# *Invitro* and *invivo* studies on controlled release microspheres of Simvastatin

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**Abstract**. The present study involved design and *invitro –invivo* evaluation of simvastatin microspheres with ethylcellulose as controlled release polymer. Simvastatin – ethylcellulose microspheres were prepared by water-in-oil-in-oil double emulsion solvent diffusion method and evaluated for entrapment efficiency, *in vitro* drug release behavior, particle size and size distribution. The formula was optimized by changing the polymer-drug ratio, surfactant concentration for secondary emulsion process and stirring speed of emulsification process. The designed microspheres were spherical, free flowing and size distribution was between 24-48 μm. The entrapment efficiency and percentage yield were 83.67% & 84.31% respectively. The drug release was controlled for 12 h. The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicated that the release is diffusion controlled mechanism. Absence of drug-polymer interaction was also evidenced by IR & DSC thermograms. *In vivo* pharmacodynamics study of the optimized formulation proved that prolongation of drug release by presently designed microspheres.

**Keywords**: Ethyl cellulose, *in vitro-in vivo*, microspheres, simvastatin, w/o/o double emulsion

## 1. Introduction

Microspheres can be defined as solid approximately spherical and polymeric particles ranging in size from 1 to 100 μm. They are made from polymeric, waxy or other protective materials such as natural, semisynthetic and synthetic polymers and are used as drug carrier matrices for drug delivery. Simvastatin (a hypolipidemic class of drug) is a water insoluble drug and is a HMG-CoA reductase inhibitor. It is widely used in the treatment of hyperlipidemia and dyslipidemia. The half life of the drug is 3 hrs, usual oral dosage regimen is 5-80 mg/ day. Thus to achieve maximum therapeutic effect with a low risk of adverse effects and to improve patient compliance a controlled release formulations of simvastatin is required (Swarbrick J., 2001). Though statins have been associated with adverse side effects including hepatic toxicity and myopathy including rhabdomyolysis , the use of a controlled drug delivery system may eliminate them ( Bottorff M B., 2006). So far, many film coated tablets of simvastatin were available in the market. But no other approaches like extended release formulations are attempted, which provide more advantages than the conventional tablets.

### 1.1. Objectives

The purpose of the present work was to prepare and evaluate oral controlled release microparticulate drug delivery system of simvastatin using ethyl cellulose by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion method with high entrapment efficiency and extended release. To optimize the various processes and formulation parameters such as drug-polymer ratio, surfactant concentration and stirring speed for maximizing the entrapment and prolong release. To evaluate the drug content, *in vitro, invivo* drug release, drug-polymer interactions, micromeritic properties, shape and surface morphology.

## 2. Materials and methods

## 2.1. Materials

Simvastatin was received as a gift sample from KREBS Pvt. Ltd., India, Ethyl cellulose (14 cps viscosity grade), dichloromethane, acetonitrile, light paraffin and pet. ether were also used in the study. All the reagents and solvents used were of analytical grade and were obtained from S.D. Fine Chem. Ltd., Mumbai, India.

## 2.2. Preparation of microspheres by double emulsion solvent diffusion method

Simvastatin microspheres were prepared according to the given formulae in table 1 by the w/o/o double emulsion solvent diffusion method.  After agitation a fine emulsion (primary w/o emulsion) was formed, then  it  was slowly added to light liquid paraffin containing span 80 as a surfactant with constant stirring for 2 h.  The pet ether (10 ml.) was added to harden the formed microspheres.  The resulting microspheres were separated by decantation, by repeated washing with pet ether. Different ethylcellulose: simvastatin ratios (1:0.01, 1:0.02, 1:0.04, 1:0.08 & 1:0.16) were used in order to investigate the effect of polymer: drug ratio on release and the entrapment efficiency of microspheres. Various concentrations of span 80 (0.1%, 0.3% and 0.5%) and various stirring speeds (300,500 and 700 rpm) were used to investigate the influence of surfactant concentration and effect of stirring rate on release and entrapment efficiency of microspheres

## 2.3. Characterization

### 2.3.1. Size, shape and surface analysis (Nappinnai M., 2007)

Particle sizes of all formulations were carried out by optical microscopic technique. The surface morphology of the microspheres were investigated by using scanning electron microscope model (SEM), Hitachi 5520, at 10 kv.

### 2.3.2. Flow properties

The flow properties of microspheres were characterized in terms of angle of repose, carr's index and hausner's ratio (Sinha., 2005).

### 2.3.3. Entrapment efficiency (Das MK., 2007)

The amount of drug entrapped was calculated by using the formula, drug entrapment efficiency = (experimental drug content/theoretical drug content) ×100….(1). The percentage yield of microsphere was also calculated based on the quantities of polymer, drug used in microspheres.

### 2.3.4. Interaction studies:

Drug-polymer interactions were studied by UV spectroscopy, IR spectroscopy and DSC analysis

### 2.3.5. In vitro release studies (Fude cui., 2006)

The USP paddle type dissolution test apparatus was used for all prepared formulations. Percentage drug dissolved and dissolution rate of selected prepared microspheres (F8) was compared with marketed film coated tablets of simvastatin (Ranboxy Laboratoies). All the release studies were carried out in triplicate.

### 2.3.6. Release kinetics

In order to investigate the mechanism of simvastatin release from microsphere prepared,  the release data was analyzed with the following mathematical models, zero order equation, first order equation, Higuchi equation and Korsmeyer-Peppas equation

### 2.3.7. In vivo release studies

Wistar strain of albino rats weighing 150-200g were used in the study. Except for the normal group, all other group animals were fed with high fat diet (HFD) for 7 days to confirm the induction of hyperlipidemia. The rats were then divided into 5 groups of 6 animals each (Na Young Yoon., 2008). Group I was a normal control group, served with normal diet. Group II was a fat diet control group, did not receive any treatment. Group III, IV and V received blank microspheres, reference tablet (SIMVOTIN) and test formulation along with fat diet respectively. The microspheres were administered to the rat by oral gavages. Blood samples were withdrawn from retro-orbital puncture at predetermined time intervals (Pradeep P., 2007). Plasma samples were separated by centrifugation and analyzed for TC, HDL-C and TG using in vitro diagnostic kits (Med source, India)..

## 3. Results and discussion:

Microspheres for formulations of F1, F2, F3, F4 & F5 were prepared with different drug-polymer concentrations of 0.01:1, 0.02:1, 0.04:1, 0.08:1 and 0.16:1 respectively (Table 1). The highest entrapment efficiency was achieved with F2 formulation, further increasing in drug- polymer ratio significantly decreased the entrapment efficiency (P<0.01 ANOVA test). This is because of high drug/polymer ratio caused a coarse covering, likely due to drug's residue that has not been surrounded by polymer thoroughly (Solmaz D., 2010)

TABLE 1: EFFECT OF VARIOUS PARAMETERS ON MEAN PARTICLE SIZE AND ENTRAPMENT EFFICIENCY.

| Formulation Code | Formulation Parameter | Entrapment efficiency % | % Yield | Mean Particle size µm | % Drug release |
|---|---|---|---|---|---|
| | *Ethyl cellulose : Simvastation* | | | | |
| F1 | 1:0.01 | 61.8 | 73.26 | 24 | 72.35 |
| F2 | 1:0.02 | 77.95 | 86.23 | 32 | 80.23 |
| F3 | 1:0.04 | 76.82 | 84.6 | 38 | 79.12 |
| F4 | 1:0.08 | 57.9 | 75.92 | 46 | 60.63 |
| F5 | 1:0.16 | 55.98 | 55.17 | 48 | 57.36 |
| | *Surfactant Concentration (%W/V)* | | | | |
| F2 | 0.1 | 77.95 | 86.23 | 32 | 80.23 |
| F6 | 0.3 | 54.08 | 76.47 | 29 | 77.94 |
| F7 | 0.5 | 52.04 | 70.58 | 25 | 79.34 |
| | Stirring speed of Secondary emulsification (rpm) | | | | |
| F2 | 300 | 77.95 | 86.23 | 32 | 80.23 |
| F8 | 500 | 83.67 | 84.31 | 34 | 86.37 |
| F9 | 700 | 79.08 | 78.62 | 32 | 81.32 |

F1 to F5 formulations vary in polymer to drug ratio, F2,F6 and F7 formulations vary in surfactant concentrations with constant polymer to drug ratio (1:0.02) and F2, F8 and F9 formulations differ in stirring speeds of emulsifications with same formulae.
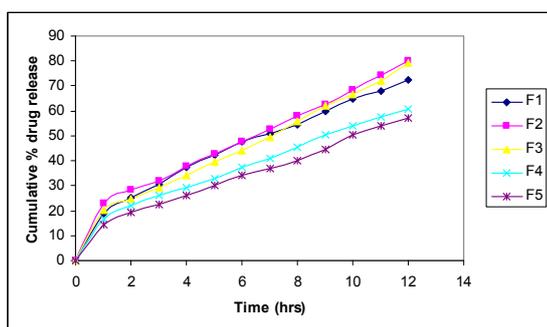


Fig. 1: Comparative *in vitro* dissolution profiles of simvastatin from microspheres prepared with different polymer – drug ratios include F1-1:0.01; F2-1:0.02; F3- 1:0.04 F4-1:0.08,andF5-1:0.16
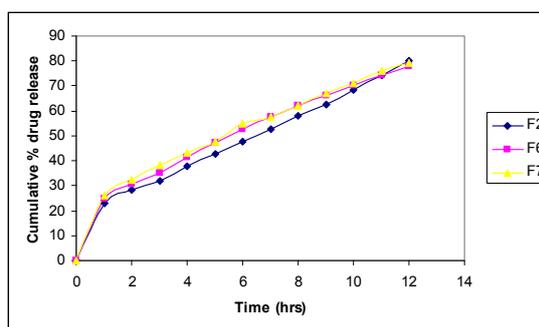


Fig.2: Comparative *in vitro* dissolution profiles of simvastatin from microspheres prepared with different concentration of surfactant span 80 include F2-0.1%; F6-0.3% and F7-0.5%.

Fig.3 : SEM Photographs showing surface morphology of simvastatin – loaded miscrospheres.

Table No. 2: Regression values of corresponding kinetic equations

| Formula-tion code | Zero order | First order | Higuchi plot | Korsemeyer Peppas plot | |
|---|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ | n |
| F2 | 0.9365 | 0.9852 | 0.9898 | 0.9715 | 0.4554 |
| F8 | 0.9516 | 0.9599 | 0.9885 | 0.9725 | 0.4917 |

Table No. 3: Micromeritic properties of the prepared microspheres.

| Code No | Angle of repose±S.D | Bulk density±S.D | Tapped density±S.D | Carr's index±S.D | Hausner's ratio±S.D |
|---|---|---|---|---|---|
| F | 24.98±0.21 | 0.4±0.24 | 0.44±0.15 | 9.0±0.43 | 1.1±0.19 |
| F2 | 28.05±0.52 | 0.48±0.18 | 0.54±0.25 | 11.1±0.36 | 1.13±0.23 |
| F8 | 20.55±0.24 | 0.58±0.26 | 0.68±0.36 | 14.3±0.51 | 1.17±0.21 |

Fig.4 : DSC thermogram of simvastatin
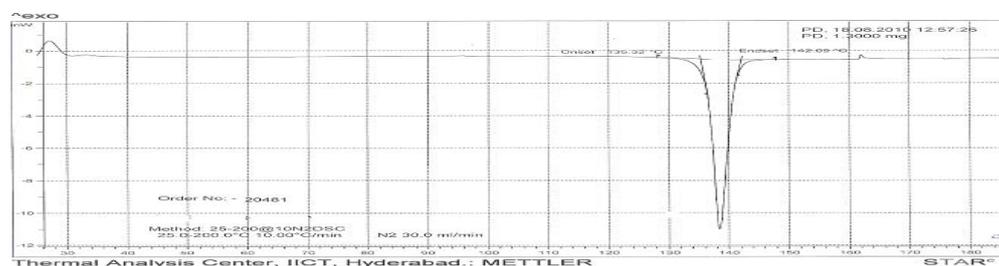


Drug release rates were decreased with increasing amounts of simvastatin in the formulation may be due to decreased entrapment efficiency with increased simvastatin in formulation. The mean particle size of these formulations F1, F2, F3, F4 & F5 was within the range of 24 μm to 48 μm.

There was significant ($P<0.01$, ANOVA test) decrease in encapsulation efficiency of microspheres with increasing the concentration of surfactant for secondary emulsification. This may be due to the fact that the increase in surfactant concentration proportionately increases miscibility of acetonitrile with liquid paraffin

(processing medium) which may increase the extraction of simvastatin into the processing medium. As the concentration of span 80 was increased, a faster drug release rate was observed (Figure 2). This may be attributed to the presence of more free drug on the surface of the microspheres with the increasing the concentration of span 80 used for secondary emulsification (Das MK., 2007).

There was significantly (P<0.01, ANOVA test) decreased entrapment efficiency (Table 1) was observed with the change of stirring speed from 500 rpm to 300 and 700 rpm may be due to the formation of larger and smaller emulsion droplets respectively ensuring the drug diffusion out of the microspheres before they harden.

The dissolution rate of selected prepared formulations F2 and F8 were compared with that of marketed conventional tablet. The F8 formulation shown prolonged release (86%) up to 12 hrs, but the marketed tablet released 97% of drug within 2.5 hrs.

Further, to confirm the mechanism of drug release, F2 and F8 were subjected to release kinetic studies and based on its respective $R^2$ values as given in table no.2, the best fit with the highest correlation coefficient was shown in Higuchi, first order equation as the regression value is closer to unity in case of First-order plot ($R^2$ = 0.9852, 0.9599). So, the release was apparently first order and the drug release from ethyl cellulose microspheres was diffusion controlled. The microspheres were found as spherical in shape and free flowing by surface topography investigation using SEM (Fig 3).

The Carr's index and Hausner's ratio were calculated, and the ranges were 9.0 to 14.3 & 1.1 to 1.17 respectively as shown in table no.3, indicated prepared microspheres possessed excellent or good flow properties. Based on the results of micromeritic properties it can be concluded that the prepared microspheres have good flow properties.

The compatibility of simvastatin with ethyl cellulose in microspheres was evaluated through UV spectroscopy, IR and, DSC analysis. The absorption bands of functional groups for drug loaded microspheres were almost similar to those obtained for the pure drug. The DSC profile of simvastatin-loaded microspheres showed a peak at the temperature corresponding to simvastatin melting point as shown in fig.4 but with the loss of its sharp appearance may be due to reduced drug crystallinity or may be due to combined melting points of drug and the components of microspheres . It confirmed that there was no interaction between drug and polymer.

Then the optimized best formulation 'F$_8$' was used for evaluation of its *in vivo* performance using rats. The *in vivo* performance of simvastatin was evaluated using its pharmacodynamics effects (Ambike A A., 2005).

In the present study, the hypolipidemic effect of simvastatin microspheres in a rat model in which hyperlipidemia was induced by a high-cholesterol diet was determined. Rats fed a cholesterol-rich diet showed an elevated TC, TG, and reduced HDL-C levels. However, the oral administration of drug loaded microspheres resulted in decreased levels of TC, TG and a slight elevation of HDL-C levels. On the other hand TC, TG, HDL-C levels with blank microspheres on hyperlipidemia were equivalent to the HFD group indicated least possible effect of blank microspheres without the drug.

## 4. Conclusion

The attempts to prepare controlled release microspheres of simvastatin with highest entrapment efficiency and prolonged release were successfully characterized by *invitro and invivo* methods, which suggested that the double emulsion- solvent diffusion method is an efficient technique for use with poorly water- soluble drug.

## 5. References

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