KINETICS OF ENCAPSULATED LITHIUM CARBONATE INTO CARNAUBA WAX MICROSPHERES

D.V.Gowda*, Rajesh.N², H.G.Shivakumar¹, Nawaz Mahammed¹ and Siddaramaiah²

¹Department of Pharmaceutics, JSS College of Pharmacy, JSS University, S.S.Nagar, Mysore -570015, India.
²Department of Polymer Science and Technology, Sri Jayachamarajendra College of Engineering, Mysore - 570006, India.

ABSTRACT

The objective of the present study was to minimise the unwanted side effects of lithium carbonate (LC) drug by kinetic control of drug release, LC was entrapped into gastro resistant, biodegradable carnauba wax microspheres using meltable emulsified dispersion cooling induced solidification technique utilizing a wetting agent. Solid, discrete, reproducible free flowing microspheres were prepared. The yield of the microspheres was up to 92.0%, having smooth surfaces, with free flowing and good packing properties, angle of repose, % Carr’s index and tapped density values were within the limit, more than 98.0% of the isolated spherical microspheres were in the particle size range of 345-365 µm were confirmed by scanning electron microscopy (SEM). The drug loaded in microspheres was stable and compatible, as confirmed by DSC and FTIR studies. The release of drug was controlled for more than 8 hours. Intestinal drug release from microspheres was studied and compared with the release behaviour of commercially available formulation Intalith CR®-450. The release kinetics followed different transport mechanisms. The drug release performance was significantly affected by the materials used in microsphere preparations, which allows absorption in the intestinal tract.

Key words: Carnauba wax microspheres, lithium carbonate, controlled release, kinetic control.
INTRODUCTION

Lithium carbonate, a mono valent cation belonging to the group of alkali metals has been widely used in the treatment of bipolar and unipolar disorder [1]. Lithium is used to treat certain mood disorders, such as schizoaffective disorder and aggressive behavior and emotional instability in adults and children. Rarely is lithium taken to treat depression in the absence of mania. Considering the long regimen of anti maniac therapy, the administration of lithium carbonate was reported to induce adverse side effects on gastro intestinal tract (GIT) as well as hepatic, pancreatic, renal, endocrine, nervous, cardiac and hematological systems [2]. To achieve maximum therapeutic effect with a low risk of adverse effects, controlled released preparations are preferred [3]. 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 [4,5]. Delivering the drug in the intestinal milieu from wax/fat microspheres could be manipulated by suitable coating techniques [6]. Extensive clinical experience and well-controlled studies have shown that lithium carbonate is more effective than chlorpromazine in the treatment of acute maniac phase of mixed bipolar disorder [7]. Major advantages of lithium carbonate over the other antipsychotics are decreased subjective sedation, lack of extra pyramidal reactions, absence of tardive dyskinesia and the biochemical specificity of lithium to normalize biochemical defects [8, 9]. The chief characteristics of enteric coating are their impermeability to gastric juices but susceptibility to intestinal juices [10, 11]. Lithium carbonate should be dosed at least three doses with a maintenance dose per day. Due to its low therapeutic index, the frequency of adverse effects may be dose related [12, 13]. A controlled release dosage form of lithium salt is preferable than the conventional dosage form of lithium, because there is a considerable saving in nurses and pharmacists time [14]. As demonstrated by pharmacokinetic studies on lithium carbonate, the ingestion of a single controlled release enteric coated tablet is effective even when administered once a day [15, 16]. These findings suggested that kinetic control is effective for preventing the toxicity of lithium carbonate. Previous experimental results demonstrated that waxes are biocompatible, non-immunogenic material used for the entrapment of drug, used for controlling drug release in the intestinal tract [17, 18]. The objectives of the present study
are to formulate, characterize and study the *in vitro* drug release from carnauba wax microspheres loaded with lithium carbonate. The pattern of drug release from the carnauba wax microspheres is compared with that of the commercially available enteric coated oral formulation Intalith CR – 450.

**MATERIALS AND METHODS**

Lithium carbonate USP Grade was obtained as a gift sample from Micro Labs, Bangalore, India. It is a white, light alkaline powder with molecular formula Li$_2$CO$_3$ and molecular weight 73.89. Carnauba wax was obtained from Shri Ram Sons Wax Pvt. Ltd, New Delhi, India. It is a yellow in colour, having melting point 181.4°F. It is insoluble in water. Tween 80 was obtained from Loba Chemie Pvt. Ltd., Mumbai, India. It is Amber colored viscous liquid, readily soluble in water. All other chemicals and solvents were of analytical grade.

**Preparation of wax microspheres**

9 gm of carnauba wax was melted separately in china dish using water bath. Drug (3 gm) previously passed through sieve no.100 was dispersed in the melted wax mass and stirred to obtain a homogeneous melt. These individual mixtures were poured into 150 ml of pH 10.9 Ammonia buffer solution (in order to minimize the solubility of drug), which was previously heated to a temperature higher than melting point of wax/fat (> + 5°C). Span 20 (2.0% w/w) for the mixture containing carbauna wax. The whole mixture was mechanically stirred at 900 rpm using a stirrer (RQ-127A) fitted with a 4- blade impeller of 53 mm diameter. Spherical particles are produced due to dispersion of molten wax in the aqueous medium. The mixture was stirred continuously at 900 rpm at a higher temperature (> + 5°C) of the melting point of wax for 3 min. The temperature of the mixture in the beakers was cooled rapidly to 10°C by the addition of cold water. The resultant solid spheres collected by filtration were extensively washed with water to remove any drug and surfactant residues. Air drying was carried out at room temperature for 48 h produced discrete, free flowing solid microspheres.

**Size analysis of microspheres**

The separations of the microspheres in to various size fractions were carried out by sieve analysis technique and SEM analyzed the size of microspheres.
Micromeritic properties

Tap density of the prepared microspheres was determined using tap density tester and % Carr’s index (% I ) was calculated. Angle of repose was assessed to know the flowability of wax microspheres.

Scanning electron microscopic studies (SEM)

SEM photographs were taken using scanning electron microscope (Model Joel-LV-5600, USA). The photographs were subjected for morphological characteristics and to confirm spherical nature of the microspheres.

Sphericity determination

To determine the sphericity, the tracings of wax microspheres (magnification 45 X) were taken on a black paper using Camera Lucida, (Model-Prism type, Rolex, India) and circulatory factor was calculated \[^{[19]}\]. The sphericity of microspheres was calculated using the equation,

\[ S = \frac{p^2}{(12.56 \times A)}. \]

Where A is area (cm\(^2\)) and p is perimeter (cm)

Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out on DuPont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina discs of DuPont Company) 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. The runs were made in triplicate.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of pure drug, empty microspheres and drug loaded microspheres were obtained using KBr pellet method (6000 kg/cm\(^2\)). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (JASCO-4100, Japan).

Drug loading Efficiency

Drug incorporated wax microspheres of each batch was selected and powdered with pestle and mortar. Drug was extracted from wax microspheres using 0.1 N HCl, filtered and analyzed for drug content after suitable dilution. Estimation of lithium was
accomplished by atomic absorption spectrophotometer (Perkin-Elmer, USA) at the wavelength of 670.8 nm.

**Stability studies**

The optimized formulation was subjected to stability studies. The stability studies were carried out by storing the microspheres in capsules kept in a glass bottle at 25°C / 60 % RH 30°C / 65 % and RH 40°C / 75 for 90 days. These samples were collected on 15th, 45th and 90th day respectively and at regular intervals for changes in their physical appearance. The drug content was estimated through atomic absorption spectrophotometer (Perkin-Elmer, USA).

**In vitro studies**

USP XXI dissolution apparatus type II was employed to study percentage of drug release from various formulations prepared. Encapsulations of the drugs-loaded microspheres were avoided, as dissolution of shell will add one more parameter to the result. Accurately weighed quantities of drug (lithium carbonate - 450 mg equivalent to a commercial preparation - Intalith CR®-450 mg tablet,) loaded microspheres of each batch were taken in 900 ml dissolution medium (lithium carbonate - 2 h in pH 1.2 HCl buffer and 6 h in pH 7.4 tris chloride buffer BET) 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 of 30 min for first 4 h and at 60 min interval for the next 4 h. Release studies were carried out in triplicate.

**RESULTS AND DISCUSSIONS**

**Preparation of wax microspheres**

Evidence have shown in the recent years that waxy materials have the physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen. In the present study, a modified novel meltable dispersion emulsified cooling induced solidification method was employed using inert carnauba wax and non-toxic solvents to entrap the drug, furthermore various parameters were characterized for drug and wax ratio, stirring speed and time, amount of surfactant added, volume of the aqueous phase used, effect of pH on drug entrapment, temperature of the aqueous phase and
rapid cooling studies. Therefore the influence of the above parameters was highlighted. When the pH value of the external aqueous phase was highly alkaline, 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 7.2, the percent of drug loading reduced from 13.23 to 3.2%, 14.12 to 3.0% , 15.23 to 3.2%, 12.43 to 2.8% 11.65 to 2.6% for F₁, F₂, F₃, F₄ and F₅ formulations. The present study reveals that 150 ml of aqueous phase suitable for producing the ideal spherical microspheres. Resultant microspheres did not have any surface irregularities and are non aggregated. As the volume of external phase increased, the yield was reduced and the resultant microspheres were irregularly shaped. When the volume of the aqueous phase was less than 150 ml, the resultant microspheres were highly aggregated in nature and highly impossible to distinguish as individual microspheres. In order to avoid the formation of irregularly shaped larger particles, in the present method, 150 ml of aqueous phase was used. Incorporation of drug into carnauba wax microspheres required the addition of tween 80 as a 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 a failed, as it resulted in an aggregate cake like mass during the solidification of wax. This may be due to repulsion resulting from high interfacial tension between the hydrophobic waxy material and external aqueous phase. It was found that tween 80 having hydrophobicity and lipophobicity balance (HLB) value of 15 was suitable to increase substantially dispersion of waxy material in external aqueous phase and promote drug incorporation in the wax microspheres. The batches were tested to obtain an optimal surfactant concentration, various concentrations ranging from 0.8 to 2.3 % (w/w) of the total formulation were selected. Discrete microspheres with good flow properties using an optimum concentration of surfactants 1.4 % w/w (tween 80) were used. Concentrations of tween 80 ranging from 0.8 to 2.3 % w/w failed to produce reproducible microspheres. The resultant wax microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres. A similar surfactant concentration was reported for carnauba wax and bees wax microspheres prepared by melttable dispersion method.
Temperature of the aqueous phase was maintained at 5\(^{0}\)C higher than the melting point of the carnauba wax in the corresponding formulations. From SEM studies it was observed that the resultant microspheres were free from surface irregularities, except some wrinkles. It was also observed that when the temperature of the aqueous phase was less than the 5\(^{0}\)C than the melting point, the big flakes were produced. 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 be due to reduced melting point of the waxy materials. SEM photographs also indicate the presence of the crystals on the surface of the microspheres. The resultant microspheres were unsuitable for pharmaceutical uses. Hence an optimum 1:3 ratio was used to prepare microspheres \[^6,11, 20\] (Table 1). The average size of the microspheres ranged between 339 to 357. A stirring speed of 900 rpm and stirring time of 3 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 900 to 1100 rpm there was a decrease in the average size of the spheres and recovery yield of the microspheres. It is due to small sized wax microspheres, which were lost during successive washings. When the stirring speed was lower than 900 rpm, larger pellets were formed. It was also found that an increase in stirring time, from 2 to 4 min (at a stirring speed of 900 rpm), there was a decrease in the recovery yield of microspheres. When the stirring time lower than 2 min. A little amount of melted material adhered to the sides of the beaker during the cooling process results in reduction of yield.

**TABLE 1. DRUG AND WAX RATIO FOR THE PREPARED MICROSPHERES FORMULATIONS**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug (gm)</th>
<th>Carnauba wax (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F(_1)</td>
<td>2.8</td>
<td>8.8</td>
</tr>
<tr>
<td>F(_2)</td>
<td>2.9</td>
<td>8.9</td>
</tr>
<tr>
<td>F(_3)</td>
<td>3.0</td>
<td>9.0</td>
</tr>
<tr>
<td>F(_4)</td>
<td>3.1</td>
<td>9.1</td>
</tr>
<tr>
<td>F(_5)</td>
<td>3.2</td>
<td>9.2</td>
</tr>
</tbody>
</table>
**Micromeritic properties**

Micro particulate drug delivery systems are formulated as single unit dosage forms in the form of capsule or tablet. Such microparticulate systems should possess the better and adequate micromeritic properties. The obtained micromeritic properties are given in (Table 2). The values of angle of repose were well within the range, indicating reasonable good flow potential for the microspheres. The tapped density values ranged between 0.45 g/cm$^3$ to 0.52 g/cm$^3$. The results of % compressibility index ranges from 9.31% to 15.02%, suggests good flow characteristics of the microspheres. The better flow property indicates reasonable and good flow potential of prepared microspheres.

**TABLE 2. MICROMERITIC PROPERTIES OF THE DRUG LOADED WAX MICROSPHERES**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average size (µm)</th>
<th>Yield (%)</th>
<th>Angle of repose ($\theta^0$)</th>
<th>% Compressibility index</th>
<th>Tapped density (g/cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_1$</td>
<td>339</td>
<td>83.51</td>
<td>26.31</td>
<td>9.31</td>
<td>0.45</td>
</tr>
<tr>
<td>F$_2$</td>
<td>345</td>
<td>86.21</td>
<td>27.12</td>
<td>10.34</td>
<td>0.47</td>
</tr>
<tr>
<td>F$_3$</td>
<td>352</td>
<td>90.32</td>
<td>27.98</td>
<td>12.54</td>
<td>0.50</td>
</tr>
<tr>
<td>F$_4$</td>
<td>354</td>
<td>88.54</td>
<td>24.89</td>
<td>11.67</td>
<td>0.49</td>
</tr>
<tr>
<td>F$_5$</td>
<td>357</td>
<td>87.92</td>
<td>25.98</td>
<td>15.02</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values shown in the table mean percent of 3 batches (n = 3)

**Scanning electron microscopy (SEM)**

SEM photographs showed that the wax/fat microspheres were spherical in nature, had a smooth surface with inward dents and shrinkage, which is due to the collapse of the wall of the microspheres (Fig.1). photograph reveal the absence of crystals of the drug on the surface of microsphere, indicating uniform distribution of the drug within the microspheres and further indicate that low molecular weight wax/fat produce
better quality microsphere than that of high molecular weight waxes. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product\(^{[20]}\). The sphericity factor obtained for the microspheres nearer to the value 1, thereby confirming the sphericity of the microsphere.

**Figure. 1**

SEM microphotographs of wax microspheres loaded with Lithium carbonate

**Differential scanning calorimetry (DSC)**

DSC studies were performed on pure drug, empty and drug-loaded microspheres have shown sharp endothermic peaks. Lithium carbonate exhibits a sharp endothermic peak at 618.28°C presented in (Fig.2). It was observed that absence of the endothermic peak of the drug at 618.28°C in the drug loaded wax microspheres indicates that the drug is uniformly distributed at molecular level in the microspheres\(^{[3]}\).

**Figure. 2**

DSC thermograms of carnauba wax, pure lithium carbonate and lithium.
Fourier transform infrared spectroscopy (FTIR)

The characteristic bands for important functional group of pure drug empty microspheres and drug-loaded microspheres were identified. FTIR spectra showed that the characteristics bands of lithium carbonate were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and wax used. Peak at 2915 cm\(^{-1}\) due to CO\(_3\) stretching, 1715 cm\(^{-1}\) due to C=O stretching, 1081 cm\(^{-1}\) OH deformation and 860 cm\(^{-1}\) due to carbonyl stretching. A comparison and interpretation of this region in our spectra agrees with their conclusions (figure 3).

![FTIR spectra of carnauba wax, pure drug and F3 formulation.](image)

**Figure. 3**

FTIR spectra of carnauba wax, pure drug and F3 formulation.

Drug loading Efficiency

The percent of drug loading in the formulations was found to be in the range of 13.12 % to 14.78 %. It was low in the formulation F\(_4\) and more in F\(_3\). The percent of encapsulation efficiency was found to be 85.12 % in formulation F\(_3\) and the results are presented in (Table 3).
TABLE 3. DRUG LOADING EFFICIENCY OF WAX MICROSPHERES

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug loading (mg)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁</td>
<td>13.28</td>
<td>81.32</td>
</tr>
<tr>
<td>F₂</td>
<td>13.89</td>
<td>82.93</td>
</tr>
<tr>
<td>F₃</td>
<td>14.78</td>
<td>85.12</td>
</tr>
<tr>
<td>F₄</td>
<td>13.12</td>
<td>81.10</td>
</tr>
<tr>
<td>F₅</td>
<td>13.56</td>
<td>82.62</td>
</tr>
</tbody>
</table>

Values shown in the table mean percent of 3 batches (n=3)

**Stability studies**

Stability studies for the optimized formulation F₃ were performed to ascertain whether the drug undergo any change or degradation during its shelf life. The samples were checked for changes in physical appearance and drug content at regular intervals. The obtained results are given in (Table 4). From the results, the drug was stable in the prepared formulations for the study period.

**In vitro studies**

From the release studies it was observed that, there is no significant release of drug at gastric pH from wax microspheres. At the end of 8th h, *in vitro* drug release from F₃ (83.32%), was slower than Intalith CR®-450 (86.98%) in the intestinal environment as shown in (Fig 4). Drug was released in a biphasic manner consisting of initial fast release followed by a slow release in intestinal pH from the wax microspheres. The decreased *in vitro* drug release from wax microspheres might be due to more hydrophobicity and influence of molecular weight of wax. The *in vitro* drug release was considerably retarded from the wax microspheres when compared Intalith CR®-450. The rate of drug release followed first order release kinetics and numerical data fitted into Peppa's model showed that, the mechanism of drug release from wax microspheres (Table 5)
was non fickian diffusion. After an initial burst effect, the subsequent release of drug from microspheres was slow, and the influence of molecular weight. It is also noticed, a small variation in the value of k, k = 0.0152 in F₃, while it was 0.0139 for F₅. An intermediate value of k 0.0148, 0.0147 and 0.0145 was observed for formulation F₁, F₂ and F₄, respectively.

**TABLE 4. STABILITY STUDIES FOR DRUG CONTENT OF FORMULATION F₃**

<table>
<thead>
<tr>
<th>Stability condition</th>
<th>Sampling (days)</th>
<th>Drug content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 °C / % RH</td>
<td>15</td>
<td>99.12</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>99.07</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>99.02</td>
</tr>
<tr>
<td>30 °C / % RH</td>
<td>15</td>
<td>99.09</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>99.04</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>99.02</td>
</tr>
<tr>
<td>40 °C / % RH</td>
<td>15</td>
<td>99.05</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>99.01</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>98.96</td>
</tr>
</tbody>
</table>

Values shown in the table mean percent of 3 batches (n=3)

Cummulative % release of lithium carbonate from wax microspheres and Intalith CR® - 450 in the gastric and intestinal environment.
TABLE 5. *IN VITRO* RELEASE KINETIC PARAMETERS FOR CARNAUBA WAX MICROSPHERES

<table>
<thead>
<tr>
<th>Formulation</th>
<th>n</th>
<th>k</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1$</td>
<td>0.532</td>
<td>0.0148</td>
<td>0.9957</td>
</tr>
<tr>
<td>$F_2$</td>
<td>0.547</td>
<td>0.0147</td>
<td>0.9971</td>
</tr>
<tr>
<td>$F_3$</td>
<td>0.524</td>
<td>0.0152</td>
<td>0.9986</td>
</tr>
<tr>
<td>$F_4$</td>
<td>0.535</td>
<td>0.0145</td>
<td>0.9956</td>
</tr>
<tr>
<td>$F_5$</td>
<td>0.576</td>
<td>0.0139</td>
<td>0.9973</td>
</tr>
</tbody>
</table>

Values shown in the table mean percent of 3 batches (n=3)

CONCLUSION

The method is quite simple, rapid, and economical and does not imply the use of toxic organic solvents. The drug release from the carnauba wax microspheres was found sufficient for oral delivery and the drug release profile was significantly affected by the properties of wax used in the preparation of microspheres. The drug release through first order kinetics and followed by non-fickian diffusion. The study also revels as the pH of external aqueous phase alkaline the solubility of drug reduced and the encapsulated amount of the drug were significantly increases. The study results demonstrate the potential use of wax for the fabrication of controlled delivery devices for many water soluble drugs.

ACKNOWLEDGEMENT

The authors are highly grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for sanctioning the financial grant to carry out this research work.

REFERENCES


5. G. Campisi, G. Giandalia, V. De Caro, C. Di Liberto, P. Arico, L.I. Giannola: A new delivery system of clobetasol-17-propionate (lipid-loaded microspheres 0.025%) compared with a conventional formulation (lipophilic ointment in a hydrophilic phase 0.025%) in topical treatment of atrophic/erosive oral lichen planus. A Phase IV, randomized, observer-blinded, parallel group clinical trial. British Journal of Dermatology, 2004; 150:5, 984-990.


9. Dominique Januel, Marie-France Poirier, Françoise D’alche-Biree, Michel Dib, Jean-Pierre Olié and Study group. Multicenter double-blind randomized parallel-group clinical trial of efficacy of the combination clomipramine (150 mg/day) plus lithium carbonate (750 mg/day) versus clomipramine (150 mg/day) plus placebo in the treatment of unipolar major depression. J. Defective Disorders. 2003; 76: 191-200.


For Correspondence:
D.V.Gowda
E-mail- dvg5@lycos.com
Mob: +919663162455