An ideal therapeutic drug delivery system is supposed to present and maintain optimum drug concentration within the body for required time period (1, 2). Efficacy of drug is directly influenced by its bioavailability and bioavailability can also be enhanced to raise therapeutic compliance by decreasing dose frequency and associated side effects (3). The new era of pharmaceutical research is accompanied with evolution of novel delivery systems with higher drug efficacy, safety, stability and patient compliance. Thus administration way (drug delivery system) of a drug can also have significant influence on its efficacy. The use of novel sustained release formulations is advantageous for consistent plasma drug concentration profile, and reduction in concentration relevant side effects along with a better therapeutic outcome, enhanced physical stability, dual loading ability for lipophilic and hydrophilic drugs, suitable and economical for large scale production. Also, the patient compliance is enhanced because of once-daily dose of sustained release formulation of a shorter-acting medicament (2).

Stable angina pectoris is most commonly prevailed chronic illness and the existing conventional drug delivery systems for the cure of it cannot generate the optimum therapeutic response for a longer time period. Thus, fluctuation and missing of dose is more common (4). Such situation surely demands sustained release or controlled release drug delivery of a medicament for achieving increased control of heart rate (5). Exploitation of lipid-based microparticulate drug delivery systems is found to be the most hopeful strategy out of numerous available
strategies (4, 5). Realization of that drug bioavailability can be increased by administering a drug in lipid based microparticulate system provided a ground for such systems to gain a lot of attention in pharmaceutical research during previous decades (6). The improved bioavailability is associated with the formation of lipid droplets on dispersion of SLMs (solid lipid microparticles) which assist absorption of drug and increase the permeability of drug through gastrointestinal membrane (6, 7).

The SLMs are one of the sustained release formulations employed for the situations which demand sustained plasma drug concentration for a prolonged time period and can successfully administer semi-synthetic, biological and synthetic drugs (8). The SLMs can limit plasma drug level fluctuation, reducing frequency of dose, lowering side effects and enhancing patient compliance (9). The SLMs can be formulated by various methods like melt emulsification congealing technique, solvent evaporation, coacervation, spray drying and ionotropic gelation and their size usually varies from 1 to 1000 micrometers (8).

The SLMs are prepared from solid lipids like bees wax (BW) because of their very good biocompatibility and biodegradability (8). The lower melting points of solid lipids make the preparation of SLMs easy (6). The natural and biological nature, lack of toxicological risk of the wax and ease of production make SLMs much better than polymeric microparticles. As drug carriers, BW can make drug release sustained and enhance drug bioavailability (8).

Different factors like concentration and type of lipid, microencapsulation technique, concentration and type of surfactant, stirring speed and stirring time affect the various properties of formulation of SLMs (10). The conventional experimentation for optimization needs a variation of one variable while keeping all others at constant level that may lack the elaboration of interaction of different variables at the same time and also cause the excessive consumption of time and excipients. The effect of different controlled independent factors in different combinations on different responses can be studied with the help of central composite rotatable design (CCRD) which is a statistical procedure for optimization of formulations and is usually employed with the help of software like Design Expert (10, 11). CCRD provides a good graphical representation of results for a particular response in the form of 3D and contour plots and it also suggests a model equation for a specific response as a function of various controlled independent variables (12).

Ivabradine hydrochloride (Iva) is a novel bradycardic agent employed for the symptomatic management of hypertension and stable angina pectoris (4). Iva causes a decrease of chemokine-induced PI-3 kinase activity by inhibiting T cell migration. Ivabradine is responsible for reduction of the heart rate by a mechanism that is different from that β-blockers and calcium channel blockers, two commonly prescribed anti-anginal drugs. It is classified as a cardiotonic agent. The plasma half-life of Iva is about 2 h demanding frequent dosing and its bioavailability is about 38% (13).

In the present study, bees wax was employed as release retarding material for the preparation of controlled release ivabradine loaded SLMs by melt emulsification method which is also called as hot emulsion congealing technique. With the help of CCRD - a response surface methodology - different SLMs formulations were designed keeping concentration of BW, surfactant concentration and stirring speed as independent variables while percentage recovery (Y1) and entrapment efficiency (Y2) as set as dependent variables/responses. Wax and drug compatibility were evaluated with the help of FTIR, DSC and XRD. The prepared SLMs were evaluated for particle size, rheological behavior, morphological appearance, zeta potential, in vitro drug release at pH 1.2 and pH 6.8. The release profiles were evaluated with the help of different kinetic models like zero order, first order, Higuchi and Korsmeyer Peppas models. The effect of formulation factors on Y1 and Y2 of SLMs was also statistically analyzed and the results were presented in the form of 3D and contour plots.

EXPERIMENTAL

Materials

Ivabradine was generously gifted by Nabi Qasim Pharmaceuticals (Pvt.) Ltd. and Highnoon Laboratories Pvt. Ltd., Lahore, Pakistan. Potassium dihydrogen phosphate and Tween 20 were purchased from Merck, Germany. Bees wax (Sigma Aldrich, USA), potassium bromide (Fischer Scientific, UK) of IR grade cellulose dialysis tube (Sigma-Aldrich, USA) were also purchased. All of the used chemicals and reagents were of analytical grade.

Experimental design (CCRD)

In this study, the formulation factors investigated for their impact on various responses of SLMs were lipid concentration, concentration of surfactant and stirring speed as shown in Table 1. Dependent
variables also called as Responses for this study interval include percentage yield (Y1) and entrapment efficiency (Y2). A statistical software “Design Expert (version 8.0.6.1 Stat-ease, Inc.)” was used to generate the representative combinations of these three factors at five levels and the entire design consisted of 20 runs of experiments out of which 17 formulations were prepared. It was because 6 runs were at the central point (similar composition) out of which three were formulated (Table 2).

**Preparation of SLMs (hot emulsion congealing technique)**

Iva loaded SLMs were prepared by the melt emulsification method which is also known as hot emulsion congealing technique. Bees wax (BW) was heated up to its melting point (65-80°C) and a specific amount of drug was dissolved in it. At the same time, an aqueous solution of surfactant Tween-20 (T-20) was made and maintained at 75°C. The solution of drug in lipid maintained at 75°C was then added to the hot aqueous surfactant solution with continuous homogenization/stirring at different speeds for 30 min using a high-speed homogenizer (Yellow Line Ost Basic Company, Germany). Later, the resulting pre-emulsion was poured into cold water (1–4°C) and stirred with a magnetic stirrer. The SLMs were allowed to recrystallize at room temperature, filtered by using 0.45 µm filter paper and dried at room temperature by using desiccators.
Every batch for each formulation was prepared three times which were then combined before further analysis. The same procedure was repeated to yield 17 formulations by varying three variables like BW concentration, surfactant concentration and stirring speed (Table 1, Table 2). The volume of aqueous phase and drug concentration were kept constant (14).

**CHARACTERIZATION**

**Fourier Transform Infrared Spectroscopy (FTIR)**

The compatibility of Iva with BW was analyzed with the help of FTIR (IR Prestige 21 spectrophotometer, Shimadzu, Japan). The FTIR spectra of drug alone, bees wax alone, physical mixture of drug-wax and drug loaded SLMs were recorded. The samples for analysis were prepared by making a pellet from mixture of sample plus KBr disks (2 mg sample and 200 mg KBr). The resolution was set at 2 cm⁻¹ and scanning range kept within 400–4000 cm⁻¹. The hydraulic pressure of 150 kg/cm² was used in the study (15).

**Differential scanning calorimetry (DSC studies)**

The drug-polymer interaction was also performed with the help of thermal analyzer (SDT Q600 TA, USA). The DSC analysis of ivabradine, bees wax, blank solid lipid microparticles and ivabradine loaded SLMs was performed to examine any possible drug-wax interaction. Bees wax, ivabradine, their physical mixture and drug loaded SLMs were finely triturated and the prepared sample was then heated at a heating rate of 10°C/min from 0 to 220°C in a sealed aluminum pan. The flow of nitrogen was maintained at 40 mL/min. The reproducibility of results was checked by running every sample in triplicate (12, 15).

**Entrapment efficiency (EE)**

The amount of Iva entrapped in SLMs was also calculated. For this purpose, a specified amount of SLMS were crushed and then dispersed in a phosphate buffer (pH 7.4) for 24 h with continuous stirring. The solution was diluted up to 5 times by use of phosphate buffer (pH 7.4) after filtration. The sample was run on UV-visible spectrophotometer (Pharma Spec 1700 Shimadzu, Japan) and absorbance was observed at 286 nm. The calculation of drug amount encapsulated in the SLMs was performed by using the following formula (14-16):

\[
EE = \frac{\text{Actual drug amount}}{\text{Theoretical drug amount}} \times 100
\]

**Percentage yield**

For percentage yield, the weight of finally prepared and dried SLMs was taken and the obtained weight was divided by total amount of all of the solid components employed to prepare SLMs. The percentage yield was calculated by following formula (16):

\[
\text{Percentage yield} = \frac{\text{Weight of obtained SLMs}}{\text{Total weight of all solid components}}
\]

**Rheological studies**

Rheological studies concern with evaluation of flow properties of formulations. Appropriate flow behavior of SLMs is mandatory if SLMs are to be converted in tablet or capsule or any other dosage form. These studies were as follows:

**Bulk density**

For calculation of bulk density, the pre-weighed amount (M) of SLMs was poured in graduated cylinder. The bulk volume (V) occupied by SLMs was observed from the cylinder. The bulk density was calculated with the help of the following formula (17):

\[
\text{Bulk density} = \frac{M}{V_b}
\]

where "Vₙ" is the amount of bulk volume and "M" is the mass of SLMs.

**Tapped density**

For the measurement of tapped density, the graduated cylinder having pre-weighed amount of SLMs was tapped for specified number of tapings/time so that SLMs had attained a plateau condition. The tapped volume (Vₜ) occupied by the SLMs after tapping was observed (17). The calculation of tapped density was performed with the help of the following formula:

\[
\text{Tapped density} = \frac{M}{V_t}
\]

where "Vₜ" is the tapped volume of SLMs.

**Carr's compressibility index**

The compressibility of SLMs is also an indicator of flow behavior. The Carr’s index (I) was determined by the following formula (17);

\[
I = \frac{V_b - V_t}{V_t} \times 100
\]

where "Vₙ" is the normal bulk volume occupied by SLMs and "Vₜ" is the tapped volume of SLMs. The value of Carr’s index from 12 to 19% indicate good flow character while its value greater than 21% suggest poor flow properties (17).
Hausner’s ratio

Hausner’s ratio is a ratio between tapped density and bulk density of a material. It was also applied to calculate flow behavior of SLMs.

\[
\text{Hausner ratio} = \frac{\rho_t}{\rho_b}
\]

where \(\rho_t\) is the tapped density and \(\rho_b\) represents the bulk density of SLMs. The value of ratio greater than 1.25 shows poor flow behavior and a value less than 1.25 indicate good flow property (17).

Angle of repose

The funnel method was used to calculate angle of repose. A specified amount of SLMs was passed through funnel on a plain sheet of paper. The falling microparticles made a heap on the sheet of paper. The height (h) and radius (r) of the heap were measured and these values were used to determine angle of repose with the help of the following formula:

\[
\tan \theta = \frac{h}{r}
\]

Free flow behavior of SLMs will be confirmed from a value of angle of repose less than 30° (17).

Surface morphology

The shape and surface morphology of the three selected SLMs formulations was examined by using scanning electron microscope (JSM-840, Joel Instruments, Tokyo, Japan). The sample of SLMs was placed on a double adhesive tape in drops form and adhesive tape was then struck to an aluminum stub. After evaporation from this prepared sample, the stubs were coated with gold to make them electrically conductive under an argon atmosphere. The photomicrographs of SLMs were obtained at 500◊ magnification at 10 kV (14).

In vitro drug release study

The release of drug from the prepared SLMs was examined in phosphate buffer (pH 6.8) as a medium and 0.1 M HCl (pH 1.2) as medium at 37 ± 0.5°C with a rotating speed of 50 rpm using USP type-II dissolution apparatus (PT-DT7, Pharma Test, Germany). For each formulation, sample of SLMs equivalent to 5 mg of Iva was added in cellulose dialysis tube containing 5 mL of dissolution medium. The tube was then attached with paddle to maintain sink condition in dissolution vessel. The study was performed for 12 h at pH 6.8 and for 2 h at pH 1.2. After a specified time interval (30 min), 5 mL of sample was withdrawn from each dissolution vessel and an equal volume (5 mL) of dissolution medium, which was freshly prepared and pre-warmed at 37°C was added in the same vessel. The dissolution samples withdrawn from vessel were diluted up to 100 times with distilled water and the absorbance of diluted sample was measured at 286 nm using the UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) to determine the content of Iva (14, 18). The concentration of Iva was calculated with the help of standard calibration curve which was generated before start of in vitro drug release study with the help of known dilutions of Iva. The drug release study was performed with every formulation in triplicate.

Kinetics of drug release

In order to study drug release mechanism, the release data were analyzed with the help of different models of kinetics like zero order, first order, Korsmeyer-Peppas, Hixson-Crowell and Higuchi’s models which were as follows (18):

\[
F_t = K_0t
\]

\[
\log F = \log F_0 - \frac{K_1}{2.303} t
\]

\[
F = K_Ht^{1/2}
\]

\[
F_0^{1/3} - F_t^{1/3} = K_{HC} \times t
\]

\[
\frac{M_t}{M_\infty} = K_{Pe} t
\]

Zero and first order rate constants are denoted by \(K_0\) and \(K_1\) respectively. Similarly, \(K_H\) and \(K_{HC}\) represent Higuchi and Hixson-Crowell rate constants. The concentration of drug at time t was denoted by \(F/F_0\), and initial concentration of drug was indicated by \(F_0\). \(K_{Pe}\) denotes Korsmeyer- Peppas rate constant while n represent an exponent of drug release mechanism. The value of n less than 0.43 shows Fickian mechanism while value of n greater than 0.43 and less than 0.85 indicate non-Fickian drug release mechanism. The value of n greater than 0.85 suggests slow erosion plus diffusion drug release mechanism (18).

Zeta potential and particle size measurements

The charge on prepared drug loaded SLMs formulation was determined by measuring the electrophoretic mobility of SLMs in U-shaped tube at 25°C with the help of Malvern Zetasizer (Zetasizer Ver System; Malvern Instruments Ltd., Malvern, UK). Average size and size distribution of drug loaded SLMs were also measured. SLMs were added in a cuvette containing deionized water and then it was placed in Zetasizer for size measurement (19).

X-ray powder diffraction studies

X-ray diffraction studies were performed to know the effect of melt emulsion coconaling microencapsulation process on crystallinity of drug.
The samples were subjected to irradiation with monochromatized X-rays of Cu-Kα by using D8 advance X-ray diffractometer (Bruker AXS, Madison, WI, USA) at a current of 40 mA by using rays with a voltage of 40 kV. Scanning of the samples like Iva, BW and drug loaded SLMs were conducted at a scan rate of 2°/min in the diffraction angel (2θ) range from 0 to 45° (8, 19).

**Stability studies**

The stability studies of SLMs stored in airtight glass bottles at 25 and 4°C was conducted. The drug contents and dissolution of SLMs were analyzed after a period of 1, 2, 3 months to evaluate SLMs stability. The dissolution analysis for stored SLMs formulation was performed thrice.

**RESULTS AND DISCUSSION**

Waxes have widely been used for the development of sustained release drug delivery systems because of their poor aqueous solubility (14) and they can also exhibit pH sensitive character (3, 8). SLMs of ivabradine were formulated by applying hot melt emulsion congealing method. The prepared SLMs were found to be free flowing and white in

---

Figure 1. FTIR spectra of ivabradine (A), bees wax (B), ivabradine loaded SLMs (C) and blank SLMs (D)
color. The employed method was found to be easy and rapid. Surfactant (Tween 20) was utilized from 0.5 to 3% w/v to facilitate the wetting of hydrophobic material (BW). Stirring speed also influenced preparation and characteristics of SLMs. In present study, stirring speed from 1000 to 4000 rpm had been employed.

**FTIR spectroscopy**

FTIR study indicated good compatibility between Iva and BW. The individual spectra of Iva and BW have been compared with FTIR spectrum of prepared SLMs. The FTIR spectra of Iva, BW, and prepared SLMs are shown in Figure 1. The characteristic aliphatic C-N and alkanes C=C stretches were observed at 2940.91 cm⁻¹ and 1105 cm⁻¹, respectively, in the individual FTIR-spectrum of ivabradine and in the spectrum of Iva loaded SLMs. The particular aromatic peaks for aromatic C-C and C-H functional groups of ivabradine have also been observed at 1633 cm⁻¹ and 1469 cm⁻¹, respectively, in the spectrum of drug loaded SLMs. The spectra of drug loaded SLMs have also indicated the prominent stretches for alkenes (R-CH₂-CH₃) groups. FTIR spectra have not shown absence or shift of any principal peaks of the drug or BW neither in the FTIR spectrum of physical mixture of Iva and BW nor in the FTIR spectrum of formulated SLMs. The absence of any new peak in the FTIR spectrum of prepared SLMs and in FTIR spectrum of physical mixture of Iva-BW indicates the intactness of drug and BW. These results indicated the compatibility of the wax polymers for preparation of SLMs of Iva (20).

**Differential scanning calorimetry (DSC)**

The compatibility of drug and BW was checked with the help of differential scanning calorimetry. DSC thermograms of Iva, BW, blank SLMs and drug loaded SLMs are presented in Figure 2. The drug (Fig. 2-B) showed sharp endothermic peak at a temperature of 195°C indicat-
ing its melting. The particular endothermic peaks corresponding to melting point of BW were clearly visible at a temperature of 65°C for BW alone (A) and prepared blank SLMs (C) as shown in Figure 2. The specific peak relevant to melting of Iva was also found in drug loaded SLMs at temperature of 195°C. In drug loaded SLMs, the particular peaks corresponding to melting points of Iva and BW were visible at temperature of 66°C and 196°C, respectively, indicating the compatibility of wax and drug (1, 14, 20).

Percentage yield

For each of the SLMs formulation, the percentage yield was also determined (Table 2). It was found that percentage yield of SLMs was greatly influenced by the concentrations of BW and surfactant (T-20). The results for percentage yield (Y1) was also analyzed statistically as response of independent variables like BW, T-20 and stirring speed with the help of Design Expert. Results (Table 2) indicated significant variation of percentage yield (Y1) from 53% to 90%. Higher concentration of BW along with lower T-20 (F2, F5, F6) showed less than 60% percentage yield. Lower yield of SLMs may be associated with higher aggregation of wax because of less stabilization of lipid droplets at lower concentration of T-20 (18). Higher BW along with higher T-20 (F7, F8, F10) was found to be a good condition because greater than 80% percentage yield was observed in these SLMs. Similar findings were published in the literature (16-18) for biodegradable microparticulate system.

Entrapment efficiency (EE)

EE for each SLM formulation was also assessed and the results are shown in Table 2. A great variation from 29 to 78% in EE was observed. The EE increased with an increase in concentration of BW provided that there must be an equal increase in surfactant-T20 concentration as observed for F1, F7 and F10. Stirring speed also showed a positive impact on the EE but after a certain limit there was a decline in EE with higher stirring speed (3392 rpm) and it may be due the fact that very high speed produced turbulence and loss of SLMs (18, 21). The formulations F2 and F6 had the same concentrations of BW and T-20 but both formulations have different EE because of stirring speed difference used in preparation of these formulations. The formulations with less percentage of BW or T-20 at lower homogenization speed (F15, F17) showed minimum EE. Higher concentration of T-20 alone could not increase EE as observed for F3 (27%), F13 (35%), F17 (35%) because less amount of wax polymer was available to enhance entrapment of Iva. So, increase in EE depended on both the enhancement of BW concentration and surfactant T-20 concentration. It is suggested that higher concentration of T-20 not only prevent the loss of drug in the external phase but also responsible for the stabilization of lipid microparticles in external phase.

Optimization of data and model validation

A mathematical relationship was established between the independent factors (X1, X2, X3) and responses (Y1: percentage yield, Y2: entrapment efficiency) and a second order polynomial equation representing the impacts of these factors alone and their interaction on responses was obtained.

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \]

where ‘Y’ denoted the studied dependent response. The intercept \( b_0 \) represents the average of all outcomes from 17 runs while regression coefficients (\( b_j \)) are the calculated average value of response \( Y \) by changing one factor alone and changing two factors at the same time. The coded level of independent factors were denoted by \( X_1, X_2 \) and \( X_3 \) and indicated the average value of response obtained from changing one factor from low to high level. The interaction terms for combination of factors were denoted by \( X_1X_2, X_1X_3 \) and \( X_2X_3 \) and they indicated the average change in response occurred due to change of two factors at the same time (12). A negative or positive sign showed the antagonistic or synergistic impact of the factor/factors on the response (10). The equations obtained for \( Y_1 \): percentage yield and \( Y_2 \): entrapment efficiency are given below:

\[ Y_1 = 89.15 - 4.26X_1 + 8.51X_2 - 3.84X_3 - 7.88X_1X_2 + 1.68X_1X_3 + 1.62X_2X_3 - 5.55X_1^2 - 2.95X_2^2 \]

\[ Y_2 = 59.95 + 16.44X_1 + 2.26X_2 + 4.42X_3 + 0.32X_1X_2 - 1.12X_1X_3 + 0.62X_2X_3 - 3.57X_1^2 - 1.28X_2^2 \]

The ANOVA was applied with the help of software Design Expert (version 8.6.1. Stat-Ease Inc.) in order to statistically validate the polynomial equations and model. For \( Y_1 \), \( X_i \) has a negative impact on percentage yield indicating that increase in wax concentration caused a decrease in percentage yield while for \( Y_2 \) (entrapment efficiency), \( X_i \) had a positive impact indicating that increase of wax concentration caused a significant increase in EE. For \( Y_1 \) and \( Y_2 \) (surfactant concentration) has positive sign indicating positive influence on both responses. Three dimensional surface graphs and two dimensional contour plots indicating the
Figure 3. Contour (a) and 3D plots showing the impact of bees wax concentration ($X_1$) and stirring speed ($X_3$) on response $Y_1$: percentage yield

Figure 4. Contour (a) and 3D plots showing the impact of surfactant concentration ($X_2$) and stirring speed ($X_3$) on response $Y_1$: percentage yield

Figure 5. Contour (a) and 3D plots showing the impact of bees wax concentration ($X_1$) and surfactant concentration ($X_2$) on response $Y_1$: percentage yield

Figure 6. Contour (a) and 3D plots showing the impact of bees wax concentration ($X_1$) and stirring speed ($X_3$) on response $Y_2$: entrapment efficiency
impact of factors (X_1-X_3) on responses are presented in Figures 3 to 8.

Significance of the model was analyzed with the help of analysis of variance (ANOVA) applied at 5% significance level and a p-value less than 0.05 indicated that model was significant. Both responses followed quadratic model because they showed a good fit with quadratic model instead of linear model. The F values for Y_1 and Y_2 were found as 23.60 and 66.80, respectively, indicating that model was significant (p < 0.0001) in both cases. For Y_1, the p-values of X_2 was < 0.0001 showing that X_2 (surfactant concentration) was a significant term. Similarly, the “Prob > F” at a level of less than 0.05 showed that model terms were significant. For Y_2, significant terms were X_1, X_2, X_3, X_1X_2, X_1^2, X_2^2 while for Y_1, X_1, X_2, X_3, X_1^2 were found to be significant terms (11).

Surfactant concentration played a vital role in the development and stabilization of droplets in emulsion which finally contributed towards an increase in percentage yield of SLMs. At lower concentration of surfactant, the yield remained very low because higher wax concentration with lower surfactant concentration caused more aggregation and accumulation of wax instead of SLMs production.

Similarly, the p-values for X_3 and X_1 were found to be 0.0021 and 0.0040 suggesting that both terms were significant for Y_1. For Y_2, the p-values for X_1, X_3 were found to be less than 0.0001 suggesting that these three factors have significant impact on Y_2.

The predicted R^2 values for Y_1 (0.8365) and Y_2 (0.8757) were found to be very close to the adjusted R^2 values of Y_1 (0.9146) and Y_2 (0.9689), respectively. Signal to noise ratio was measured from adequate precision and its values for Y_1 (13.775) and Y_2 (29.471) greater than 4 showed the adequacy and suitability of model for both responses (22).

**Rheological properties**

Rheological studies deal with flow behavior of SLMs formulations and the outcomes of rheological analysis are presented in Table 3. There was no significant difference among the SLMs formulations, however the wax-surfactant ratio had shown its effect on flow behavior. Higher surfactant concentration and lower BW concentration had positive influence on the flow behavior of SLMs. The value of Car’s index for all SLM formulations ranged from 9 to 16 indicating a better flow character of SLMs. The outcomes from angle of repose had also verified the excellent flow character of SLMs because for
most of the formulations, the value of angle of response remained less than 20°. The value of angle of repose ranged from 15 to 24° indicating excellent and good flow parameters for prepared SLMs. Similar findings from the results of Hausner’s ratio had confirmed the good rheological behavior of SLMs. It remained less than 1.5 for all of the formulations suggesting the good flow behavior of SLMs (14, 17).

**Particle size and zeta potential**

The particle size, size distribution and zeta potential of selected SLM formulation (F1) were determined with the help of Malvern Zetasizer. Formulation F1 was selected because this formulation has offered a better control over the Iva release. The curve regarding size distribution and a zeta potential curve of selected SLM formulation (F1) are shown in Figures 9 and Figure 10, respectively. The size distribution of SLMs ranged from 300 µm to 500 µm while the major fraction (55%) of the SLMs have an average size of 400 µm. Particle size was greatly influenced by the concentration of polymer (BW) and the formulation F1 was prepared with higher concentration of the BW (3% w/v) showing a large size and wider size distribution. Similarly, stir-

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Bulk density</th>
<th>Tapped density</th>
<th>Angle of repose</th>
<th>Hausner’s ratio</th>
<th>Carr’s index</th>
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<tbody>
<tr>
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<td>1.09</td>
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</tr>
<tr>
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<td>0.523</td>
<td>0.665</td>
<td>19</td>
<td>1.08</td>
<td>12</td>
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</table>

Figure 9. Particle size distribution of prepared SLMs formulation F1
ring speed may also be the influencing factor for size of microparticles and here a higher speed (2500 rpm) was found to be optimum for sufficiently mixing immiscible wax microparticles with aqueous phase and preventing the aggregation of the hydrophobic polymeric microparticles (23). The surfactant at a concentration level of 1.75% w/v had also contributed towards the yield of micron size SLMs (16, 19) because the SLMs preparation without surfactant during preliminary stage was failed.

The stability of the SLMs was deduced from the measurement of zeta potential which is an electri-

Figure 10. Zeta potential distribution of SLMs formulation F1

Figure 11. XRD patterns of bees wax (a), drug (b) and prepared SLMs (c)
cal/charge potential at the shear plane. The particles with higher zeta potential could be regarded as having better storage stability. In current study, the zeta potential of F1 was observed in the range of -30 mV to -52 mV (Figure 10) indicating that SLMs should have better stability. The presence of high intensity of negative charge would generate electrostatic repulsion between microparticles causing prohibition of aggregation of SLMs (23).

X-ray powder diffraction studies

In this study, XRD patterns for Iva, BW, and Iva loaded SLMs were recorded. The X-ray powder diffraction patterns of SLMs along with those of raw crystals of drug and bees wax are shown in Figure 11. Significant reduction in the peak intensities was observed in the XRD patterns of SLMs which suggests reduced crystallinity of the drug (8). A comparison was made between Figures 11(a), 11(b) and 11(c), the particular peaks of Iva and BW had been clearly observed in Figure 11(c). The XRD analysis of Iva loaded SLMs (Figure 11c) revealed sharp as well as scattered peaks indicating that a fraction of Iva was changed into amorphous form while formulating SLMs. There was a complete absence of any prominent alteration in the diffraction position relevant to Iva or BW. The peaks for Iva were observed at 20 of 20°, 25° and 30° indicating the crystalline nature of Iva (Figure 11b). The study discovered that formulation steps of Iva loaded SLMs have not produced any unfavorable effects on Iva (23).

Surface morphology of SLMs

The prepared SLMs were found to be discrete and spherical in shape (Fig. 12) and no significant difference in morphology of SLMs was observed. Three different image of three SLMs formulations were taken to know the influence of concentration of surfactant, BW and stirring speed on the morphology of SLMs. SEM images revealed that the microparticles of formulation F13 (Fig. 12-c) were having more smooth and uniform surface as compared to microparticles of formulation F6 (Fig. 12-a). This effect could be associated with the fact that F13 have higher concentration of T-20 (2.5%) with lower BW (1%) while F6 contained lower amount of T-20 (1%) with higher concentration of BW (2.5%). Surfactant played a significant role not only in formation but also in stabilization emulsion droplet of BW (25). The SLMs like F4 containing equal percentage of SA and BW (Fig. 12-b) were also spherical in shape. Furthermore, it was also evident from the SEM images that stirring speeds, like 1600 rpm for F13, 2500 rpm for F4 and 3392 rpm for F6 were sufficient to yield spherical microparticles and this speed range had prevent the formation of large clumps or aggregates of
BW (14, 25). However, some cracks and holes were also present on the surface of prepared SLMs of F4.

In vitro drug release study

It was expected that SLMs made of BW impede the release of drug at the gastric fluid pH and control the release in the pH of intestinal fluid. The graphical presentations of release profiles of all SLM formulations at pH 1.2 and pH 6.8 are shown in Figures 13 to 15. The release of Iva varied from 54 to 90% in different SLMs formulations at pH 6.8. The graphs show that release of drug from F1 was almost 54% after 12 h because it has 3% of polymer-BW while F3 having 0.5% of BW showed 90% drug release after 12 h at pH 6.8. Formulations (F3, F4, F5, F8, F9, F12, F13, F15, F16) with lower concentrations like 0.5, 1.006, 1.75% of BW presented rapid release of Iva even more than 70% after 12 h. The formulations F1, F2, F6, F7, F10 containing higher concentration (2.5 and 3%) of BW showed very slow and sustained release of Iva even less than 65% at pH 6.8 after 12 h.

It is evident from Figures 12-14 that the time of dissolution/drug release is strongly associated with total concentration of the BW used in SLMs preparation and time required for the drug release (p ≤ 0.05) was increased with the increasing amount of the BW (14, 23). The major factor that is contributing in controlling the drug release was the amount of BW. By increasing the proportion of wax in SLMs, the spread and penetration of water molecules in BW would be less which ultimately caused slower release of drug. Furthermore, higher concentrations of BW induce more hydrophobic character (18) to SLMs as observed for the formulations F1, F2, F6, F7, F10. Moreover, a minimum rate of drug release was observed at pH 1.2 that suggests the gastroprotective effect of BW (24). It is evident from release graphs (Figs. 13-15) that maximum 10-15% of the drug release was observed for SLMs at pH 1.2. For F1 and F2, about 9% of drug release was observed after 2 h. This behavior could be associated with insoluble nature of BW and BW had successfully modify/control the IVA release from SLMs (24).

The release data of SLM formulations were also evaluated using different kinetic models. The drug release constant (k) and regression coefficient (R²) obtained from zero order, Higuchi and first order, Hixson Crowell and Korsmeyer-Peppas mod-

![Figure 14. Cumulative % drug release (a) at pH 6.8 and (b) at pH 1.2 for Formulations F6-F10](image)

![Figure 15. Cumulative % drug release (a) at pH 6.8 and (b) at pH 1.2 for formulations F11-F17](image)
els are indicated in Table 4. It was clearly seen that the data could be comparatively better fitted in zero order model as the $R^2$ values of zero order were found greater as compared to that obtained from the first order, Hixson Crowell and Haguchi models. The data suggested the sustained release mechanism of drug release from prepared SLMs, which indicates that drug release was independent of remaining drug concentration (18). According to Korsmeyer-Peppas model, the value of $n$ was found to be greater than 0.85 which was indicative of diffusion along with erosion of wax polymer (14).

### Stability studies

The drug contents from SLMs formulations after 3 months were found to be within specified limits.

### CONCLUSION

SLMs of Iva were prepared by using BW as drug release controlling material with the help of melt emulsification congealing method. FTIR and DSC studies have confirmed the lack of any drug wax interaction and XRD studies showed that Iva retained its crystalline nature while encapsulating in waxy SLMs. SLMs showed suitable rheological properties. The SLMs showed a size distribution ranged from 300 to 500 µm. They showed good physical stability as indicated from negative charge in the range of -30 mV to -52 mV. SEM study depicted smooth spherical shape of SLMs.

Percentage yield ($Y_1$) and EE ($Y_2$) showed a significant variation depending upon the concentration of BW, surfactant and stirring speed. Quadratic model was validated and synergistic or antagonistic effect of combination of different variables on SLMs was successfully presented with the help of contour and 3D plots. In vitro drug release observed form 54 to 90% at pH 6.8 and was significantly ($p \leq 0.005$) influenced by BW concentration. No significant release at pH 1.2 was observed showing gastroprotective effect of BW. The release mechanism followed the zero order drug kinetics and $n \leq 0.85$ showed that drug release followed a slow erosion and diffusion mechanism. The study proved the influence of formulation variables on various features of SLMs. Furthermore, sustained release SLMs will offer possible benefits of controlling heart rate for 24 h in patients with chronic stable angina and will be helpful to minimize side effects and cost of treatment for patients.

### Table 4. Results of various kinetics models for SLMs at pH 6.8.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zero order kinetics</th>
<th>First order kinetics</th>
<th>Higuchi kinetics</th>
<th>Hixon-Crowell kinetics</th>
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