GAVIN PUBLISHERS

Journal of Pharmaceutical and Pharmacological Sciences

Research Article

Gangurde HH, et al., J Pharma Pharma Sci: JPPS-113.
DOI:10.29011/2574-7711/100013

Microencapsulation and *Invitro* Characterization of Acrylate Microspheres for Controlled Release of Ambroxol Hydrochloride

HemantHGangurde*, Nayana S Baste, Mayur A Chordiya,Chandrasekhar D Upasani

Department of Pharmaceutics, SSDJ College of Pharmacy, Nasik, Maharashtra, India

***Corresponding author:** Hemant H Gangurde, SSDJ College of Pharmacy, Neminagar, Chandwad-423 101, Nasik, Maharashtra, India. Tel: +919423115957; Email: hhgangurde@gmail.com

Citation: Gangurde HH, Baste NS, Chordiya MA, Upasani CD (2016) Microencapsulation and InvitroCharacterization of Acrylate Microspheres for Controlled Release of Ambroxol Hydrochloride.J Pharma Pharma Sci: JPPS 113. DOI:10.29011/2574-7711/100013

Received Date: 10 December, 2016; **Accepted Date:** 25 December, 2016; **Published Date:** 03 January, 2017

Abstract

The aim of present study was to formulate microspheres of Ambroxol hydrochloride by solvent evaporation technique using acrylic polymers Eudragit RS100 and Eudragit RL100. Both the polymers were compared for microencapsulation efficiency, drug content and *in vitro* drug release. X-RD, DSC and FTIR confirmed the absence of any drug polymer interaction. All the batches yielded microspheres with excellent topographical characteristic. The results of two ways ANOVA suggested statistically significant effect of aluminium tristearate and polymer concentration on encapsulation efficiency and drug release.

Keywords: Ambroxol hydrochloride; Eudragit RS100; Eudragit RL100; Solvent Evaporation Method

Introduction

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.[1-3]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[4-7].

In the present study, we examine the potential for the sustained delivery of Ambroxol by forming microspheres. These multiparticulate solid dosage forms have a number of advantages such as more uniform distribution of the drug in the gastrointestinal tract, more uniform drug absorption, reduced local irritation and elimination of unwanted intestinal retention of polymeric material[8].Microencapsulation of hydrophilic drugs have one major disadvantage of low loading efficiency. One method of ensuring high entrapment efficiency of is to use W/O emulsion solvent evaporation method which having hydrophobic processing medium[9].Eudragit RL100 and Eudragit RS100 are hydrophobic copolymers synthesized from acrylic acid and methacrylic acid esters, with RL having higher content of functional quaternary ammonium groups than RS[10]

Ambroxol hydrochloride microspheres were prepared by solvent evaporation technique using Eudragit RS100 as well as Eudragit RL100 as a matrix-forming polymer. Aluminium tristearate was used as a dispersing agent[11].The main objectives of this study were to formulate microspheres of Ambroxol hydrochloride and to investigate the effect of formulation parameters such as polymer concentration and variation of dispersing agent on microsphere properties. Simultaneously both the polymers were compared for surface topology, microencapsulation efficiency, and drug release. The microsphere cross-linking mechanism and topological properties have been characterized by FTIR, DSC, XRD, and SEM.

Materials and Methods

Materials

The materials used were Ambroxol hydrochloride (Glen mark Pharmaceutical Ltd, Nasik, Maharashtra, India), Eudragit RS100 and Eudragit RL100 (Rohm Pharma, USA), aluminium tristearate (Ipca Laboratories, Mumbai, Maharashtra, India),

acetone and methanol (Fine Chemicals, Mumbai, India). All other chemicals and solvents were of analytical grade.

Methods

Preparation of Microspheres

Ambroxol hydrochloride and Eudragit RS100 were dissolved in an acetone-methanol mixture. The dispersing agent was added, and the mixture was stirred at 500 rpm in a water bath on a magnetic stirrer 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 35˚C for 4 h at 400 rpm, and the organic solvent, acetone-methanol, were 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**[**11]. Formulations of microspheres are given in (Table 1).

Batch code†		Variable level	
		Polymer concentration (%)‡	Dispersing agent concentration (%)‡
RL1	RS1	10	3
RL2	RS2	15	3
RL3	RS3	20	3
RL4	RS4	10	2
RL5	RS5	15	2
RL6	RS6	20	2
RL7	RS7	10	1
RL8	RS8	15	1
RL9	RS9	20	1
	† RS: EudragitRS100 and RL: Eudragit RL100 ‡ The concentrations of dispersing agents and polymer were calculated from dispersed inner phase volume (%w/v). Each formulation contained 1 g of Ambroxol HCl.		

Table 1: Formulations of Ambroxol Hydrochloride Microspheres.

Percentage Yield Value of Microspheres

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

Drug Entrapment Efficiency

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

Encapsulation efficiency = (Drug entrapped/ Theoretical drug content) × 100. (1)

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 several minutes using an ultrasonic bath. Then aliquot of the microspheres suspension was added into recirculation unit, which was subsequently circulated 3500 times per minute. Each sample was measured in triplicate for the analysis. Particle size was expressed as equivalent volume diameter. The particle size distribution was also expressed in terms of SPAN factor determined as:

$$SPAN = \frac{d_{90} - d_{50}}{d_{10}} \quad (2)$$

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 (XRD)

X-ray powder diffractometry was carried out to investigate the effect of microencapsulation process on crystallinity of drug. Powder XRD was carried out using XRD (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 XRD 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

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 buffers 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 spectrophotometric ally. 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, Higuchi models, and Baker Lonsdale. The rate constants were also calculated for the respective models.

Results and Discussion

Microspheres Morphology and Drug Encapsulation

The shape and surface morphology of microspheres were observed by scanning electron microscopy. Eudragit RS100 microspheres have spherical, discrete, non-porous structure with rugged polymeric surface (Figure 1) whereas Eudragit RL100 microspheres were spherical, discrete, with distinct pores on the surface and also showed accumulation of free aluminium tristearate particles on the surface. (Figure 2).

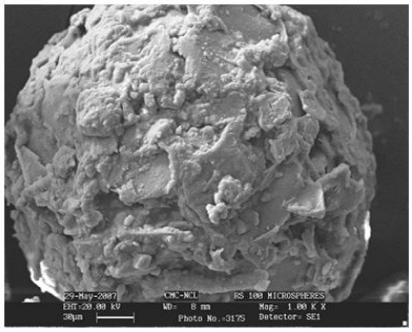

Figure 1: Scanning electron micrograph of Optimized RS2 microspheres at 1.00 KX.

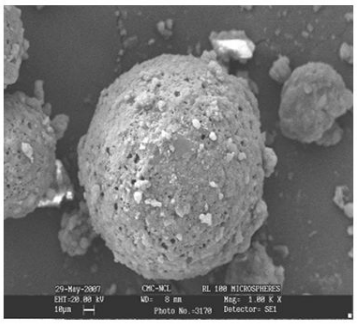

Figure 2: Scanning electron micrograph of optimizedRL6 microspheres at 1.00 KX magnifications.

The method showed good encapsulation efficiency. Percent drug encapsulated was found to be in a range of 82-95% for Eudragit RS100, and 76-93% for Eudragit RL100. From (Table 2) data it was observed that with increase in polymer concentration drug encapsulation efficiency was increased. Eudragit RL100 showed low encapsulation efficiency as compared to Eudragit RS100 because it is more permeable than RS100. Drug encapsulation efficiency was slightly increased as the aluminium tristearate concentration was increased because dispersing agent reduces the interfacial tension between the two immiscible phases of the emulsion and reduces the extent of collision and coalescence between the microspheres during their solidification[15].

Batch code	YV (%)	EE (%)	d †	SPAN
RS1	82.26	82.862±0.548	27.67±0.127	1.373
RS2	92.24	83.461±0.403	26.62±0.204	1.555
RS3	83.55	86.427±1.236	30.48±0.089	1.525
RS4	87.96	89.758±0.619	38.11±0.094	1.246
RS5	85.72	90.982±0.645	29.32±0.249	1.588
RS6	92.89	91.407±0.528	32.46±0.042	1.642
RS7	96.25	92.625±0.765	43.01±0.346	1.424
RS8	90.59	93.827±1.380	35.63±0.076	1.566
RS9	85.03	95.424±0.636	38.33±0.418	1.282
RL1	86.66	76.16±0.509	28.79±0.035	1.518
RL2	85.00	78.72±0.858	23.08±0.287	1.350
RL3	84.52	81.75±0.502	32.50±0.548	1.498
RL4	86.91	82.31±0.657	39.20±0.426	1.431
RL5	98.96	86.70±0.799	29.48±0.054	1.324
RL6	97.4	88.26±0.758	31.84±0.016	1.598
RL7	89.35	91.42±0.009	40.34±0.388	1.337
RL8	74.43	92.56±0.825	32.96±0.064	1.495
RL9	85.60	93.60±0.848	44.18±0.092	1.447

* YV indicates yield value; EE, encapsulation efficiency (n=3)
† Values shown represent the equivalent volume diameter (µm). (n=3)

Table 2: Physical Properties of Microspheres.

Effect on Particle Size

Particle size analysis done by laser diffraction revealed that Eudragit RS100 microspheres were in the range of 26.62- 43.01 µm with SPAN factors ranging between 1.24-1.64 whereas Eudragit RL100 microspheres were in the range of 23.08- 44.18 µm with SPAN factors ranging between 1.32-1.59. (Table 2) 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. Because this increased concentration of polymer solution increases viscosity of inner phase droplets and gives difficulty in dispersion and subdivision of droplets[14]. But the variations of the concentrations of aluminum tristearate did not affect the particle size of microspheres. SPAN factors for all the batches ranges in between 1.24-1.64, which indicates narrow size of distribution.

In vitro Drug Release

In vitro dissolution results showed that the microspheres prepared with a different core-coat ratio gave better-sustained action. Eudragit RS100 and Eudragit RL100 gave sustained action over 10 h and 4 h respectively. It was seen that the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices. From Figure 3 and 4 it was observed that an inverse relationship exists between polymer concentration 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. Drug release from Eudragit Rs100 microspheres was slow as compared to Eudragit RL100 microspheres this is due to the fact that Eudragit RL00 contains more functional quaternary ammonium groups (10%) than Eudragit RS100 (5%) gives the microspheres membrane a more open structure. Moreover, Eudragit RL100 is strongly hydrophilic which causes easy diffusion of the dissolution medium and hence good leaching of the drug. Due to strong permeability and greater porosity of Eudragit RL100 the release of drug was more as compared to the Eudragit RS100[16].

In case of effect of dispersing agent on drug release it was seen that drug release decreases with increasing concentration of aluminium tristearate at a constant polymer concentration. The decrease in drug release is due to hydrophobicity of the dispersing agent. The increasing amount of dispersing agent led to accumulation of free aluminium tristearate particles on to the surfaces of microspheres.

In vitro dissolution results showed that the RS2, RL6 microspheres gave better-sustained action up to a period of 12 h. Hence these formulations were optimized and studied for compatibility between drug and polymer.

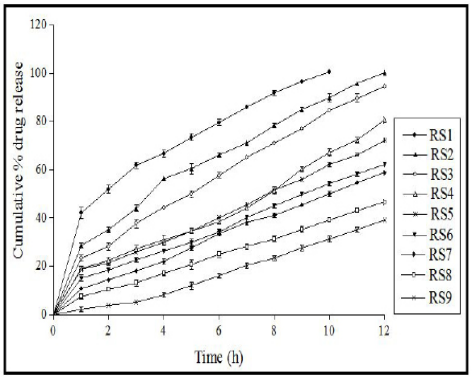

Figure 3: *In vitro* dissolution profile of Ambroxol hydrochloride loaded Eudragit RS100 microspheres.

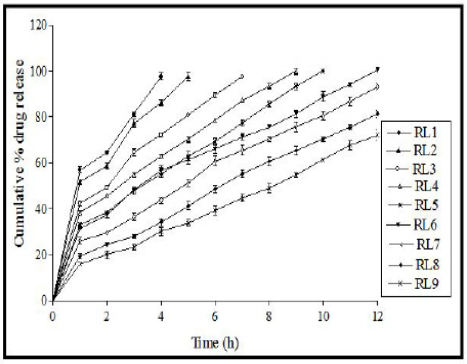

Figure 4: *In vitro* dissolution profile of Ambroxol hydrochloride loaded Eudragit RL100 microspheres.

Release Kinetics

From (Table3) all the Eudragit RS100 microspheres except RS1 and RS2 follows zero order kinetics (R^2>0.9823) whereas the remaining two gives best fit with Higuchi's equation (R^2>0.9919). Whereas for Eudragit RL100 microspheres all the formulations showed best fit with zero order kinetics (R2>0.9749)

The release mechanism of Ambroxol hydrochloride from various formulations was determined by computing release exponent values 'n' from Korsmeyer Peppas equation. From n value, RS1 microspheres showed Fickian diffusion and remaining RS2 to RS9 anomalous type, which refers to a combination of both diffusion and erosion controlled-drug release whereas RL1 to RL3 showed Fickian diffusion and RL4 to RL9 showed anomalous type. The marketed preparation showed best fit with Higuchi's equation (R^2=0.9945) and exponent value of 0.54 indicating transport mechanism was anomalous type. The values of K showed decreasing trend perceptible with increasing level of either polymer or aluminium tristearate. It is already documented in literature that K is trait function of polymer properties such as solubility, viscosity and molecular weight[17].

Kinetic models					
Batch code	Zero order		Higuchi model		Peppas model
	R^2	K_0 (%mg/h)	R^2	Kh (%mg/$h^{1/2}$)	n
RS1	0.986	5.838	0.9974	31.793	0.3847
RS2	0.9882	5.961	0.9919	28.942	0.5278
RS3	0.9974	5.931	0.9828	27.325	0.5991
RS4	0.9823	5.142	0.9281	23.324	0.6431
RS5	0.9962	4.470	0.9561	20.882	0.5828
RS6	0.9971	3.936	0.9629	17.981	0.6084
RS7	0.9976	3.991	0.9722	16.985	0.7297
RS8	0.9987	3.246	0.967	13.452	0.7654
RS9	0.9899	3.070	0.9387	11.298	0.803
RL1	0.9749	10.135	0.9362	48.755	0.3812
RL2	0.9836	9.192	0.9677	43.688	0.4068
RL3	0.9909	7.868	0.9868	36.840	0.4433
RL4	0.9981	6.830	0.985	33.290	0.4586
RL5	0.9993	6.701	0.9774	31.569	0.5051
RL6	0.9901	5.732	0.9873	29.003	0.4743
RL7	0.9933	5.575	0.981	26.881	0.5675
RL8	0.9961	5.170	0.9747	23.503	0.6176
RL9	0.9962	4.674	0.9578	20.799	0.6384

Table 3: In vitro Release Kinetic Parameters of Ambroxol Hydrochloride Loaded Eudragit Microspheres

Similarity factor (f2) and difference factor (f1) were calculated for optimized microspheres considering marketed capsule as the reference standard. It was found that f1 and f2value for RS2 were 66.21 and 4.66 whereas for RL6, 64.56 and 4.50 respectively. This suggested that microspheres RS2 and RL6 showed similarities of dissolution profiles with that of marketed capsule. (Figure 5)

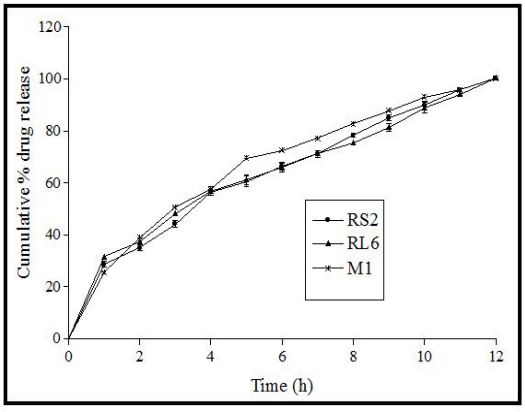

Figure 5: Comparative *in vitro* dissolution profile of optimized RS2 and RL6 microspheres with marketed capsule M1.

$t_{70\%}$,of RS2, RL6 and marketed capsule was 6.32h, 6.87h, and 6.34h respectively which suggested that microspheres RS2, RL6 showed release profiles comparable with that of marketed capsule.

Using two-way ANOVA statistically significant difference of both the polymer on drug release, encapsulation efficiency and drug release was found. (p=0.001-0.023). The effect of aluminium tristearate on the paricle sizes obtained by both polymers was not found statistically significant. (p >0.05) but on encapsulation efficiency and drug release it was significant. (p= 0.0014-0.020)

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 RS2, RL6 microspheres even if reduced in intensity. This declination of drug crystallinity reduces the intensity of peaks[18].Typical X-RD patterns of Ambroxol hydrochloride loaded Eudragit RS100 and Eudragit RL100 microspheres are shown in (Figure 6).

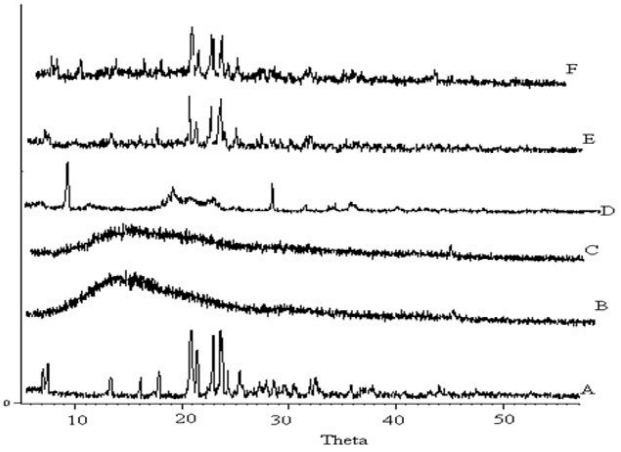

Figure 6: X-ray diffract grams of Ambroxol hydrochloride (A), EudragitRS100 (B), Eudragit RL100 (C), Aluminium tristearate (D), RS100 microspheres (E), RL100 microspheres. (F)

Differential Scanning Calorimetry (DSC)

The characteristic endothermic peak for Ambroxol hydrochloride was obtained at 243.0^{0}C, which was also obtained in RS2, RL6 microspheres with slender change. For RS2 it obtained at 238.5^{0}C, for RL6 at 232.6^{0}C, which showed, that drug is dispersed in microspheres. Typical DSC patterns of Ambroxol hydrochloride loaded Eudragit RS100 and Eudragit RL100 microspheres are shown in (Figure 7).

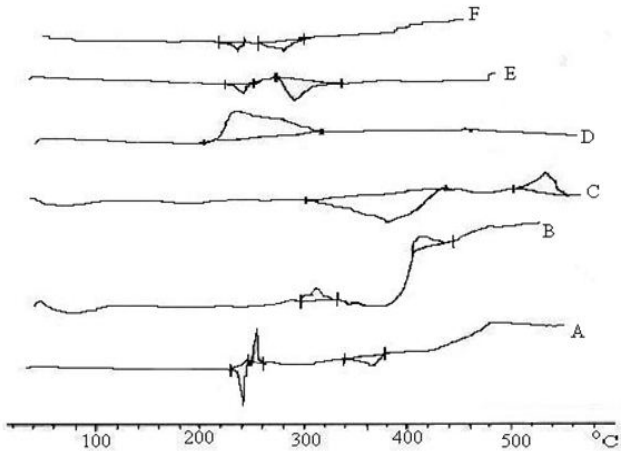

Figure 7: X-ray diffract grams of Ambroxol hydrochloride (A), EudragitRS100 (B), Eudragit RL100 (C), Aluminium tristearate (D), RS100 microspheres (E), RL100 microspheres. (F)

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 of RS2, RL6 and EC2 microspheres which indicated that absence of any polymer drug interaction. Typical FTIR patterns of Ambroxol hydrochloride loaded Eudragit RS100 and Eudragit RL100 microspheres shown in (Figure 8).

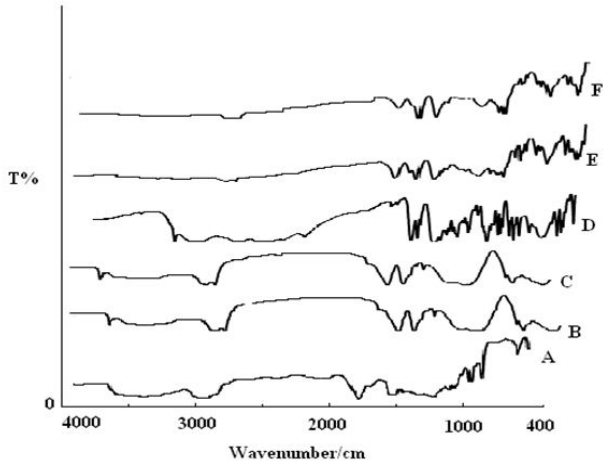

Figure 8: FTIR spectra of Ambroxol hydrochloride (A), EudragitRS100 (B), Eudragit RL100 (C), Aluminium tristearate (D), RS100 microspheres (E), RL100 microspheres. (F)

Conclusion

In conclusion, the attempt to microencapsulate Ambroxol hydrochloride was successful. The method showed good encapsulation efficiency with high yield value. Aluminium tristearate and polymer concentration were clearly effective on encapsulation efficiency, and in vitro drug release. Particle size was affected by only polymer concentration not aluminium tristearate. Due to low permeability Eudragit RS100 showed more encapsulation efficiency and slow drug release as compared to Eudragit RL100.

Acknowledgments

The authors are grateful to Dr. D.V. Derle, Principal NDMVP's College of Pharmacy, Nashik for his valuable guidance and Glen mark pharmaceuticals Ltd. Nasik for providing gift sample of Ambroxol hydrochloride.

References

1. Lee HJ, Joung SK, Kim YG, Yoo JY, Han SB (2004) Bioequivalence assessment of Ambroxol tablet after a single oral dose administration to healthy male volunteers. Pharm Research 49: 93-98.

2. Villacampa J, Alchntar F, Rodriguez JM, Morales JM, Herrera J, et al. (2003) Pharmacokinetic properties of single-dose loratadine and Ambroxol alone and combined in tablet formulations in healthy men. Clin Ther 25: 2225-2232.

3. British Pharmacopoeia (2002) University Printing House, Cambridge 211.

4. Kubo W, Miyazaki S, Dairaku M, Togashi M, Mikamib R, et al. (2004) Oral sustained delivery of Ambroxol from in situ-gelling pectin formulations. Int J Pharm 271: 233-240.

5. Itoh K, Wataru K, Fujiwara M, Hirayama T, Miyazaki S, et al. (2006) The influence of variation of gastric pH on the gelation and release characteristics of in situ gelling pectin formulations. Int J Pharm 312: 37-42.

6. Basak SC, Jayakumar RBM, Lucas MKP (2006) Formulation and release behavior of sustained release Ambroxol hydrochloride HPMC matrix tablet. Indian J Pharm Sci 68: 594-598.

7. Gangurde HH, Chavan NV, Mundada AS, Derle DV, Tamizharasi S (2011) Biodegradable Chitosan-Based Ambroxol Hydrochloride Microspheres: Effect of Cross-Linking Agents. J Young Pharm 3: 9-14.

8. Anal AK, Stevens WF, Lopez CR (2006) Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. Int J Pharm 312: 166-173.

9. Sahoo SK, Mallick AA, Barik BB, Senapati PC (2004) Formulation and *in vitro* evaluation of Eudragit microspheres of stavudine. Trop J Pharm Res 4: 369-375.

10. Biswanath S, Tamilvanan S (1995) Characterization of *in vitro* release of sulphamethizole from Eudragit RL100 microspheres. Indian Drugs 32: 176-183.

11. Horoz BB, Kilicarslan M, Yuksel N, Baykara T (2006) Influence of aluminium tristearate and sucrose stearate as the dispersing agent on physical properties and release characteristics of Eudragit RS microspheres. AAPS Pharm SciTech 7: 16.

12. Gavini E, Hegge AB, Rassu G, Sanna V, Testa C, et al. (2006) Nasal administration of carbamezepine using chitosan microspheres: *in vitro*/ *in vivo* studies. Int J Pharm 307: 9-15.

13. Gohel MC, Amin AF (1999) Studies in the preparation of diclofenac sodium microspheres by emulsion solvent evaporation technique using response surface analysis. Indian J Pharm Sci 61: 48-53.

14. Costa P, Lobo JMS (2001) Modeling and comparison of dissolution profiles. Eur J Pharm Sci 13: 123-133.

15. Horoz BB, Kilicarslan M, Yuksel N, Baykara T (2004) Effect of dispersing agents on the characteristics of Eudragit microspheres prepared by a solvent evaporation method. J Microencap 21: 191-202.

16. Bhanja RS, Pal TK (1994) *In vitro*release kinetics of salbutamol sulphate microcapsules coated with both Eudragit RS100 and Eudragit RL100. Drug Dev and Ind Pharm 20: 375-386.

17. Singh B, Agarwal R (2002) Design development and optimization of controlled release microcapsules of diltiazem hydrochloride. Indian J Pharm Sci 64: 378-385.

18. Desai KGH, Park HJ (2005) Preparation of cross-linked chitosan microspheres by spray drying: effect of cross-linking agent on the properties of spray dried microspheres. J Microencap 22: 377-395.