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Original Research Article

Verapamil hydrochloride nanoparticles formulated with chitosan and sodium alginate by an ionic gelation method

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Abstract

Purpose: *To formulate chitosan-based nanoparticles for extended release of verapamil so as to reduce its dosing frequency.*

Methods: *Nanoparticles of chitosan and sodium alginate loaded with verapamil HCl were synthesized using the ionotropic gelation method. The formulations were optimized by cross linking of tripolyphosphate with chitosan, and $CaCl_2$ with sodium alginate at different concentrations. The ionic gelation method was used to produce seven different formulations. The best formulation for nanoparticles preparation involved using 0.06 % sodium alginate with 18 mM $CaCl_2$ and of chitosan: TPP (5:1 ratio). The compatibility of verapamil HCl with sodium alginate and chitosan was tested using FTIR.*

Results: *Verapamil nanoparticles had a direct correlation with the polymer concentrations used. A 5:1 ratio of chitosan:TPP resulted in a particle size of 173 nm, and high drug entrapment. The size of verapamil nanoparticles prepared with sodium alginate was 186 nm (p = 0.02). Cross-linking agent played an important role in the preparation of the nanoparticles. Scanning electron micrographs showed that the nanoparticles were coalesced with one another, and had rough surfaces. Chitosan-based formulations had an entrapment efficiency in the range of 38.63 – 50.58 %, while the entrapment efficiency of sodium alginate-based formulations was 11.70 – 40.57 % (p 0.43).*

Conclusion: *Verapamil nanoparticles have been successfully formulated by ionic gelation method using natural polymers. The nano-formulations did not exhibit polymer-drug interactions, but manifest extended drug-release profiles.*

Keywords: *Chitosan, Sodium alginate, Verapamil hydrochloride, Tri-polyphosphate, Nanoparticles*

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INTRODUCTION

Verapamil belongs to the class of calcium channel blockers, and it produces its action by relaxation of arterial smooth muscle, resulting in reduced cardiac contractility. Clinically, verapamil is utilized for the management of essential hypertension, cardiac arrhythmias and vasospastic angina [1]. The oral dose of verapamil ranges from 20 to 40 mg/kg/day, usually administered into three to four spilt

doses. In a chronic disease such as hypertension, there is need to administer this drug for a long period of time [2]. However, long term administration of verapamil is associated with poor patient compliance. In order to improve patients' responses, research has been focused on designing modified-release dosage forms of verapamil. Nanotechnology provides a potential for developing a variety of strategies aimed at addressing formulation challenges [3,4]. Polymeric nanoparticles are promising drug delivery systems capable of providing extended release, as well as targeted delivery of therapeutics. Nanoparticles improve drug absorption through the biological membranes. These particles also enhance drug safety [5].

There are number of methods for the preparation of polymeric nanoparticles. Methods are selected according to the nature of the polymer and the drug. Chitosan is cationic polysaccharide-based polymer that is biodegradable, biocompatible and muco-adhesive nature. Owing to its favorable properties, it is widely used in the formulation of designs [6]. In this research work, verapamil was encapsulated in chitosan and sodium alginate nanoparticles prepared using ionic gelation method. The nanoparticles not only masked the bitter taste of verapamil, but also provided extended release of the drug that ultimately reduced dose frequency.

EXPERIMENTAL

Material

The material used were verapamil hydrochloride (Searle Pharma), sodium alginate, chitosan, tripolyphosphate (TPP), and calcium chloride.

Formulation of verapamil nanoparticles using chitosan

Verapamil HCl nanoparticles were prepared using Ionic Gelation method. Chitosan (0.05%; v/v) was dissolved in acetic acid solution with continuous stirring for 24 h to produced Chitosan solution of concentration 2.5 mg/ml. Then verapamil hydrochloride (0.1 mg/mL) was added to the chitosan solution. Tri-polyphosphate (TPP) solution (0.25 mg/mL) solution was prepared and added drop-wise at a flow rate of 0.3 mL/min) to the Chitosan – verapamil solution. Varying concentration of chitosan and TPP were used to prepare the superlative solution for nanoparticle preparation (Table 1). The nanoparticles suspensions were left to gel at room temperature for 30 min [7].

Table 1: Composition of Verapamil hydrochloride nanoparticles formulations by chitosan

Formulation Code	Verapamil Hydrochloride (mg/mL)	Polymer (Chitosan: TPP)
F1	0.1	4:1
F2	0.1	5:1
F3	0.1	6:1

Preparation of verapamil nanoparticles using sodium alginate

Sodium alginate solutions of different concentrations [(0.03, 0.06 and 0.12% (w/v)] were prepared by adding the required amounts of polymer in distilled water, with gentle stirring at room temperature. Verapamil hydrochloride (0.1 mg/mL) was added to the sodium alginate solution, while stirring with a magnetic stirrer, resulting in a homogenizes sodium alginate solution. Calcium chloride solutions (18 and 36 mM) were prepared and added drop wise to the verapamil-sodium alginate solution (Table 2). The mixture was stirred during the reaction time, and the resultant suspension was centrifuged for 20 min [8].

Determination of mean particle size and Zeta potential

The nanoparticle size and Zeta potential of the formulations were determined using Malvern Zeta-Sizer ZS (Malvern Instrument, Worcestershire, United Kingdom). Diluted samples of the nanoparticles formulations were placed in disposable poly-styrene cuvettes and the scatter intensity of each samples was measured at 25^{o}C.

Table 2: Composition of verapamil hydrochloride nanoparticles formulations by sodium alginate

Formulation code	Verapamil hydrochloride (mg/mL)	Polymer (sodium alginate %)	Calcium chloride (mM)
SA1	0.1	0.03	18
SA2	0.1	0.06	18
SA3	0.1	0.12	18
SA4	0.1	0.03	36
SA5	0.1	0.06	36
SA6	0.1	0.12	36

The mean particle size values were determined from the mean hydrodynamic diameter (MHD) and poly dispersity index (PDI).

Shape and surface morphology

Scanning electron microscope (SEM) was used to evaluate the surface morphologies and shapes of verapamil nanoparticles. The nanoparticles suspensions were air dried on petri dishes at 8ºC for 3 days. Each powdered nanoparticles sample was spread on a double-side carbon dust and the dried samples was then positioned in a sample carrier in a cylindrical shape. The nanoparticles were examined under a scanning electron microscope (SEM) [9].

Entrapment efficiency

To determine entrapment efficiency, each prepared nanoparticles suspension was centrifuged at 15000 rpm and the supernatant was taken up in a separate tube. A standard drug solution was prepared in chitosan/sodium alginate system containing the same amount of drug present in the nanoparticles suspension. The absorbance values of the supernatant and the standard solution were read spectrophotometrically in a UV visible spectrophotometer at λ max of 278 nm. Drug entrapment (E) was calculated as in Eq 1.

$$E (\%) = \{(As – Sp)/As\}100 \quad \ldots\ldots\ldots\ldots (1)$$

where As and Ap are the absorbance of standard and supernatant solutions, respectively.

Fourier transforms infrared spectroscopy (FTIR)

To visualize the interaction and compatibility between verapamil HCl and polymer, FTIR spectroscopy was performed on all polymers and drug. The spectra of pure drug, polymers and nanoparticles containing both the drug and the polymers in formulation were recorded. A small amount of sample (2 mg) was mixed with KBr (200 mg) and the mixture was ground for 3-5 min. The resolution was set at 1 cm^{-1}, while the range used for scanning of materials was set at 4000 – 400 cm^{-1}.

In vitro drug release studies

Regular dialysis technique was used to study *in-vitro* release of verapamil from the nanoparticles. Nanoparticles equivalent to 5mg were introduced into a dialysis bag containing phosphate buffer solution (inner media/compartment), and the bag was subsequently sealed and placed in a larger vessel containing 90 ml of phosphate buffer solution [10,11]. Sample were taken from the outer compartment at 1-hour intervals and analyzed in a UV-visible spectrophotometer at λ_{max} 278 nm.

RESULTS

Particle size

Verapamil-loaded chitosan and sodium alginate nanoparticles were in the size range of 173 and 186 nm, respectively. The results are shown in Table 3.

Table 3: Particle size and polydispersity of the formulations

Formulation code	Mean particle size (± SD, nm)	PDI
F1	-	-
F2	173 ±12	0.48
F3	490±136	0.27
SA1	35±2.5	1.00
SA2	186±17.4	0.38
SA3	965±212	0.31
SA4	-	-
SA5	154±57	0.40
SA6	682±165	0.65

Shape and surface morphology

Chitosan and Sodium alginate nanoparticles result of scanning electron microscope (SEM) shows that nanoparticles coalescence with one another and their average particle size increased during the drying and thus their surface was rough (Figure 1).

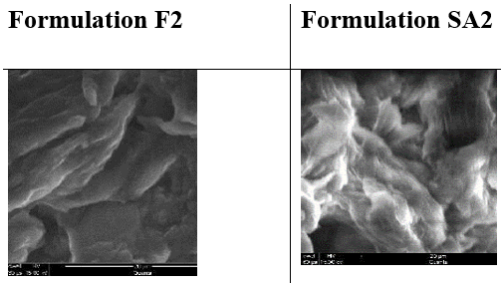

Figure 1: SEM images for formulations F2 and SA2 at x5000

Entrapment efficiency of verapamil nanoparticles

As shown in table 4, the entrapment efficiency values of the verapamil nanoparticle prepared from chitosan were in the range of 38.6 – 50.5 %,

while the corresponding values for sodium alginate-derived nanoparticles were in the range of 11.7 – 40.5 %.

Nanoparticles formulations with chitosan F3 had the highest entrapment of 50.5 %, while nanoparticle formulation with sodium alginate SA3 showed highest concentration 40.5 %.

FT-IR spectra

Results from FT-IR spectroscopy demonstrated that all characteristics peaks of verapamil HCl were present in the combined spectrum of polymer and pure drug. There were no interactions among the polymer, drug and crosslinking agent. Moreover, there were no significant changes in the IR peaks of verapamil HCl, when mixed with the natural polymers; sodium alginate and chitosan as well as calcium chloride, acetic acid and sodium tripolyphosphate. These observations indicates that the pure drug was compatible with the polymers, a condition which is necessary to producing a stable, safe and efficacious formulation. These results are shown in Figure 2.

In vitro *drug release*

Maximum release of nanoparticles in phosphate buffer pH 7.4 was observed for up to 10 hours. Drug release from the nanoparticles decreased with increase in polymer concentration. The dissolution profiles for formulation F2, SA2 and SA5 were evaluated, and a graph of drug release against time was plotted (Figure 3). Drug release for SA2 formulation was the highest. In contrast, drug release was gradually decreased for formulations F2 and SA5. Drug release profile indicates the relationship between the concentration of polymer and drug release property. Increases in polymer concentration decreased the release of drug from the nanoparticles. The reverse was the case with respect to entrapment efficiencies of the nanoparticles. Cumulative *in vitro* drug release of formulations F2, SA2 and SA5 were compared by plotting a graph of time against drug release, as shown in Figure 3. The result showed the drug release patterns of all predesigned formulations. These graphs show a direct relationship between fraction of drug dissolved and time. During time interval of 6-8 h, drug profile showed maximum absorbance, but thereafter, variation in drug absorbance was observed.

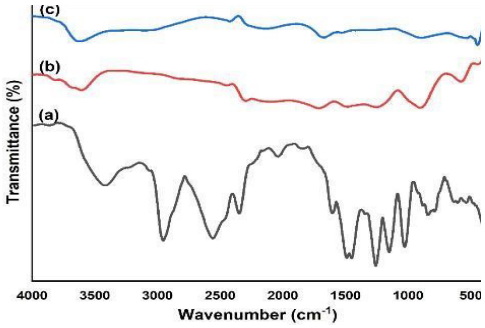

Figure 2: FT-IR spectrum of verapamil and verapamil nanoparticles a) FT-IR spectrum of verapamil HCL, b) FT-IR spectrum of chitosan nanoparticles, c) FT-IR spectrum of sodium alginate nanoparticles

Drug release kinetics

Data from *in vitro* release studies of all formulations were fitted into the Korsmeyer-Peppas model using DD Solver software. The result indicated that the drug release followed the KorsKorsmeyer-Peppas model due to higher regression coefficient values in Korsmeyer-Peppas model. The F2 value of n was 18.36, while SA2 and SA5 values of n were 15.4 and 14.0, respectively (Figure 4).

Table 4: Entrapment efficiency of Verapamil nanoparticles

Formulation code	Absorbance (483 nm) Drug in polymer	Absorbance (483 nm) supernatant of nanoparticles	EE (%)
F2	1.615	0.991	38.63
F3	1.615	0.798	50.58
SA1	1.708	1.508	11.70
SA2	1.708	1.335	21.83
SA3	1.708	1.015	40.57
SA4	1.708	1.708	N.A
SA5	1.708	1.463	14.3
SA6	1.708	1.279	25.18

Table 5: Application of the kinetic models on dissolution profile of all the formulations

Kinetic order /model		Formulation						
		F1	F2	SA1	SA2	SA3	SA5	SA6
Zero	K_0	10.86	11.23	10.99	10.89	10.04	10.47	10.0
	K_0	0.898	0.90	0.90	0.95	0.94	0.94	0.96
First	K_I	0.209	0.222	0.21	0.20	0.17	0.18	0.16
	R^2	0.94	0.93	0.94	0.92	0.95	0.98	0.89
Higuchi	K_H	28.714	26.66	29.0	28.58	26.44	27.41	25.9
	R^2	0.882	0.88	0.89	0.86	0.88	0.84	0.79
KorsemeyerPeppas	R^2	0.058	0.061	0.05	0.05	0.05	0.05	0.04
	KKP	0.971	0.97	0.97	0.96	0.98	0.95	0.92
	N	18.17	18.36	18.7	15.40	15.33	14.0	10.6
Hixson- Crowell	KHC	0.95	0.95	0.73	0.82	0.78	0.85	0.96
	R^2	0.74	0.75	0.96	0.97	0.97	0.96	0.96

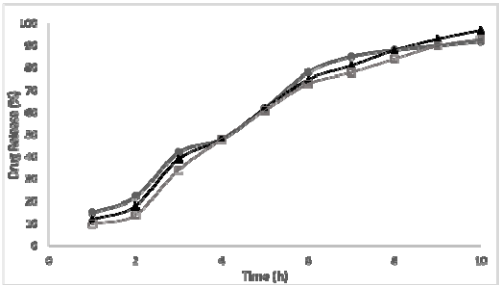

Figure 3: In-vitro drug release profile of nanoparticle formulations F2, SA2, SA5

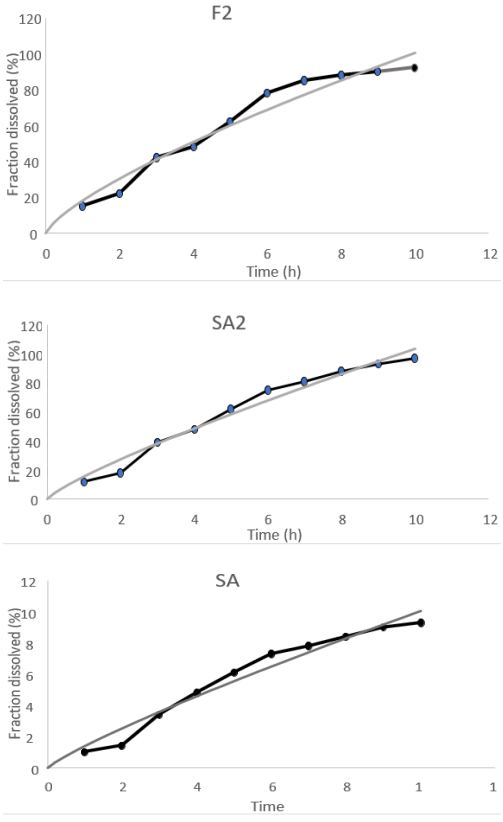

Figure 4: Korsmeyer-Peppas plots for verapamil nanoparticles

DISCUSSION

Chitosan and sodium alginate were used to prepare verapamil hydrochloride nanoparticles via ionic gelation method. The polymeric verapamil nanoparticle formulations had differences in average particle size and polydispersity index. With chitosan and sodium alginate verapamil nanoparticles, the particle size and polydispersity index were 173 and 186 nm, respectively. Polymer concentration and presence of cross linker markedly affected the particle size and polydispersity of nanoparticles. High polymer concentration and low cross linker led to increased particle size.

The entrapment efficiency of verapamil nanoparticles prepared with chitosan was two-fold higher than that of sodium alginate. Drug entrapment efficiency in nanoparticles prepared using ionic gelation method with sodium alginate was directly proportional to polymer concentration. An increase in the concentration of alginate improved the drug entrapment. The low entrapment efficiency could be due to variation in pH or stirring speed during the addition of cross-linking solution to the polymer solution [12,13]. Alginate particles are quite sensitive to pH: they swell in alkaline solutions and shrink in acidic solutions. Alginate is hydrophilic, and it is difficult to arrange alginate particles in an oil-water phase during the preparation process, leading to unstable emulsions [14].

Chitosan has high biodegradability and biocompatibility. The amino groups of chitosan molecules interact with cations to form a cationic polymer, and easily create complexes with nanoparticles in water, which facilitates drug carriage. A porous structure and large specific surface area help chitosan to load drugs with high capacity [14]. The release rate of verapamil depended mainly on the concentration of the polymer. Drug release from the nanoparticles was found to decrease with increase in polymer

concentration. Increased polymer concentration build up cross-linking between the polymer and cross-linking agent, thereby retarding the release of the entrapped drug.

Maximum release of nanoparticles in phosphate buffer pH 7.4 was observed for up to 10 hours. The increased verapamil release occurred because, due to their smaller particle size, the nanoparticles dissolved and diffuse into the buffer solution more easily, leading to enhanced release of verapamil from the nanoparticles. The physical interactions between the polymers and verapamil facilitated the absorption and distribution of the drug, thereby more easily controlling its release. Chitosan has been used as a polymer to produce microsphere of verapamil hydrochloride by spray-drying method, thereby enhancing the bioavailability of the drug [15]. The results of this study indicated that the drug release followed the KorsKorsmeyer-Peppas model (diffusion/ relaxation model) due to higher regression coefficient values in Korsmeyer-Peppas model. The value of n for F2 formulation was 18.36, while for SA2 and SA5, values of n were 15.4 to 14.0, respectively. This result means that the release of verapamil from F2 nanoparticles into pH 7.4 solution resulted from changes in surface area and particle size. Drug release mechanism was a combination of many processes, including swelling and dissolution of polymers, as well as the diffusion and dissolution of the drug. These results are in agreement with verapamil release from the other polymers in other studies [16]. The formulation of polymeric nanoparticles made from alginate and chitosan as drug carriers have been on the increase due to their good adhesion, biodegradability, and biocompatibility. Alginate particles are mixed with chitosan to improve the hydrophilic properties of alginate, but the biological compatibility and pH sensitivity of alginate are still retained. It is well known that combination of the carboxyl groups of alginate and the protonated amino groups of chitosan form a polymer-electrolyte complex (PEC) [14].

CONCLUSION

Nanoparticles of verapamil hydrochloride have been successfully formulated by the ionic gelation technique using two different natural polymers, namely, sodium alginate and chitosan at different ratios with cross linking agent calcium chloride and sodium tripolyphosphate, respectively. These formulations also exhibit extended drug release of entrapped drug from the particles. The drug and polymers are compatible as they not interact.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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