**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
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 70°C 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 150nmX4.6 mm) and 10 mmole Acetate buffer of pH-Acetonitrile mobile phase in the ratio 55:45 at a flow rate of 1 ml/minute4. Eluents were monitored at a fixed λmax 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. Ritonavir concentrations were determined using ODS column (Zorbax 150 mmX4.6 mm) 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.

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:1:1 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 centric tubes and 20:1 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).

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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, NPs (Figure 2). In FT-IR studies it has been observed that the major peak of C=O stretching at 1526.12 cm⁻¹ of
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 R1 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\(^6\). 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).

**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

<table>
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<tr>
<th>Drug</th>
<th>polymer ratio</th>
<th>Time (hr)</th>
<th>% Release</th>
<th>Time (hr)</th>
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</table>

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.