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

Hemant H Gangurde*, 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

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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 (Glenmark 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.

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 = \((\text{Drug entrapped/ Theoretical drug content}) \times 100\).

Particle Size Analysis of Microspheres

Average particle diameter and size distribution of microspheres were determined by laser diffraactometry using a Master-sizer 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:

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

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 nm 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, CuKa 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 spectrophotometrically. The study was performed in triplicate. Dissolution study was also conducted for marketed capsule Mucolite® 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).

Table 2: Physical Properties of Microspheres.

| Batch code | YV (%) | EE (%) | d † | SPAN |
|------------|--------|--------|-----|------|
| RS1        | 82.26  | 82.86±0.548 | 27.67±0.127 | 1.373 |
| RS2        | 92.24  | 83.46±0.403 | 26.62±0.204 | 1.555 |
| RS3        | 83.55  | 86.42±1.236 | 30.48±0.089 | 1.525 |
| RS4        | 87.96  | 89.75±0.619 | 38.11±0.094 | 1.246 |
| RS5        | 85.72  | 90.98±0.645 | 29.32±0.249 | 1.588 |
| RS6        | 92.89  | 91.40±0.528 | 32.46±0.042 | 1.642 |
| RS7        | 96.25  | 92.62±0.765 | 43.01±0.346 | 1.424 |
| RS8        | 90.59  | 93.82±1.380 | 35.63±0.076 | 1.566 |
| RS9        | 85.03  | 95.42±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)

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].
**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.

**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 ($R^2>0.9749$).

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 |
|------------|------------|---------------|--------------|
|            | R² | K₀ (%mg/h) | R² | Kh (%mg/h₁/₂) | 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 f₂ value 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) 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 stearate on the particle 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 5: Comparative in vitro dissolution profile of optimized RS2 and RL6 microspheres with marketed capsule M1.
Figure 6: X-ray diffract grams of Ambroxol hydrochloride (A), Eudragit RS100 (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°C, which was also obtained in RS2, RL6 microspheres with slender change. For RS2 it obtained at 238.5°C, for RL6 at 232.6°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), Eudragit RS100 (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), Eudragit RS100 (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.

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