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Effect of Chitosan on Ethyl Cellulose and Eudragit RS 100 Microspheres

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The aim and objective of the present investigation is to study the effect of chitosan on the various characteristics of Eudragit RS 100 and ethyl cellulose microspheres which were formulated by o/o emulsion, solvent evaporation method using methanol, acetone, light liquid paraffin system. Effect of chitosan was evaluated on several aspects such as particle size, drug content and entrapment, morphology, drug-polymer interaction and *in vitro* release study. It was found that chitosan had great influence on the various properties of ethyl cellulose and Eudragit RS 100 microspheres containing lamivudine sulphate as drug candidate.

Key Words: Chitosan, Eudragit RS 100, Ethyl cellulose, microspheres, Lamivudine sulphate.

INTRODUCTION

Recent years have seen an ever increasing interest in the application of novel materials in the medical and pharmaceutical fields, whether as prostheses or in medical devices designed for contact with the biological environment of the living body. Eudragit RS 100 is a copolymer of acrylic and methacylic acid esters with a low content of quaternary ammonium groups, present as salts to increase the permeability of lacquer films[1]. It offers water insoluble but permeable film coatings, independent of pH values. Ethyl cellulose is basically a hydrophobic coating agent for tablets and granules to modify the release of drug, to mask an unpleasant taste or to improve the stability of a formulation. It is also used as binder and filler in tablets. Chitosan is form of fiber, chemically processed from crustacean shells which are not well digested in the human body and as it passes through the digestive tract, out in the stool[2]. In the present study, the effect of chitosan on various characteristics of microspheres, when blended with ethyl cellulose/Eudragit RS 100 to get microspheres is studied. Lamivudine sulphate (candidate drug) is an antiretroviral drug [nucleoside reverse transcriptase inhibitors (NRTIs)] approved by FDA on November 17, 1995 for the use with zidovudine and again in 2002 as a once in a day dosed medication. Lamivudine in combination with other drugs is used to treat HIV in parents with AIDS and hepatitis B[3].

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EXPERIMENTAL

Lamivudine sulphate is a gift sample from Alkem, Mumbai, India. Eudragit RS 100 and chitosan are from Rohm Pharma, GmbH, Darmstadt, Germany and Ethyl cellulose is from Dr. Reddy's Lab., Hyderabad, India. All other materials used were of pharmaceutical grade.

Formulation of lamivudine microspheres only with Eudragit RS 100/ethyl cellulose by solvent evaporation method[4]: 1 g of Eudragit RS100/ethyl cellulose was dissolved in 14 mL acetone using a magnetic stirrer (Remi Equipments, model 2MIH). Pure lamivudine (1 g dissolved in 4 mL methanol) and dispersing agent, magnesium stearate or aluminum stearate (150 mg) were dispersed in the polymer solution (aluminum/magnesium stearate were not used in case of ethyl cellulose microspheres instead, 5 mL of Span 80 was added in the continuous phase). The resulting dispersion was then poured into 500 mL beaker, containing the mixture of 260 mL liquid paraffin light and 30 mL *n*-hexane, while stirring in a mechanical stirrer (Remi Motors, Model No. RO-123R, Mumbai) continuously for 3 h at 600 rpm, until acetone evaporated completely, after which, the formed microsphere were filtered, washed with 4-5 times in 50 mL *n*-hexane and dried at room temperature for 24 h in a desiccator. Spherical microspheres with polymer:drug ratio, 1:1 were obtained.

Formulation of lamivudine microspheres with Eudragit RS 100 and Chitosan by o/o emulsion and solvent evaporation technique[4]: Eudragit RS100 (750 mg) was dissolved in organic solvents containing 4 mL acetone, 1.5 mL methanol. Chitosan (250 mg) was dispersed in Eudragit RS100 solution and stirred by magnetic stirrer for 15 min. Drug (250 mg) was added and stirred for 10 min after that magnesium stearate (75 mg) was added and stirring was continued for further 15 min (aluminum stearate/magnesium stearate are not added in case of ethyl cellulose microspheres instead, 5 mL of Span 80 is added in the continuous phase). Then the above dispersion was added in the form of thin strip into the continuous phase containing light liquid paraffin 100 mL while stirring at 750 rpm for 4 h, after which, the resulting microspheres were filtered and washed with *n*-hexane 3 times (30 mL) and then air dried at room temperature.

Drug content and entrapment efficiency analysis of prepared microspheres: 50 mg of crushed and powdered microsphere was taken in 50 mL distilled water in a volumetric flask. The flask was stopped tightly and kept on mechanical shaker for 24 h. Then solution was filtered and 1 mL of filtrate was diluted to 10 mL with 7.4 pH buffer solution and was assayed in UV-vis spectrophotometer (Elico SL-159, Ahmedabad) at 269.6 nm to find out the lamivudine content and lamivudine entrapment of microspheres.

Scanning electron microscopy (SEM) analysis: The samples for the SEM analysis were prepared by sprinkling the microspheres on the one side of double adhesive stub. The stub was then coated with fine gold dust. The coated samples were then exposed under Jeol superprobe (JxA-8100) to get magnified high resolution view of the grains and their microstructural peculiarities.

Fourier transform infrared spectroscopy: Drug-polymer inter-actions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and microspheres with and without Chitosan using FTIR Shimadzu (Model No. 8400S). Samples were prepared in KBr discs (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 2 cm^{-1}.

***In vitro* release studies[5]:** The *in vitro* release studies of the drug incorporated microspheres were carried out at 37 ± 0.5 ºC, 100 rpm for 6 h in the USR-XX 8 basket dissolution apparatus with phosphate buffer saline (pH 7.4) as dissolution medium. 5 mL of aliquots were withdrawn at specified time intervals and replaced by equal volume of fresh dissolution medium. The aliquots were analyzed spectrophometrically at 269.6 nm to obtain the amount released with respect to time.

RESULTS AND DISCUSSION

Lamivudine microspheres were formulated by o/o emulsion, solvent evaporation method using acetone, methanol and light liquid paraffin system with and without Chitosan.

The yield of various formulations was in the range of 50-86 %. Microspheres with chitosan had bigger size than microsphere without chitosan (Table-1). Chitosan which is dispersed in acetone for particles measures the total amount of solid substances dispersed in the same volume of the linear phase. Therefore, chitosan microsphere which are formed from droplets of the same size in acetone/light liquid paraffin emulsion have great average diameter than microsphere without chitosan.

TABLE-1
PARTICLE SIZE DRUG CONTENT AND ENTRAPMENT EFFICIENCY OF VARIOUS MICROSPHERES

Microspheres	Particle size (μm)	Drug content (%)	Entrapment efficiency (%)
Eudragit RS 100	135.3	11.5530	47.76515
Eudragit RS 100 and Chitosan	196.2	13.0883	52.35353
Ethyl cellulose	110.7	7.4646	29.85750
Ethyl cellulose and Chitosan	165.9	8.6388	43.19440

The drug content and drug entrapment efficiency increases when chitosan is used with ethyl cellulose or eudragit (Table-1). This may be due to the fact that with chitosan there occurs in increase in the size of microsphere which results in decrease in total surface for drug to escape from the resulting microspheres to the external medium.

From the SEM study it may conclude that the resulting microspheres are white, spherical with rough and irregular surface (Fig. 1).

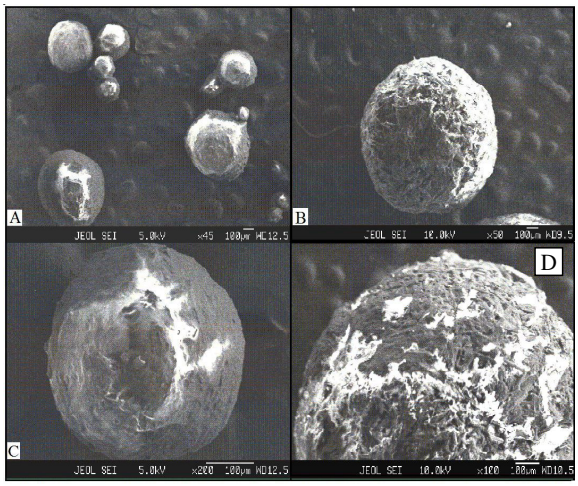
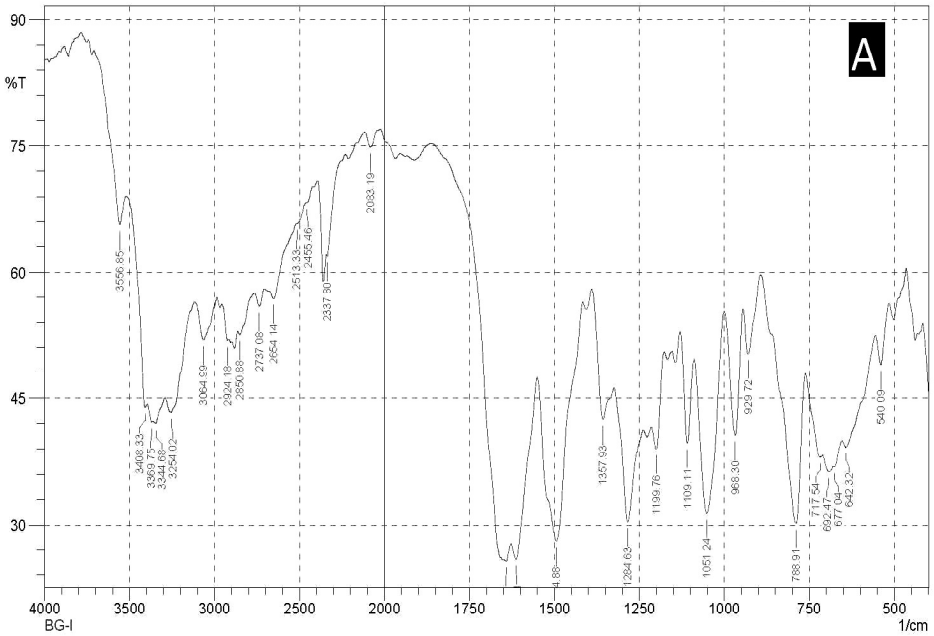
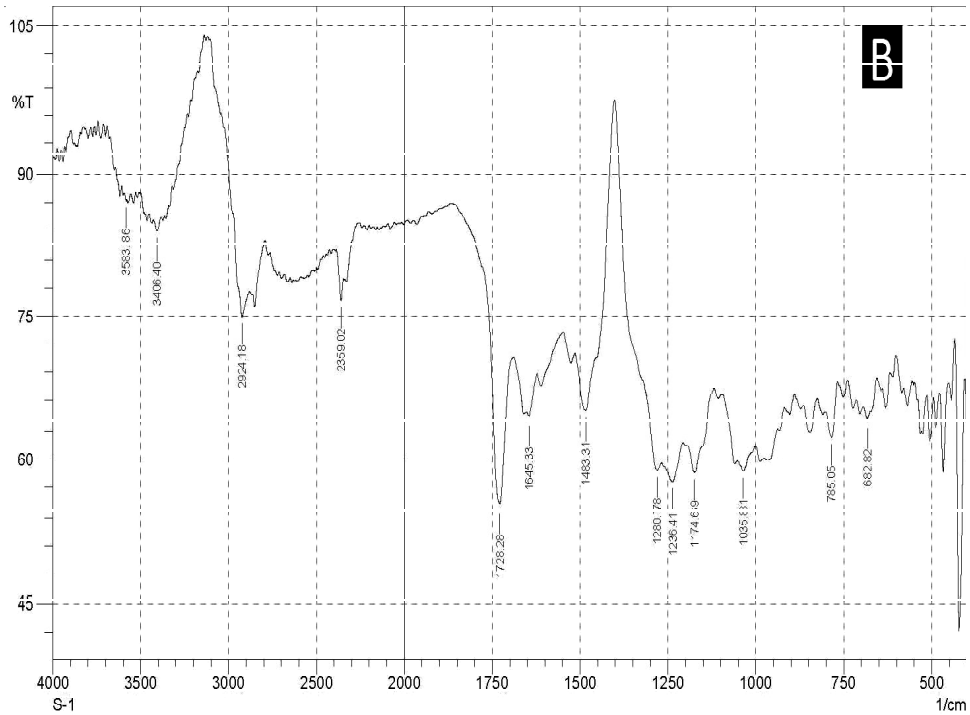


Fig. 1. SEM Photographs (A) Eudragit RS 100 microsphere (B) Edudragit RS 100 with Chitosan microsphere (C) ethyl cellulose microsphere (D) ethyl cellulose with Chitosan microsphere

The FTIR study shows prominent peaks at 3369 ν(NH_2) symmetrical stretching, 3344.68 (NH_2) asymmetrical stretching, 3254 ν(OH) stretching, 1641 ν(C=O) stretching, 1199 ν(C-O-C) stretching, 1284 ν(C-N) stretching which was also shows at the same region confirming no interaction of drug in the formulation (Fig. 2).

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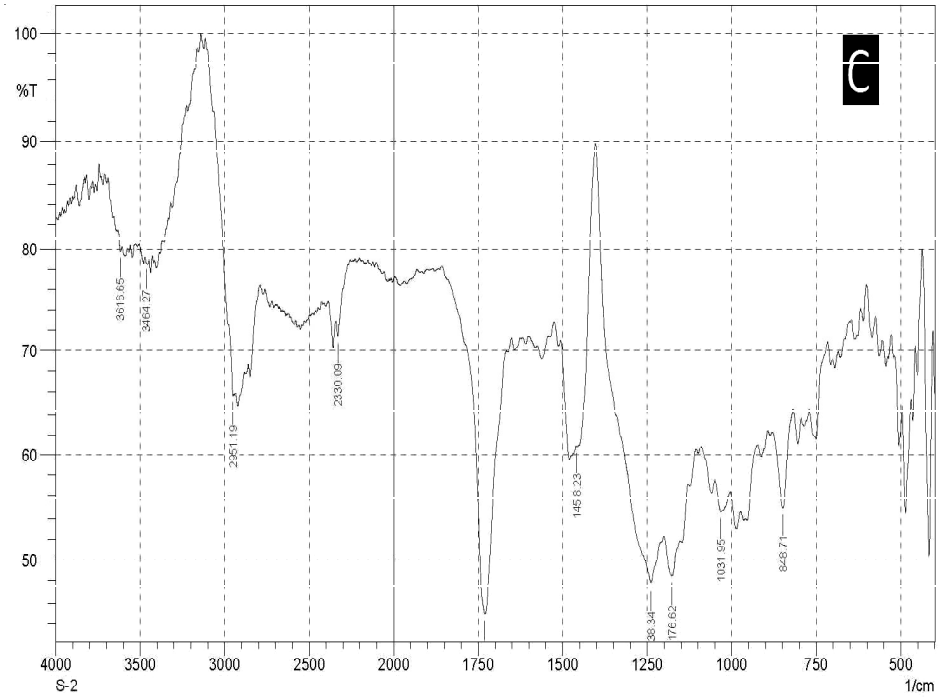
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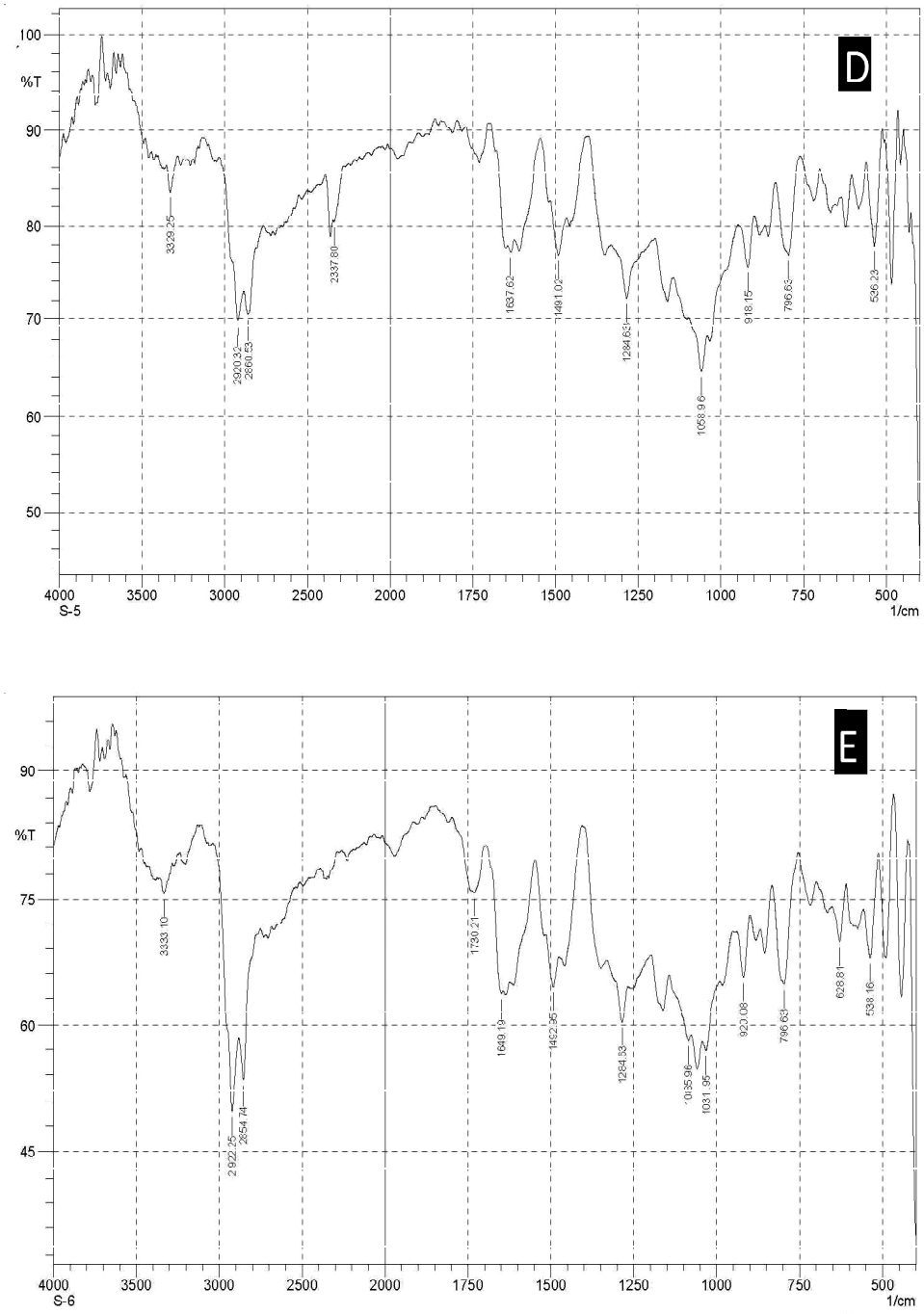

Fig. 2. FTIR Spectrum (A) lamivudine (B) Eudragit RS 100 microsphere-without chitosan (C) Eudragit RS 100 microsphere-with chitosan (D) ethyl cellulose microsphere-without chitosan (E) ethyl cellulose microspheres with chitosan

The *in vitro* release study revels that chitosan had prominent effect on the release of the microspheres. For ethyl cellulose microsphere without chitosan, the amount of lamivudine release in 6 h was found to be 74 % without an initial burst effect. For Eudragit RS 100 microspheres, the amount of release of lamivudine in 8 h was found to be 84 % without an initial bursting effect. When microspheres alongwith chitosan were used the dissolution rate became more faster (Fig. 3). Both Eudragit and ethyl cellulose are insoluble and non-swellable in aqueous environment but chitosan gets dissolved in the aqueous environment of phosphate buffer saline. The aqueous solubility of chitosan creats pore in the microspheres, thus, releasing the drug at a faster rate.

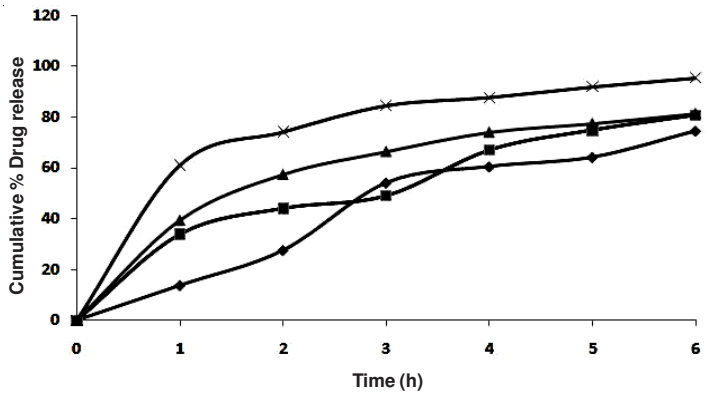

Fig. 3. Comparative dissolution profile of microspheres containing ◆ Eudragit RS 100, ■ Eudragit RS 100 and chitosan, ▲ ethyl cellulose and ✕ ethyl cellulose and chitosan

Conclusion

It was found that chitosan had great influence on the various properties of prepared ethyl cellulose and Eudragit RS 100 microspheres containing lamivudine sulphate as drug candidate.

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