Formulation and Evaluation of Floating Microspheres of Verapamil Hydrochloride.

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ABSTRACT

Microencapsulation by the solvent evaporation method is a complex process, which can be influenced by many process parameters like solvent evaporation rate, temperature, solubility of polymer, drug and excipients in both emulsion phases, dispersion stirring rate, viscosity, solubility, volume and volume ratio between the inner and outer phases, the quantity of polymer and drug and the physico-chemical properties and concentration of the stabilizers. In this present study Floating microspheres were prepared satisfactorily by solvent evaporation technique using Carbopol, Eudragit S100 and HPMC K4M. The entrapment efficiency was found to be good and that was above 90% and the in vitro buoyancy was more than 54% after 10 hours indicated satisfactory performance of proposed formulations. The percent buoyancy was found to be 83% in formulation. The mean particle size of microspheres was in the range of 0.221 ± 0.53 to 1.211 ± 0.81 mm depending upon the type of polymer used. The particle size increased significantly as the speed of stirrer decreased. The flow properties of all the prepared microspheres were good as indicated by low angle of repose and low compressibility index. In-vitro release of microspheres of Verapamil hydrochloride was found to be 91% optimized batch in a proper manner within 12 hours.

KEYWORDS: Solvent evaporation, Verapamil hydrochloride, Carbopol, Eudragit S100 and HPMC K4M.

INTRODUCTION:

Various microsphere preparation methods were reported which can be used to incorporate drug in microspheres. Due to microscopic size, microspheres can be used alone or incorporated in other drug delivery systems and are suitable for various routes of application. Microencapsulation by the solvent evaporation method is a complex process, which can be influenced by many process parameters, e.g. solvent evaporation rate,2 temperature,3 solubility of polymer, drug and excipients in both emulsion phases,4 dispersion stirring rate,5 viscosity, solubility, volume and volume ratio between the inner and outer phases,6 the quantity of polymer and drug,7 and the physico-chemical properties and concentration of the stabilizers. Verapamil hydrochloride is a calcium channel blocker that is an anti-arrhythmia agent. It has been shown to be effective and safe alone or in combination, in patients with hypertension and/or coronary artery diseases. Verapamil hydrochloride is having faster absorption of drug in stomach with higher concentrations entering in plasma and hence improving its bioavailability.8 So there is need to formulate novel dosage form of Verapamil hydrochloride which will be gastroretentive and will increase the absorption of drug. Floating drug delivery is able to prolong the gastric retention of microspheres and thereby possibly improve oral bioavailability of Verapamil hydrochloride. The aim of this work is to develop and evaluate floating microspheres of Verapamil hydrochloride whose physicochemical properties and short half-life make it suitable candidate for floating drug delivery system.

MATERIALS AND METHODS:

Materials:

Verapamil Hydrochloride was obtained as gift sample from Emcure Pharmaceuticals Pvt. Ltd., Pune (India). Dichloromethane, Ethanol, Carbopol, Eudragit S100 and HPMC K4M were purchased from Rajesh Chemicals, Mumbai (India). All chemicals used were of analytical grade.

Methods:

Formulation of Floating microspheres

Floating microspheres loaded with Verapamil Hydrochloride was prepared using Solvent Evaporation method using Carbopol, Eudragit S100 and HPMC K4M. Drug and polymer in proportion of 1:1 was dissolved in 1:1 mixture of solvent system of dichloromethane and ethanol. This clear solution was poured slowly in 150 ml liquid
paraffin containing Tween 80. The emulsion was continuously stirred for three hours at speed of 800-1000 rpm. Floating microspheres were collected, filtered and dried at room temperature.

Sixteen formulations were prepared by using various polymers with same concentrations of drug and three different polymers. The powder blend (drug with polymer) was co-dissolved at room temperature into a mixture of ethanol and dichloromethane (1:1% v/v) with vigorous agitation to form uniform drug-polymer dispersion. This was slowly poured into the dispersion medium consisting of light liquid paraffin (150ml) containing tween80. The system was stirred using over head propeller agitator at 800-1000 rpm and room temperature over a period of 3-4 hrs, to ensured complete evaporation of the solvent. The liquid paraffin was decanted and the microspheres were separated by filtration through a whatmann filter paper, washed thrice with 180 ml of n-Hexane and air dried for 24 hrs. All batches were prepared in triplicate. Bead size was determined manually by the method of microscopy (table 1).

**Table 1: Formulation Codes with quantities**

<table>
<thead>
<tr>
<th>FC</th>
<th>Drug</th>
<th>Ethanol</th>
<th>DCM</th>
<th>HPMC</th>
<th>Carbapol</th>
<th>ES-100</th>
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<tr>
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<td>100mg</td>
<td>5ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
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<td>-</td>
<td>5ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>100mg</td>
<td>5ml</td>
<td>-</td>
<td>100mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>100mg</td>
<td>5ml</td>
<td>-</td>
<td>-</td>
<td>100mg</td>
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</tr>
<tr>
<td>F5</td>
<td>100mg</td>
<td>5ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100mg</td>
</tr>
<tr>
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<td>-</td>
<td>5ml</td>
<td>100mg</td>
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<td>F7</td>
<td>100mg</td>
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<td>100mg</td>
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<td>F8</td>
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<td>100mg</td>
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<td>-</td>
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<td>2.5ml</td>
<td>-</td>
<td>-</td>
<td>100mg</td>
</tr>
<tr>
<td>F13</td>
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<td>2.5ml</td>
<td>2.5ml</td>
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<td>-</td>
</tr>
<tr>
<td>F14</td>
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<td>2.5ml</td>
<td>100mg</td>
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<td>F15</td>
<td>100mg</td>
<td>2.5ml</td>
<td>2.5ml</td>
<td>100mg</td>
<td>100mg</td>
<td>100mg</td>
</tr>
<tr>
<td>F16</td>
<td>100mg</td>
<td>5ml</td>
<td>5ml</td>
<td>100mg</td>
<td>100mg</td>
<td>100mg</td>
</tr>
</tbody>
</table>

**FC:** Formulation Code, **DCM:** Dichloromethane, **ES-100:** Eudragit RS 100, **HPMC:** Hydroxy propyl Methyl Cellulose-K4M

**Evaluation of Microspheres**

**Yield and Entrapment Efficiency**

The prepared microspheres were collected and weighed. Percentage Yield was determined by following formula.

*Percentage Yield = (Actual weight x 100)/ Theoretical Weight*

Weighed quantity of microspheres (50 mg) were triturated and transferred to 100 ml of 0.1N HCl. The solution was stirred for 8 hours at 500 rpm. Then the solution was filtered with 0.45 micron membrane filter. By making suitable dilutions the drug content was determined spectrophotometrically at 278 nm by using UV-Visible spectrophotometer, UV-1800, Shimadzu, Japan.

**Entrapment efficiency (EE) for each batch was determined by following formula.**

*Percentage Entrapment Efficiency = (Drug loading / Theoretical drug loading) x 100*

**Micrometric properties**

Diameters of the dried microspheres were measured by optical microscopy. Bulk density and tap density was determined according to following method:

A 50 ml glass cylinder was weighed and filled with 30 ml of sample and reweighed. The opening was secured with parafilm. The cylinder was gently reversed once and the powder was carefully leveled without compacting. Bulk volume was determined after one mechanical tap on a tap density tester (Dolphin™). Tap volume was measured after 2000 taps. Each analysis was repeated twice. Values of bulk density and tap density used to calculate Carrs index. The flow behavior of microspheres were determined by Angle of repose. Fixed funnel method was used for determination of angle of repose.

**In vitro Buoyancy studies**

The floating microspheres (100 mg) were spread over the surface of the dissolution medium (simulated gastric fluid (SGF), pH 1.2 containing 0.02 %w/v of Tween 20) that was agitated by a paddle rotated at 100 rpm. After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately. After drying, each fraction of the microspheres was weighed and their buoyancy was calculated by the following equation.

*Percent Buoyancy = [Qf / (Qf + Qs)] x 100*

Where, Qf and Qs are the weight of the floating and the settled microspheres respectively.

**Scanning Electron Microscopy**

The surface morphology of microspheres were determined by Scanning Electron Microscopy using a JEOL JSM-6360 scanning microscope (Germany). Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres were taken by random scanning of the stub.

**X-Ray Diffraction Analysis (XRD)**

X-Ray diffraction pattern of pure drug, physical mixture and opti-
Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transforms Infrared spectroscopy of pure drug, and optimized batch F16 was recorded using Jasco V5300 (Jasco, Japan) FTIR system using potassium bromide (KBr) pellet method. Each spectrum was derived from single average scans collected in the region 4000 to 400 cm⁻¹.

Differential scanning calorimetry (DSC)

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure drug, and optimized batch F16. The study was carried out using a TA Instruments, USA, Model: SDT 2960. The 2 mg of sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-300 ºC at heating rate of 10 ºC /min under nitrogen flow of 30 ml/min.

Fourier Transform Infrared Spectroscopy (FTIR)

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In-Vitro Dissolution Study

The in vitro release of Verapamil hydrochloride microspheres was measured using USP-1 basket type dissolution apparatus. About 100 mg of Verapamil hydrochloride microsphere were placed in the basket. The volume of dissolution medium was 900 ml and maintained at 37±0.5 ºC at a rotation speed of 100 rpm. The dissolution mediums were 0.1N HCl for 2 hours and 6.8 pH phosphate buffer for further 10 hours. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals and replaced by 5 ml of fresh dissolution medium immediately. The samples were assayed via UV-1800 Shimadzu UV-Visible spectrophotometer at 278 nm after filtration through a 0.45 µm membrane filter. All dissolution tests were performed in triplicate.

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from beads11. The kinetic models used were zero order, second order, Higuchi and Hixon-Crowell model.

For zero order release kinetics equation is,\[ Q = K_o t \], Where, \( Q \) = amount of drug released per unit surface area, \( K_o \) = Zero order release rate constant and \( t \) = time.

For first order release kinetics equation is, \[ \ln q/t = -K_1 t \], Where, \( q \) = Amount of drug released per unit surface area, \( K_1 \) = First order release rate constant, \( q_0 \) = Initial amount, \( C_s \) = Saturation Solubility and \( C \) = Concentration and \( t \) = time.

For Hixon Crowell release kinetics equation is, \[ W_{Wt}^{1/3} - W_{Wt}^{1/3} = KHt \], Where, \( W_{Wt} \) = Initial weight of the particles, \( W \) = Weight of the particles, \( K_{HC} \) = Hixon Crowell release rate constant and \( t \) = time.

For Higuchi release kinetics equation is, \[ Q = K_{HG}t^{1/2} \], Where, \( Q \) = Amount of drug released per unit surface area of the dosage form, \( W \) = Initial amount, \( C_s \) = Saturation solubility of the drug in the surrounding liquid, \( K_{HG} \) = Higuchi release rate constant, \( A \) = Conc. of the drug in the matrix, and \( t \) = time.

RESULTS AND DISCUSSION:

Preparation of Microspheres:

In selection of drug to polymer ratio total three ratios were tried and it has been observed that out of the drug: polymer ratio 1:0.5, 1:1, 1:1.5 the better results were obtained for 1:1 ratio. For the ratio of 1: 0.5 drug content was less than 40% and for the ratio of 1:1.5 the viscous solution was formed so for further study 1:1 ratio was finalized. Experiment was performed at different rpm (400, 600, 800) from that at 800 rpm roughly spherical microspheres was formed. When rpm was decreased large microspheres were formed and vice versa. So 800 rpm was finalized for all study. It has been observed that formulations F1, F2, F3, F5, F6, F7, F8, F9, F10, F12 were not satisfactory but formulations F4, F11, F13, F14, F15, F16 given satisfactory yield so only these formulations were evaluated.

Evaluation of Microspheres

The percentage yield, percentage entrapment efficiency, micrometric properties and Percentage buoyancy of microspheres is shown in table no 2. The percentage yields were found to be 84 % to 93 %. The maximum yield of microsphere was 93% in HPMC K4M and carabap polymer. The loss of material during solvent evaporation method may be due to material adhering to the propeller of stirrer. Formation

Table 2: Evaluation parameters of the microspheres

<table>
<thead>
<tr>
<th>FC</th>
<th>Yield (%)</th>
<th>EE (%)</th>
<th>PS (mm)</th>
<th>BD (g/ml)</th>
<th>TD (g/ml)</th>
<th>HR (%)</th>
<th>CR (%)</th>
<th>AOR (%)</th>
<th>B (%)</th>
</tr>
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<tbody>
<tr>
<td>F4</td>
<td>90 ± 2</td>
<td>46 ± 2</td>
<td>1.21±0.81</td>
<td>0.281±0.0002</td>
<td>0.331±0.003</td>
<td>1.18±0.04</td>
<td>14.01±0.9</td>
<td>23.14±0.6</td>
<td>54.36±0.64</td>
</tr>
<tr>
<td>F11</td>
<td>86 ± 1</td>
<td>98 ± 2</td>
<td>0.396±0.71</td>
<td>0.279±0.003</td>
<td>0.325±0.006</td>
<td>1.16±0.02</td>
<td>14.15±0.8</td>
<td>22.02±0.5</td>
<td>56.79±0.82</td>
</tr>
<tr>
<td>F13</td>
<td>93 ± 1</td>
<td>82 ± 23</td>
<td>0.389±0.62</td>
<td>0.275±0.002</td>
<td>0.320±0.005</td>
<td>1.16±0.02</td>
<td>11.6±1.1</td>
<td>22.07±0.6</td>
<td>78.25±0.94</td>
</tr>
<tr>
<td>F14</td>
<td>84 ± 2.5</td>
<td>67 ± 2.5</td>
<td>0.221±0.52</td>
<td>0.271±0.003</td>
<td>0.319±0.005</td>
<td>1.17±0.03</td>
<td>15.04±0.9</td>
<td>24.3±0.3</td>
<td>72.39±0.48</td>
</tr>
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</table>

batch F13 shows higher percent yield as compared to other batches and formulation batch F11 shows higher percent entrapment efficiency compared to other batches. Values of Carr’s Index range were ranges from 8 % to 15 % and that of Hausner’s ratio from 1.16 to 1.23 indicated good flow. All formulations floated for more than 10 hours over the surface of the dissolution medium without any apparent gelation. The microspheres showing lower densities influence buoyancy and they were retained for longer than 10 hours, which helped in improving the bioavailability of the drugs. F16 batch shows good percent buoyancy up to 12 hours. The SEM photographs of the microspheres shows that microspheres were spherical with porous surface and slightly aggregated as shown in figure 1.

Figure 1: Scanning electron microphotograph of batch F16 at different magnification

XRD, DSC and FTIR spectra of drug and formulation F16 is as shown in figure 2, 3 and 4 respectively. Drug and formulation has exhibited identical spectra indicting absence of any interaction of the drug with the excipients.

The in vitro drug released is as shown in figure 5. For F16 batch
Manisha Vijaysinh Mane et al. / Journal of Pharmacy Research 2014, 8(10), 1498-1502

Percentage drug released up to 12 hours was found to be 91.93%. The F4 and F11 batch showed 88.93% and 92.81% drug release within 4 hours respectively. F13 batch showed 90.18% drug release within 8 hours. F14 batch showed 94% drug release within 6 hours. F15 batch showed 90.78% drug release within 9 hours. So F16 batch was found to be optimized batch because it has given proper release within 12 hours. From the release kinetics study (Table 3) it has observed that all formulations follow Higuchi model which indicated that drug release is from porous matrix.

CONCLUSION:
The microspheres were prepared satisfactorily by solvent evaporation technique. The entrapment efficiency was found to be above 90% along with the in vitro buoyancy was more than 54% after 10 hours indicated satisfactory performance of proposed formulations. The particle size has increased significantly as the speed of stirrer decreased. The flow properties of all the prepared microspheres were good as indicated by low angle of repose and low compressibility index. In vitro release of Verapamil hydrochloride from microspheres was found to be 91% for optimized batch (F16) sustained up to 12 hours. In vitro release data fitted into various kinetic models suggest that the release of Verapamil hydrochloride from floating microspheres obeyed Higuchi model.

ACKNOWLEDGMENT:
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REFERENCES:

Source of support: Nil, Conflict of interest: None Declared

![Figure 5: In-Vitro drug release profile of all formulation batch](image-url)

**Table 3: Release kinetics study**

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>n values of mathematical models for dissolution profiles.</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Hixson-Crowell</th>
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<tr>
<td>F4</td>
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<td>F11</td>
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