

Design, evaluation and optimization of microcapsules of leflunomide with Eudragit[®] RL100 and Eudragit[®] RS. 100 by solvent evaporation technique

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Leflunomide, an immune modulatory prodrug that is rapidly converted to its active metabolite possibly in the gut wall, plasma and in the liver was microencapsulated by the solvent evaporation technique using a nonaqueous solution of polymethacrylate polymer to achieve its release from the microcapsules at a slower rate. At the optimal condition of process variables such as stirring speed, temperature of the medium, drug polymer ratio and ratio of light liquid paraffin and heavy liquid paraffin, maximum encapsulation efficiency was obtained. These microspheres were free-flowing in nature, discrete and uniform spherical in size, as evident by scanning electron microscopy. The *in vitro* release experiments were carried out in the simulated intestinal fluid (pH 7.2 phosphate buffer) using United States Pharmacopoeia (USP) XXII apparatus II. The data obtained from the dissolution profiles were compared using different kinetics models and the regression coefficients were compared.

Key words: Dissolution profile release kinetics, leflunomide, microcapsules, polymethacrylate, regression coefficient

INTRODUCTION

Despite tremendous advancement in the drug delivery system, the oral route remains the preferred route for the administration of therapeutic agents and because of the low cost of therapy and ease of administration, it leads to higher levels of patient compliance. The conventional oral dosage form, such as tablets and capsules, provides specific drug concentration in systemic circulation without offering any control over drug delivery and also causes great fluctuations in the plasma drug levels. The designs of oral-controlled drug delivery system should be primarily aimed to achieve more predictable and increased bioavailability.

Leflunomide^[1] is one of the new drugs used in the treatment of rheumatoid arthritis.^[2] It works by suppressing the immune system because rheumatoid arthritis is caused by damage from an overacting immune system. It is available for oral administration as a tablet. Leflunomide is widely used in the treatment of pain management. Its control

release dosage form is still not available. But, microcapsules have been widely accepted as a means to achieve oral- and parenteral-controlled release drug delivery systems.^[3] With the help of some polymeric substances such as chitosan, polyacrylate, polymethacrylate and ethyl cellulose, we prepared the sustained release formulations. Therefore, an attempt was made to design and evaluate microcapsules^[4,5] of Leflunomide using Eudragit[®] RS. 100^[6] in combination with Eudragit[®] RL 100.^[7,8] The prepared microcapsules were evaluated for size distribution, drug entrapment efficiency and drug release behavior in phosphate buffer at pH 7.2 to match the target release profile and possible release mechanism. Fourier transform infrared (FT-IR) studies showed no drug polymer interaction, as evident from FT-IR spectra. Scanning electron microscopy (SEM) studies clearly reveal the smoothness of the spherically shaped particles.

MATERIALS AND METHODS

Materials

Leflunomide was gifted from Aventis Pharma Limited, Bombay, India and Eudragit[®] RL 100 and Eudragit[®] RL 100 were received from Albert Devid Limited, Kolkata, India as gift samples. All other ingredients used were of analytical reagent grade obtained commercially and were used as such without further purification.

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DOI: 10.4103/0973-8398.59957

Methods

Preparation of polymethacrylate microcapsules

Leflunomide and Eudragit (RL 100 and RS 100 in the ratio of 4:1) were used in the ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 giving, the batch numbers B1, B2, B3, B4 and B5, respectively.

Polymers were dissolved in acetone by stirring with a magnetic bar. To this, measured quantities of the drug were added and allowed to dissolve thoroughly but the total concentration was kept constant. The temperature was maintained at 15°C to minimize the rapid evaporation of acetone. Stirring was continued until a smooth polymer solution was formed. The resulting slurry was poured at a constant and steady stream in liquid paraffin also maintained at 15°C, which was being stirred at 800 rpm with an electrical stirrer (Remi, Bombay, India). The liquid paraffin had an absolute viscosity of 87.1 cp at 30°C (determined by the falling sphere method) obtained by blending heavy liquid paraffin with light liquid paraffin in the ratio of 1:1. The slurry was converted into spherical microcapsules. Stirring was continued for a sufficient period of time to evaporate the acetone at ambient temperature. Petroleum ether was added to the extract and the residual amount of acetone to rigidize^[9] the resultant microcapsules. The microcapsules were separated by filtration through a 100 mesh nylon cloth and washed with three to four portions of 100 ml cold petroleum ether to remove the adhering liquid paraffin and dried at 40-45°C for 4 h.

Content of leflunomide in the microcapsules

1. Twenty milligrams of accurately weighed microcapsules were suspended in 5 ml of USP phosphate buffer (pH 7.2) and stirred for 30 min in a mechanical shaker. The solution was filtered and analyzed spectrophotometrically (Shimadzu UV/visible spectrophotometer Model UV-1700) at 260 nm. Reliability of the method was judged by conducting recovery analysis using a known amount of the drug with or without polymer.^[10]

Particle size analysis

Microcapsules were separated into different size fractions by sieving for 30 min using a nest of standards sieves in a sieve shaker.

Fourier transform infrared study

Determination of interaction between drug and polymer were performed using FT-IR analysis. FT-IR of leflunomide blank, polymer blank and drug-loaded polymer were studied with potassium bromide pellets using a Perkin-Elmer model 883 spectroscope in the range of 400-4000/cm and the resolution was 2/cm at the Indian Institute of Chemical Biology, Kolkata.

Scanning electron microscopy study

For morphology and surface characteristics, prepared microcapsules were coated with gold in an argon atmosphere. The surface morphology of the microspheres was then studied by an SEM (model quanta 200 mk 2 make Fer Netherland) at the Bose Institute, Kolkata.

In vitro drug release study

In vitro drug release studies were carried out for all products in USP type II^[11] fitted with six rotating baskets (Campbell Electronics, Mumbai, India). The release of leflunomide from the microcapsules to the surrounding sink solution of pH 7.2 phosphate buffer media was studied. The concentration of leflunomide was determined spectrophotometrically at 260 nm and cumulative amount of the drug released was determined from the calibration curve. The dissolution studies were conducted on microcapsules in different particle size ranges. However, for the purpose of this reporting, the dissolution kinetics of the optimum size of 25 mesh was taken up for detailed kinetic studies.

Kinetics assessment of release data

The data obtained from the *in vitro* dissolution studies were analyzed in terms of different kinetics models (Pseudo first order, Zero order and Higuchi Model) and regression coefficients were compared.

RESULTS AND DISCUSSION

Drug entrapment and content uniformity studies

No significant differences in drug loading for microspheres made of different polymer solution viscosity were noted. However, the drug loading increases as the concentration of polymer is increased relative to drug concentration. The analysis of drug content showed a maximum entrapment efficiency of $91.7 \pm 0.62\%$ at the drug polymer ratio of 1:5. Overall, drug entrapments were found to range between $84.4 \pm 0.22\%$ and $91.7 \pm 0.62\%$.

Initially, solvents for polymer, concentration of polymer solution, temperature of the system and viscosity of liquid paraffin were optimized. The polymer concentration as well as the solvent concentration were assessed such that it would be easily pourable in the processing vessel and no pin holes would be present on the surface of the microcapsules. Liquid paraffin of high viscosity posed resistance to the microcapsules to assume a spherical shape. Low viscosity of the liquid paraffin led to gradual adherence of the microcapsules, resulting in the formation of an agglomerated mass. Application of heat to flash off acetone led to sudden agglomeration of the microcapsules and formation of pin holes in the microcapsules due to rapid evaporation of acetone. The uniformity of drug contents in each batch indicated reproducibility of the manufacturing method.

Particle size analysis

Results of sieve analysis revealed that microcapsules prepared were confined within 10-40-mesh sieve sizes and maximum amount of microcapsules were retained by 25-mesh sieve [Table 1]. The particle size of the microspheres using a different ratio of drug polymers differs significantly at the same stirring speed. There was formation of microspheres with large and irregular sieve due to an increase in solution

Table 1: Weight percentage distribution of different particle sizes of different batches

| Sieve no. | B1 | B2 | B3 | B4 | B5 |
|-----------|-------------|-------------|-------------|-------------|-------------|
| 10 | 4.1 ± 0.12 | 5.3 ± 0.21 | 6.2 ± 0.13 | 6.6 ± 0.22 | 6.7 ± 0.12 |
| 20 | 15.4 ± 0.08 | 16.4 ± 0.19 | 16.7 ± 0.11 | 17.4 ± 0.31 | 20.1 ± 0.23 |
| 25 | 46.1 ± 0.22 | 45.7 ± 0.31 | 48.3 ± 0.15 | 49.0 ± 0.16 | 51.2 ± 0.11 |
| 30 | 22.2 ± 0.09 | 21.5 ± 0.11 | 19.5 ± 0.22 | 19.0 ± 0.21 | 18.8 ± 0.10 |
| 40 | 12.2 ± 0.12 | 11.1 ± 0.16 | 9.3 ± 0.08 | 8.0 ± 0.13 | 9.2 ± 0.14 |

viscosity of the polymers. Hence, higher agitation speed is required to prepare microspheres of the same size as that of single polymers alone. Particle size distribution of different batches containing different concentration of polymer are shown in Table 1 and Figure 1.

Fourier transform infrared studies

FT-IR spectra study shows no change in the fingerprint region of pure drug spectra. This confirms the absence of drug to polymer interaction [Figure 2]. FT-IR spectra revealed that there was no such interaction between the drug and the polymers used for microsphere formulation.

Scanning electron microscopy study

SEM study shows that the particles made of Eudragit RL 100 and Eudragit RS. 100 were spherical.

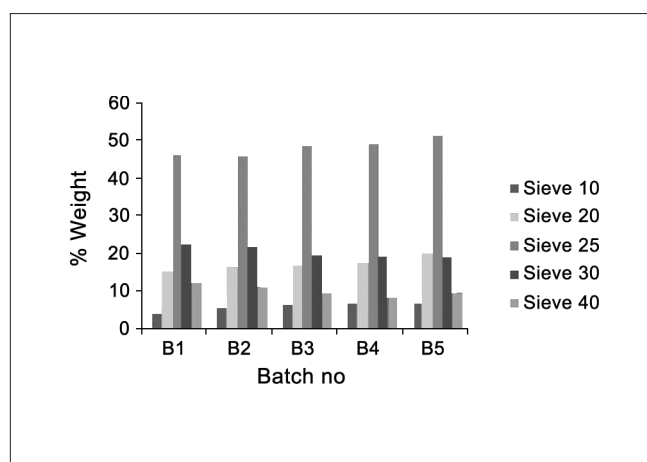
The SEM image of leflunomide-containing microcapsules is shown in Figure 3. The microcapsules were discrete, free-flowing and spherical. There was hardly any more visible, indicating a total enveloping of the core by the coat. But, at a higher concentration, pores were observed, which might have been formed during the solvent evaporation process. Presence of pores were detected on the surface, which increased in size and number with respect to time after dissolution, indicating leaching of the drug through these channels [Figure 3a and b].

In vitro dissolution studies

In vitro dissolution studies of all batches of microspheres were shown in Table 2. Microspheres made of Eudragit RS. 100 and RL100 showed good flow properties and maximum releasing tendency. Dissolution profiles of leflunomide from polymethacrylate microcapsules in pH 7.2 are depicted in Figure 4. The release of drug from microcapsules was gradual without producing a dose dumping effect and it was sufficiently prolonged as the coating thickness is increased. Table 2 shows that for the drug: Polymer ratio 1:1 and 1:2, linearity was best by using pseudo first order kinetics. The difference between first order diffusion models was noted to be minimal. For drug: Polymer ratio 1:3, 1:4 and 1:5, the best fit kinetic model was square root equation, i.e. Higuchi matrix diffusion. The combination polymer at polymer to polymer ratio 1:2 helps to leach out the drug from its matrices and exhibits an initial rapid drug release for the first 2-3 h and then a slower drug release, which can be best explained by Higuchi's spherical matrix release.

Table 2: Regression coefficient of different formulations in different models of kinetics

| Drug: polymer | Zero order | First order | Higuchi matrix diffusion |
|---------------|------------|-------------|--------------------------|
| B1 1:1 | 0.9974 | 0.9387 | 0.9787 |
| B2 1:2 | 0.9937 | 0.9446 | 0.9797 |
| B3 1:3 | 0.9891 | 0.9531 | 0.9945 |
| B4 1:4 | 0.9865 | 0.9465 | 0.9985 |
| B5 1:5 | 0.9892 | 0.9419 | 0.9973 |

**Figure 1: Sieve analysis**

Thus, on overall analysis of the kinetic data, it may be inferred that the release of leflunomide from the microcapsules was predominantly diffusion rate controlled and followed the Higuchi equation.

CONCLUSION

The present investigation of microcapsules of leflunomide was successfully prepared by using two polymers of different permeability characteristics (Eudragit RS. 100 and Eudragit RL 100). Eudragit RS. 100 has a firm binding capacity and Eudragit RL 100 has a tendency to loosen the firmness to hold the drug in microcapsules. Microspheres prepared with Eudragit RS. 100 alone have the drug release for a longer period of time with an initial slow release at the first period of time, with an initial slow release at the first hour and then controlled release for the remaining period of time. But, microspheres made of both the polymers at different ratios exhibited a satisfactory drug release pattern.

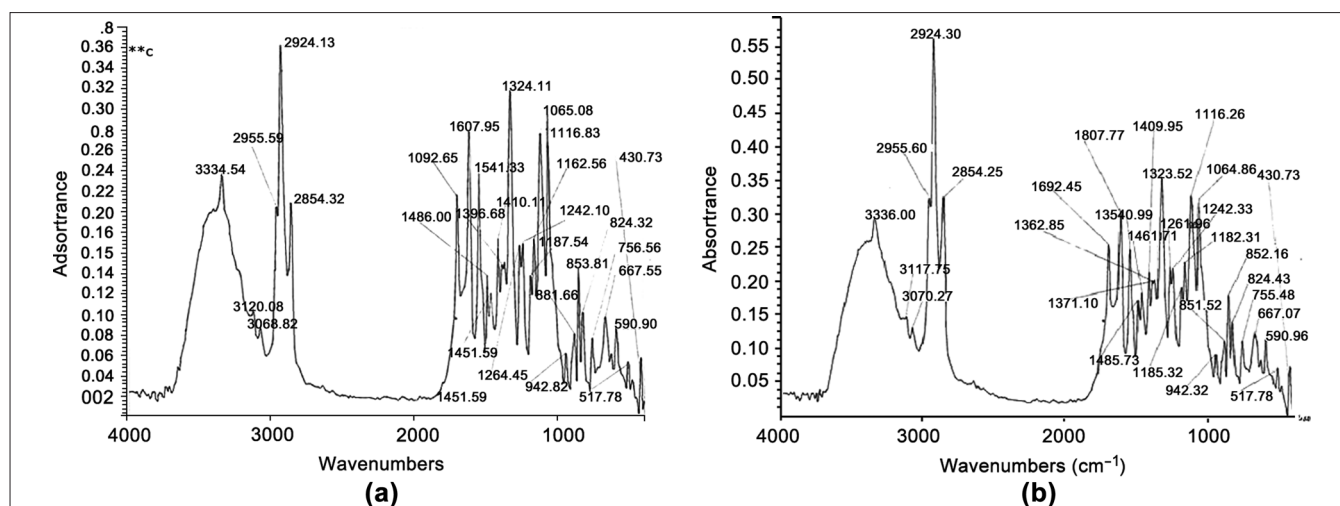


Figure 2: (a) Drug leflunomide; (b) Drug with polymer

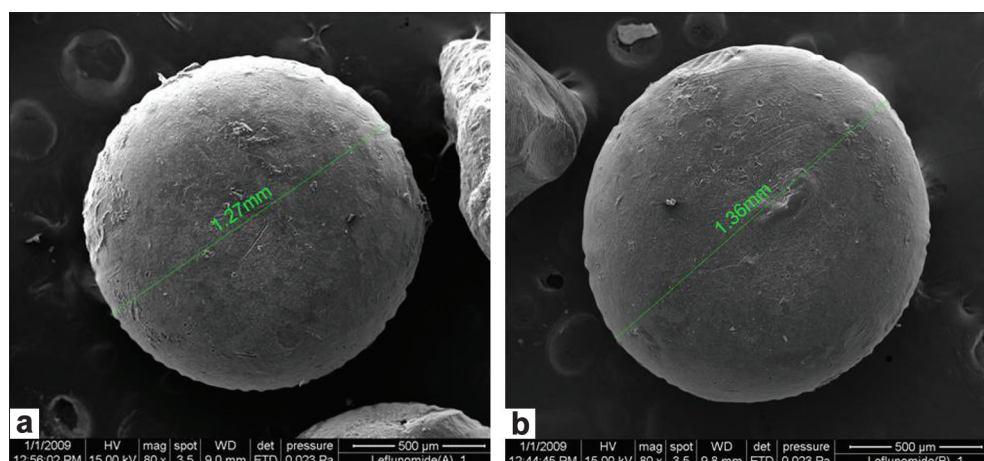


Figure 3: (a) Leflunomide microsphere after dissolution ($\times 80$); (b) Leflunomide microsphere before dissolution ($\times 80$)

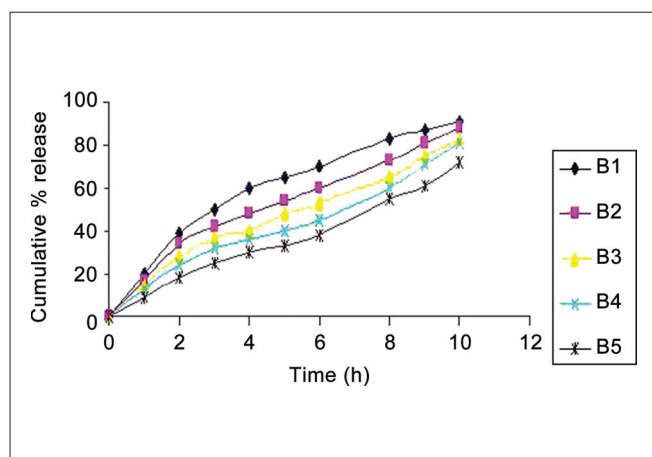


Figure 4: Dissolution profiles of leflunomide

Microcapsule formulations offer several advantages over other sustained release systems, especially matrix-type tablets, because they can be widely distributed throughout the gastrointestinal tract and produce a local high concentration of the drug at the absorption site. Therefore,

it may be concluded that drug-loaded microspheres are a suitable delivery system for leflunomide with a new choice of an economical, safe and more bioavailable formulation in the management of rheumatoid arthritis.

ACKNOWLEDGEMENT

We acknowledge the help received from the following organizations, i.e. Indian Association of Cultivation of Sciences, Government of India, Kolkata; Bose Institute, Kolkata; Central Drug Laboratory, Kolkata, for providing their active support in FT-IR, SEM and other analytical studies. We also acknowledge other supporting staff of our institute for providing all other facilities.

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Source of Support: Nil, **Conflict of Interest:** None declared.