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ORIGINAL ARTICLE

Encapsulation of cyclodextrin complexed simvastatin in chitosan nanocarriers: A novel technique for oral delivery

Amber Vyas · Shailendra Saraf · Swarnlata Saraf

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Abstract The purpose of the present work was to design and investigate the potential of novel hydroxylpropyl-beta-cyclodextrin (HP-β-CD) and chitosan nanocarriers (NCs) for effective delivery of model, poorly water soluble drug simvastatin. The prepared system was characterized for particle size, particle size distribution (PDI), zeta potential, differential scanning calorimetery, x-ray diffraction, encapsulation efficiency and drug release studies. The results revealed that among the selected ratios of tripolyphosphate/chitosan, ratio 1:4 and 1:5 proved to be optimum in terms of particle size, particle distribution and drug release profile. The average size of nanoparticles increased from 516 to 617 and 464 to 562 nm for ratio 1:4 and 1:5 with increase in drug/HP-β-CD amount. To assess interactions and whether the simvastatin was incorporated in the NCs in its crystalline or amorphous form DSC and XRD were performed. These results suggest that the encapsulation process produces a marked decrease in crystallinity of simvastatin and/or confers to a nearly amorphous state of drug in NCs. Results reveled that with increase in the amount of HP-β-CD/drug the final loading of the NCs increased due to increased solubilization of simvastatin in the presence of HP-β-CD. The in vitro release profile of prepared NCs showed initial fast release (burst effect) followed by a delayed release pattern. In conclusion, these nanocarriers constitute a novel and efficient system for encapsulation and oral delivery of poorly soluble drugs.

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Hydroxylpropyl-beta-cyclodextrin (HP-β-CD) · Nanocarrier · Chitosan · Tripolyphosphate · Novel drug delivery system

Introduction

According to recent estimates, nearly 40% of new chemical entities are rejected because of poor biopharmaceutical properties. Also new compounds being licensed for clinical use continues to fall. So it is conceivable that the future of the industry may lie in not just striving to make equivalent products, but actually manufacturing improved medicines. However conventional approaches available are not able to circumvent problem of poor bioavailability with controlled delivery. Thus the delivery of poorly soluble drugs is served unfavourably by conventional dosage forms, and poor solubility continues to compromise bioavailability by limiting the level of molecular drug available. Therefore, developing a drug delivery system that can delivery such drugs into the body in a sufficiently bioavailable form is still a challenging task for researchers and academicians.

In the recent years, nanoparticle technology has emerged as a strategy to tackle such formulation problems associated with poorly water-soluble and poorly water- and lipid-soluble drugs [1–4]. The reduction of drug particles to the nanoscale increases dissolution velocity and saturation solubility, which leads to improved in vivo drug performance [5, 6]. Presently, polymer nanoparticles from biodegradable and biocompatible polymers are being widely investigated as a carrier for drug delivery [7]. Polymer nanoparticles are expected to be adsorbed in an intact form in the gastrointestinal tract after oral administration [8]. The hydrophilic nanoparticles generally have longer circulation in blood [9].

Such systems could not only control the rate of drug administration that prolongs the duration of the therapeutic effect but also deliver the drug to specific sites. Most of nanoparticles prepared from water-insoluble polymers involve organic solvents that can be harmful to the drug stability. Moreover, their preparation methods are complex and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent [10].

Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties such as biocompatible, biodegradable, nontoxic, and inexpensive. Chitosan has the ability to increase membrane permeability, both in vitro [11–13] and in vivo [14]. From a biopharmaceutical point of view, chitosan has the potential of serving as an absorption enhancer across intestinal epithelial for its mucoadhesive and permeability enhancing property [15]. Chitosan microparticles and nanoparticles made by chemical cross-linking are not preferred owing to their physiological toxicity [16].

Chitosan is polycationic and can interact with negatively charged species such as tripolyphosphate. This characteristic can be employed to prepare cross-linked chitosan nanoparticles. The interaction of chitosan with tripolyphosphate leads to formation of biocompatible cross-linked chitosan nanoparticles [16]. Tripolyphosphate is a nontoxic and multivalent anion. It can form either inter- or intramolecular links between positively charged amino groups of chitosan and negatively charged counter-ion of tripolyphosphate [17–19].

Besides the use of polymeric nanocarriers as drug delivery systems, the above mentioned barriers could also be overcome via addition of cyclodextrins (CDs). Cyclodextrins are water-soluble cyclic carbohydrate compounds with a hydrophobic core and are a relatively new class of compounds for solubilising drugs. There is evidence that cyclodextrins improve the bioavailability of drugs through a number of routes. Cyclodextrins and their derivatives have been used as solubilizers to enhance the loading capacity of liposomes, microparticles and nanoparticles [20]. In practice, cyclodextrin derivatives such as hydroxypropyl beta cyclodextrin (HP-β-CD) are preferred to natural cyclodextrins for drug formulation because they have higher water-solubility and a better biocompatibility profile [21].

Thus on the basis of available information we aimed to create a kind of new biodegradable nanocarriers for the incorporation of HP-β-CD complexed simvastatin as model drug and evaluate their potential as delivery systems. This novel delivery system was suppose to increased dissolution of poorly water soluble model drug simvastatin by HP-β-CD complexation and controlled delivery via. encapsulation in chitosan nanocarrier.

Materials and methods

Materials

Simvastatin was a kind gift from Ranbaxy, India; Hydroxypropyl-β-cyclodextrins (HP-β-CD) with an average molar substitution degree (MS = 0.6) was gifted by Roquette (Lestrem, France). Chitosan (Low molecular Weight, water soluble) was kindly supplied by Marine Chemicals, Cochin; India. Tripolyphosphate was supplied by Loba Chemie, Mumbai, India. All other reagents were of analytical grade.

Solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors [22]. Excess amount of drug (simvastatin) was added to 25 mL aqueous solution (pH 6.8) containing varying concentrations of HP-β-CD (from 0 to 10 mM/L) in absence and presence of fixed amount of chitosan (0.3% w/v) in screw-capped vials. The contents were stirred for 48 h at 30 ± 1 °C on rotatory shaker. Preliminary "time-dependence" experiments were performed which showed that the equilibrium was reached after 48 h stirring period. After equilibrium, the samples were filtered suitably diluted with methanol and absorbance was read at 240 nm to determine drug concentration using UV-spectrophotometer (Shimadzu UV-1700, Pharmaspec, Tokyo, Japan). Methanol was used as blank solution for analysis of samples.

The presence of HP-β-CD and/or chitosan did not interfere with the assay. The apparent stability constant ($K_{1:1}$) and complexation efficiency (CE) [23] values was calculated from the initial straight portion of the phase solubility diagram using the following equation:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})}$$

$$CE = \left[\frac{D \cdot CD}{CD}\right] = S_0 \cdot K_{1:1} = \frac{\text{Slope}}{(1 - \text{Slope})}$$

where, S_0 is the intrinsic solubility of drug in the absence of chitosan and HP-β-CD at 30 °C.

Nanocarriers (NCs) preparation

Nanocarriers (NCs) were prepared using the ionotropic gelation method [24]. Chitosan/Tripolyphosphate nanocarriers encapsulating HP-β-CD complexed simvastatin were prepared according to the procedure previously developed by Calvo et al. [25, 26]. NCs were spontaneously obtained

via ionotropic gelation between the positively charged amino groups of chitosan and negatively charged tripolyphosphate [27].

Briefly to 8 mL chitosan solution (0.3% w/v) varying amount of HP-β-CD and simvastatin (always in 1:1 ratio as determined by phase solubility studies) were incubated under magnetic stirring. The cross-linking agent tripolyphosphate was added to this solution (Tripolyphosphate/Chitosan w/w ratios of 1:1–1:8) leading to the controlled gelation of chitosan in the form of NCs. Blank NCs were also prepared by same method as discussed above in order to see effect of drug and HP-β-CD on various physiochemical parameters of prepared system. The NCs were isolated in tarred eppendorff vials by centrifugation in a glycerol bed (16,000$\times g$, 30 min, room temperature) and then resuspended in 0.5 mL of pure water by shaking using a vortex. Glycerol was used for centrifugation to enhance the resuspensability of centrifuged NCs. Supernatants were collected for determination of the amount of unbound drug. The production yield was calculated comparing the actual weight with the theoretical weight of the NCs. Every sample was prepared in triplicate and the results represent the average value. The purified NCs were freeze dried.

Nanocarrier characterization

Physicochemical characterization of NCs

Size measurements, zeta potential and morphological characterization of NCs

Dynamic light scattering (DLS) (Malvern, Autoszer 4700) was used to measure the hydrodynamic diameter and size distribution (PDI, polydispersity index). All measurements were done at 25 °C (90°). The zeta potential of nanocarriers was measured on a zeta potential analyzer (Brookhaven, USA). All measurements were performed in triplicate. NCs systems morphology such as shape and occurrence of aggregation phenomena was studied by scanning electron microscopy (SEM). Samples of NCs were mounted on metal stubs, gold coated under vacuum and then examined on a JEOL JSM-840 SEM (10 kV, Japan).

X-ray diffractometry

X-ray diffractometry (XRD) was used to investigate the physical form (crystalline or amorphous) of drug dispersion within the chitosan matrix of the nanocarriers. The XRD patterns of chitosan, simvastatin, HP-β-CD and prepared nanocarriers containing HP-β-CD complexed simvastatin were recorded on an x-ray diffractometer (PW 1729, Philips, Eindhoven, Netherlands). The samples were irradiated with monochromatized Cu Kα radiation and analyzed between 2 and 50° (2θ). The voltage and current used were 40 kV and 36 mA, respectively.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was performed using Shimadzu-Thermal Analyzer DT 40. Samples 2–6 mg were accurately weighed and were heated in aluminum pans at a rate of 10 °C min^{-1}. The measurements were performed at a heating range of 50–350 °C temperature range under a nitrogen flow of 20 mL/min. All samples were analyzed in duplicate.

Evaluation of drug encapsulation

The determination of the drug loading of the NCs was made indirectly from the calculation of the unbound drug which remained dissolved in the NCs suspension medium. The drug loaded NCs were centrifuged as described above in preparation of NCs and the amount of unbound drug (free drug) was measured in the clear supernatant at 240 nm using UV spectrometry (Shimadzu Pharma Spec UV-1700). The corresponding calibration curves were produced using the supernatant of blank nanoparticles. Each sample was measured in triplicate. The drug loading capacity (LC) and association efficiency (AE) of the nanoparticles were calculated according to the following equations:

$$\%LC = \frac{\text{Total drug} - \text{Free Drug}}{\text{Nanocarrier Weight}} \times 100$$

$$\%AE = \frac{\text{Total drug} - \text{Free Drug}}{\text{Total Amount of Drug}} \times 100$$

Drug release studies

For the release experiments, isolated NCs were resuspended in phosphate buffer (pH 6.8) (2 mg/mL of NCs) and maintained under agitation, at 37 ± 0.5 °C. Samples of the release medium were withdrawn at suitable intervals. The aliquots were centrifuged at 16,000g for 30 min and the drug released from NCs was determined by UV spectrophotometry. These experiments were performed under sink conditions according to the solubility of the drug in the presence of HP-β-CD.

Result and discussion

Phase solubility studies

Simvastatin is practically insoluble in water [28, 29]. It was found that the peak of drug (absorbance) was clear and

prominent after dilution with methanol as compared to aqueous solution. The solvent used for λ max determination of simvastatin in official books and in many published articles is methanol. Dilution of aqueous samples of simvastatin with methanol for analysis of drug amount and concentration is reported in literature [30–32]. Thus for having accurate analysis of drug samples were diluted with methanol after equilibrium.

Figure 1 shows the phase solubility diagram of drug in absence and presence of fixed chitosan concentration (0.3% w/v) and varying HP-β-CD concentrations at 30 ± 1 °C. The phase solubility diagram of simvastatin were found to be of linear AL type, as classified by Higuchi and Connors. This suggests a 1:1 stoichiometry of simvastatin and HP-β-CD complex over the concentration range (0–10 mM). The solubility of simvastatin increased up to 11.5 fold at 10 Mm/L of HP-β-CD concentration. However there was a little increase, up to 13.1 fold (1.6%) in solubility of simvastatin in presence of ternary system (i.e. drug, HP-β-CD and 0.3% w/v of chitosan). The apparent complexation constants ($K_{1:1}$) calculated from phase solubility diagram without chitosan was found to be 824 M^{-1}. But a marked decrease of apparent complexation constants ($K_{1:1}$) 298 M^{-1} in presence of ternary system (i.e. drug, HP-β-CD and 0.3% w/v of chitosan) was observed. Although there was decrease in stability constant but the value 298 M^{-1} still falls in the range of stability constant value showing improved dissolution and hence better bioavailability (200–5,000 M^{-1}) of stable complex. The results of decrease in stability constant with addition of hydrophilic polymer were in agreement with previously reported results for the complex of gliburide with HP-β-CD in the presence of chitosan [33] and for complex of triclosan and furosemide with HP-β-CD in the presence of chitosan [34] On the contrary the results of present study differs from the earlier reported results for solubilization of fenofibrate by cyclodextrin complexation [35] and for celecoxib complexation with HP-β-CD [36] respectively i.e. increase in stability constant by addition of hydrophilic polymers. However possibility of decrease in stability constant with addition of hydrophilic polymer in some cases is also reported [37].

Also results for solubility of simvastatin in presence of ternary system i.e. little increase in solubility were quite far from previous reports claiming the synergistic solubilizing and complexing effects of cyclodextrins in the presence of hydrophilic polymers (chitosan in this case) [35, 36, 38, 39]. However the apparent increase in drug solubility is due to the fact that the negative effect of chitosan in the drug HP-β-CD complexation is counteracted by its inherent solubilising effect. These results support results previously reported for the complex of gliburide with HP-β-CD and fursemide with HP-β-CD in the presence of chitosan [33, 34]. However the complexation efficiency (CE) values for complexes without chitosan and in presence of ternary system (i.e. drug, HP-β-CD and 0.3% w/v of chitosan) were found to be 0.03626 and 0.077609 observed at 10 Mm/L of HP-β-CD concentration.

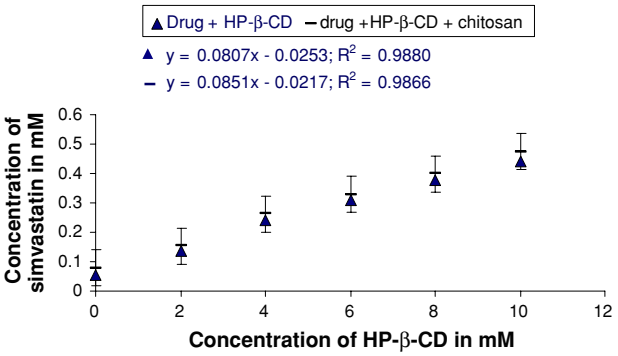

Fig. 1 Phase solubility studies without and in presence of 0.3% chitosan

Nanocarriers (NCs) preparation

Fixed concentration of chitosan and tripolyphosphate was selected on the basis of preliminary studies (not presented here) done by using varying concentration (0.2–0.8% w/v) of chitosan and tripolyphosphate 0.5–2.5 mg/mL respectively. Results of preliminary studies demonstrated that too high and too low concentration of chitosan and tripolyphosphate are not suitable for nanoparticles formation. Thus a moderate conc. i.e. of chitosan 0.3% w/v and tripolyphosphate 2 mg/mL were selected for further studies. As the ratio between tripolyphosphate and chitosan is critical and controls the size and the size distribution of the NCs [25]. Thus effect of tripolyphosphate/chitosan ratio on the size and other physiochemical parameters of the NCs were studied in order to find the optimum ratio that result in NCs of small size and narrow size distribution. The size characteristics have been also reported to affect the biological performance of CS nanoparticles [40]. Hence the tripolyphosphate/chitosan ratio was optimized in presence of fixed amount (50 mg) of HP-β-CD and simvastatin (in 1:1). Table 1 shows effect of varying tripolyphosphate/chitosan ratio on different physiochemical parameters of nanocarriers.

Result presented in Table 1 shows that tripolyphosphate/chitosan ratio 1:1 (F-1) does not result in nanocarrier formation whereas NCs formed with tripolyphosphate/chitosan 1:2 (F-2) have higher tendency to agglomerate into clumps. NCs formed by tripolyphosphate/chitosan ratio 1:3 (F-3) were not completely spherical in shape, and had a rough surface with weak tendencies to agglomerate as compared to other ratio. But NCs formed with tripolyphosphate/chitosan 1:4 (F-4) and 1:5 (F-5) (Figs. 2, 3) had

Table 1 Effect of varying ratio of tripolyphosphate/chitosan on particle size, polydispersity index (PDI), zeta potential and percent yield of nanocarriers prepared by using 0.3% w/v of chitosan and 2 mg/mL tripolyphosphate

Formulation	Tripoly-phosphate/ chitosan ratio	Particle size (nm)	PDI	Zeta potential (in mV)	Percent yield
F-1	1:1	–	–	–	–
F-2	1:2	612 ± 4	0.79	+30.4 ± 0.5	35 ± 8
F-3	1:3	569 ± 1	0.65	+30.1 ± 1.0	39.5 ± 6
F-4	1:4	516 ± 5	0.52	+29.7 ± 0.3	46 ± 4
F-5	1:5	464 ± 3	0.47	+29.2 ± 1.6	52 ± 4
F-6	1:6	578 ± 6	0.67	+28.6 ± .08	59 ± 2
F-7	1:7	U.R[a]	–	–	–
F-8	1:8	U.R.[a]	–	–	–

Amount of HP-β-CD/ Drug (in 1:1) in all above formulation was constant i.e. 50 mg

[a] Unfeasible ratio

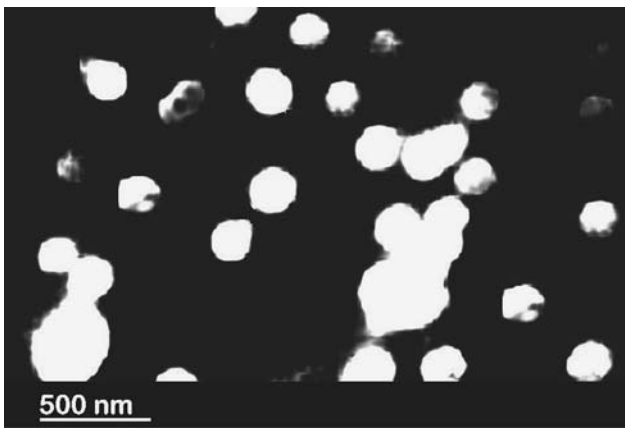

Fig. 2 SEM image of formulation F-4

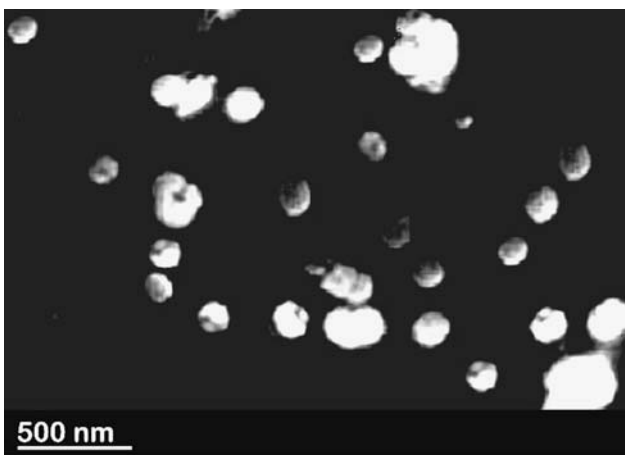

Fig. 3 SEM image of formulation F-5

more spherical shape, smoother surface and discrete with negligible clumping as compared to NCs formed from above mentioned ratios. Also the NCs formed from tripolyphosphate/chitosan ratio 1:6 (F-6) were too having uneven surface morphology and large size. However tripolyphosphate/chitosan ratios 1:7 (F-7) and 1:8 (F-8) were not found suitable ratios for formation of NCs. It is evident from the results shown in Table 1 that as the ratio of tripolyphosphate/chitosan is increased from 1:1 to 1:5 nanocarriers with smaller sizes are produced. But when tripolyphosphate/chitosan ratio increased beyond 1:5, it results in formation of large size NPS. This increase in size may be due to in-sufficient tripolyphosphate responsible for poor gelation of chitosan solution. Based on above observations tripolyphosphate/chitosan ratio in formulation F-4 and F-5 were found optimum for NCs formation in terms of size, size distribution, morphology and percent yield. Figures 2 and 3 shows the SEM images of the nanocarrier formulation F-4 and F-5.

The obtained results of decreasing size with increase in tripolyphosphate/chitosan ratio (up to 1:5) is in accordance with result for chitosan nanoparticles loaded with dorzolamide and pramipexole [41]. It supports the fact that tripolyphosphate is a poly-functional cross-linking agent that can create five ionic cross-linking points with amino groups of chitosan [41, 42]. As a result, the tripolyphosphate/chitosan ratio in formulation F-5 (i.e. 1:5) lead to the most efficient cross-linking of amino groups producing the smallest size particles (464 nm) (Table 1). It is obvious from Figs. 2, 3 and Table 1 that the NCs formulation F-4 and F-5 were of nano-size range and exhibited a positive zeta potential. The particle sizes of drug loaded chitosan nanocarrier ranged between 612 to 464 nm. Also formulation F-4 and F-5 showed lowest PDI, indicating narrow size distribution of NCs. Hence NCs formulation F-4 and F-5 was selected for further studies.

Afterwards effect of absence and presence of cyclodextrin and varying amount of HP-β-CD and drug (in 1:1) was evaluated on entrapment efficiency, drug loading and other physiochemical parameters (viz. size, zeta potential,

& yield) of nanocarriers formulation F-4 and F-5. Table 2 shows the effect on various selected parameters.

When the drug/HP-β-CD amount was varied from 50 to 200 mg, the average size of nanocarriers was increased from 516 to 617 nm and 464 to 562 nm for formulation NCs-1 and NCs-A, respectively (Table 2). The difference or increase in size due to increase in amount of HP-β-CD/drug (in 1:1) leads to a drug proportion dependent increase of their size compared with the blank (without drug) nanocarriers. The reason can be reduction of ionic interactions between tripolyphosphate and chitosan during NCs formation because of the presence of the drug molecules [41]. The above results are in agreement with previously reported data [43, 44].

SEM results (not presented) reveled that presence of cyclodextrin have minor effect on size of NCs (Table 3) however no noticeable change in the NCs appearance was observed. These results support the finding of triclosan and furosemide chitosan NCs having a sponge like structure justified by their formation by an ionic gelation process [34]. It is also obvious from results that the differences in zeta potential of the NCs prepared without or in presence of increasing HP-β-CD/drug amount are minor (Tables 2, 3). So based on above results it can be assumed that cyclodextrins do not interfere with the NCs formation process.

It is clear from results mentioned in Table 2 that as amount of HP-β-CD/drug is increased the NCs yield also increases noticeably. So it can be assumed that increased yield is not only due to increased entrapment drug in presence HP-β-CD but as well as due to entrapment of HP-β-CD into chitosan nanocarriers (Tables 2, 3). As the amount of HP-β-CD/drug increased, the final loading of the NCs increased due to increased solubilization of simvastatin in the presence of HP-β-CD. However this increase in the final loading was accompanied by decrease in the drug entrapment efficiency. The results supports previous findings, that with increase in loading the entrapment efficiency for nifedipine loaded chitosan microspheres decreased [45]. It is obvious from results in Table 2 that as the amount of HP-β-CD/drug increased, the association efficiency (AE) decreased from 26.6% to 19.4%, 29.8% to 21.3% and loading capacity increased up to the values of 7.9, 8.2 for formulations NCs-4 and NCs-D respectively. Therefore, from these results we could conclude that, the use of cyclodextrins enhance the solubility of poorly soluble drug as well as increase drug loading capacity of HP-β-CD containing chitosan nanocarriers. Finally formulations NCs-4 and NCs-D was selected for drug release studies based on highest loading efficiency.

X-ray diffractometry

Figure 4 shows the XRD patterns of simvastatin, chitosan, HP-β-CD and nanocarrier formulations NCs-4 and NCs-D. High intensity peaks for pure drug were obtained at 6.89°, 9.54°, 11.98°, 15.02°, 15.96°, 16.14°, 17.99°, 23.23°, 25.56°, 27.79°, 31.52° and 32.06° (2θ) revealing that the drug is present in crystalline form. However, no characteristic peaks of drug could be detected in the nanoparticles, irrespective of the simvastatin/chitosan ratio (1:4, 1:5). However, small peaks at 11.32, 17.52, 22.62 and a reduced peak at 18.2 (peak of pure HP-β-CD 18.2) can be attributed to the crystalline structures of chitosan and HP-β-CD respectively. Simvastatin probably formed a molecular dispersion or an amorphous nanodispersion within the chitosan matrix of the nanocarriers [41, 46, 47] as well as the reduced signals of XRD may also be attributed to a possible complexation of simvastatin inside the HP-β-CD during NCs formation process, suggesting the possible interaction such as hydrogen bonding between simvastatin and HP-β-CD. The formation of amorphous drug nano dispersion in chitosan matrix is also in accordance with previously published results [41, 47–51].

Table 2 Effect of presence of cyclodextrin and varying amount of HP-β-CD and drug (in 1:1) on particle size, polydispersity index (PDI), zeta potential, yield, Association efficiency (A.E.) and Loading capacity (L.C.) on nanocarriers prepared by tripolyphosphate/chitosan ratio 1:4 and 1:5

Formulation	Amount of HBPCD/drug (in 1:1)	Tripoly-phosphate/chitosan ratio	Particle size	PDI	Zeta potential	Percent yield	A.E. (in %)	L.C. (in %)
NCs-1	50	1:4	516 ± 5	0.52	+29.7 ± 0.3	46 ± 8	26.6 ± 1.2	4.2 ± 0.06
NCs-2	100	1:4	548 ± 5	0.43	+29.2 ± 0.4	51 ± 6	23.5 ± 0.8	5.3 ± 0.05
NCs-3	150	1:4	585 ± 3	0.56	+29.9 ± 0.2	58 ± 3	21.2 ± 0.5	6.7 ± 0.03
NCs-4	200	1:4	617 ± 1	0.58	+28.7 ± 0.2	64 ± 2	19.4 ± 0.3	7.9 ± 0.02
NCs-A	50	1:5	464 ± 3	0.47	+29.2 ± 1.6	53 ± 4	29.8 ± 1.1	4.8 ± 0.04
NCs-B	100	1:5	502 ± 3	0.59	+28.2 ± 1.4	57 ± 3	26.6 ± 0.7	6.1 ± 0.04
NCs-C	150	1:5	539 ± 2	0.55	+28.9 ± 1.5	62 ± 2	24.1 ± 0.4	7.3 ± 0.03
NCs-D	200	1:5	562 ± 1	0.58	+29.2 ± 1.1	67 ± 2	21.3 ± 0.2	8.2 ± 0.03

Table 3 Effect of presence and absence of HP-β-CD and drug on particle size, polydispersity index (PDI), zeta potential, Association efficiency (A.E.) and Loading capacity (L.C.) on nanocarriers prepared by tripolyphosphate/chitosan ratio 1:4 and 1:5

NCs type	Tripoly phosphate/ chitosan ratio	Particle size	PDI	Zeta potential	A.E. (in %)	L.C. (in %)
Without HP-β-CD	1:4	572 ± 3	0.58	29.5 ± 0.3	32.4 ± 0.8	2.9 ± 0.04
Without Drug	1:4	478 ± 2	0.48	29.1 ± 0.2	–	–
Without HP-β-CD	1:5	526 ± 2	0.50	28.8 ± 0.4	34.7 ± 0.5	3.6 ± 0.02
Without Drug	1:5	392 ± 1	0.45	28.5 ± 0.3	–	–

Differential scanning calorimetry

The Differential scanning calorimetry (DSC) curves of the pure drug simvastatin showed a single endothermic peak at 140 °C (Fig. 5) corresponding to the melting of drug. The DSC profile of nanocarrier formulation NCs-4 and NCs-D (Fig. 5) shows broad but low intensity peak at 140 °C accompanied by endothermic peaks is due to the Tg relaxation enthalpy of the chitosan. These results, taken together, suggest that the encapsulation process produces a marked decrease in crystallinity of simvastatin and/or confers to this drug a nearly amorphous state. However, it should be taken into account that quantification of low content of amorphous/crystalline phase by DSC or other analytical techniques proved to be ineffective [52].

Drug release studies

Comparison of the release profile of pure drug with the release profiles of drug entrapped nanocarrier formulations NCs-4 and NCs-D showed sustained release of drug entrapped in nanocarriers. Sustained drug release from the nanoparticles is important, as it will increase drug bioavailability and prolong the therapeutic effect. Simvastatin release from both the formulations NCs-4 and NCs-D follows a biphasic release pattern. These profiles are characterized by an initial fast release phase (burst release)

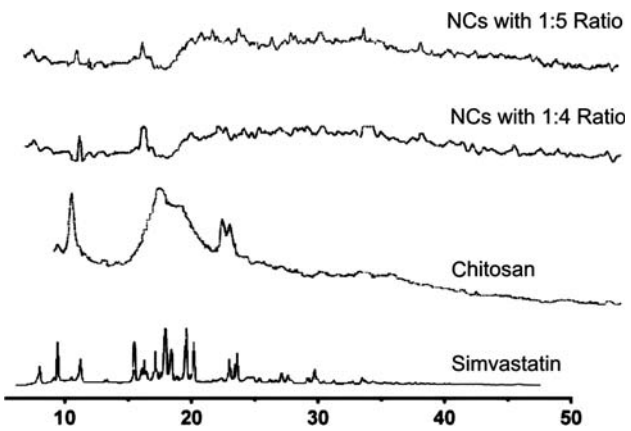

Fig. 4 XRD patterns of simvastatin, chitosan, HP-ß-CD and prepared nanocarriers

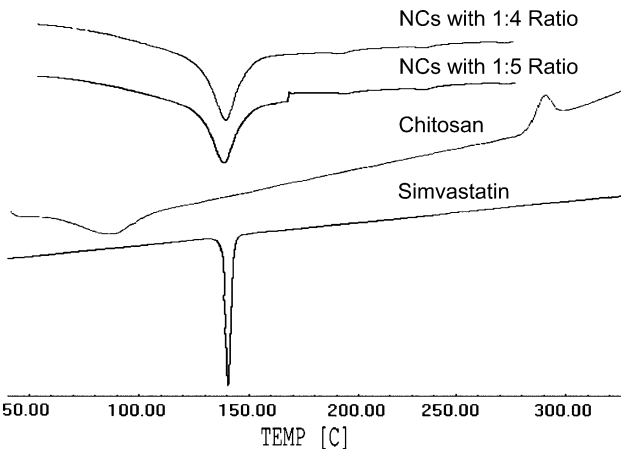

Fig. 5 Differential scanning calorimetry (DSC) curves

followed by a delayed release and the plateau level (between 37% and 32% release) is obtained approximately after 2 h for formulations NCs-4 and NCs-D and was maintained for as least 24 h. The burst release lasted for 30 min, and during this period approximately 28% and 23% of the drug was released from NCs formulations NCs-4 and NCs-D respectively. However 42% and 36% release was observed after 6 h. The results are in close agreement with recent studies in drug-loaded chitosan nanoparticles [34].

The initial fast release might be the result of the rapid dissolution of the drugs crystals located at or close to the surface of the NCs. These results suggest release of some amount of drug from drug to cyclodextrin complexes by simple diffusion through the polymer network. After the burst release period, the decrease in release rate can be attributed to change of release mechanism to diffusion through the chitosan matrix. Furthermore the release of simvastatin from the NCs in the buffer is much slower than the release of pure drug in the same medium, indicating that the entrapment of simvastatin in chitosan nanocarrier can effectively sustain the rate of drug release (Fig. 6). On basis of above mentioned result and assuming that the HP-β-CD complexed simvastatin chitosan nanocarriers will exhibit similar sustained release profiles in vivo, suggests that simvastatin nanocarriers could be further considered as a sustained, oral drug delivery system for simvastatin.

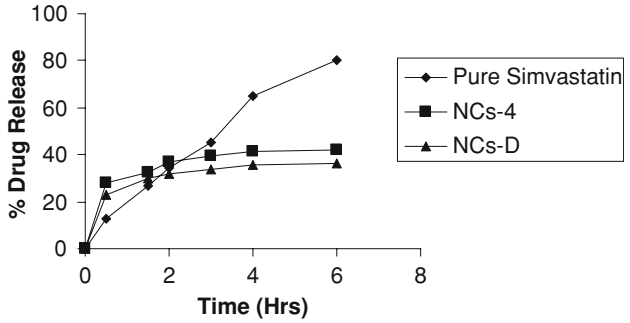

Fig. 6 Drug release studies of pure drug and its comparison with prepared NCs formulations NCs- 4 and NCs- D

Conclusion

In conclusion, this work showed that nanocarriers encapsulating HP-β-CD complexed simvastatin can be prepared by cross-linking of chitosan with tripolyphosphate. The prepared NCs formed are able to encapsulate hydrophobic compounds designated to oral delivery. These results confirms that addition of HP-β-CD enhanced the solubility of poorly soluble drug as well as increase drug loading capacity and also allow to foresee some other potential applications with similar active substance having a structural homology. Finally, it could be interesting to study potential of nanocarriers as possible candidates for having mucoadhesive and permeability enhancing property (because of chitosan) should also be investigated to prove their dual nature.

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References

1. Radtke, M.: Nanopure TM: pure drug nanoparticles for the formulation of poorly soluble drugs. New Drugs **3**, 62–68 (2001)
2. Muller, R.H., Bohm, B.H.L.: Nanosuspensions. In: Muller, R.H., Bentia, S., Bohm, B.H.L. (eds.) Emulsions & nanosuspensions for the formulation of poorly soluble drugs, pp. 149–174. Medpharm Scientific Publishers, Stuttgart, Germany (1998)
3. Muller, R.H., Jacobs, C., Kayser, O.: Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. **47**(1), 3–19 (2001)
4. Liversidge, E.M., Liversidge, G.G., Cooper, E.R.: Nanosizing: a formulation approach for poorly water-soluble compounds. Eur. J. Pharm. Sci. **18**(2), 113–120 (2003)
5. Rabinow, B.E.: Nanosuspensions in drug delivery. Nat. Rev. Drug Discov. **3**(9), 785–796 (2004)
6. Patravale, V.B., Date, A.A., Kulkarni, R.M.: Nanosuspensions: a promising drug delivery strategy. J. Pharm. Pharmacol. **56**(7), 827–840 (2004)
7. Gref, R., Minamitake, Y., Perracchia, M.T., Trubeskoy, V., Torchilin, V., Langer, R.: Biodegradable long-circulating polymeric nanospheres. Science **263**, 1600–1603 (1994)
8. Florence, A.T., Hillery, A.M., Hussain, N., Jani, P.U.: Nanoparticles as carriers for oral peptide absorption: studies on particle uptake and fate. J. Control. Release **36**, 39–46 (1995)
9. Allemann, E., Gurny, R., Deolker, E.: Drug loaded nanoparticles: preparation methods and drug targeting issues. Eur. J. Pharm. Biopharm. **39**, 173–191 (1993)
10. Tiyaboonchai, W.: Chitosan nanoparticles: a promising system for drug delivery. Naresuan Univ. J. **11**(3), 51–66 (2003)
11. Aspden, T.J., Mason, J.D., Jones, N.S.: Chitosan as a nasal delivery system: the effect of chitosan solutions on in vitro and in vivo mucociliary transport rates in human turbinates and volunteers. J. Pharm. Sci. **86**, 509–513 (1997)
12. Lehr, C.M., Bouwstra, J.A., Schacht, E., Junginger, H.E.: In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. **78**, 43–48 (1992)
13. Dumitriu, S., Chornet, E.: Inclusion and release of proteins from polysaccharide-based polyion complexes. Adv. Drug Deliv. Rev. **31**, 223–246 (1998)
14. Takeuchi, H., Yamamoto, H., Niwa, T., Hino, T., Kawashima, Y.: Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm. Res. **13**, 896–901 (1996)
15. Janes, K.A., Calvo, P., Alonso, M.J.: Polysaccharide colloidal particles as delivery systems for macromolecules. Adv. Drug Deliv. Rev. **47**, 83–97 (2001)
16. Bhumkar, D.R., Pokharkar, V.B.: Studies on effect of PH on cross-linking of chitosan with sodium tripolyphosphate: a technical note. AAPS PharmSciTech. **7**(2), E1–E6 (2006)
17. Aral, C., Akbuğ a, J.: Alternative approach to the preparation of chitosan beads. Int. J. Pharm. **168**, 9–15 (1998)
18. Mi, F.L., Shyu, S.S., Chen, C.T., Schoung, J.Y.: Porous chitosan microsphere for controlling the antigen release of Newcastle disease vaccine: preparation of antigen-adsorbed microsphere and in vitro release. Biomaterials **20**, 1603–1612 (1999)
19. Shu, X.Z., Zhu, K.J.: A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled drug delivery. Int. J. Pharm. **201**, 51–58 (2000)
20. Duchê ne, D., Ponchel, G., Wouassindjewe, D.: Cyclodextrins in targeting- Application to nanoparticles. Adv. Drug Deliv. Rev. **36**(1), 29–40 (1999)
21. Vyas, A., Saraf, S., Saraf, S.: Cyclodextrin based novel drug delivery systems. J. Incl. Phenom. Macrocycl. Chem. **62**(1–2), 23–42 (2008)
22. Higuchi, T., Connors, K.A.: Phase-solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965)
23. Brewster, M.E., Loftsson, T.: Cyclodextrins as pharmaceutical solubilizers. Adv. Drug Deliv. Rev. **59**, 645–666 (2007)
24. Shu, X.Z., Zhu, K.J.: Chitosan/gelatin microspheres prepared by modified emulsification and ionotropic gelation. J. Microencapsul. **18**, 237–245 (2001)
25. Calvo, P., Remuña′n-Lo′ pez, C., Vila-Jato, J.L., Alonso, M.J.: Novel hydrophilic chitosan–polyethylene oxide nanoparticles as protein carriers. J. Appl. Polym. Sci. **63**, 125–132 (1997)
26. Calvo, P., Vila-Jato, J.L., Alonso, M.J.: Evaluation of cationic polymer-coated nanocapsules as ocular drug carriers. Int. J. Pharm. **153**, 41–50 (1997)
27. Bodmeier, R., Chen, H., Paeratakul, O.: A novel approach to the oral delivery of micro- or nanoparticles. Pharm. Res. **6**, 413–417 (1989)
28. Ambike, A.A., Mahadik, K.R., Paradkar, A.: Spray-dried amorphous solid dispersions of simvastatin a low Tg drug: in vitro and in vivo evaluations. Pharm. Res. **22**, 990–998 (2005)

29. Kang, B.K., Lee, J.S., Chon, S.K., Jeong, S.Y., Yuk, S.H., Khang, G., Lee, H.B., Cho, S.H.: Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int. J. Pharm. **274**, 65–73 (2004)
30. Rajput, S.J., Raj, H.A.: Simultaneous spectroscopic estimation of ezetimibe and simvastatin in tablet dosage forms. Indian J. Pharm. Sci. **69**, 759–762 (2007)
31. Arayne, M.S., Sultana, N., Hussain, F., Ali, S.A.: Validated spectrophotometric method for quantitative determination of simvastatinin pharmaceutical formulations and human serum. J. Anal. Chem. **62**, 536–541 (2007)
32. Bhatia, N.M., Deshmukh, D.D., Kokil, S.U., Bhatia, M.S.: (2009) Simultaneous spectrophotometric estimation of simvastatin and ezetimibe in tablet formulation. J. Chem. **6**(2), 541–544. http://www.e-journals.in/
33. Zerrouk, N., Corti, G., Ancillotti, S., Maestrelli, F., Cirri, M., Mura, P.: Influence of cyclodextrins and chitosan, separately or in combination, on glyburide solubility and permeability. Eur. J. Pharm. Biopharm. **62**, 241–246 (2006)
34. Maestrelli, F., Garcia-Fuentes, M., Mura, P., Jose′ Alonso, M.: A new drug nanocarrier consisting of chitosan and hydoxypropylcyclodextrin. Eur. J. Pharm. Biopharm. **63**, 79–86 (2006)
35. Patel, A.R., Vavia, P.R.: Effect of hydrophilic polymer on solubilization of fenofibrate by cyclodextrin complexation. J. Incl. Phenom. Macrocycl. Chem. **56**, 247–251 (2006)
36. Chowdary, K.P.R., Srinivas, S.V. Influence of hydrophilic polymers on celecoxib complexation with hydroxypropyl β-cyclodextrin. AAPS PharmSciTech. **7**(3), E-1–E-6 (2006)
37. Bibby, D.C., Davies, N.M., Tucker, I.G.: Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. Int. J. Pharm. **197**, 1–11 (2000)
38. Loftsson, T., Frioriksdottir, H., Siguroardottir, A.M., Ueda, H.: The effect of water-soluble polymers on drug–cyclodextrin complexation. Int. J. Pharm. **110**, 169–177 (1994)
39. Ammar, H.O., Salama, H.A., Ghorab, M., Mahmoud, A.A.: Formulation and biological evaluation of glimepiride–cyclodextrin–polymer systems. Int. J. Pharm. **309**, 129–138 (2006)
40. Pan, Y., Li, Y.J., Zhao, H.Y., Zheng, J.M., Xu, H., Wei, G., et al.: Int. J. Pharm. **349**, 139–147 (2002)
41. Papadimitriou, S., Bikiaris, D., Avgoustakis, K., Karavas, E., Georgarakis, M.: Chitosan nanoparticles loaded with dorzolamide and pramipexole. Carbohydr. Polym. **73**, 44–54 (2008)
42. Zhang, H., Oh, M., Allen, C., Kumacheva, E.: Monodisperse chitosan nanoparticles for mucosal drug delivery. Biomacromolecules **5**(6), 2461–2468 (2004)
43. Fernandez-Urrusuno, R., Calvo, P., Remunan-Lopez, C., Vila-Jato, J.L., Alonso, M.J.: Enhancement of nasal absorption of insulin using chitosan Nanoparticles. Pharm. Res. **16**, 1576–1581 (1999)
44. Kim, D.G., Jeong, Y.I., Choi, C., Roh, S.H., Kang, S.K., Jang, M.K., Nah, J.W.: Retinol-encapsulated low molecular water-soluble chitosan nanoparticles. Int. J. Pharm. **319**, 130–138 (2006)
45. Dhawan, S., Singla, A.K.: Nifedipine loaded chitosan microspheres prepared by emulsification phase separation. Biotech. Histochem. **78**, 243–254 (2003)
46. Gupta, P., Bansal, A.K.: Devitrification of amorphous celecoxib. AAPS PharmSciTech. **6**, E223–E230 (2005)
47. Kausbal, A.M., Gupta, P., Bansal, A.K.: Amorphous drug delivery systems: molecular aspects, design, and performance. Crit. Rev. Ther. Drug Carrier Syst. **21**(3), 133–193 (2004)
48. Karavas, E., Georgarakis, E., Sigalas, M.P., Avgoustakis, K., Bikiaris, D.: Investigation of the release mechanism of a sparingly water-soluble drug from solid dispersions in hydrophilic carriers based on physical state of drug, particle size distribution and drug-polymer interactions. Eur. J. Pharm. Biopharm. **66**(3), 334–347 (2007)
49. Karavas, E., Georgarakis, E., Docoslis, A., Bikiaris, D.: Combining SEM, TEM, and micro-Raman techniques to differentiate between the amorphous molecular level dispersions and nanodispersions of a poorly water-soluble drug within a polymer matrix. Int. J. Pharm. **340**, 76–83 (2007)
50. Shah, B., Kakumanu, V.K., Bansal, A.K.: Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. J. Pharm. Sci. **95**, 1641–1665 (2006)
51. Jun, S.W., Kim, M.S., Kim, J.S., Park, H.J., Lee, S., Woo, J.S., Hwang, S.J.: Preparation and characterization of simvastatin/hydroxypropyl-β-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process. Eur. J. Pharm. Biopharm. **66**(3), 413–421 (2007)
52. Boonsongrit, Y., Mitrevej, A., Mueller, B.W.: Chitosan drug binding by ionic interaction. Eur. J. Pharm. Biopharm. **62**(3), 267–274 (2006)