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DEVELOPMENT OF ALGINATE-BASED NANOPARTICULATE DRUG DELIVERY SYSTEM FOR ANTI HIV DRUG RITONAVIR

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ABSTRACT

Ritonavir is an important protease inhibitor for HIV treatment. But this useful drug possesses certain shortcomings i.e. short life as well as side effects. The aim of this study is to improve the therapeutic effect of the drug by altering the delivery system. Slow release ritonavir-loaded nanoparticles in alginate, biodegradable hydropolymer, were prepared by *in-situ* nanoemulsion-polymer crosslinking approach. Different formulations varying in the drug loading solvent phase were prepared. Four different drug-loading solvents were used. However among them dichloromethane provided maximum load of drug in nanoparticles i.e. 15.203%. Ritonavir loading was confirmed by Fourier Transform Infra-red Spectrophotometer (FT-IR) and quantitated by High Performance Liquid Chromatography (HPLC). Prepared nanoparticles appeared slightly elongated with a dense drug core in transmission electron microscopy studies. Hydrodynamic diameter of nanoparticles was 220 ± 1.7 nm. Sustained diffusive drug release was observed *in vitro*, depending on drug polymer ratio; alginate nanoparticles are able to deliver 30 to 80% of the loaded drug by the end of 24 hour. The nanoparticles prepared with 1:6 drug polymer ratio show better release pattern and control the drug release over a period of 24 hour. The release followed Higuchi kinetics rather than first order kinetics, indicating diffusion controlled drug release. The nanoparticulation technique developed can be a good choice for the development of different sustained protease inhibitor drug carriers.

Keywords: *Ritonavir, nanoparticles, alginate, dichloromethane*

INTRODUCTION

Nanoparticles, composed of polymeric amphiphiles, have been considered to provide the site-specific delivery of drugs because polymeric amphiphiles can solubilize various hydrophobic drugs, increased bioavailability, and reside in a stable manner in the blood circulation[1] Ritonavir is a protease inhibitor that provides adequate and durable suppression of viral load and sustained improvement in $CD4^+$ cell counts for HIV-1 infected patients[2,3] . The drug Ritonavir, however, is practically insoluble in water, has a relatively short plasma half life of about 3 hours and the drug is extensively metabolized by the hepatic cytochrome P_{450} system, almost exclusively by the CYP3A isozyme[4] . At steady state, ritonavir is approximately 98% bound to plasma protein[5]. Ritonavir protein binding remains constant over the range of observed concentrations after 600 mg oral dose twice daily, and is similar between healthy volunteers and HIV positive patients. The most common adverse event in adults associated with ritonavir were diarrhoea and pancreatitis[6]. In view of several pharmacokinetic limitations of the protease inhibitor and its increasing multifarious therapeutic requirements, a suitable sustained release formulation of ritonavir loaded nanoparticles (NPs) in biodegradable material was perceived.

EXPERIMENTAL

Materials

Sodium alginate (viscosity 250 cps, 2% m/V in water) was purchased from Sigma Chemicals (USA). Ritonavir was obtained as a gift from Cipla India Limited. All organic solvents and water used were of high performance liquid chromatography (HPLC) grade (Merck/ Spectrochem, India). All other reagents of analytical grade were procured from Merck or Spectrochem and used as received.

Preparation of ritonavir-loaded alginate nanoparticles

Ritonavir was encapsulated in calcium alginate by the *in situ* nanoemulsification-polymer cross-linking method. Thus, 5 mg of the drug, ritonavir, was taken in 10 mL of different drug loading solvents and emulsified under sonication at 20 kHz in 30 mL of 0.1% *m*/*V* aqueous solution of sodium alginate, using polyoxyethylene sorbitan mono-oleate (Tween 80) as emulsifier. Stabilizer, glycerol (10 mL), was then added dropwise to produce the nanoemulsion. Calcium chloride solution (2 mol L^{-1}, 3 mL) was added into the reaction mixture under continuous stirring speed between 1000 rpm to 1500 rpm to effect cross-linking of the NPs produced. The reaction mixture was cured

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for 24 h at room temperature (25 °C). NPs were then separated by ultra centrifugation (Remi K24A) at 20,000 rpm, 0 °C, 60 min. NPs thus obtained were washed with 50 mL of HPLC grade water, recentrifuged and harvested in micro centrifuge tubes. These mass dried over fused phosphorus pentoxide under reduced pressure (0.1 mm Hg) at 70ºC and the dried mass of NPs was preserved in vacuum desiccators at 4 °C for further evaluations. The drug to polymer ratio (D:P) used in all cases was 1:6 (*m/m*) and a series of batches were prepared using four different drug encapsulating solvents, *viz*., dichloromethane, n-hexane, isopropyl alcohol and 1,2-dichloroethane to optimize drug loading.

Characterization of nanoparticles (NP)

Particle size distribution in Photon Correlation Spectroscopy (PCS).

The particle size and size distribution of the prepared NPs were measured using Photon Correlation Spectroscopy, PCS (Zetasizer 1000HS, Malvern Instruments, UK) with a 4 mW He–Ne laser beam at a wavelength of 633 nm at 25 °C and at a scattering angle of 90°. Aliquots from each preparation batch were sampled in PCS cuvettes and NPs were then examined for equivalent diameters and size distribution.

Size distribution in TEM

Transmission electron microscopy, TEM (FEI Technai 12 BioTwin, The Netherlands) with a CCD camera Megaview III soft imaging system was employed to visualize and record the nature and the size distribution of nanoparticles. A generalized protocol was used for TEM studies. A drop of water suspension of the NCs was mounted on a carbon coated copper grid (CCG) and air-dried. NPs were stained with 2% uranyl acetate after washing with buffer solution (pH 4.0, phosphate) and micro graphed at 80–100 kV.

Drug loading

Reverse phase HPLC method was used to determine the total entrapment of ritonavir.

Ritonavir concentrations were determined using ODS column (Zorbax 150mmX4.6 mm) and 10 mmole Acetate buffer of pH-Acetonitrile mobile phase in the ratio 55:45 at a flow rate of 1 ml/minute[8]. Eluents were monitored at a fixed λ_{max} of 239 nm. A 20 µl volume of sample through rheodyne injector port was injected each time and peak area was recorded by computed system. HPLC elution time for ritonavir was 7 minutes. Standard curve equation was Y=5014.1 X - 24.219 (R^2 = 0.9993) Where Y= HPLC peak area; X= Concentration in mg/ml, R= correlation coefficient. Prepared ritonavir loaded nanoparticles were digested in 10 ml of 1% w/v sodium citrate. The mixture was sonicated at 30 KHz for 15 minutes and the solution was centrifuged by cold centrifuge (Remi K24A) at 0°C, 15,000 rpm. 20µl of filtrate was injected into HPLC system. 6 samples from each batch were analyzed in

FT-IR Analysis

Infrared spectra of alginate nanoparticles loaded with ritonavir were recorded in Nicolet Impact 410 using potassium bromide pellets. Ritonavir powder was mixed with dried KBr (FT-IR grade) palletized at 15 psi and spectra was recorded between 4000-650 cm^{-1} using high-energy ceramic source and DLATGS detector. Similarly the nanoparticles prepared and the sodium alginate powder was analyzed in FT-IR for spectral analysis comparison.

In-vitro *drug release*

The release of the drugs from the nanoparticles core into the surrounding sink solution was carried out at 7.4 pH values by USP30 dissolution rate testing apparatus (type I) (Model V-Scientific, DA-6D, India), using 900ml of bath fluid (100mM Phosphate Buffer, pH 7.4), prewarmed and maintained at 37°C±1°C. A stainless steel wire basket at 50 rpm rotation speed basket system was used and drug release is measured under sink condition. 0.1ml aliquots are sampled out at regular time intervals, filtered in centricon tubes and 20µl is injected into the HPLC system. The sample withdrawn was replaced each time with 1ml of fresh buffer mixture. HPLC conditions are as described earlier and the standard curve similarly prepared is used for data analysis with necessary correction for the dilution factors.

RESULTS AND DISCUSSION

Ritonavir loaded alginate Nanoparticles were prepared following oil in water nanoemulsion and in-situ polymer crosslinking method. Four different formulations of different drug encapsulating solvents e.g 1,2 dichloromethane (d.c – 9.08), n hexane (d.c – 1.89), 1,2- dichloroethane (d.c – 10.36), Isopropyl alcohol (d.c –1.6) were used. The different drug encapsulating solvents gave different drug payload.

Ritonavir nanoparticles were analyzed in HPLC and expressed as w/w percentage drug loading (Table1). Formulation R_1 provided the best loading for ritonavir at 15.2034%, where Formulation R_2, Formulation R_3, Formulation R_4 however carry drug payload of 4.687%, 8.534%, 7.541% respectively. Therefore encapsulated nanoparticles of Formulation R_1 were used throughout the other experimental conditions.

Table 1. Formulation Design and Drug payload for Ritonavir loaded Calcium alginate Nanoparticles

Sample	Solvent(Oil phase (10ml each))	Polymer solution (0.1%) ml	Surfactant (mg)	Cross linker (1M) ml	Drug loading ± SE (%)
Formulation R_1	Dichloromethane	30	0.7	3.0	15.2034±2.66
Formulation R_2	n hexane	30	0.7	3.0	4.687±0.1979
Formulation R_3	Isopropyl Alcohol	30	0.7	3.0	8.534±0.2997
Formulation R_4	1,2 dichloroethane	30	0.7	3.0	7.541±0.2710

*Mean ±SE, n = 6

Drug –Polymer interaction by Fourier Transform Infra - red Spectrophotometer (FT-IR)

FT-IR studies at 280 scans were recorded for the pure powdered ritonavir (Figure 1), formulation R_1 NPs (Figure 2). In FT-IR studies it has been observed that the major peak of C=O stretching at1526.12 cm^{-1} of

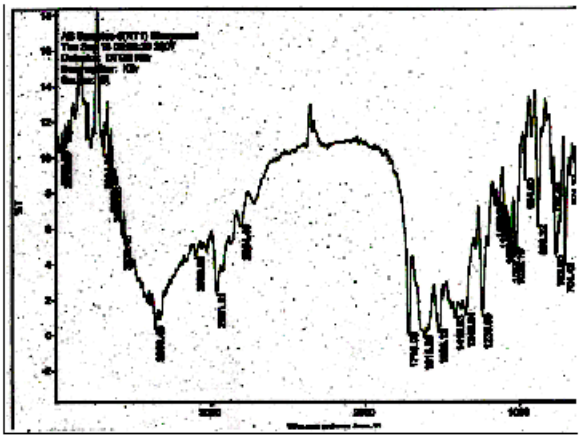
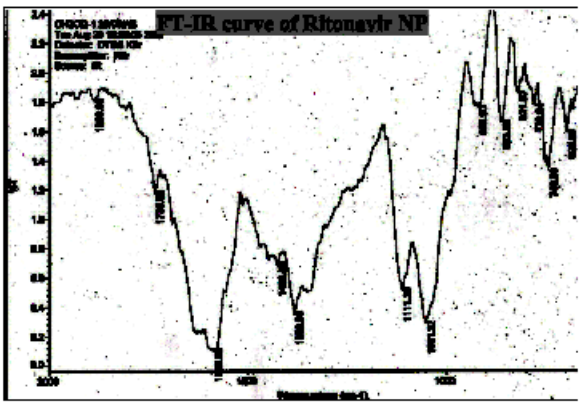


Fig. 1: FT-IR spectra for pure ritonavir powder (C=O stretching at1526.12 cm^{-1})



lation R_1 (C=O stretching at1535.90 cm^{-1})

pure ritonavir complies with the peaks of ritonavir NP at C=O stretching at 1535.90 cm^{-1}.

The observation revealed that there were no drug-polymer chemical interactions. Nanoparticles prepared in Formulation R_1 can therefore be generally conceived as a central amorphous drug core that is encoated with calcium alginate polymer layer.

Particle Size distribution

Both the size and size distribution can strongly influence nanocapsular drug delivery[9]. The small size (100-300nm in diameter) of the colloids is expected to promote passive targeting through permeable vasculature in tumors (EPR effect), while the hydrophobic pockets allowed drug-loading[10].

Size distributions for R_1 formulation were studied in PCS and the mean PCS diameters were directly recorded as intensity-weighted. PCS size distributions of formulation R_1 provided a Gaussian size distribution with an average nanoparticle diameter of 225 nm (Figure 3). Size distribution in formulation R_2 was partially skewed, average diameter was 434.8 nm.

Formulation R_3 and formulation R_4 produced larger particles of average PCS diameters of 585.2 nm and 819.8 nm, respectively (not shown in figure).

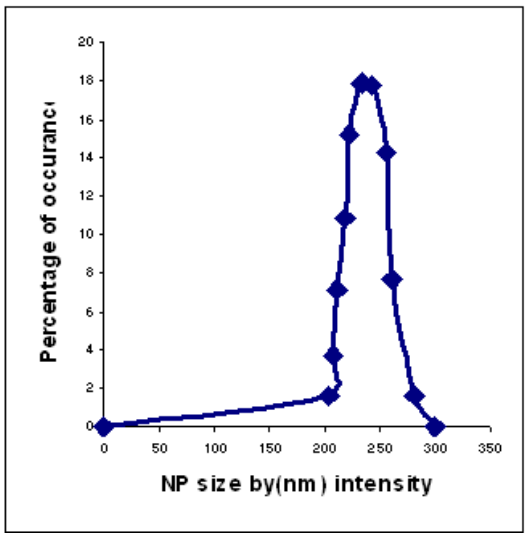


Fig. 3: *PCS particle size distribution of ritonavir nanoparticles formulations R_1 using (dichloromethane).*

TEM is a 2D image of a 3 dimensional nanoparticles while PCS provides NPs hydrodyanamic diameter in terms of equivalent sphere. Transmission electron microscopy of uranyl acetate stained formulation R_1 NPs were slightly elongated with a dense central core for encapsulated ritonavir (Figure 4). Average TEM diameter for formulation R_1 was observed to be 220 nm from 200 counts in four observation plates. TEM diameters though appeared relatively larger they were within the higher ranges of PCS observations. Both PCS and TEM are independent techniques of observation, but were sufficiently informative and complementary to each other[11].

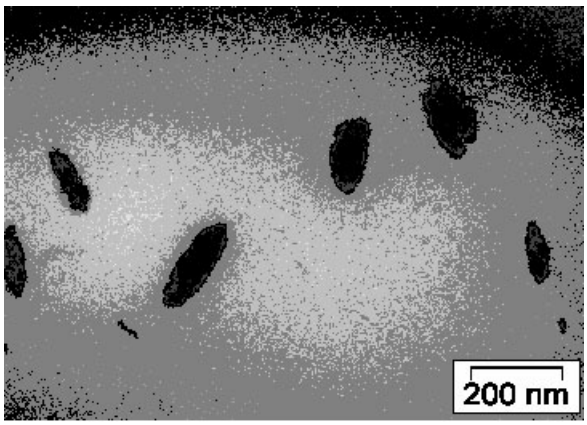


Fig. 4:*TEM micrograph of formulation R_1 (using dichloromethane) nanoparticles.*

In-vitro release

The in vitro release data were applied to various kinetic models to predict the drug release mechanisms. The best fit with highest correlation coefficients (r) was shown (Table 2) by the Higuchi rather than first order and zero order models. The plot of cumulative percentage release versus square root of time

Table 2: *Coefficient and release rate of ritonavir from ritonavir nanoparticles*

Batch No	Drug/ polymer ratio	Time for 25% release(h)	Time for 50% release(h)	First order model log(Q_0- Q) vs t		Higuchi model		Logc/logt
				r^2	release rate (h^{-1})	r^2	release rate (%-$h^{-1/2}$)	r^2
1	1:6	0.5	5	0.996	0.138	0.999	4.42	0.972
2	1:3	1.5	12	0.974	0.427	0.994	11.97	0.972
3	1:2	12	-	0.962	0.720	0.982	12.88	1.000

produces high linearity. This clearly indicates that the release followed Higuchi model. Furthermore, a plot of log percentage release against log time (Figure 5) revealed a high level linearity, which confirms ritonavir release from alginate nanoparticles, is diffusion controlled.

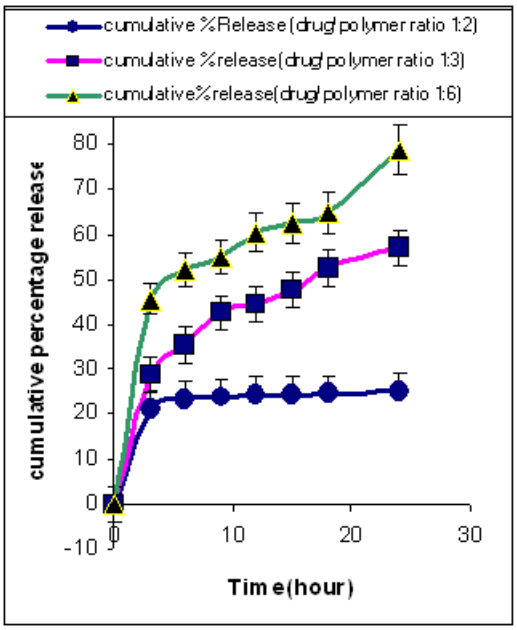


Fig. 5: *In vitro release of ritonavir from ritonavir nanoparticles (encapsulating solvent dichloromethane)*

Conclusion

Our study shows that an efficient nanoparticle formulation can be developed for ritonavir to ensure better encapsulation and release of the drug. A significant loading of nanoparticles was achieved using the dichloromethane as a drug encapsulating solvent. The *in-vitro* release of ritonavir from alginate nanoparticles followed a diffusion control mechanism. This strategy can potentially serve as a useful targeted drug delivery system for eradicating the viral sanctuaries in patients infected with HIV-1/AIDS.

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REFERENCES

1. Kataoka K, *et al.,* J Control Release. 2000; 64:143.
2. Turner BG, Summers MF. J Mol Biol. 1999; 285, 1.
3. Fauci AS, *et al*. Science. 1988; 239: 617.
4. Ho DD, *et al*. Engl J Med. 1987; 317: 278.
5. Chu CM, *et al*. Thorax. 2004; 59: 252.
6. Cvetkovic RS, Goa KL. Drugs .2003; 63: 769.
7. Rajanorivony M, *et al*. J Pharm Sci. 1993; 82(9): 912.
8. U.S. Pharmacopoeia 24, NF 19. United States Pharmacopoeial Convention. Rockville. 2001, p 1610.
9. Vinogradov SV, *et al*. Adv Drug Deliv Rev. 2002; 54: 135.
10. Schiffelers RM, *et al*. Biochem Biophys Acta. 1999; 1421: 329.
11. Bootz A, *et al*. Eur J Pharm Biopharm. 2004; 57: 369.