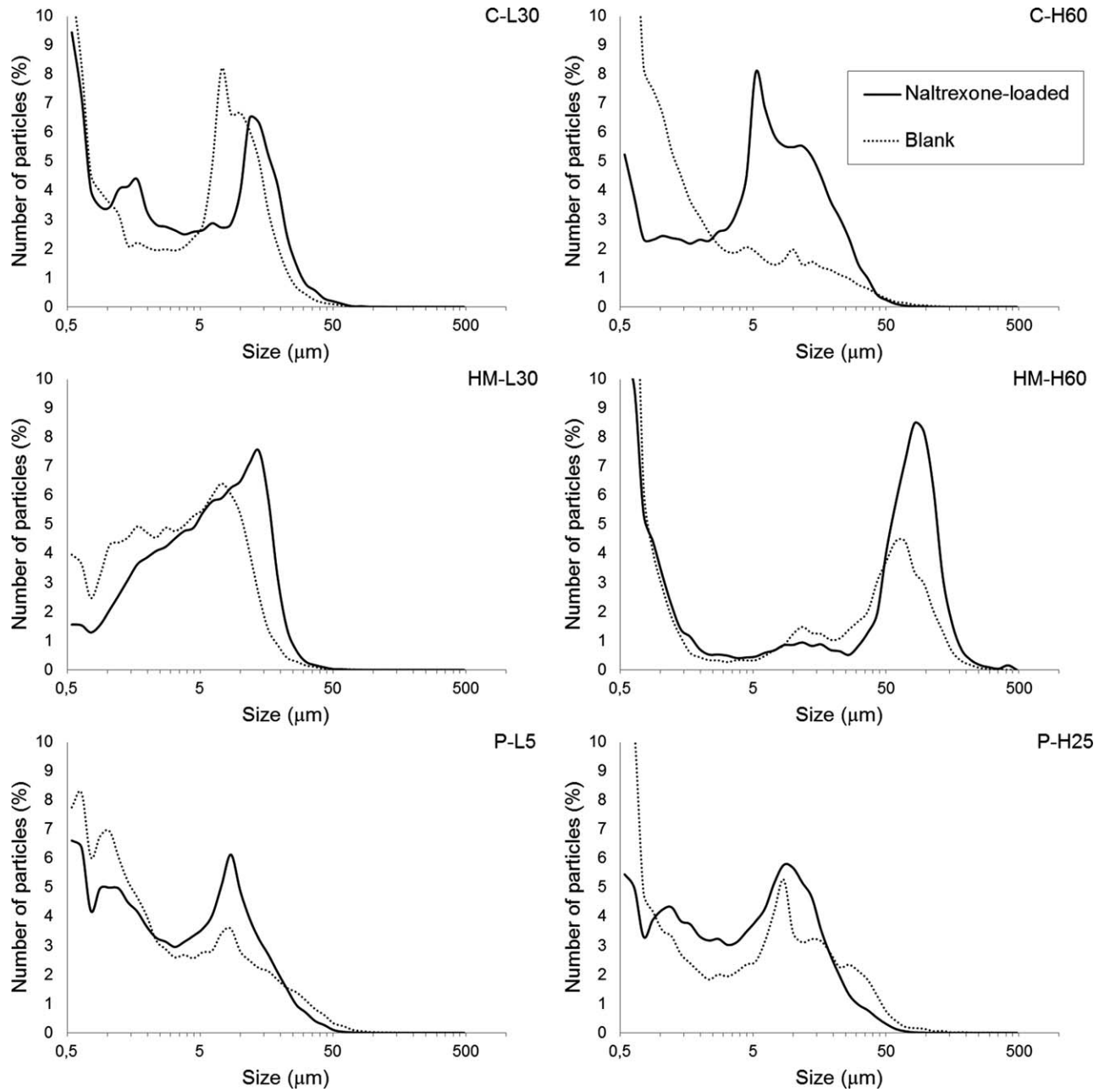

Figure 3. Effect of the presence of drug on the size distribution of chitosan, HM-chitosan and PLGA microspheres (the preparation conditions of each sample are described in Table I). The solid and dashed lines represent the NTX-loaded and the blank microspheres, respectively.

recover the microparticles having a pore size of 0.8 μm. In suspension, these smaller particles may dissociate from the larger particles and were hence detected by the Optical Particle Sizer.

In the case of chitosan and PLGA particles, the profile differed slightly between formulations, but in general all the preparation conditions led to the production of polydisperse samples, with a high presence of particles in the range 5–25 μm. The differences observed between formulations were not found to be statistically significant with respect to size and size distribution.

In contrast, clear differences were observed between formulations prepared from HM-chitosan. The polymer concentration was observed to play an important role. In general, the conditions used in HM-L30 and HM-L60 formulations yielded smaller particles with profiles comparable to chitosan and PLGA. However, the size distribution of these samples also shows the presence of larger structures (up to 50 μm in HM-L30 and 100 μm in HM-L60) that are possibly large aggregates, as shown by SEM (Figure 2).

The formulations that stand out compared with the others are HM-H30 and HM-H60, which produced microparticles with sizes up to 200 and 350 μm, respectively. The size difference between HM-chitosan microparticles is probably related with differences in the viscosity of the dispersed phase. In particular, the higher concentration of HM-H30 and HM-H60 probably

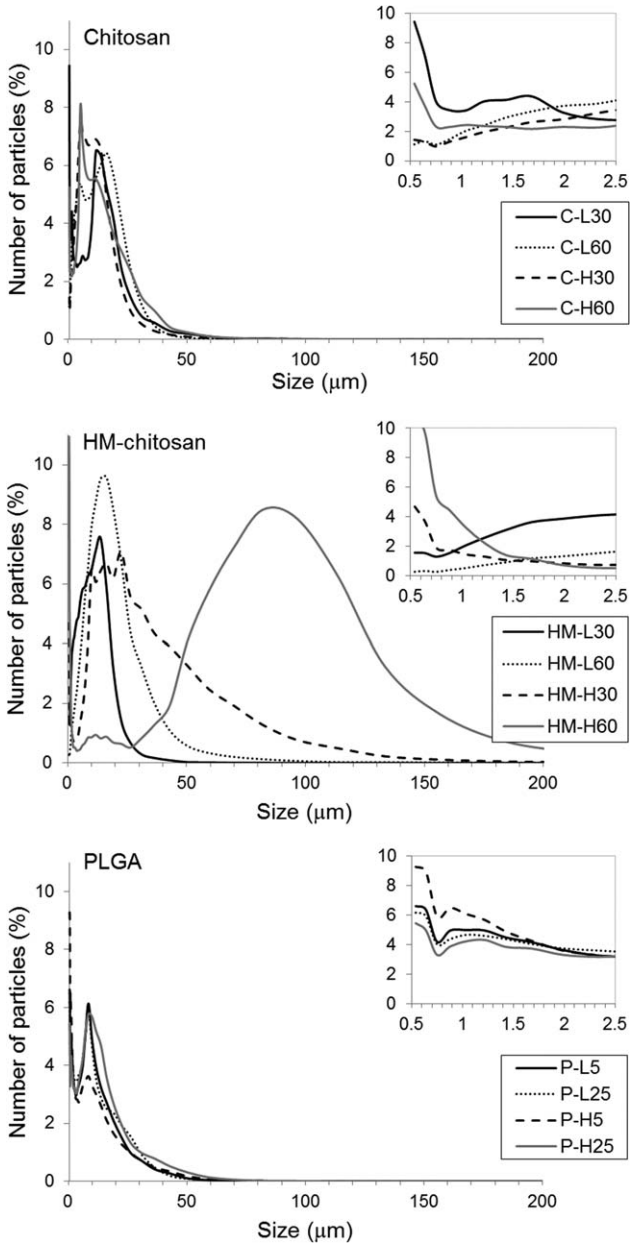

Figure 4. Size distribution of naltrexone-loaded microparticles prepared from chitosan, HM-chitosan and PLGA. The inset shows the number of particles smaller than 2.5 μm. Samples were prepared as described in Table I.

created high viscosity dispersed phases that were difficult to divide by the shear forces present in the mixture. The largest particles were obtained at higher temperatures (HM-H60), which explains the higher loading capacity of the particles in this case (Table I).

Viscosity Measurements of Chitosan and HM-Chitosan Solutions

In the emulsion-solvent evaporation method, one of the critical factors that define the size of the droplets in the emulsion is the viscosity of the dispersed phase. The general tendency is that high viscosity solutions are more difficult to disrupt into small droplets, thereby yielding larger particles.[30] In this work, it was hypothesized that the different size distributions obtained for chitosan and HM-chitosan microparticles could be related with the viscosity of the polymer solutions. For this reason, the viscosities of chitosan and HM-chitosan solutions were determined at different concentrations and temperatures, as shown in Figure 5.

First of all, it is clear that chitosan solutions were less viscous than the corresponding ones prepared using HM-chitosan. This effect is very prominent for the highest polymer concentration (1.5 wt %). The viscosity of semi-dilute solutions of unmodified- and HM-chitosan samples has previously been attributed to both entanglement effects and hydrophobic associations.[20,31] The viscosity has also been shown to increase as the hydrophobicity of the polymer increases, due to the high tendency of the hydrophobic groups to associate.[20,31] The association of the hydrophobic moieties results in aggregates that act as cross-links between the polymer chains, causing the formation of an interconnected network.[32,33]

Figure 5 also shows that, in most cases, the viscosity decreased with increasing temperature. This is explained by the fact that the intermolecular associations are broken to some extent at higher temperatures, due to the enhanced mobility of the chains.[31] However, the viscosity of the HM-chitosan sample prepared at a concentration of 1.5 wt % is less affected by the temperature increase, because of strong entanglements and association effects.

The viscosity of the polymer solutions confirmed the dependency of particle size on the viscosity of the dispersed phase. As mentioned previously, the high viscosity of 1.5 wt % HM-chitosan solutions led to the formation of large droplets in the emulsion, ultimately causing the formation of particles with sizes up to 200 and 350 μm (for HM-H30 and HM-H60, respectively). The opposite relationship can also be established, i.e. the lower viscosity of chitosan solutions, as well as HM-L-30 and HM-L60, on average led to the formation of smaller particles.

As a whole, the higher viscosity of HM-chitosan solutions can additionally help to explain the higher drug encapsulation efficiency of these samples, in agreement with a superior degree of polymer–polymer and polymer–drug interactions. One interesting case is that of HM-H60, where the highest drug loading was observed (EE = 86.5%, LC = 28.8%, Table I). Here, the concentrated solution retained its high viscosity at elevated temperatures, leading to the formation of large particles, with a high loading capacity. It is anticipated that the fast solidification rate of the particles at the high temperature additionally contributed to rapidly entrap the drug within the carrier.

In Vitro Drug Release Studies

The *in vitro* release profile of NTX from the different types of polymeric microparticles is shown in Figure 6.

Chitosan microparticles generally showed a pronounced burst release during the first 6 h—55 to 69% of the drug was released within this period—followed by a second stage, where the drug was slowly released or remained virtually constant over time.

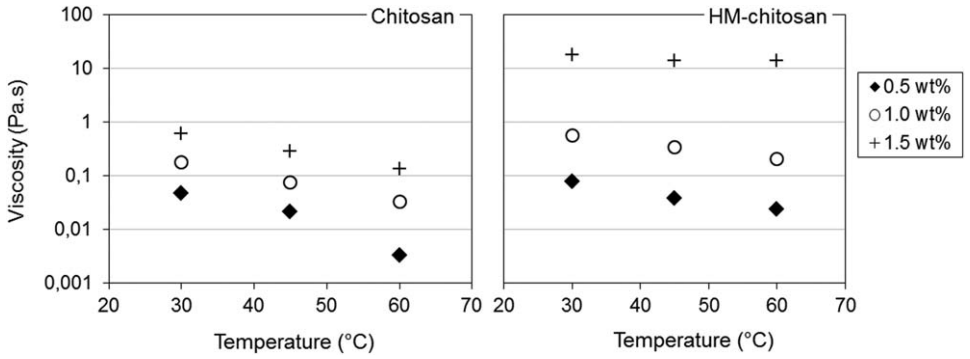

Figure 5. Temperature-dependencies of the shear viscosity of chitosan and HM-chitosan solutions prepared in 1% (v/v) acetic acid at the polymer concentrations of 0.5, 1.0, and 1.5 wt % (shear rate 0.4 s^{-1}).

The initial burst release is quite commonly observed in controlled drug delivery systems that are unable to retain the drug located at the surface of the particles. The constant release stage that follows, reflects a slower release of the drug, which is usually caused by both diffusion through the matrix and degradation of the polymer.[34]

A similar release profile was observed among the different chitosan-based microparticles. However, the release rate was slightly faster for C-L30, and slower for C-H30. Considering that the size distribution among the different chitosan formulations was quite similar for particles larger than 2.5 μm (Figure 4), it is likely that the differences observed here are related to the fraction of particles smaller than this threshold (inset in Figure 4). As a matter of fact, C-L30 was found to contain the largest presence of small particles and accordingly this was the sample that showed the fastest release, among the chitosan formulations. The opposite trend was observed for C-H30. The effect of particle size on the release rate is well-known and established irrespective of the release mechanism. Specifically, smaller particles have a high surface area-to-volume ratio, which causes a higher drug flow to the external medium, whereas the small distance from the surface to the core also facilitates water penetration.[35]

A substantial reduction in the initial burst release was observed for HM-chitosan microparticles. One possible reason for this observation might be the lower proportion of small particles in most HM-chitosan formulations, as compared to chitosan microparticles (the exception being C-H60 compared with HM-H60; inset plots in Figure 4).

When HM-chitosan was used, only 23–40% of NTX was released within the first 6 h, depending on the formulation. After this initial release, NTX was very slowly and continuously released for more than 50 days, particularly for HM-L30 and HM-L60. Only 13–26% of the total drug content was released during this time period, which suggests that HM-chitosan microparticles can constitute promising carriers in long-term applications. Furthermore, it is possible that the slower release rate is related to the presence of the C_{12} groups. These hydrophobic groups can act as cross-linkers due to the hydrophobic associations, while stronger polymer-drug interactions are also expected to occur, as pointed out previously. Furthermore, the presence of the hydrophobic groups can contribute to restrict water penetration. The importance of HM-chitosan hydrophobic groups in the improved drug retention (as compared with the unmodified chitosan) is depicted in Figure 7.

Particles prepared according to HM-L60 and HM-H60 conditions showed a very similar release profile and the slowest *in vitro* release rate. Interestingly, these samples were very different in terms of size distribution (Figure 4) and morphology (Figure 2). HM-L60 was characterized by a relatively narrow size distribution, with most particles being smaller than 50 μm. In spite of this reasonably small size, the presence of particles < 2.5 μm was the lowest of all samples (inset in Figure 4). With respect to HM-H60, the size distribution was predominantly bimodal, including very large particles (average $\sim$90 μm), and a significant presence of particles smaller than 2.5 μm. In other words, the low proportion of small particles in HM-L60, and the high proportion of large particles in HM-H60 may explain the slower release rate in both cases, in agreement with an average low surface area-to-volume ratio, and a larger distance from the core to the surface of the particles.[35] Furthermore, both of them were prepared at a high temperature (60°C), and it is likely that the fast solvent removal resulted in a rapid solidification of the droplets. It may also be hypothesized that at the same time that the solvent evaporated, the extent of hydrophobic associations increased, prompting the formation of a dense network with limited possibilities for drug diffusion. Conversely, the slow evaporation rate used to prepare HM-L30 and HM-H30 microparticles may have induced the formation of more porous structures. Similar findings have been reported for microspheres based on a hydrophobic dextran derivative.[36] In that study, particles prepared at a lower solvent evaporation rate were formed slowly, and demonstrated large pores and cracks at the surface. These microspheres were shown to release the drug faster than the microspheres prepared at higher temperatures.[36]

In addition to the different release rates, the differences in morphology and size between the samples may also cause them to have different fates *in vivo*.[37]

Finally, PLGA microspheres showed a very low burst release, followed by a continuous drug release that reached 72–94%, depending on the formulation, within 16 days. PLGA-based controlled delivery systems have been extensively investigated, due to the polymer's hydrophobicity, long-term stability, and its

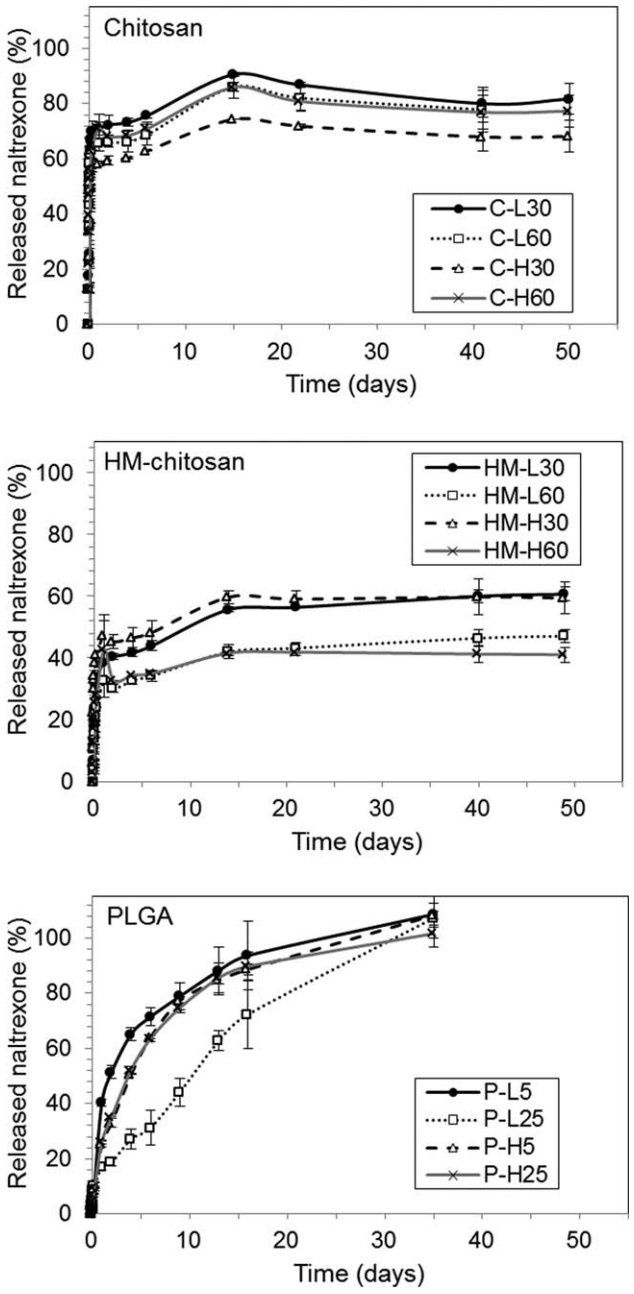

Figure 6. Influence of the microparticle preparation conditions on the *in vitro* release of naltrexone. Error bars = max/min values, $n = 2$. The preparation conditions are described in Table I.

biocompatibility and biodegradability.[38,39] In this work, we used PLGA with a LA/GA composition of 50:50, since this is the most commonly used PLGA composition in drug delivery.[38] However, this copolymer also degrades relatively fast, and other compositions may be employed for improved polymer stability.[38,40] After the second day, the drug release % (relative to the total loaded NTX) from PLGA microparticles was comparable with that observed for HM-chitosan, while the latter provided a slower release rate at longer times.

In spite of the different conditions used to produce the PLGA microparticles, similar release profiles were observed for three of the formulations, namely P-L5, P-H5, and P-H25. These results are explained by the similar EE and LC (Table I) and similar size distribution (Figure 4) between these samples. The rate of NTX release was significantly lower for particles prepared according to P-L25. This is probably due to the low drug loading of these particles (Table I). For poly(L-lactide) (PLA) microspheres containing NTX,[41] decreasing the drug content similarly resulted in a significant reduction in the drug release, due to the lower presence of NTX at the surface of the particles. In the case of P-L25, the processing conditions had an important influence on this outcome. The low polymer concentration and the fast evaporation rate formed a number of large pores at the surface of the particles, as discussed before. As a consequence, a high amount of NTX was probably eliminated from the surface during the particle washing step, which accounts for the low EE. The low presence of drug at the surface of the microparticles also explains the reduced burst release in this case.

The results discussed above should additionally be interpreted from the point of view of the development of an implant allowing for a long-term drug release. In this context, burst release should be kept to a minimum, whereas the drug should be continuously released over an extended period of time, through a combination of diffusion and erosion mechanisms.

HM-chitosan microparticles demonstrated a lower burst release in the initial moments of the experiment as compared to chitosan microparticles, which suggests that the chemical modification improved the controlled delivery properties of the polymer. PLGA microparticles revealed the lowest burst effect, but subsequent drug release was significantly faster than for HM-chitosan. The faster release of NTX from PLGA particles is probably due to the hydrolytic cleavage of the polymer's ester bonds.[42] From HM-chitosan microparticles, the slower release rate suggests that the drug is mostly released by diffusion, to the external medium.

It should further be emphasized that in cases where an incomplete drug release was observed—particularly for chitosan and HM-chitosan microparticles—it is expected that higher release rates would be observed *in vivo*.

Chitosan is known to be hydrolytically degraded by enzymes present in human body fluids, such as lysozyme[43,44] and N-acetyl-β-D-glucosaminidase.[43] For injectable formulations, it has also been suggested that chitosan nano- and micro-structures may be taken up and degraded by macrophages.[44] The degradation rate is also known to depend strongly on the degree of acetylation of the polymer.[43,44] In a study on the oral delivery of insulin, chitosan particles were shown to be enzymatically degraded over time in simulated fluids containing pepsin, trypsin or chymotrypsin.[45] Interestingly, an HM-chitosan derivative containing succinyl and lauryl moieties revealed improved drug protection properties and drug release characteristics in relation to the native chitosan.[45]

With respect to our work, the *in vitro* release experiment did not aim at providing a result representative of the release from the microparticles *in vivo*, but rather to provide a relative result, allowing comparison of the drug retention properties of the

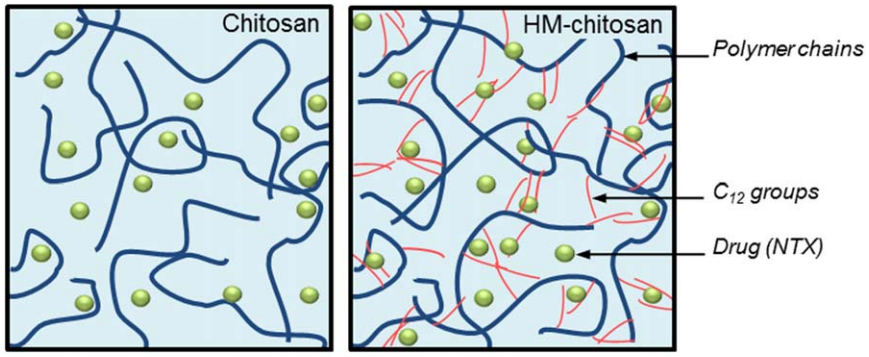

Figure 7. Schematic illustration of chitosan/HM-chitosan networks in the presence of NTX. Within the chitosan network, NTX is mostly physically entrapped due to chain entanglement. In the HM-chitosan network, the hydrophobic intra- and inter-molecular interactions are also very important, forming cross-linking points due to the presence of the C_{12} groups. In this case, both the entanglements and the hydrophobic interactions are responsible for NTX retention. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

polymers under simple incubation conditions. The results from this experiment suggest that the C_{12} groups in HM-chitosan may provide the polymer with enhanced drug delivery properties *in vivo*, due to its higher mechanical strength and expectedly an improved resistance to enzymatic activity and hydrolysis.

CONCLUSIONS

HM-chitosan and chitosan microparticles were manufactured in higher yields than PLGA microparticles. HM-chitosan particles showed a higher encapsulation efficiency of NTX. The encapsulation efficiency was further improved when high polymer concentrations were used, and when the solvent was evaporated at high temperatures. In addition, the drug loading caused the number of small particles to decrease and the amount of large particles to increase, in comparison with the blank microparticles. In most cases, particle size was smaller than 50 μm, with a large number of small particles produced when PLGA was used as the matrix.

HM-chitosan microparticles showed a lower burst release than chitosan microparticles. It is hypothesized that the C_{12} groups hydrophobically associate in the aqueous medium to form additional cross-linking points in the network, thereby contributing to increased NTX retention. Furthermore, the presence of the hydrophobic groups may limit the water diffusion through the pores, and promote strong interactions with the drug molecules. The release rate of HM-chitosan particles was even lower when the samples contained a higher proportion of larger particles, or a smaller amount of small particles. Furthermore, it is additionally hypothesized that when the particles were prepared at higher temperatures compact structures of low porosity are formed. PLGA particles showed a markedly lower burst release in the first hours. However, after a few days, these were shown to be less able to control drug release, as compared to HM-chitosan microparticles.

In conclusion, the results from this study indicate that HM-chitosan can be a promising material for a sustained delivery of drugs such as NTX.

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