Improved bioavailability through floating microspheres of lovastatin

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ABSTRACT

Background and purpose of the study: Lovastatin is an antihyperlipidemic agent which has low bioavailability due to the extensive first pass metabolism. It was sought to increase gastric retention of lovastatin by development of a sustained release gastroretentive drug delivery system leading to reduced fluctuation in the plasma concentration and improved bioavailability.

Methods: Floating microspheres were prepared by emulsion solvent diffusion technique, using various polymers and their blends. The in vitro performance was evaluated for drug-polymer compatibility, percent yield, particle size, drug entrapment efficiency, in vitro onset and duration of floatation, in vitro drug release as well as in vivo determination of serum cholesterol level.

Results: The mean particle size of microspheres was observed to be between 6.9 to 9.5 μm and the maximum particle size was around 50 μm. In vivo studies of the selected batches indicated lower level of serum cholesterol compared to the marketed tablet at the same dose but was not significant.

Major conclusion: The data obtained in this study suggested that a microparticulate floating dosage form of lovastatin can be successfully designed to yield controlled delivery with improved therapeutic efficacy.

Keywords: Floating drug delivery system, In vitro drug release, Microsphere

INTRODUCTION

Floating microspheres have been used as gastro-retentive system for controlled drug delivery (1), separation of incompatible substances (2), improvement in dissolution and bioavailability of drugs (2), protection of compound from atmospheric decomposition (2), masking unfavorable odor and taste (3), sustained and prolonged release of the drug (4), and as a bioadhesive microsphere for intestinal (5) and nasal administration (6), radiopaque hydrogel microspheres for X-ray contrast properties (7), drug targeting to a specific site or a particular organ in the body (e.g. lung) and even intracellular structures such as lysosomes and nucleus (8) and magnetically responsive microsphere system to localize the carriers and the entrap agents at specific in vivo targeting area (8). Lovastatin, an antihyperlipidemic agent inhibits the production of cholesterol by the liver. It lowers overall blood cholesterol as well as blood LDL cholesterol levels and undergoes extensive first pass metabolism. As a consequence of extensive hepatic extraction of lovastatin, the availability of the drug to the general circulation is low and variable (9). Moreover, conventional oral dosage form offers no control over drug delivery leading to fluctuations in plasma drug level. One of the approaches to improve the retention of oral dosage form in the stomach is to deliver lovastatin through hollow microspheres a non-effervescent gastroretentive drug delivery system to reduce fluctuation in the plasma concentration and to improve bioavailability. Eudragit L 100 and Eudragit S 100 are Poly(methacrylic acid, methyl methacrylate) 1 : 1 and Poly(methacrylic acid, methyl methacrylate) 1 : 2 respectively and therefore their solubility characteristics vary at different pH. Methocel K15 MP and ethocel standard 45 Pare cellulose derivatives with different aqueous solubility. Cyclodextrin has been used in the formulation to improve aqueous solubility of lovastatin. The present work is an effort to improve the bioavailability of lovastatin utilizing the floating microspheres for gastroretentive drug delivery. The objectives were preparation of floating microspheres of lovastatin by using pure polymers and blends of polymers, characterization and evaluation of the drug loaded floating microspheres and in vivo blood cholesterol level study of the formulated floating microspheres.

MATERIAL AND METHODS

Lovastatin was obtained as a gift sample from Sun
Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using the formula:

\[
\% \text{ yield} = \frac{\text{mass of microspheres obtained}}{\text{total wt. of drug and polymer used}} \times 100
\]

**Encapsulation efficiency**

A sample of 10 mg of microspheres was accurately weighed and dissolved in 10 ml of 0.1 N HCl. It was kept for 24 hrs to allow the dissolution of drug in the medium. After 24 hrs a specific dilution of 10 μg/ml was prepared for all batches and was assayed spectrophotometrically at 239 nm against a suitable blank. All determinations were carried out in triplicates. The encapsulation efficiency was then calculated using the formula:

\[
\% \text{ EE} = \left( \frac{\text{mass of incorporated drug in sampled microspheres}}{\text{mass of drug used for preparation of sampled microspheres}} \right) \times 100
\]

**Particle size and size distribution study**

The prepared microspheres were evaluated for particle size and size distribution by light microscopy using a calibrated eyepiece micrometer and a stage micrometer.

**Drug polymer compatibility study by DSC**

DSC Studies of drug loaded microspheres of different polymers were carried out by heating the samples from 40°C to 300°C at a rate of 10°C/minute using Differential Scanning Calorimeter.

**In vitro buoyancy study**

The floatation studies were carried out to ascertain the floating behavior of various polymer combinations. Micro balloons (10 mg) were dispersed in 0.1N

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**Figure 1.** Preparation of floating microspheres of Lovastatin using emulsion solvent diffusion method.

Dichloromethane (25 ml) and Ethanol (25 ml) were mixed

Lovastatin (drug) and Polymer (Cyclodextrin in some batches) were added dropwise with stirring

to 200 ml of 0.2 % SLS or to 200 ml of 1 % PVA

stirring for 2 hrs at 500 rpm

Microspheres were filtered washed and dried

Pharmaceutical Ind. Ltd (Mumbai, India). Eudragit S-100 and Eudragit L-100 were obtained from Röhm GmbH & Co. KG. (Darmstadt, Germany). Methocel K15MP and Ethocel standard 45 P were obtained from (Dow Inc), (USA). All other reactants were analytical grades. The microspheres were prepared by using emulsion solvent diffusion method as described by Kawashima et al, (1) and shown in figure 1. The drug, lovastatin and the polymer (Cyclodextrin was added as complexing agent in some batches) were dissolved or dispersed in a mixture of dichloromethane (25 ml) and ethanol (25ml) at room temperature and dropped into 200 ml of 0.2% sodium lauryl sulphate (batch A1-A14) or to 200 ml of 1%w/v of PVA (batch B1-B7) with constant stirring at 500 rpm for 2 hrs employing a propeller type agitator. The formed microspheres were filtered, washed with distilled water and dried at room temperature in a desiccator.

**Scanning of lovastatin**

A test solution was prepared (10μg/ml) and absorbance was measured at different wavelengths from 180 to 400 nm and maximum absorbance was determined at a wavelength of 239 nm.

**Preparation of standard plot of lovastatin in 0.1N HCl**

Stock solution of 100 μg/ml was prepared in 0.1N HCl and diluted to obtain concentrations of 0, 2, 4, 6, 8 &10 μg/ml and analyzed spectrophotometrically at 239 nm against suitable blank. The results were plotted to obtain calibration equation (Absorbance=0.06452*Conc.) and correlation coefficient (0.99941).

**Evaluation of floating microspheres**

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For Standard (S): 20 μl standard + 1000 μl working reagent.

The study was performed in batches (n=3). Blood samples were collected by one hours (Table 3). Wistar rats were used for the estimation of serum cholesterol level using autoanalyzer (Erba Chem-5-plus V2, Erba Diagnostics Mannheim Gmbh, Germany). An oral dose of 25mg/kg of the drug was calculated using the equation generated from standard curve (9).

**Stability study**

Stability study was carried out on formulated microspheres after storing at 40°C and 75% relative humidity for one month and then drug content was analyzed.

**RESULTS AND DISCUSSION**

The percentage yield for the batches was found to be greater than 55% whereas three batches A-6 (87%), A-13 (79%) and A-14 (75%) had a yield higher than 75% which may be attributed to the presence of HPMC or Eudragit S-100 which yielded uniform dispersion in dichloromethane / ethanol mixture and precipitated in their absences.

The encapsulation efficiency of the formulations varied from 42.3 to 51.2%. The lower encapsulation efficiency may be attributed to the drug leaching in the presence of cyclodextrin as well as partitioning of the drug in aqueous and nonaqueous phases of the mixture.

The thermograms obtained after DSC are shown in figure 3 for ethyl cellulose, methyl cellulose, β-cyclodextrin, pure drug, batch A-12 and batch B7 respectively. DSC indicated no physicochemical interaction or any other polymorphic change in the formulation (Table 1).

In vitro drug release study from the prepared microspheres was carried out for 4 hrs and the drug release was studied with respect to zero order and Higuchi matrix release parameters. Higher value of R² indicates higher co-relation coefficient for the best fit drug release model. It was observed that the drug release from the microspheres followed Higuchi matrix release (Table 2) which was in accordance with the expected result that the drug is embedded in polymer matrix and the release of drug takes place by diffusion from this matrix gel network. The drug release from different batches A1 to A7, A8 to A14 and B1 to B7 are shown in figures 4-6 respectively. Best four batches selected on the basis of their in vitro release were evaluated for their in vitro floating time and it was observed that about 63-72% of microspheres were floating even after one hours (Table 3).

The mean particle size of microspheres was observed to be between 6.9 to 9.5 μm although the maximum particle size were around 50 μm in batch A-12 and A-14 as shown in table 4. It was observed that the presence of β-cyclodextrin resulted in smaller particle size whereas microspheres prepared with methocel K15MPand HPMC yielded larger particle size with polydisperse properties.

The in vivo study showed insignificant results in controlling total cholesterol level in Wistar rats than the marketed standard (tablet). Based on student t-test value of 2.128 at p=0.05 the results are positive but insignificant (Table 5).

For Blank (B): 20 μl distilled water + 1000 μl working reagent.
For Standard (S): 20 μl standard + 1000 μl working reagent.
For Test (T): 20 μl serum + 1000 μl working reagent. The mixture incubated for 10 min at 37°C and absorbance was read at 510 nm (filter 1) and against the reagent blank (filter 2) using autoanalyser (15, 16).

HCl (20 ml, pH 1.2). The layer of floating and at the bottom microspheres were separated after 2 hrs and dried overnight. The buoyancy was determined by the weight ratio of floating to the total microspheres (11-14).

**In vitro release study**

In vitro release study of lovastatin from prepared microspheres was carried out using USP XXIII dissolution apparatus with paddle (Type 2) (in triplicates). The dissolution medium was 900 ml of 0.1N HCl solution (37 °C) and 0.2% SLS solution (rpm=50). The weighed amounts of microspheres were filled in the capsules and kept in wire mesh in order to avoid flotation. Five milliliters of sample was withdrawn at every hour and replaced with an equal volume of fresh dissolution medium and analyzed at λ_max 239 nm (9). The in vitro dissolution studies were carried out for a period of 4 hrs as it was speculated that any drug won’t stay for more than 4 hrs in gastric environment. The content of drug was calculated using the equation generated from standard curve (9).

**In vivo studies**

In vivo studies were performed for the selected best batch (A-4). Wistar rats were used for the estimation of serum cholesterol level using autoanalyzer (Erba Chem-5-plus V2, Erba Diagnostics Mannheim Gmbh, Germany). An oral dose of 25mg/kg of the body weight was administered and the cholesterol levels were measured in the test and formulation batches (n=3). Blood samples were collected by sacrificing of animals at specific time intervals and serum was separated. The study was performed taking lovastatin tablets as standard.

| Sample No | Name                  | Peak Temp. (°C) |
|-----------|-----------------------|-----------------|
| 1         | Lovastatin            | 173             |
| 2         | β-Cyclodextrin         | 141.06          |
| 3         | Ethocel               | 88.78           |
| 4         | Methocel              | 144.6           |
| 5         | A-12 (Methocel, Lovastatin) | 144, 172 |
| 6         | B-7 (Ethocel, Lovastatin) | 88.78, 173.05 |
Figure 2. Morphological characteristics of the best selected four batches
Figure 3. DSC thermogram of the pure drug, pure Ethocel standard 45, pure Methocel K15MP, pure β-Cyclodextrin, Batch A12 and Batch B7.

Figure 4. In vitro drug release from Batches (A1-A7) at different time intervals.

Figure 5. In vitro drug release from Batches (A8-A14) at different time intervals.
Figure 6. In vitro drug release from batches (B1-B7) at different time intervals.

Table 2: The batch specification of different batches of formulated microspheres and corresponding R² value.

| Batch No. | Drug (mg) | Cyclodextrin (mg) | Polymers (mg) | R² value | R² value |
|-----------|-----------|-------------------|---------------|----------|----------|
|           |           |                   |               | Zero order Equation | Higuchi Equation |
| A-1       | 100       | -                 | ES-100 (400)  | 0.9106   | 0.9744*  |
| A-2       | 100       | 50                | ES-100 (350)  | 0.9168   | 0.9846*  |
| A-3       | 100       | 100               | ES-100 (300)  | 0.9041   | 0.9640*  |
| A-4       | 100       | 100               | ES-100 (200)  | 0.9206   | 0.9751*  |
| A-5       | 100       | 100               | ES-100 (150)  | 0.9059   | 0.9597*  |
| A-6       | 100       | 150               | ES-100 (250)  | 0.7300   | 0.9437*  |
| A-7       | 100       | 150               | ES-100 (200)  | 0.9677*  | 0.9673   |
| A-8       | 100       | -                 | EL-100 (400)  | 0.8388   | 0.9870*  |
| A-9       | 100       | 100               | EL-100 (300)  | 0.8132   | 0.9661*  |
| A-10      | 100       | -                 | HPMC (400)    | 0.9373   | 0.9684*  |
| A-11      | 100       | 100               | MC (300)      | 0.6700   | 0.9052*  |
| A-12      | 100       | -                 | MC (400)      | 0.7289   | 0.9443*  |
| A-13      | 100       | 100               | HPMC (150)    | 0.8314   | 0.9852*  |
| A-14      | 100       | 100               | HPMC (400)    | 0.8597   | 0.9675*  |

* The best fit model

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|           |           |                   |               | Zero order equation | Higuchi equation |
| B-1       | 100       | -                 | ES-100 (400)  | 0.8289   | 0.9778*  |
| B-2       | 100       | -                 | MC (400)      | 0.8453   | 0.9818*  |
| B-3       | 100       | 100               | HPMC(300)     | 0.8159   | 0.9773*  |
| B-4       | 100       | -                 | HPMC (400)    | 0.8151   | 0.9784*  |
| B-5       | 100       | -                 | MC: HPMC (1:1)| 0.7645   | 0.9596*  |
| B-6       | 100       | -                 | EC(400)       | 0.8625   | 0.9736*  |
| B-7       | 100       | 100               | EC(300)       | 0.8557   | 0.9752*  |

* The best fit model
an outer shell composed of the drug and polymer. They appeared to be hollow presumably because of the rapid escape of volatile solvent from the polymer matrix. This hollow nature was also responsible for the rapid escape of volatile solvent from the polymer matrix. This hollow nature was also responsible for stability study was carried out on formulated microspheres after storing at 40°C and 75% relative humidity for one month and no appreciable change were found in drug contents.

Lovastatin, a lipid lowering agent has oral bioavailability of only 5% and based on physicochemical nature of the drug it was expected that its oral bioavailability may be enhanced if the duration of stay in GIT is increased. To overcome the low water solubility, cyclodextrin was added as a complexing agent to some batches to increase the solubility of the drug in GIT fluid prior to absorption. The solubility and dissolution rates of lovastatin inclusion complexation (17). Microspheres showed a particle size range between 3-61 μm. Batch no. A-4 (containing the ES-100 polymer and EL-100 along with cyclodextrin) released 42.3% of drug. Drug entrapment in microspheres was higher for batch A-1 (51.2%) and A-4 (49.71%). Maximum buoyancy was shown by A-12 (methocel K15MP was used as the polymer) although batch A-4 showed buoyancy in more than 30% of microspheres even after 4 hrs. In vivo study for best selected batch (A-4) was performed and lower level of cholesterol was obtained by using this formulation compared to the standard tablet but the results did not pass the student t-test and requires further investigation.

**CONCLUSION**

Lovastatin, a lipid lowering agent has oral bioavailability of only 5% and based on physicochemical nature of the drug it was expected that its oral bioavailability may be enhanced if the duration of stay in GIT is increased. To overcome the low water solubility, cyclodextrin was added as a complexing agent to some batches to increase the solubility of the drug in GIT fluid prior to absorption. The solubility and dissolution rates of lovastatin were significantly increased by using cyclodextrin inclusion complexation (17). Microspheres showed a particle size range between 3-61 μm. Batch no. A-4 (containing the ES-100 polymer and EL-100 along with cyclodextrin) released 42.3% of drug. Drug entrapment in microspheres was higher for batch A-1 (51.2%) and A-4 (49.71%). Maximum buoyancy was shown by A-12 (methocel K15MP was used as the polymer) although batch A-4 showed buoyancy in more than 30% of microspheres even after 4 hrs. In vivo study for best selected batch (A-4) was performed and lower level of cholesterol was obtained by using this formulation compared to the standard tablet but the results did not pass the student t-test and requires further investigation.

**REFERENCES**

1. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J Pharma Sci. 1992; 81: 135-140.
2. Burgass DJ, Hickey, AJ, Swarbrick J, Boylan JC. Microsphere process and Technology. In: Swarbrick J, Boylan JC, ed. Encyclopedia of Pharmaceutical Technology, New York: Marcel Dekker; 1995; 1-29.
3. Sugao H, Yamazaki S, Shiozawa H, Yano K. Taste masking of nitter drug powder without loss of bioavailability by heat treatment of wax coated microspheres, J Pharm. Sci. 1998; 87: 96-100.
4. Orienti I, Aiedeh K, Gianasi E, Bertasi V, Zecchi V, Indomethacin loaded chitosan microspheres:

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**Table 3. In vitro buoyancy behavior of different batches at different time intervals.**

| Sample No | Batch No. | Percent of floating microspheres±SD (n=3) |
|-----------|-----------|-------------------------------------------|
|           |           | After 1 hr | After 2 hrs | After 4 hrs |
| 1         | A-1       | 63.2±0.4   | 44.5±0.6   | 26.2±0.3   |
| 2         | A-4       | 66.3±0.6   | 53.3±0.4   | 31.7±0.8   |
| 3         | A-12      | 72.1±0.3   | 57.7±0.6   | 38.3±0.5   |
| 4         | A-14      | 69.3±0.2   | 51.3±0.3   | 33.5±0.6   |

**Table 4: Particle size distribution of selected batches of microspheres containing lovastatin.**

| Sample No | Batch no. | Mean Particle Size (μm) | Size Range (μm) |
|-----------|-----------|-------------------------|-----------------|
|           |           |                          | 0-10  | 10-20 | 20-30 | 30-40 | 40-50 |
| 1         | A-1       | 9.3                     | 73    | 15    | 8     | 4     | -     |
| 2         | A-4       | 6.9                     | 87    | 9     | 2     | 2     | -     |
| 3         | A-12      | 9.25                    | 69    | 22    | 4     | 3     | 1     |
| 4         | A-14      | 9.5                     | 75    | 14    | 3     | 7     | 1     |

**Table 5: Serum cholesterol level of the standard and the formulation with respect of time.**

| Sample No | Time (hrs) | Cholesterol level for two groups |
|-----------|------------|---------------------------------|
|           |            | Standard | Formulation |
| 1         | 0.5        | 143.3    | 137.4       |
| 2         | 1          | 134.2    | 123.7       |
| 3         | 2          | 129.3    | 116.2       |
| 4         | 3          | 118.4    | 112.11      |
| 5         | 4          | 103.7    | 91.3        |
Correlation between erosion process and release kinetics, J. Microencapsul. 1996; 13: 463-472.
5. Chickering DE 3rd, Harris WP, Mathiowitz E, A microtensinometer for the analysis of bioadhesive microspheres, Biomed instrum Technol. 1995; 29: 501-512.
6. Bjork, E, Edman P, Degradable starch microspheres as a nasal delivery system for insulin, Int. J. Pharm. 1988; 47: 233-238.
7. Thanoo BC, Jayakrishnan A, Radioopaque hydrogel microspheres, J. Microencapsulation. 1989; 16: 233-244.
8. Polard E, Lecorre P, Chevanne F, and Le Verage R, In vitro and in vivo evaluation of polylactide and polylactide-coglycoside microspheres of morphine for site specific delivery, Int. J. Pharm. 1996; 134: 37-46.
9. USP28-NF23 Page 1156. Pharmacopeial Forum: Volume No. 29 (5) page 1525.
10. Joseph NJ, Laxmi S, Jayakrishan A, A floating type oral dosage form for piroxicam based on hollow microspheres: in vitro and in vivo evaluation in rabbits, J Control Release. 2002; 79: 71-79.
11. Yang L, Fassihi R, Zero order release kinetics from self correcting floatable configuration drug delivery system, J Pharm Sci. 1996; 85:170-173.
12. Fell John T, Whitehead L, Collet JH, Prolonged gastric retention using floating dosage forms, Pharm Tech. 2000; 3: 82-90.
13. Whitehead L, Collet JH, Fell JT, Amoxicillin release from a floating dosage form based on alginates, Int J Pharm 2000; 210:45-49.
14. Hilton AK, Deasy PB, In vitro and in vivo evaluation of an oral sustained release floating dosage form of amoxicillin trihydrate. Int J Pharm. 1992; 86:79-88.
15. Patel JK, Patel RP, Amin AF, Patel MM, Formulation and Evaluation of Mucoadhesive Glipizide Microspheres, AAPS PharmSciTech. 2005; 6: E49-55.
16. Meyer BJ, Hammervold T, Rustan AC, Howe PR, Dose-Dependent Effects of Docosahexaenoic Acid Supplementation on Blood Lipids in Statin-Treated Hyperlipidaemic Subjects, Lipids. 2007; 42:109-115.
17. Mehramizi A, Asgari ME, Pourfarzib M, Bayati Kh, Dorkoosh FA, Rafiee-Tehrani M, Influence of β-cyclodextrin complexation on lovastatin release from osmotic pump tablets (OPT), DARU. 2007;15: 71-78.