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CONTROLLED RELEASE MICROSPHERES OF AMBROXOL HYDROCHLORIDE: EFFECTS OF FORMULATION PARAMETERS

Nayana S. Baste*, Dilip V. Derle[1], Charulata T. Nemade, Vandana R. Daga

Department of Pharmaceutical Sciences, S.S.D.J.College of Pharmacy, Neminagar, Chandwad, Nasik, 423 101, Maharashtra, India.

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***Correspondence for Author**
Nayana S. Baste
Department of Pharmaceutical Sciences, S.S.D.J.College of Pharmacy, Neminagar, Chandwad, Nasik, 423 101, Maharashtra, India.

ABSTRACT

The present work was undertaken to examine the potential for the controlled release of ambroxol hydrochloride by forming microspheres. The effect of formulation variables such as polymer concentration and variation of dispersing agent on microsphere properties such as average particle size, encapsulation efficiency and drug release from the microspheres were studied. Microspheres were prepared by solvent evaporation technique using ethyl cellulose as a matrix-forming agent. Morphological and physicochemical properties of microspheres were investigated by scanning electron microscopy, X-ray diffractometry, differential scanning calorimetry, and Fourier transform infrared spectroscopy. SEM revealed that microspheres were spherical, discrete having smooth and non-porous structure. Encapsulation efficiency of microspheres was found to be 81-94% and drug release was extended up to 12 h. The infrared spectra and differential scanning calorimetry thermographs showed stable character of ambroxol hydrochloride in the drug-loaded microspheres and revealed the absence of any drug-polymer interactions. X-ray diffraction patterns showed that there was decrease in crystallinity of the drug. The best-fit release kinetics was achieved with zero order kinetics and drug release was found to be diffusion controlled. Encapsulation efficiency and drug release was affected by both, concentration of polymer and presence of aluminium tristearate. Particle size was affected by polymer concentration not aluminium tristearate.

Keywords: Ambroxol hydrochloride, Ethyl cellulose, Solvent evaporation method.

INTRODUCTION

O/W emulsion solvent evaporation process mainly used for microencapsulation of water-insoluble and water-soluble drugs. The major drawback of this method is the low encapsulation efficiency of water-soluble drug because the drug can diffuse from the organic dispersed phase into the aqueous continuous phase, which results in poor entrapping.[1] To solve this problem mainly W/O emulsion solvent evaporation method is used which having hydrophobic processing medium.

In the present investigation, ambroxol hydrochloride and ethyl cellulose were used as model water-soluble drug and water-insoluble polymer, respectively, for the preparation of microspheres. Ambroxol is an active N-desmethyl metabolite of the mucolytic bromohexine. It is indicated for acute and chronic disorders of respiratory tract, where there is copious thick secretion or mucus production. It has biological half-life of 3-4 h. It is absorbed in throughout GIT. Its bioavailability is 70-72%. Usual initial dose of ambroxol hydrochloride is 30 mg three times a day. Therefore to reduce frequency of dosing as well as to increase bioavailability and enable better compliance, formulating sustained release dosage form is necessary.[2-4] In literature several sustained release formulations of ambroxol hydrochloride have been reported that are based on tablet, capsule or sol dosage forms allowing once daily administration.[5-7] In the present study, we examine the potential for the sustained delivery of ambroxol hydrochloride by forming microspheres. This multiparticulate reservoir system is more reliable in their biopharmaceutical behavior such as distribute throughout the digestive tract and give uniform drug absorption.[8]

Ethyl cellulose, a non-biodegradable and biocompatible polymer, is an extensively studied encapsulating material for the controlled release of microspheres.[9] It is widely used in oral formulations as a hydrophobic coating agent for tablet and granules. Ethylcellulose coatings are used to modify the release of a drug, to mask the unpleasant taste, to improve the stability of a formulation.[10]

The main objective of this study was to investigate the effect of formulation parameters such as polymer concentration and variation of dispersing agent on microsphere properties such as average particle size, encapsulation efficiency and drug release from the microspheres.

MATERIALS AND METHODS

Materials

Ambroxol hydrochloride and ethyl cellulose were obtained from Glenmark Pharmaceutical Ltd. (Nasik, India) as a gift samples. Aluminium tristearate obtained as gift samples from Ipca Laboratories, Mumbai. Acetone and methanol were purchased from S.D. Fine Chemicals (Mumbai, India). All other chemicals and solvents were of analytical grade.

Methods

Microspheres were prepared by solvent evaporation method.[11] Ambroxol hydrochloride and ethyl cellulose were dissolved in an acetone-methanol mixture. The dispersing agent was added, and the mixture was stirred at 500 rpm on a magnetic stirrer by maintaining temperature at 10 °C. The mixture was then poured rapidly into liquid paraffin, previously cooled to 10 °C while being stirred at a speed of 400 rpm on remi three-blade propeller stirrer. The resulting emulsion was stirred at 400 rpm by maintaining temperature 35 °C for 4 h. The organic solvent, acetone-methanol, was completely removed by evaporation. The solidified microspheres were filtered, washed 6 times with an aliquot of 50 ml n-hexane, then washed with water and dried under vacuum at room temperature overnight, and stored in a desiccator. Formulations of microspheres are as follows.

Table 1. Formulations of ambroxol hydrochloride ethyl cellulose microspheres*

Formulation code**	Variable level	
	Polymer concentration (%) #	Dispersing agent concentration (%) #
EC1	10	3
EC2	15	3
EC3	20	3
EC4	10	2
EC5	15	2
EC6	20	2
EC7	10	1
EC8	15	1
EC9	20	1

* Each formulation contained 1 gm of ambroxol hydrochloride

** EC: Ethyl cellulose

The concentrations of dispersing agents and polymer were calculated from dispersed inner phase volume (wt/vol %).

Percentage Yield Value of Microspheres

The percentage yield value of microspheres was determined from the ratio of amounts of solidified total microspheres (practical yield value) to total solid material used in the inner phase (Theoretical yield value) multiplied by 100

Yield Value = Practical yield value/Theoretical yield value X 100

Drug Entrapment Efficiency

Weighed quantity of microspheres were crushed and suspended in distilled water to extract the drug from microspheres. After 24 h, the filtrate was assayed spectrophotometrically at 244.4 nm for drug content. The encapsulation efficiencies were calculated by using the following relationship:

Encapsulation efficiency = (Drug entrapped /Theoretical drug content) × 100.

Particle Size Analysis of Microspheres

Average particle diameter and size distribution of microspheres were determined by laser diffractometry using a Mastersizer Micro V 2.19 (Malvern Instruments, Malvern, UK). Approximately 10 mg of microspheres were dispersed in 10 ml distilled water containing 0.1% tween 80 for 2min. using an ultrasonic bath. Then aliquot of the microspheres suspension was added into recirculation unit, which was subsequently circulated 3500 times per min. Particle size was expressed as equivalent volume diameter. The analysis was carried out in triplicate. The particle size distribution was also expressed in terms of SPAN factor determined as:

$$\text{SPAN} = \frac{d_{90} - d_{50}}{d_{10}}$$

where d_{10}, d_{50} and d_{90} are the diameter sizes and the given percentage value is the percentage of particles smaller than that size. A high SPAN value indicates a wide size distribution.[12]

Scanning Electron Micrography (SEM)

The microspheres were scanned using scanning electron microscope (Leica-Stereoscan-440). For the SEM, the microspheres were mounted directly on to the SEM sample stub using double sided sticking tape, and coated with gold film thickness of 200 mm under reduced pressure of 0.001 mm of Hg. The shape and surface characteristic of the microspheres was observed under electron micro analyzer and photographs were taken using SM 4504 camera.

X-ray Diffractometry (X-RD)

X-ray powder diffractometry was carried out to investigate the effect of microencapsulation process on crystallinility of drug. Powder X-RD pattern were recorded on X-RD (Philips-PW-1050) with filter Ni, CuKα radiation, voltage 40 kV and a current of 20 mA. The scanning rate employed was 1°/min over the 5° to 50° diffraction angle (2θ) range. The X-RD patterns of drug powder, polymer, aluminium tristearate and drug-loaded microspheres were recorded.

Differential Scanning Calorimetry (DSC*)*

The DSC analysis of pure drug, polymer, aluminium tristearate and drug-loaded microspheres were carried using DSC (Mettler TC 11, TA Processor) to evaluate any possible drug polymer interaction. The samples (6 mg each) were placed into a pierced aluminium sample container. The studies were performed under a static air atmosphere in the temperature range of 50 °C to 500 °C, at a heating rate of 10 °C/min. The peak temperatures were determined after calibration with standard.

Fourier-Transform Infrared Spectroscopy (FTIR)

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, polymer, aluminium tristearate and drug-loaded microspheres using FTIR spectrophotometer (Jasco FTIR-410). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 2/cm.

Drug Release Studies

Microspheres equivalent to 75 mg ambroxol hydrochloride were filled in a capsule[13] and *in vitro* drug release was studied using USP Apparatus I with 900 ml of dissolution medium at 37.5 ± 0.1 °C for 12 h at 100 rpm. 0.1N HCl (pH 1.2) was used as dissolution medium for the first 2 h, followed by pH 6.8 phosphate buffer for further 10 h. 5 ml of sample was withdrawn after every hour, and was replaced with an equal volume of fresh dissolution medium. Collected samples were analyzed at 244.4 nm by spectrophotometrically. The study was performed in triplicate. Dissolution study was also conducted for marketed capsule Mucolite^{R} SR. (M1)

Release Kinetics

Data obtained from *in vitro* release studies were fitted to various kinetics equations[14] to find out the mechanism of drug release from microspheres. The kinetics models used were zero

order, first order, and Higuchi models. The rate constants were also calculated for the respective models.

RESULTS AND DISCUSSION

Microspheres Morphology and Drug Encapsulation

SEM study shows that particles were spherical, discrete having smooth and non-porous structure. (Figure1) The method showed good encapsulation efficiency. Percent drug encapsulated was found to be in a range 86-95%. It was observed that with increase in polymer concentration drug encapsulation efficiency was increased. Drug encapsulation efficiency was slightly decreased as the aluminium tristearate concentration was increased because optimum level of dispersing agent decrease the interfacial tension between the lipophilic and hydrophilic phases of the emulsion and simplify the formation of microspheres also this dispersing agent provides a thin protective layer around the droplets and reduces the extent of their collision and coalescence.[15]

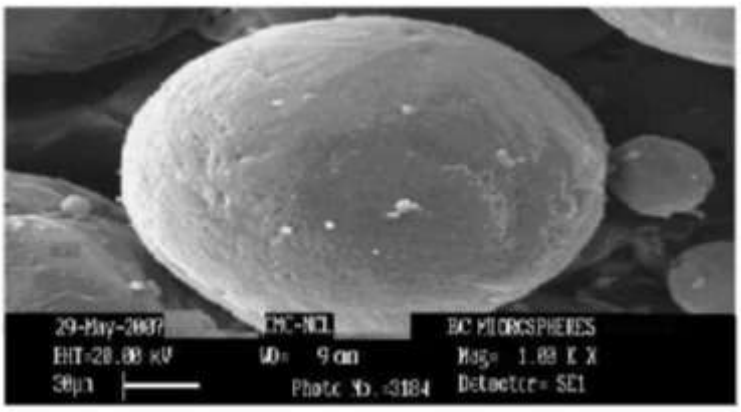

Figure 1: Scanning electron micrograph of optimized EC2 microsphere at 1.00 KX magnification

Effect on Particle Size

Particle size was found to be in the range of 29.22- 38.37 µm with SPAN factors ranging between 1.20-1.55. It was found that the size of microspheres was increased as the concentration of inner phase polymer was increased while the concentration of dispersing agent was kept constant. This can be ascribed to an increased in the viscosity of internal phase caused coalescence of inner phase droplets. But the variations of the concentrations of aluminum tristearate did not affect the particle size of microspheres. SPAN factor ranged in between 1.20-1.55, indicating narrow size of distribution.

Table 2. Physical properties of ambroxol hydrochloride loaded ethyl cellulose microsphere

Formulation code	Yield value (%)	Encapsulation efficiency (%)*	Particle size# (µm)	SPAN
EC1	84.76	81.51±0.580	29.22±0.024	1.439
EC2	83.63	83.12±1.873	32.22±0.126	1.552
EC3	86.17	84.90±0.687	34.38±0.473	1.459
EC4	86.26	85.43±0.770	34.56±0.861	1.519
EC5	92.12	87.89±1.891	36.82±0.068	1.476
EC6	96.14	89.33±0.912	37.42±0.193	1.443
EC7	90.58	90.24±1.169	36.97±0.752	1.362
EC8	88.12	92.76±0.837	37.68±0.045	1.204
EC9	76.48	94.20±0.552	38.37±0.086	1.426

Values shown represents the equivalent volume diameter (µm). (n=3)

* Mean ± S.D., n=3

Drug Release Behavior

In vitro dissolution results showed that the microspheres prepared with a different core-coat ratio gave better-sustained action over 11 h. Figure 2 clearly illustrates that the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices. An inverse relationship was observed between polymer content and drug release rate from the prepared microspheres. In all cases of polymers it was seen that microspheres containing 10% polymer released the drug more rapidly, while those with 20% polymers exhibited a relatively slower drug release profile.

In case of effect of dispersing agent on drug release it was seen that the slowest release of ambroxol hydrochloride from microspheres observed with formulations containing 3% aluminum tristearate at all polymer concentrations, emphasizing the effect of the hydrophobicity of the dispersing agent on the drug release. At a constant polymer concentration, the higher the amount of aluminium tristearate in the microspheres, the lower the drug release. *In vitro* dissolution results showed that the EC6 microspheres gave better-sustained action up to a period of 12 h. Hence it was considered as an optimized and used for compatibility study between drug and polymer.

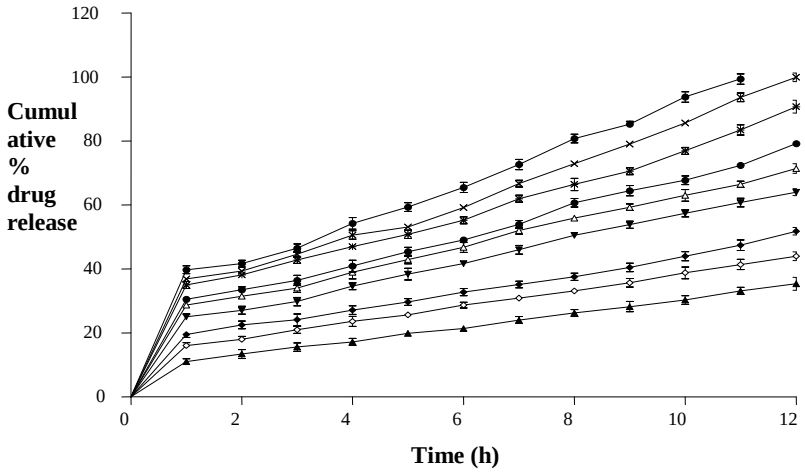

Figure 2. In vitro dissolution profile of ambroxol hydrochloride loaded ethyl cellulose microspheres.

Release Kinetics

All the formulations showed best fit with zero order kinetics with R^2 ranging from 0.9898-0.999. The release mechanism of ambroxol hydrochloride from various formulations was determined by computing release exponent values 'n' from Korsmeyer Peppas equation. It would appear that all the formulations except EC9 showed n value ranged from 0.39-0.42, which showed Fickian diffusion whereas in case of EC9 drug transport mechanism was anomalous type (n=0.48) that is summation of both diffusion and dissolution. The value of K varies between 1.84-5.42, which clearly showed that as the concentration of polymer or dispersing agent increased there is distinct decreasing trend of K. This characteristic of K was already reported in literature.[16]

The marketed capsule showed best fit with Higuchi's equation having R^2 0.9945 and exponent value of 0.54 indicating transport mechanism was anomalous type.

Table 3. *In Vitro* release kinetic parameters of ambroxol hydrochloride loaded ethyl cellulose microspheres

Formulation code	Kinetic models							
	Zero order		First order		Higuchi model		Peppas model	
	R^2	K_0 (%mg/h)	R^2	K_0 (h^{-1})	R^2	K_h (%mg/$h^{1/2}$)	R^2	n
EC1	0.9931	5.421	0.8254	0.287	0.95	29.973	0.9147	0.4137
EC2	0.9898	5.253	0.8492	0.198	0.9387	28.855	0.91	0.4245
EC3	0.9912	4.649	0.8754	0.154	0.9437	26.203	0.9181	0.3939

EC4	0.994	4.058	0.8218	0.103	0.953	22.874	0.9228	0.4021
EC5	0.9975	3.559	0.9807	0.082	0.9677	20.620	0.9358	0.392
EC6	0.9973	3.246	0.9881	0.068	0.9678	18.483	0.9366	0.4119
EC7	0.9934	2.693	0.9771	0.044	0.9523	14.956	0.9343	0.3959
EC8	0.999	2.328	0.994	0.036	0.9718	12.764	0.9556	0.424
EC9	0.998	1.841	0.9943	0.028	0.9614	9.572	0.9638	0.4806

Similarity factor (f_2) and difference factor (f_1) were calculated for optimized microspheres (EC6) considering marketed capsule as the reference standard. It was seen that microspheres EC6 have f_2 value 51.89 and f_1 value 8.19 respectively. This suggested that microspheres showed similarities of dissolution profiles with that of marketed capsule.

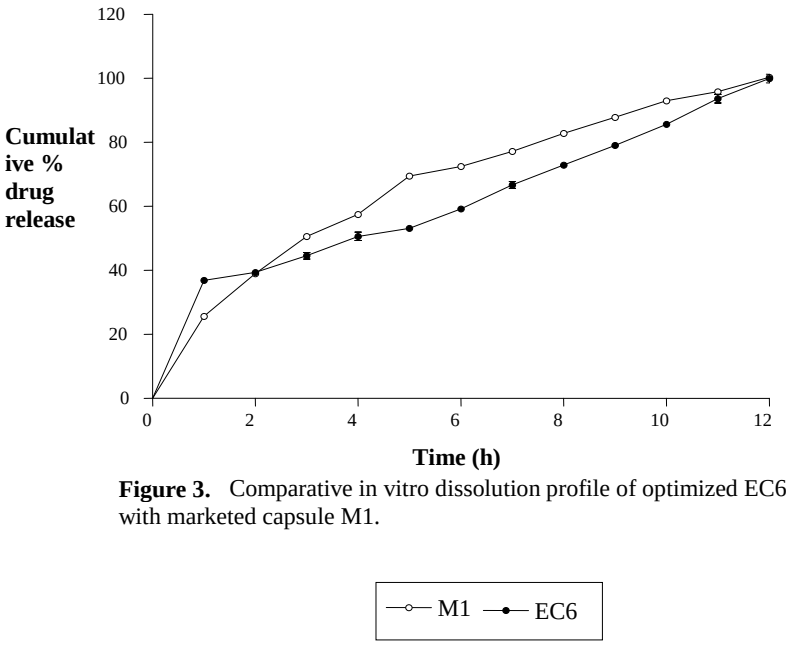

Figure 3. Comparative in vitro dissolution profile of optimized EC6 microspheres with marketed capsule M1.

Using two way ANOVA statistically significant difference of ethyl cellulose concentration on drug release, encapsulation efficiency and drug release was found. ($p < 0.0001$). The effect of aluminium tristearate on the paricle sizes was not found statistically significant. ($p > 0.05$) but on encapsulation efficiency and drug release it was significant. ($p = 0.0005$-0.0001).

X-ray Diffractometry (X-RD)

Characteristic crystalline peaks of ambroxol hydrochloride were observed at 2θ of 12.13, 6.84, 5.64, 5.08, 4.34, 4.20, 3.94, 3.82, 3.71, 3.65, 3.31, 3.24, 3.16, 3.05, 2.95, 2.81, 2.62, 2.42 and 2.06 indicating the presence of crystalline ambroxol hydrochloride. Peaks of ambroxol chloride are also present in EC6 microspheres even if reduced in intensity. Typical

diffraction patterns of ambroxol hydrochloride loaded ethyl cellulose microspheres are shown in figure 4. The decreased intensity of peaks is due to decrease in drug crystallinity.[17] This indicates that ambroxol hydrochloride is present in the ethyl cellulose microspheres with reduced crystallinity.

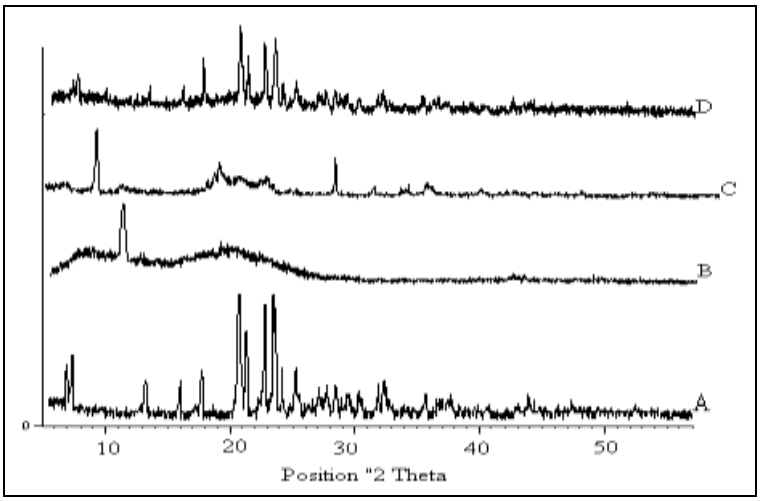

Figure 4. X-ray diffractograms of ambroxol hydrochloride (A), ethyl cellulose (B), Aluminium tristearate (C), ethyl cellulose microspheres (D).

Differential Scanning Calorimetry (DSC)

The characteristic endothermic peak for ambroxol hydrochloride was obtained at 243.0 °C, which was also obtained in EC6 microspheres at 238.6 °C, which showed, that drug is dispersed in microspheres. Typical DSC patterns of ambroxol hydrochloride loaded ethyl cellulose microspheres are shown in figure 5.

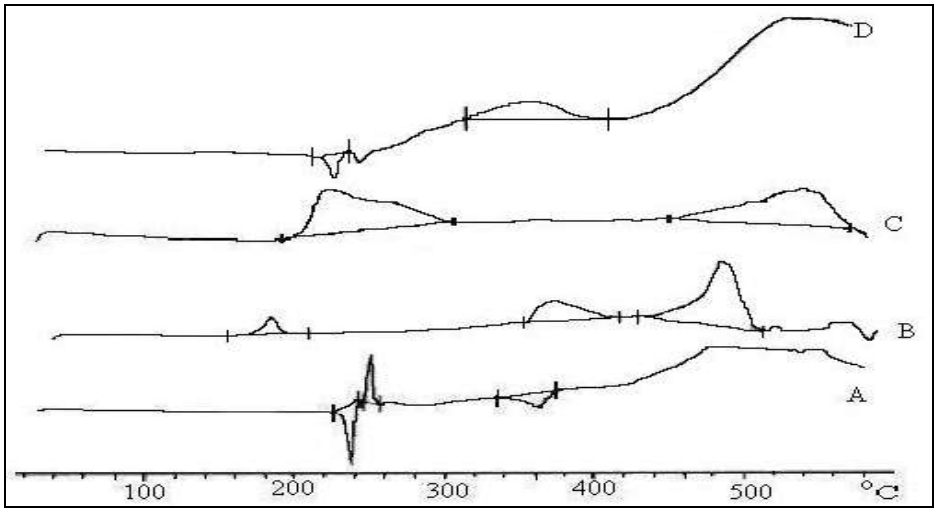

Figure 5. DSC thermograph of ambroxol hydrochloride (A), ethyl cellulose (B), Aluminium tristearate (C), ethyl cellulose microspheres (D).

Fourier Infrared Spectroscopy (FTIR)

The characteristic peaks of aromatic NH2, aliphatic NH, aliphatic OH and aromatic C=C of pure drug were almost identical with those EC6 microspheres which indicated absence of any polymer drug interaction. Typical FTIR patterns of ambroxol hydrochloride loaded ethyl cellulose microspheres are shown in figure 6.

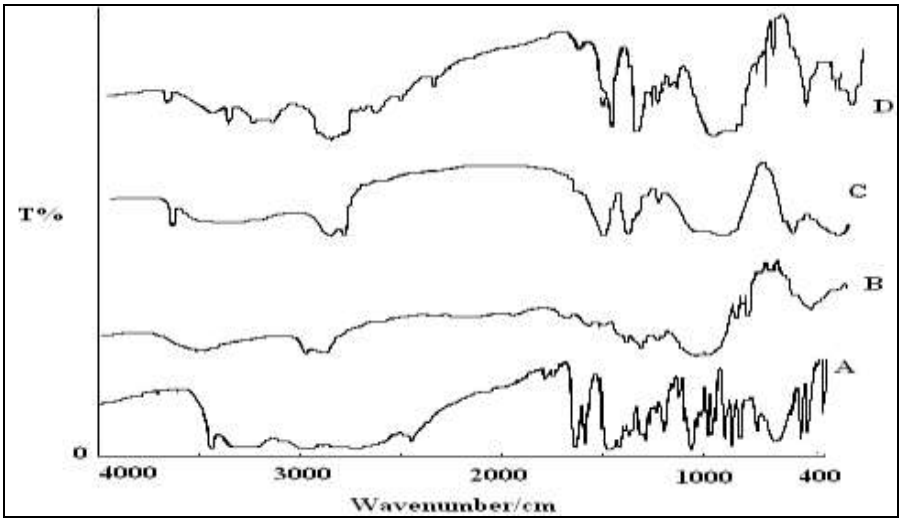

Figure 6. FTIR spectra of ambroxol hydrochloride (A), ethyl cellulose (B), Aluminium tristearate (C), ethyl cellulose microspheres (D).

CONCLUSION

Ambroxol hydrochloride microspheres were prepared successfully using solvent evaporation technique. The method showed good encapsulation efficiency with high yield value. Polymer concentration and aluminium tristearate showed statistically significant effect on encapsulation efficiency and drug release. The assessment of the release kinetics revealed that drug release from ambroxol hydrochloride microspheres followed zero order kinetics. It was suggested that mechanism of drug release from microspheres was diffusion-controlled.

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