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Formulation and Physico-chemical Evaluation of Polystyrene Nanoparticles Containing Cefotaxime Sodium

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Polystyrene nanoparticles containing cefotaxime were prepared by emulsion polymerization in continuous aqueous phase. Scanning electron microphotograph showed that the morphological structures of the nanoparticles are discrete, spherical and uniform in size. The drug content in the polystyrene nanoparticles were considerably increased proportionately with increasing polymer concentration. The infrared spectroscopic analysis revealed that there was no significant chemical interaction between the polymer and the drug. The *in vitro* release studies carried out across the artificial membrane indicated that the release of the drug from the nanoparticles followed zero order kinetics.

Nanoparticles are novel drug delivery systems that can successfully deliver a drug at optimum dose at the required site of action. This polymeric colloidal solid particles posses a size range of 1-1000 nm and have been extensively studied[1] both as targeted and as sustained drug delivery system. Nanoparticles are classified into two main types namely nanospheres and nanocapsules. Nanospheres are polymeric matrices in which the drug is dissolved or dispersed while the nanocapsules are of polymer wall entrapping an oily core in which the drug is dissolved[2]. It is a potential drug delivery system for various classes of drugs that include anticancer, antimalarial, antiinflammatory[3], antiviral, hormones and antibiotics[4,5] and many were reported to have an increased therapeutic efficacy.

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Cefotaxime sodium is one of the third generation antibiotics. Development of resistance to certain antibiotics is a common difficulty faced during antibacterial chemotherapy. The main reason for the development of such antibiotic resistance is due to the low uptake of the drug by the microorganisms. Therapeutic activity of ampicillin was shown to be dramatically enhanced after its binding to nanoparticles in an experiment conducted in acute salmonellosis[6] in vivo. Hence a pilot experiment was conducted to prepare polystyrene nanoparticles containing cefotaxime sodium and the physico-chemical properties were evaluated.

Cefotaxime sodium (CFS) and polystyrene (PST) used for this work were the gift samples provided by Evergreen Vet Care products, Tamilnadu. Dichloromethane (DCM, analytical grade) was procured from E. Merck Ltd., Mumbai and acetonitrile analytical grade, from Ranbaxy, SAS Nagar. All other solvents used were of HPLC grade. Scanning electron microscope (Hitachi 5450, Japan), FTIR (Perkin Elmer Paragon 2000 series), UV/Vis spectrophotometer (Elico SL 159), sonicator (Vibracell) were the equipments used at the various stages of this study.

Cefotaxime-loaded polystyrene nanoparticles (CEFNP) prepared by emulsion polymerization[7] in continous aqueous phase. CFS was dissolved in 1% solution of Tween 80; whereas the polymer was dissolved in DCM. The drug solution and polymeric solution were emulsified at 15° using a magnetic stirrer for 10 min. This solution was sonicated for 20 min at 15°. The nanoparticles of cefotaxime sodium were separated by fractional centrifugation[8] using a cooling centrifuge and redispersed in phosphate buffer saline pH 7.4. Three batches of nanoparticles were prepared with varied drug: polymer ratios of 1:1, 1:2, and 1:3 coded as CEFNP-1, CEFNP-2 and CEFNP-3, respectively.

Particle size analysis[9] was carried out using scanning electron microscopy[10] (Hitatchi S-450 operated at 20KV) for five batches and hundred particles were counted at random. Finally an average was calculated from the observed value of five batches. Drug content was determined by taking 1 ml of the redispersed CEFNP suspension. To this suspension, 1 ml of aqueous potassium dihydrogen phosphate solution (30 mM) was added and the mixture was centrifuged at 33,000 g (Remi, C-24 cooling centrifuge) at 15°. The clear supernatant was removed and analysed spectrophotometrically at 254 nm and the drug content and drug recovery[11] were calculated. Infrared spectroscopic analysis[12] was carried out to study the mechanism of attachment of the drug to the polymer; and also the intactness of the attachment. The IR spectrum of pure CFS, pure PST and CEFNP were taken, interpreted and compared with each other.

The prepared CEFNP were transferred to dialysis tubes with an artificial membrane. In this 10 ml of phosphate buffer pH 7.4 was added and subjected to dialysis by immersing the dialysis tube to a receptor compartment containing 250 ml of phosphate buffer pH 7.4. The contents of this receptor compartment were agitated constantly using a magnetic stirrer and temperature was maintained at 37°. For 24 h, at different intervals, 5 ml samples were withdrawn from the receptor compartment and the amount of drug released was determined spectrophotometrically at 254 nm. After each withdrawal, equal volume of fresh buffer pH 7.4 was added to the receptor compartment. The studies were also carried out for free drug for 6 h.

CEFNP with varying proportions of CFS:PST were prepared. The particles were morphologically spherical in shape and discrete. The scanning electron microphotograph of CEFNP is shown in fig. 1. The particle size gradually increased with increase in the proportion of the PST. The increase in monomer concentration in this emulsification medium facilitates the diffusion of more monomers into the micelles which might have caused a considerable increase in particle size.

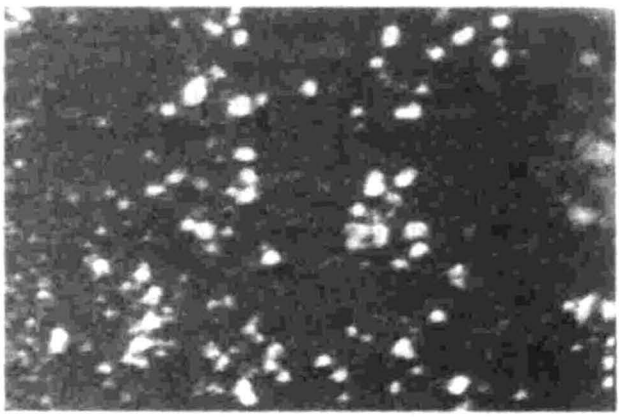

Fig 1 : Scanning electron microphotograph of CEFNP Magnification of 5000x

The drug content was determined by centrifugation method and it was maximum in CEFNP -3 as 42.02%, decreased to 35.7% in CEFNP-2 and 33.7% in CEFNP-1 (Table

TABLE 1: CHARACTERIZATION OF CEFNP

Batch Code	CFS : PST ratio	Particle Size nm (±sd)	Drug Content (%)	Drug Recovery (%)
CEFNP-1	1:1	300 (±40)	33.7	58.0
CEFNP-2	1:2	500 (±20)	35.7	68.1
CEFNP-3	1:3	800 (±80)	42.0	83.2

CEFNP- Cefotaxime loaded polystyrene nanoparticles, CFS-Cefotaxime, PST-Polystyrene, Particle size in nanometers(± standard deviation)

1). The concentration of PST was not increased beyond 1:3, because the particle size increased proportionately with increase in polymer ratio and the formulation can no more considered as nanoparticles.

The IR spectroscopic studies indicated that the functional group responsible for the antibacterial action of the CFS remained unaltered. Hence the drug was expected to get dispersed on the polymeric matrix of the nanoparticles. Increase in drug content observed with increase in the polymer proportion also suggest physical adsorption of this drug on to the polymer.

The *in vitro* release profile of CEFNP across an artificial dialysis membrane is shown in fig. 2. The release of the drug from the CEFNP was slow and constant. CEFNP-1 released about 50.6% of the drug at the end of 10 h, whereas CEFNP-2 and CEFNP-3 released 42.3% and 38.3%, respectively. At the end of 20 h the CEFNP-1, 2 and 3 released about 98.1%, 97.5% and 97.2% of the drug, respectively. The increase in polymer content although caused considerable variation in the drug content of the nanoparticles, but it caused a little variation in the total release of the drug. However the varied polymer content exhibited alterations in the release pattern. The constant and slow release of antibiotics will be highly useful in maintaining a constant drug plasma concentration thereby improving the therapeutic efficacy and reducing development of resistance. Based on the above studies it is possible to conclude that nanoparticles can serve as a successful drug delivery system for cefotaxime sodium and most probably for other cephalosporins as well.

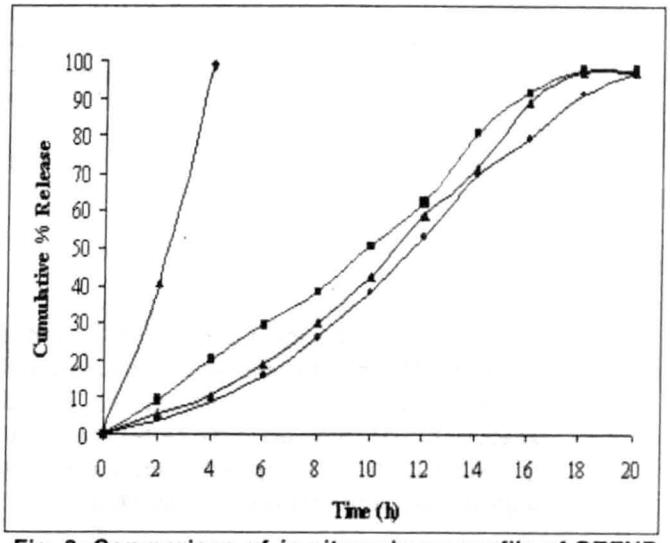

Fig. 2: Comparison of *in vitro* release profile of CEFNP with free drug.

Free drug (- ◆ -), CEFNP-1 (- ■ -), CEFNP-2 (- ▲ -), CEFNP-3 (-●-).

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