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Study on encapsulation of Ranolazine in bees wax microspheres: Preparation, characterization and release kinetics of microspheres

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ABSTRACT

The objective of this work to prepare and evaluate beeswax microspheres loaded with ranolazine and minimizes the unwanted side effects of ranolazine by controlled release of drug. Evidence has shown in the recent years that waxes have the physical properties suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen. Ranolazine was entrapped into gastro resistant, biodegradable beeswax wax microspheres using meltable emulsified dispersion cooling induced solidification technique utilizing a wetting agent. Solid, discrete, reproducible free flowing microspheres were obtained. The yield of the microspheres was up to 93 %. More than 96.0 % of the isolated microspheres were of particle size range 115 to 855 µm. The obtained angle of repose, % Carr's index and tapped density values were well within the limits, indicating had smooth surface, free flowing and good packing properties for the prepared microspheres. Scanning Electron Microscopy photographs and calculated sphericity factor confirms that the prepared formulations are spherical in nature and size range 345-355. Drug loaded in wax microspheres was stable and compatible, as confirmed by DSC and FTIR studies. The prepared formulations were analyzed quantitatively for the amount of encapsulated drug. It was observed that, there is no significant release of drug at gastric pH. The drug release was controlled more than 8hr. Intestinal drug release from wax microspheres was studied and compared with the release behavior of commercially available oral formulation Caroza®500 tablet. The release kinetics followed different transport mechanisms. The drug release performance was greatly affected by the material used in microsphere preparations, which allows absorption in the intestinal tract.

Key words: Ranolazine, Beeswax microspheres, Characterization, Release kinetics
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INTRODUCTION

Ranolazine is a novel anti anginal agent belonging to the group of piperazine acetamide has been widely used in the treatment of cardiovascular diseases, including arrhythmias, variant and exercise-induced angina, and myocardial infarction [1]. The solubility of ranolazine is relatively high at the low pH that occurs in the stomach. The high acid solubility

property of ranolazine results in rapid drug absorption and clearance, causing large and undesirable fluctuations in plasma concentration of ranolazine and a short duration of action, thus necessitating frequent oral administration for adequate treatment [2]. The administration of ranolazine induces adverse effects on GIT as well as hepatic, pancreatic, renal, endocrine, nervous, cardiac and hematological systems [3]. To achieve maximum therapeutic effect with a low risk of adverse effects, controlled released preparations are preferred [4]. The growing interest in controlled drug delivery release is because of its benefits like increased patient compliance due to reduced frequency of administration and less undesirable side effects. The side effects could be lowered by controlling the drug release and by adjusting the absorption rate.This can be achieved by employing suitable modifications in the manufacturing process [5]. Delivering the drug in the intestinal milieu from wax microspheres could be manipulated by suitable coating techniques [6]. Extensive clinical experience and extended release studies have shown that ranolazine is more effective than atenolol amlodipine or diltiazem, that ranolazine can reduce both anginal frequency and nitroglycerin consumption [7]. Major advantage of ranolazine over other traditionalantianginal agents is its lack of effect on blood pressure and heart rate. This unique property not only provides alternatives for patients who cannot tolerate the hypotensive and negative inotropic actions of traditional agents (beta blockers, calcium channel blockers, nitrates), but also provides clinicians with an adjunctive agent that does not add to the adverse effects of a patient's current therapy [8]. The chief characteristics of enteric coating are their impermeability to gastric juices but susceptibility to intestinal juices [9]. In the present study, a novel meltable dispersion emulsified cooling induced solidification method was employed using inert wax (FDA approved) material and non-toxic solvents to entrap the drug [10]. Ranolazine should be dosed twice a day and due to its short half life and low therapeutic index, the frequency of adverse effects may be dose related [11]. As demonstrated by pharmacokinetic studies on ranolazine, the ingestion of a single controlled release dosage form is effective when administered twice a day [12,13]. A controlled release dosage form of ranolazine is preferable than the conventional dosage form, because there is a considerable saving in nurses and pharmacists time. Prepared wax microspheres loaded with ranolazine, compared with commercially available oral formulation tablet caroza®500 and drug release from the wax microspheres was studied. These findings suggested that kinetic control is effective for preventing the untoward effects of ranolazine. Previous experimental results have shown in the recent years that waxes have the physical properties suitable to prepare gastro resistant, biocompatible, biodegradable and non immunogenic materials used for the entrapment of drug, used for controlling drug release in the intestinal tract [14, 15].

MATERIALS AND METHODS

Ranolazine was obtained as gift sample from Zydus Cadila, Ahmedabad, India. Beeswax and surfactant Tween-80 was purchased from M/s Loba Chemie Pvt. Ltd., Mumbai, India. All the chemicals and solvents used were of analytical grade (Merck, Mumbai, India).

Preparation of microspheres
Beeswax wax (9 g) was melted in china dish at a temperature of 65°C and kept on water bath. Drug (3g) previously passed through sieve No.100 was dispersed in melted wax mass and stirred to obtain a homogeneous melt. This mixture was poured into 200 ml of pH 10.9 ammonia buffer solution (to minimize the solubility of drug), which was previously heated to a temperature higher than melting point of wax (>+ 5°). Tween 80 (1.0-2.0% w/w) was added to the above mixture and was mechanically stirred at 900 rpm using a stirrer (RQ-127A). Spherical particles are produced due to dispersion of molten wax in the aqueous medium. The

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mixture was stirred continuously at 900 rpm at a higher temperature (>+ 5°) of the melting point of wax for 3 min. The temperature of the mixture in the beaker was cooled rapidly and brought down to 10° by the addition of cold water. The resultant solid spheres collected by filtration were washed with water to remove any drug and surfactant residues. Air-drying was carried out at room temperature for 48 h gave discrete, solid, free flowing microspheres.

Size distribution and Micromeritic properties

Sieve analysis technique and SEM studies were carried out for size distribution of the wax microspheres respectively. Tap density of the prepared microspheres was determined using tap density tester and percentage Carr's index (%I) was calculated. Angle of repose (θ) was assessed to know the flowability of wax microspheres, by a fixed funnel method.

Scanning Electron Microscopy (SEM) and sphericity

SEM photographs were recorded using scanning electron microscope Model Joel- LV-5600, USA, at suitable magnification at room temperature. The photographs were observed for morphological characteristics and to confirm spherical nature of the microspheres. To determine the sphericity, the tracings of wax microspheres (magnification 45x) were taken on a black paper using Camera Lucida (Model -Prism type, Rolex, India) and circulatory factor was calculated by the equation, $S = p^2 / (12.56 \times A)$, where, A is area (cm^2) and p is perimeter (cm).

Fourier Transform Infrared spectroscopy (FT-IR)

The FTIR spectra for pure drug, empty microspheres and drug loaded microspheres were obtained using KBr powder method. Spectral measurements were obtained by powder diffuse reflectance on a FT– infrared spectrophotometer type Shimadzu, 8033, USA.

Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out on Du Pont thermal analyzer with 2010 DSC model. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina disc) as the reference. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10° /min.

Estimation of drug loading and *In vitro* drug release studies

For determination of drug content, 100 mg microspheres were dissolved in 100 ml of methanol. The resulting solution was analyzed spectrophotometrically at 272 nm (Shimadzu-1601, Japan) after sufficient dilution with phosphate buffer (pH 7.4).

USP XXI dissolution apparatus, type II was employed to study the percentage of drug release from the prepared formulations. Accurately weighed quantities of drug (ranolazine 500 mg equivalent to a commercial preparation – Caroza 500 mg tablet) loaded microspheres of each batch were taken in 900 ml dissolution medium (aceclofenac – 2 h in pH 1.2, hydrochloric acid buffer and 6 h in pH 7.4, phosphate buffer) and stirred at 100 rpm by maintaining at a temperature of 37 ± 0.5°. The drug concentrations were determined by withdrawing the 10 ml of aliquots using guarded sample collectors periodically at an interval for the next 4 h. Release studies were carried out in triplicate.

Comparison of dissolution profiles

A differential (f_1) and similarity factor (f_2) was calculated from dissolution data according to the following equations.

$$f_1 = \frac{\sum\limits_{t=1}^{n} |R_t - T_t|}{\sum\limits_{t=1}^{n} R_t} \times 100$$

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n}\right) \sum\limits_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

Where, f_1 -Differential factor
                f_2 - similarity factor
                n - Number of time point
                R_t - Dissolution value of the reference at 't' time
                T_t - Dissolution value of test formulation at 't' time

Differential factor f_1 was calculated by the percentage difference between the two curves at each time point and was measurement of the relative error between the two curves. The acceptable value for *f1* is 0-15.

The Similarity factor f_2 was logarithmic reciprocal square root transformation of the sum-squared error and is a measurement of the similarity in the percentage dissolution between the reference and test products. For a dissolution profile to be considered similar, the values of f_2 should be between 50 and 100. An f_2 value of 100 suggests that the test and reference profiles are identical and as the value becomes smaller, the dissimilarity between release profiles increases. The evaluation of similarity is based on the conditions of a minimum of three time points, individual values for every time point, not more than one mean values of >85% dissolved and the standard deviation of the mean should be less than 10% from the second to last time point.

RESULTS AND DISCUSSION

Recent reports have shown in the recent years that waxes have the physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen [16]. In the present study, a novel meltable dispersion emulsified cooling induced solidification method was employed using inert wax (FDA approved) material and non-toxic solvents to entrap the drug. The present method is quite different from that reported methods [17]. However in the present study, various parameters were optimized such as drug and wax ratio, stirring speed and time, amount of surfactant added, effect of pH on drug entrapment, rapid cooling studies during the preparation of wax microspheres. Therefore the influence of the above parameters was highlighted. When the pH value of the external aqueous phase was basic, the solubility of the drug was reduced and the encapsulated amount of the drug increased. The maximum drug load was obtained at pH 10.9. When pH value changes from 10.9 to 7.0, the percent of drug loading reduced from 26.49 to 12.31 % for drug loaded wax microspheres.

Incorporation of drug into wax microspheres requires the addition of a tween 80 (surfactant) at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. An attempt was made to incorporate drug in the wax microspheres without the addition of a surfactant. But the process was failed and it resulted in an aggregate cake like mass during the solidification of wax. It may be due to repulsion

resulting from high interfacial tension between the hydrophobic waxy material and external aqueous phase. It was found that surfactant having a HLB value of 15 or more was suitable to increase substantially dispersion of wax and promote drug incorporation in the microspheres. To obtain an optimal surfactant concentration, various concentrations ranging from 1.0 to 2.0 % (w/w) of the total formulation were tested. The resultant wax microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres.   Optimal concentration of Tween-80 1.8 % w/w was used to produce solid, discrete microspheres with good flow properties. A similar surfactant concentration was reported for beeswax microspheres prepared by a meltable dispersion method [14,15].

In the present study, to produce the spherical discrete microspheres, an optimum drug to wax phase ratio of 1:3 w/w was used. It was found that higher the amount of drug to wax ratio (2:3) produces aggregate masses during the cooling process. It may due to more amount of drug ratio, responsible for reduced melting point of the wax, leads to aggregate mass. SEM photographs also indicated the presence of the crystals on the surface of the microspheres and resulted microspheres were unsuitable for pharmaceutical uses.

The important factor that influences the size distribution of microspheres was stirring speed and time. An optimum stirring speed of 900 rpm and time was 6 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 900 to 1300 rpm, there was a decrease in the average size and recovery yield of the microspheres, small sized wax microspheres which were lost during successive washing and filtration. When the stirring speed was lower than 900 rpm, larger pellets were formed. An increased stirring time from 4 to 9 min (at a stirring speed of 900 rpm), decreased recovery yield of microspheres was observed. Because more stirring speed for longer duration lead to produces smaller sized microspheres and these microspheres were lost during successive washing and filtration. When the stirring time was less than 4 min, some amount of melted material was adhered to the sides of the beaker during the cooling process, resulted in lower recovery of yield. Repeat batches treated at an optimized rate mentioned above to produce reproducible sizes, showing that stirring speed and time were well controlled.

In the present study, 200ml (optimum amount) of aqueous phase was used to produce spherical microspheres. Resultant microspheres free from surface irregularities and free flowing in nature. When the volume of external phase was increased (> 200 ml) or decreased (< 200 ml), resultant microspheres having surface irregularities (confirmed by SEM photographs), sticky, aggregate and impossible to distinguish as an individual microspheres. A rapid cooling study during the preparation of wax microspheres was carried out. The temperature of the mixture was cooled rapidly (< 5 min) and brought down to $10\ ^0$C by the addition of cold water, produced microspheres were solid, discrete, free flowing in nature. When, the temperature of the mixture was cooled slowly (> 5 min) obtained microspheres were flaky, sticky, aggregate in nature, not suitable for pharmaceutical purpose.

Temperature of the aqueous phase was maintained $5\ ^0$C higher than the melting point of the wax in the corresponding formulations. From SEM studies it was observed that the resultant microspheres were from free from surface irregularities, except some wrinkles. But, when the temperature of the aqueous phase was less than the $5\ ^0$C than the melting point of the wax, big flakes were produced.

A total of five formulations are made varying amount of drug to polymer ratio as shown in Table 1.

Table 1: Percentage yield, physical appearance and micromeritic properties of drug loaded beeswax microspheres

Formulation Code	Yield (%)	Physical appearance	Angle of Repose	I (%)	Tapped Density (g/cm^3)	Particle size (µm)
A1	94.82	Free flowing	24.34	11.89	0.4765	335±1.13
A2	95.29	Free flowing	25.32	10.11	0.4798	337±0.58
A3	96.25	Free flowing	25.47	12.43	0.4532	342±1.73
A4	94.48	Free flowing	27.21	12.28	0.4760	347±2.58
A5	93.54	Free flowing	27.92	11.98	0.5012	350±2.47

Values shown in the table mean percent of 3 batches (n = 3).
RN is Ranolazine, BW is bees wax, A1= RN (0.8) : BW (3.0), A2 = RN (0.9): BW (3.0), A3 = RN (1.0) : BW (3.0), A4 = RN (1.0) : BW (3.1) , A5 = RN (1.0) : BW (3.2)

Formulations A3, A1, A2 were free flowing in nature. But formulations A4 and A5 are sticky, aggregate in nature; it might be due to more ratio of wax. The percent yield ranged from 93.54 % (A5) to 96.25 % (A3). Percentage yield and physical appearance are given in Table 1. When the drug wax ratio was 1.0:3.0, maximum yield was observed formulation A3. But increased drug ratio produces increased percent yield (A2 > A1) and increased wax ratio produces decreased percent yield (A5 < A4), it might be due to increase the viscosity of the emulsifying phase leads to separates the wax into solid flakes during cooling. Addition of tween 80, stirring speed and time increased or decreased amount also affects the percent yield. But the effect of other different factors on the percentage yield of microspheres was not clear, possibly as a result of the improper recovery of microspheres during filtration.

Sieve analysis data indicated that the prepared microspheres were in the size range of 115 to 855 µm and 53.1 to 63.4 % were of size fraction 375 µm. It was observed from SEM photographs, the obtained average sizes of the microspheres were A1 (335 µm), A2 (337 µm), A3 (342 µm), A4 (347µm) and A5 (350 µm). On the other hand increase in the drug ratio (A3 > A2 >A1) or wax ratio (A5 > A4), influence the average diameter of microspheres. This may be due to higher viscosity of the internal phase, which might have rendered higher resistance to the shearing of emulsion, there by increasing the particle size. It was not possible to avoid the formation of larger sized microspheres during rapid cooling of wax leading to hardening of the microspheres This is an agreement with literature findings and utilized higher molecular weight beeswax produced microspheres little larger size than the low molecular weight waxes [15].

Micro particulate systems should possess the better and adequate micromeritic properties. The obtained micromeritic properties of the formulations were presented in Table 1. The values of θ (angle of repose) were in the range 24.34 to 27.92 indicating good flow potential for the microspheres. The measured tapped density values ranged between 0.4532 to 0.5012 g /cm^3. The results of % I (Carr's index) range from 10.11 to 12.43 %, suggest good flow characteristics of the microspheres. The better flow property indicates reasonable and good flow potential of prepared microspheres.

SEM photographs showed that the wax microspheres were spherical in nature, had a smooth surface with inward small pores on the wall of the microspheres shown in Figure1. It may due to removal of the solvent during *in situ* drying process.
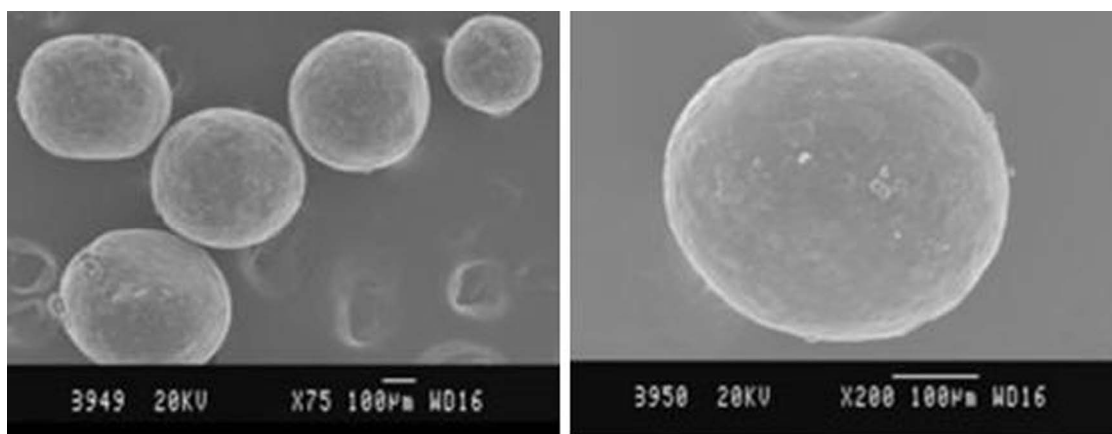
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Fig 1. SEM photographs of wax microspheres (A3). SEM Photograph of drug loaded wax microsphere showing spherical in nature. RN is Ranolazine, BW is beeswax .A3 = RN (1.0) + BW (3.0 g).

The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product. Microspheres with increase in concentrations of wax exhibited the highest size. There was a clear tendency towards microspheres size increased with concentrations of wax and this is an agreement with literature findings [15]. SEM photographs revealed the absence of crystals of the drug on the surface of microspheres indicating uniform distribution of the drug in the walls of the microspheres. The sphericity of the prepared microspheres was confirmed and the calculated sphericity values nearer to the value 1, confirming the sphericity of the microspheres.

DSC studies [17] were performed on pure drug; and drug loaded microspheres presented in Figure 2. Pure drug exhibits a sharp endothermic peak at to 122 ^{0}C. It was observed that absence of the endothermic peak at to 122 ^{0}C in the drug loaded microspheres indicated, that the drug is molecularly distributed in the microspheres.
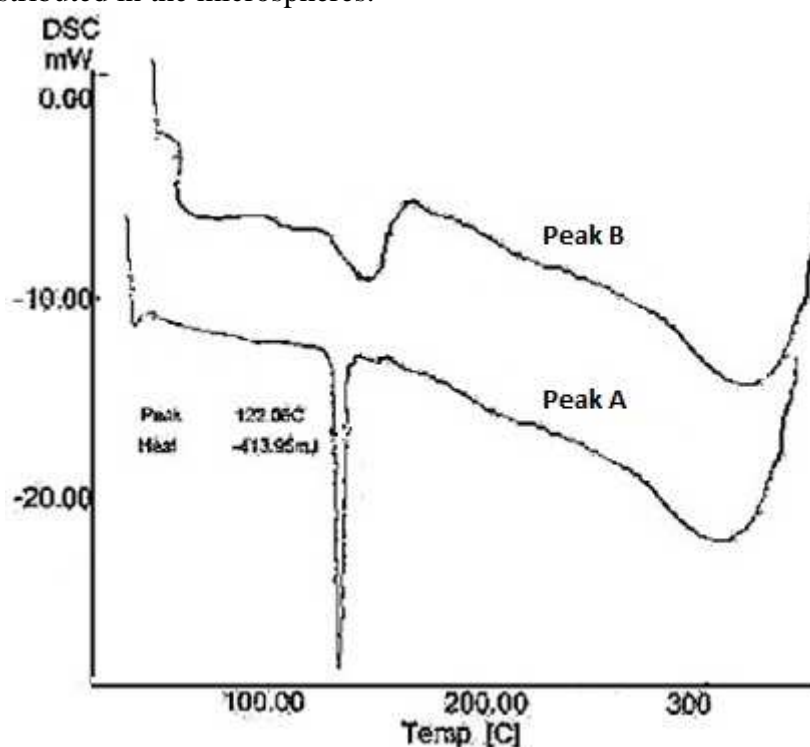

Fig 2. DSC thermograms for pure drug and wax microsphere (A3). Peak A= pure RN, peak B = A3, RN = Ranolazine, BW = beeswax, A3 = RN (1.0) + BW (3.0 g)

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From the FTIR studies the characteristic absorption bands for important functional group of pure drug and drug-loaded microspheres are identified. IR spectra at 1688.2 cm^{-1}(C=O ketone stretch), 1655.8 cm^{-1} (C=O carboxylic acid stretch), 1437.8cm^{-1} (C=C stretch aromatic),1335.8 cm^{-1} (N-H Stretch),1295.1cm^{-1} (C-N Stretch) and 1257.5 cm^{-1} (C-O stretch). FTIR spectra shown in Figure 3 showed that the characteristics bands of ranolazine were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and wax.

Fig 3.: FTIR spectra of Ranolazine and RN loaded wax microspheres (A3)
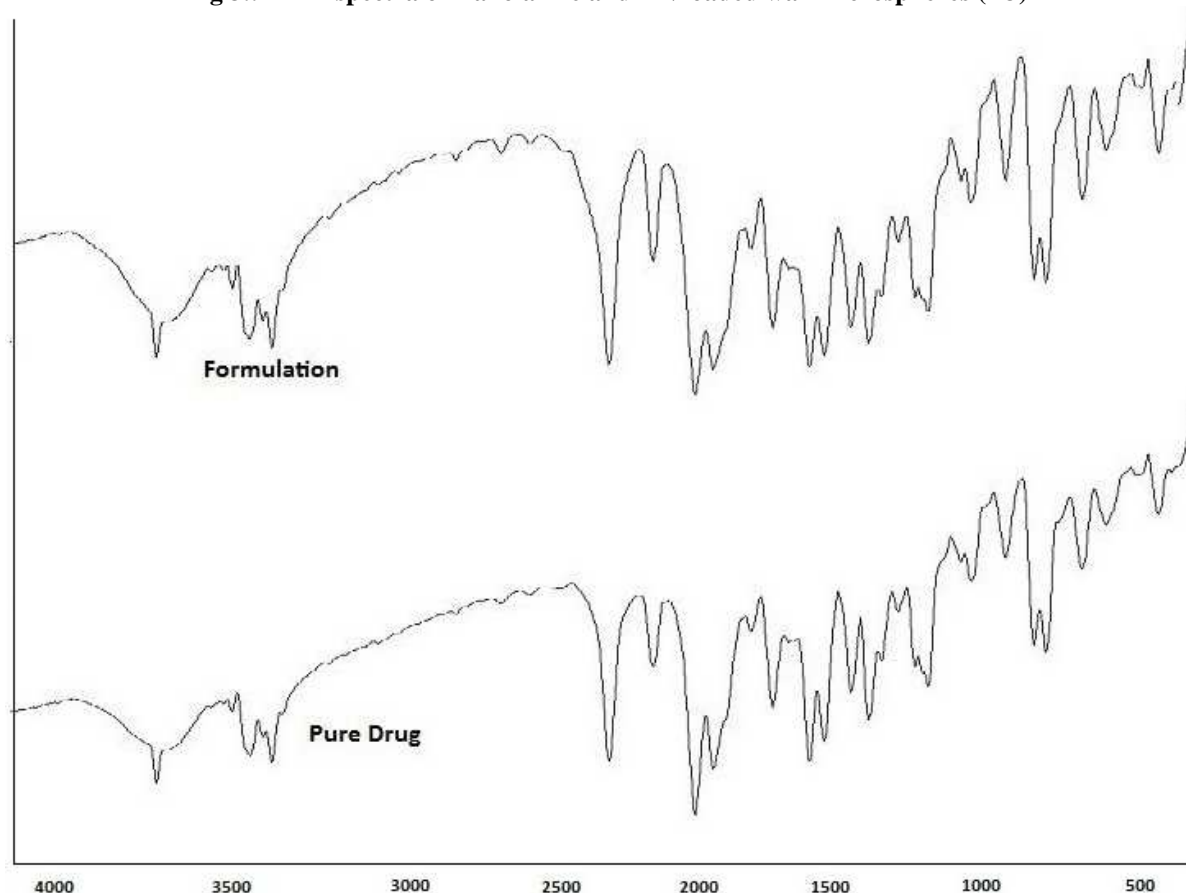
The percent of drug loading in the formulations were in the range of 23.72 % to 26.49 %. Drug loading was low in the formulation A5 and high for the formulation A3. The encapsulation efficiency (%) was found to be more for formulation A3 (86.42 %), when compared to formulation A4 (81.20 %)< A2 (84.32 %) < A1 (83.16 %) < A5 (77.42). It can be concluded that microspheres formulation A3 had more encapsulation efficiency.

From the release studies, it was observed that, there is no significant release of drug at gastric pH from wax microspheres indicates that the used wax is gastro resistant in nature [15,16]. At the end of 8th hrs, cumulative drug release (%) versus time (hrs) from formulation A3 was faster than formulations A1, A2, A4 and A5 in the intestinal environment as shown in Figure 4.

Drug was released in a biphasic manner and consisting of initial burst release followed by a slow release at intestinal pH from the wax microspheres**.** This result could be attributed to the dissolution of the drug present initially at the surface of the microspheres and rapid penetration of dissolution media to the matrix structure. However the formulation A3

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exhibited little higher burst effect, ratifying better amount of drug release. After initial burst effect, the subsequent release of drug was slow.
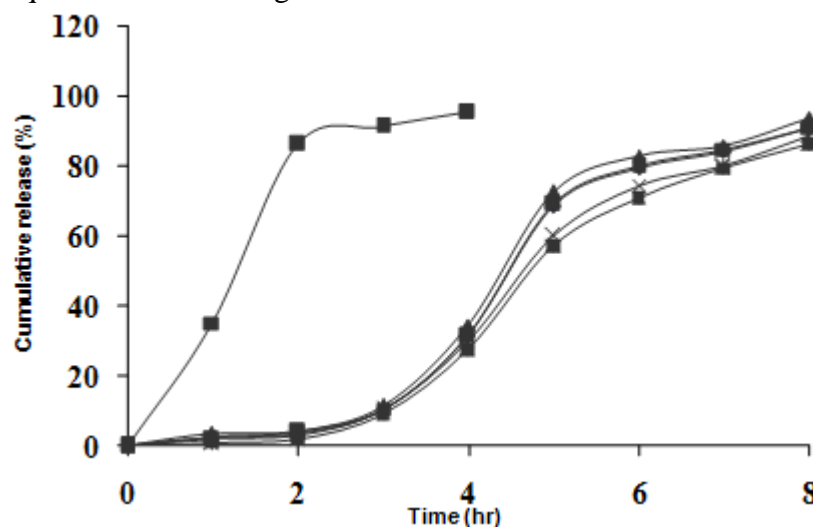

Fig 4. Cumulative % release of Ranolazine from wax microspheres and Caroza®500 in the gastric and intestinal environment against the time
A1 (—◆—), A2 (—▲—), A3 (-- ▲ --), A4 (--x--), A5 (—■—), Caroza®500(--■--).

Table 2: Formulations composition, experimented, predicted values and percentage error

Formulation Composition	Response variable	Experimented value	Predicted value	Percentage error
0.8: 3.0	$rel_{1\,hr}$	3.99	4.04	-1.237
(A1)	$rel_{8\,hr}$	91.08	90.65	0.477
	$t_{50\,\%}$	4.52	4.49	0.668
0.9: 3.0	$rel_{1\,hr}$	3.94	3.92	0.510
(A2)	$rel_{8\,hr}$	91.25	90.69	0.616
	$t_{50\,\%}$	4.55	4.50	1.111
1.0: 3.0	$rel_{1\,hr}$	3.84	3.82	0.523
(A3)	$rel_{8\,hr}$	93.99	93.94	0.053
	$t_{50\,\%}$	4.59	4.56	0.658
1.0: 3.1	$rel_{1\,hr}$	3.83	3.78	1.322
(A4)	$rel_{8\,hr}$	88.48	88.25	0.261
	$t_{50\,\%}$	5.08	5.06	0.395
1.0: 3.2	$rel_{1\,hr}$	3.75	3.73	0.536
(A5)	$rel_{8\,hr}$	86.87	86.83	0.069
	$t_{50\,\%}$	5.18	5.16	0.387

Values shown in the table mean percent of 3 batches (n = 3). $rel_{1\,hr}$; Release in 1 hr, $rel_{8\,hr}$; Release in 8 hrs, $t_{50\,\%}$;Time to 50 % drug release. RN is Ranolazine, BW is bees wax, A1= RN (0.8) : BW (3.0), A2 = RN (0.9): BW (3.0), A3 = RN (1.0) : BW (3.0), A4 = RN (1.0) : BW (3.1) , A5 = RN (1.0) : BW (3.2)

The decreased *in vitro* drug release from microspheres A1, A2, A4 & A5 was slower than that of A3 microspheres. Rate of drug release (until 8 hrs) tended to decrease with increase with in the ratio of wax, which indicates the incomplete drug release due to increased amount of wax. This is an agreement with literature findings [14]. Further increase in wax amount,

thicker wax microspheres inhibiting dissolution media penetration more strongly, resulting in significant reduction in the values of rel $_{8 \, hr}$ indicating slower drug release. The drug release was considerably retarded from wax microspheres when compared with formulation A6 (Caroza$^{®}$500). The values of $t_{50 \, \%}$ enhanced markedly from 4.52 to 5.16 hrs with further increases in the wax ratio were observed. This finding indicated considerable release retarding potential of the used wax amount.

Table 2 lists the compositions of the formulations their predicted and experimental values of all the release response, and the percentage error calculated by regression analysis method. Figure 5 (A, B, C) shows linear correlation plots between the observed and predicted values. Upon comparison of the responses with that of anticipated responses, the % prediction error varied between –1.237 to 1.322.The linear correlation plots drawn between the predicted and observed responses demonstrated values of r^2 0.9577 and 0.9923 excluding 0.9994 ($t_{50 \, \%}$), indicating excellent goodness of fit ($p < 0.05$) [18].
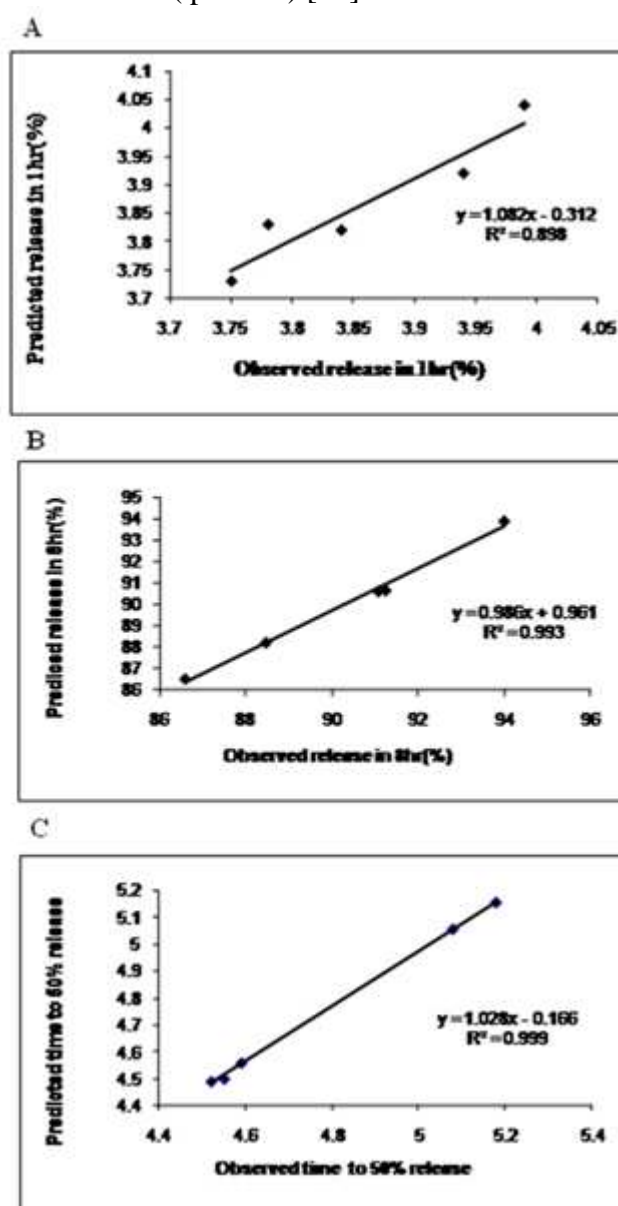

Fig 5. Linear Correlation Plots (A, B, C) between observed and predicted Values.

The rate of drug release followed first order kinetics and numerical data fitted into Peppa's equation [19]. Statistically estimated values of n at the 95 % confidence limit, the values of n are in the range of 0.41 to 0.45, indicated that the drug release from all microspheres was diffusion. In our study the results of n clearly indicated that the diffusion is the dominant mechanism of drug release from these formulations. Diffusion is related to transport of drug from the microspheres into the *in vitro* study fluid depending on the concentration of the wax used. As gradient varies, drug is released and the distance for diffusion increases. This could explain that the drug diffuses at a slower rate as the distance for diffusion increases. This is an agreement with literature findings [18]. The first order release gave consistently higher values for the correlation coefficient 0.927, 0.996, 0.947, 0.998, 0.991, 0.997 for formulations A1, A2, A3, A4, A5 and Caroza[®]500 tablet. The measured k value was different for all the microspheres in the range of 1.49 to 1.65. We found a small variation in the value of k. For instance, k =1.65 for microspheres A1, while it was smaller 1.49 for microspheres A5. Intermediate values of k = 0.00153, 0.00154, 0.00158 and 0.00161for microspheres A4, A2, A3 and formulation Caroza[®]500 tablet were observed.

Formulation F3 was compared with a marketed product Caroza[®]500 tablet presented Table 3 and differential (f_1) and similarity (f_2) factor [20] was calculated from dissolution data.

Table 3. Differential and Similarity factors for formulations F3 and Caroza[®]500 tablet

Formulations	Differential Factor ($f 1$)	Similarity Factor ($f 2$)
F 3	9.07	71.17
Caroza[®]500 tablet	6.80	62.49

The similarity factor *f2* was a logarithmic transformation of the sum-squared error of differences between the experimental drug release T_t and the ideal release R_t for over all time points 'n'. The similarity factor fit the result between 50 and 100. It approached 0 as the dissimilarity of the test and the reference profile increased, whereas, it attained 100 when the test and the reference profile were identical. The two profiles were believed to be similar when the *f*1 value of them was between 0 to 15 and *f*2 value of them was larger than 50 for which the mean deviation over all time points 'n' was less than 10% based on above equation. The value for the differential factor (*f*1 = 3.45- 10.24) and similarity factor (*f*2 = 58.42- 80.29) suggested that the dissolution profile of the prepared formulations and marketed formulation are similar. A similar calculation was reported with literature findings.
The cumulative percent drug release after stability studies from Caroza[®]500 tablet and formulation A3 were subjected for stability studies for 90 days. From the release studies, it was observed that, there is no significant change in the drug release at gastric pH from microspheres. At the end of 8 & 4hr, drug release from formulation A3 and Caroza[®]500 tablet 93.81% and 98.77 %, respectively. The cumulative percent drug release after stability studies from the above products within the range and there was no significant change in the *in vitro* drug release was indicates the encapsulated drug in stable form.

CONCLUSION

The drug release was found sufficient for oral delivery of drug. The used method was quite easy, economical, and simple and does not imply use of toxic organic solvents. The drug release profiles were significantly affected by the property of the waxy material used in the preparation of microspheres. From the present work, it can be concluded that the prepared microspheres demonstrate the potential use of wax for the development of controlled drug

delivery systems for water soluble or lipophilic drugs, which allows absorption in the intestinal tract.

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