Development and optimization of a multiple-unit controlled release formulation of a freely water soluble drug for once-daily administration

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A B S T R A C T

A controlled release resinate beads of betahistine diHCl (BHCl), a short half-life freely water soluble drug, was developed to allow once-daily administration to improve patient compliance and eliminate the risk of intolerance of the drug. BHCl–resin complex was subsequently coated with Eudragit® RS100. A 2³ full factorial design was employed for optimization and to explore the effect of Eudragit® RS100 concentration in the coating solution (X₁), the coating percentage (X₂), the speed of rotation (X₃) and the concentration of plasticizer (PEG 400) (X₄) on the release rate of the drug from the microcapsules. The extent of coating (Y₁), and the percentage drug released at given times (Y₂, Y₃ and Y₄) were selected as dependent variables. The optimization process was performed for X₁, X₂, X₃ and X₄ using target ranges of these responses determined based on target release model deduced form zero-order dissolution profile of BHCl for once-daily administration. The levels of X₁, X₂, X₃ and X₄ of optimized BHCl microcapsules are 14.42%, 50.63%, 1495 rpm and 9.94%, respectively. The calculated value of f₂ for the optimized BHCl microcapsules filled into hard gelatin capsules was 67.03 indicating that the dissolution profiles of the optimized formulation is comparable to that of the target release model. It could be concluded that a promising once-daily extended-release microcapsules of the highly water soluble drug, BHCl, was successfully designed.

1. Introduction

Betahistine is an analogue of histamine and is claimed to improve the microcirculation of the labyrinth resulting in reduced endolymphatic pressure. It is used to reduce the symptoms of vertigo, tinnitus, and hearing loss associated with Ménière’s disease. The short half life of this drug (3–4 h) necessitates frequent doses (Sweetmant, 2009). Betahistine is given by mouth as the hydrochloride. The usual initial dose is 8–16 mg three times daily taken preferably with meals; maintenance doses are generally in the range of 24–48 mg daily. It is known that betahistine has histamine-like action on the secretory cells of the gastric mucosa and that its use is inadvisable in patients with gastric irritation. It was thus advantageous to be able to design a new formulation of BHCl which allows a once-daily administration while keeping the concentration of drug within the therapeutic range and eliminating the risk of intolerance from immediate release dosage forms due to absorption of large amount of BHCl. BHCl is freely soluble in water (Sweetmant, 2009). The very high solubility of BHCl requires specific technologies in order to control the release in oral formulations.

Sustained release composition of BHCl have been formulated for instance in US 4,264,574, EP 502,642, and EP 1,158,963 but there is still the need of improved formulations, particularly with respect to the reliability, convenience and constancy in the industrial productive processes as well as formulating a once daily dosage form that approaches zero-order release characteristics. Most of the sustained release compositions of BHCl could sustain the release of the drug for only 8 h (Jean and Alain, 1981; Fabiani, 2003; Fossati, 2005).

Much of the research effort in developing novel drug delivery systems has been focused on oral controlled-release dosage forms. Among them, multiple-unit dosage forms, such as beads or microparticules, have gained much popularity for different reasons when compared to non-disintegrating single-unit dosage forms. They distribute more uniformly in the gastrointestinal tract, resulting in more reproducible release profiles, more predictable gastric emptying, more uniform drug absorption and reduced local irritation, minimized risk of dose dumping and avoid the unwanted intestinal retention of the polymeric material (Cuna et al., 2000).

Ion exchange resins (IER) are cross-linked, water insoluble, polymer-carrying, ionizable functional groups (Pongjanyakul et al., 2005). Drugs can be loaded onto the resins by an exchanging reaction, and hence, a drug–resin complex (drug resinate) is formed (Guo et al., 2009). The use of IER has occupied an important place in the development of controlled release systems because of their better drug retaining properties and prevention of dose dumping, the
major drawback of common sustained release formulations. The characteristics of IER, such as their physico-chemical stability, inert nature, uniform size, spherical shape, easier coating and reproducible drug release in ionic environment, have encouraged their use in oral drug delivery. Moreover, IER impart flexibility in designing a variety of delivery systems, such as liquids (Junyaprasert and Manwiwattanakul, 2008), beads (Motycka et al., 1985), microparticles (Ichikawa et al., 2001) and simple matrices (Anand et al., 2001).

Many grades of ion-exchange resins, varied in acid or base strength, cross-linkage and particle size, are commercially available and make it possible to control the rate of drug release over a wide range of pH. Combination of drug–resinates and microencapsulation or coating technique provides a means to further manipulate drug release profiles. Literature lacks any data about the use of drug–resinate microcapsules for the controlled delivery of BHCl.

The aim of this study was to prepare controlled release BHCl–resinate microcapsules for once daily administration. The effect of different formulation and process variables were evaluated using experimental full factorial design. The optimization procedure would aid in the preparation of controlled release system with predictable properties.

2. Materials and methods

2.1. Materials

Betahistine diHCl (BHCl) was kindly supplied by Chemipharm, Egypt. Dowex® 50WX series, polystyrene sulfonate, H+ form (Dowex® 50WX8 400 with particle size 50–75 μm and degree of cross linking 8%, Dowex® 50WX4 400 with particle size 50–75 μm and degree of cross linking 4%. Dowex® 50WX5 50 with particle size 300–840 μm and degree of cross linking 4%), Span® 80 and Polyvinyl alcohol 13000 were purchased from the Sigma–Aldrich Co., USA. EudragitCo® RS 100 was kindly provided by RÖhm Pharma, Weiterstadt, Germany. Light liquid paraffin, acetone, methylene chloride, tri-sodium orthophosphate (Na3PO4), n-hexane, and magnesium stearate were purchased from El–Nasr pharmaceutical company, Egypt.

2.2. Preparation of BHCl–resinate beads

The different types of resins were purified by rinsing three times with distilled water, two times with 95% ethanol, and then two times with distilled water again. Each treatment takes at least 8 h by a batch process. After filtration, the resin was dried in an oven at 40 °C for 24 h (Jeong and Park, 2008). The BHCl–resinate beads were prepared by a batch process, using the method described by Jeong and Park (2008). Previously purified resin particles (0.5 g dry weight) were dispersed in a 2% (w/v) drug solution (50 mL) under magnetic stirring at room temperature. In order to investigate how quickly equilibrium could be reached, 0.1 mL of supernatant was collected at predetermined intervals during complex formation at room temperature, diluted with water, and then the drug amount was quantified spectrophotometrically at λmax 261 nm. The drug–resinate beads were separated from the supernatant by filtration, washed with deionized water to remove any uncomplicated drug, and then dried in an oven at 40 °C for 24 h (Jeong and Park, 2008).

2.3. Study of the effect of resin properties on in vitro release of BHCl from drug–resinate beads

Drug release from different drug–resinate beads (Dowex® 50WX4 400, Dowex® 50WX8 400, and Dowex® 50WX4 50) was conducted according to USP 30 Apparatus II (paddle method) with the dissolution medium maintained at 37 ± 0.5 °C and mixed at 50 rpm. The dissolution medium used in this study was 250 mL 0.1N HCl (pH 1.1) for the first 2 h followed by addition of 100 mL 0.2M Na2HPO4 to change the pH to 7.4 for the rest of 24 h. 5 mL samples were withdrawn at different time intervals and replaced by equal volumes of fresh medium. The concentration of BHCl in the samples was spectrophotometrically measured at λmax 261 nm.

2.4. Preparation of coated BHCl–resinate beads using different microencapsulation techniques

The BHCl–resinate beads, showing the most sustained release of the drug, was selected to be encapsulated with Eudragit® RS 100 using solvent evaporation methods to offer the desired controlled-release profile that extended for 24 h (Torres et al., 1998). The microencapsulation process could be performed using two techniques.

(a) o/w method. The BHCl–resinate particles (coat to core ratio: 2:1) were suspended in 10 mL of a 20% (w/v) solution of the Eudragit® RS 100 in methylene chloride, followed by the emulsification in 1 mL of a 0.25% (w/v) aqueous solution of PVA, using a propeller stirrer at 500rpm. After complete evaporation of methylene chloride (approximately 3 h.), the microcapsules were isolated by vacuum filtration, washed with distilled water and air-dried for 24 h.

(b) o/l method. The BHCl–resinate particles (coat to core ratio: 2:1) were suspended in 10 mL of a 20% (w/v) solution of the Eudragit® RS 100 in acetone followed by emulsification of this phase in 100 mL of light liquid paraffin containing 1% Span® 80, and 0.1% Mg stearate. The stirrer was set at 500rpm till complete evaporation of acetone (approximately 1 h). Mg stearate was added as droplet stabilizer to overcome the problem of coalescence during solvent evaporation (Haznedar and Dortunc, 2004). The microcapsules were isolated by vacuum filtration, washed with three portions of 75 mL n-hexane and air-dried for 24 h.

2.5. Optimization of BHCl–resin microcapsules using a 24 full factorial experimental design

BHCl–resin microcapsules were prepared using a 24 full factorial experimental design in order to investigate the joint influence of formulation variables and experimental conditions using Expert-design® software. In this design, 4 factors are evaluated, each at 2 levels, and experimental trials are performed at all 16 possible combinations. The independent variables were Eudragit® RS 100 concentration in the coating solution (X1), coating percentage (X2), speed of rotation (X3), and concentration of PEG 400 (plasticizer) in the coating solution (X4) (Table 1). The extent of coating of the microcapsules (Y1), the percentage drug released after 1 h (Y2), 6 h (Y3), and 12 h (Y4) were selected as the dependent variables. Table 2 depicts the composition of the prepared tablets.

2.6. Evaluation of BHCl–resin microcapsules

2.6.1. Determination of extent of coating of the microcapsules

One hundred milligrams of BHCl–resin microcapsules were accurately weighed and washed three times with 10 mL of acetone in order to remove polymer coating (Junyaprasert and Manwiwattanakul, 2008). The remaining BHCl–resin beads were dried at 50°C for 12 h and weighed. The extent of coating was
of the coefficient of determination (kinetics of drug release from the prepared matrices. The large value calculated by the following:

\[
\text{Coating extent} = \frac{\text{BHCl–resin microcapsules weight} - \text{dried BHCl–resin beads weight}}{\text{BHCl–resin microcapsules weight}} \times 100
\]

2.6.2. Scanning electron microscope
The surface properties of selected microcapsule formulations were examined by the scanning electron microscope (Jeol JSM-6400, Tokyo, Japan). The microcapsules were fixed on a sample holder, and coated with gold palladium using sputter coater for 1 min under argon gas before electron microscopic scanning.

2.6.3. In vitro drug released from the prepared microcapsules
The in vitro drug release from the different BHCl–resin microcapsules was determined using the same procedure as previously mentioned in Section 2.3.

2.6.4. Kinetic analysis of the release data
The mean in vitro drug release data were fitted to different kinetic models: zero order (Wagner, 1969), Higuchi (Higuchi, 1961, 1963), and Korsemeyer–Peppas (Peppas, 1985) to evaluate the kinetic models: zero order (Wagner, 1969), Higuchi (Higuchi, 1961, 1963), and Korsemeyer–Peppas (Peppas, 1985) to evaluate the kinetics of drug release from the prepared matrices. The large value of the coefficient of determination \( R^2 \) indicated a superiority of the dissolution profile fitting to mathematical equations.

Table 1
Full factorial design used to optimize the formulation.

<table>
<thead>
<tr>
<th>Factors (independent variables)</th>
<th>Level used</th>
</tr>
</thead>
<tbody>
<tr>
<td>( X_1 ): Eudragit® RS 100 concentration in the coating solution (%)</td>
<td>5% 20%</td>
</tr>
<tr>
<td>( X_2 ): Coating percentage (%)</td>
<td>33% 66%</td>
</tr>
<tr>
<td>( X_3 ): Speed of rotation (rpm)</td>
<td>500 rpm 1500 rpm</td>
</tr>
<tr>
<td>( X_4 ): Concentration of plasticizer (PEG-400) in the coating solution (%)</td>
<td>0% 10%</td>
</tr>
</tbody>
</table>

Response (dependent variables) Constraints

\( Y_1 \): extent of coating – 
\( Y_2 \): % BHCl released after 1 h \( 5% \leq Y_2 \leq 11%\)
\( Y_3 \): % BHCl released after 6 h \( 27.5% \leq Y_3 \leq 42%\)
\( Y_4 \): % BHCl released after 12 h \( 60% \leq Y_4 \leq 75%\)

3. Results and discussion
The aim of this work was to develop new oral controlled release delivery system of BHCl, a freely water soluble drug, to provide zero-order controlled release for once daily administration. An ideal drug release profile (i.e., 8% in the first h and a constant drug release thereafter) was considered as a target release profile. The ion-exchange resins used in this study are strong cationic ion exchange resins consisting of sulfonic acids attached to an insoluble polysulfone divinylbenzene copolymer. Two methods have been reported for preparation of drug–resin beads, known as a column method and a batch method (Borodokin, 1993). The later procedure was used in this study to prepare BHCl–resinate beads because it is simple, quick and more suitable for very fine particles.

The dissolved BHCl exists in the protonated drug ions which can displace the hydrogen counter-ion (\( H^+ \)) at the sulfonic acid functional groups on the ion-exchange resin particle. The ion-exchange process in the preparation can be illustrated as follows:

\[
\text{Re–SO}_3^- + BH^+ \rightarrow \text{Re–SO}_3^- BH^+ + H^+
\]

where Re is an insoluble portion of resin and \( BH^+ \) is betahistine ion.

3.1. Effect of resin particle size and degree of cross linking on the loading equilibrium time
Resins of various particle sizes, and degrees of cross linking were used to investigate their effect on the equilibrium time. The weight ratio between the drug and resin was 1:2 for the loading. Fig. 1 shows the equilibrium profiles of drug loading onto different ion exchange resins. The loading of BHCl into the resins was more than 80% of the drug added. The equilibrium time was approximately 30 min for Dowex® 50WX4 50, 10 min for Dowex® 50WX8 400, and 5 min for Dowex® 50WX4 400. The difference in equilibrium time obtained was due to the influence of the degree of cross-linking and the particle size of the resins (Pongiyanakul et al., 2005). Dowex® 50WX4 50 shows the largest particle size (300–840 μm), and so it took the longest equilibrium time (30 min). According to Jeong and Park (2008), coarse particles have smaller surface area than fine particles and greater internal volume for ions to diffuse, so more time can be required to establish equilibrium. Resins with lower degree of cross linking reached equilibrium faster than resins with higher one having the same particle size (Dowex® 50WX4 400 took 5 min to reach equilibrium compared to Dowex® 50WX8 400 which reached equilibrium after 10 min). It is obvious that the amount of BHCl remaining is greater in case of Dowex® 50WX8 400 than Dowex® 50WX4 400. When an ion-exchange resin is highly cross-

Table 2
Experimental runs, independent variables, and measured responses of the 2^4 full factorial experimental design.

<table>
<thead>
<tr>
<th>Run</th>
<th>( X_1 ): Eudragit® RS 100 concentration (%)</th>
<th>( X_2 ): Coating percentage (%)</th>
<th>( X_3 ): Speed of rotation (rpm)</th>
<th>( X_4 ): PEG 400 concentration (%)</th>
<th>( Y_1 )</th>
<th>( Y_2 )</th>
<th>( Y_3 )</th>
<th>( Y_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>5</td>
<td>66</td>
<td>500</td>
<td>10</td>
<td>13.85</td>
<td>5.27</td>
<td>23.23</td>
<td>35.91</td>
</tr>
<tr>
<td>R2</td>
<td>5</td>
<td>33</td>
<td>1500</td>
<td>10</td>
<td>2.46</td>
<td>6.73</td>
<td>62.56</td>
<td>79.03</td>
</tr>
<tr>
<td>R3</td>
<td>5</td>
<td>66</td>
<td>1500</td>
<td>10</td>
<td>8.67</td>
<td>12.52</td>
<td>59.14</td>
<td>71.20</td>
</tr>
<tr>
<td>R4</td>
<td>5</td>
<td>66</td>
<td>1500</td>
<td>0</td>
<td>1.62</td>
<td>13.18</td>
<td>68.16</td>
<td>74.91</td>
</tr>
<tr>
<td>R5</td>
<td>20</td>
<td>66</td>
<td>500</td>
<td>0</td>
<td>10.44</td>
<td>0.95</td>
<td>5.84</td>
<td>7.60</td>
</tr>
<tr>
<td>R6</td>
<td>20</td>
<td>66</td>
<td>1500</td>
<td>0</td>
<td>4.90</td>
<td>2.51</td>
<td>24.50</td>
<td>35.64</td>
</tr>
<tr>
<td>R7</td>
<td>20</td>
<td>33</td>
<td>1500</td>
<td>0</td>
<td>9.97</td>
<td>2.52</td>
<td>16.58</td>
<td>24.17</td>
</tr>
<tr>
<td>R8</td>
<td>20</td>
<td>66</td>
<td>500</td>
<td>10</td>
<td>28.85</td>
<td>0.26</td>
<td>2.53</td>
<td>5.68</td>
</tr>
<tr>
<td>R9</td>
<td>5</td>
<td>33</td>
<td>500</td>
<td>0</td>
<td>8.10</td>
<td>1.35</td>
<td>10.35</td>
<td>18.84</td>
</tr>
<tr>
<td>R10</td>
<td>5</td>
<td>33</td>
<td>1500</td>
<td>0</td>
<td>2.47</td>
<td>17.53</td>
<td>75.29</td>
<td>84.79</td>
</tr>
<tr>
<td>R11</td>
<td>5</td>
<td>66</td>
<td>500</td>
<td>0</td>
<td>2.21</td>
<td>8.08</td>
<td>38.90</td>
<td>53.98</td>
</tr>
<tr>
<td>R12</td>
<td>20</td>
<td>33</td>
<td>1500</td>
<td>10</td>
<td>13.97</td>
<td>7.07</td>
<td>46.08</td>
<td>68.59</td>
</tr>
<tr>
<td>R13</td>
<td>5</td>
<td>33</td>
<td>500</td>
<td>10</td>
<td>4.63</td>
<td>4.00</td>
<td>38.01</td>
<td>60.84</td>
</tr>
<tr>
<td>R14</td>
<td>20</td>
<td>33</td>
<td>500</td>
<td>0</td>
<td>19.00</td>
<td>0.25</td>
<td>1.61</td>
<td>3.22</td>
</tr>
<tr>
<td>R15</td>
<td>20</td>
<td>66</td>
<td>1500</td>
<td>10</td>
<td>7.49</td>
<td>2.32</td>
<td>22.10</td>
<td>40.68</td>
</tr>
<tr>
<td>R16</td>
<td>20</td>
<td>33</td>
<td>500</td>
<td>10</td>
<td>14.50</td>
<td>0.27</td>
<td>3.36</td>
<td>6.98</td>
</tr>
</tbody>
</table>
linked, the diffusion of various ions can be impeded, and this will slightly increase the time required to reach equilibrium and reduce the amount of drug loaded onto IER (Jeong and Park, 2008).

3.2. Effect of resin particle size and degree of cross linking on the in vitro release of BHCl from drug–resinate beads

Fig. 2 shows the release profiles of BHCl from drug–resinate beads with Dowex® 50WX4 400, Dowex® 50WX8 400 and Dowex® 50WX4 50. It is obvious that the higher degree of cross linking of resins, the slower the release of the drug. Statistical analysis revealed that BHCl–resinate with Dowex® 50WX4 400 showed significantly faster drug release (36.47, 87.11, and 87.59%), compared to BHCl–resinate with Dowex® 50WX8 400 resinate (21.19, 70.31, and 72.78%) after 2, 4, and 6 h, respectively (*p < 0.05). This may be attributed to the swelling properties of the resin. The higher degree of cross linking resins swell less than the lower ones, and hence is more resistant to diffusion of drug molecule throughout the resin particle (Irwin, 1987). Similar results were obtained by Junyaprasert and Manwiwattanakul (2008) in a study on the preparation of diltiazem HCl resin complexes. Results also showed that decreasing the particle size results in a faster drug release. Statistical analysis revealed that drug–resinate with Dowex® 50WX4 50 showed significantly faster drug release (36.47, 87.11, and 87.59%), compared to drug–resinate with Dowex® 50WX4 50 (24.62, 70.78, and 74.96%) after 2, 4, and 6 h, respectively (*p < 0.05). This is attributed to the greater surface area exposed to the dissolution medium, which facilitates the exchange process (Jeong and Park, 2008). The in vitro release profiles of drug–resinate beads are characterized by burst release and were found to fit Higuchi diffusion model (data are not shown).

3.3. Coating of BHCl–resinate beads using different microencapsulation techniques

Based on the above results, we decided to prepare drug–resin microcapsules of BHCl that approach zero-order kinetics for once daily administration and prevent the burst release. Drug–resinate beads with Dowex® 50WX4 50, that showed the most sustained drug release, was selected to be coated with a rate-controlling membrane in order to achieve the targeted controlled release effect. Solvent evaporation processes have been used to encapsulate the drug–resinate beads into Eudragit® RS 100 microcapsule.

The in vitro release profiles of BHCl from BHCl–resin microcapsules prepared by the o/w, and o/o techniques, compared to the release profiles from uncoated drug–resinate beads are graphically illustrated in Fig. 3. The time required for complete evaporation of the solvent was reduced from 3 h in the o/w technique to 1 h in the o/o technique, which makes the process more economic and desirable. Results show that the rate of BHCl released from uncoated BHCl–resinate beads was relatively rapid. Both techniques resulted in retardation of the drug release compared to that from the uncoated resinate beads, owing to the low permeability of Eudragit® RS 100 polymer. The o/o technique resulted in a significantly lower percentage drug released compared to the o/w technique after 2, 6, and 12 h (*p < 0.05), indicating more efficient coating. The percentage BHCl released from the o/o Eudragit® coated resinate beads after 24 h was only 13.52% compared to 83.95% released from the o/w coated resinate beads.

Fig. 4a and b shows the scanning electron micrographs of BHCl resinate beads coated with Eudragit® RS 100 prepared by o/w technique. The figures revealed that the coated resinate microcapsules prepared by the o/w technique were discrete and spherical having cracked surface. In addition, some particles appear a little squeezed out and disrupted. This may be attributed to the swelling of the resinate beads in aqueous medium, which resulted in breaking and rupture of the polymer film coating. From the previous results, the o/o solvent evaporation technique was chosen for coating of BHCl–resinate beads.
3.4. Analysis of factorial design

The factorial design, a commonly used statistical approach for planning and optimization of experimental series, was used. The used design comprises a full $2^3$ factorial design. The importance and worth of experimental designs have been reported frequently in the literature, especially for pellet coating processes (Kramar et al., 2003; Akhgari et al., 2005; Ensslin et al., 2009).

The experimental runs, with independent variables and the measured responses are shown in Table 2. The independent and response variables were related using polynomial equation with statistical analysis through Design-Expert® Software. Table 3 shows the regression results of the measured responses. The values of the coefficients $X_1$, $X_2$, $X_3$, and $X_4$ are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response (Huang et al., 2004). The larger coefficient means that the independent variable has more potent influence on the response. ANOVA test was performed to evaluate the level of significance of the tested factors on the extent of coating, the percentage BHCl released from different microcapsules at 1, 6, and 12 h as well as the interactions between these factors.

A two-level experimental design provides sufficient data to fit a polynomial equation, which is in the following form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4$$

where $Y$ is the dependent variable; $b_0$ is the intercept; and $b_1$–$b_{34}$ are the coefficients for the factors $X_1$, $X_2$, $X_3$, and $X_4$ their interaction terms (Singh et al., 2008). The standardized effect of the independent variables and their interaction on the dependent variables was investigated by preparing Pareto charts (Fig. 5a–d) using Minitab® Software. These charts consist of bars, the lengths of which are proportional to the absolute value of the estimated effects, divided by the standard error. These were utilized to show the effect (in decreasing order of importance) of the independent variables and their interactions on the dependant variables. The chart includes a vertical reference line at the critical $p$ value of 0.05. An effect that exceeds the vertical line is considered to be statistically significant (Rekhi et al., 1999; Solanki et al., 2007).

### 3.4.1. The effect of formulation and process variables on the extent of coating of BHCl loaded microcapsules using a full factorial design ($2^4$)

Fig. 5a shows that the bars for the concentration of Eudragit® RS 100 in the coating solution ($X_1$), the speed of rotation ($X_2$), and the concentration of PEG 400 ($X_4$) extend after the reference line in the Pareto chart for the extent of coating ($Y_1$) indicating that these variables have a significant impact on the extent of coating of the microcapsules.

Results show that Eudragit® RS 100 concentration in coating solution showed a significant effect on the extent of coating of the microcapsules. Increasing the concentration of Eudragit® RS 100 from 5% to 20% resulted in significant increase in the extent of coating ($p < 0.0001$). This result is in agreement with the results of Junyaprasert and Manwiwattanakul (2008) in a study on the preparation of diltiazem–resin microcapsules. They reported that the extent of coating of the microcapsules increased with increasing the concentration of cellulose acetate butyrate in the coating solution.

The speed of rotation also showed a significant impact on the extent of coating of the microcapsules. Increasing the speed of rotation from 500 to 1500 rpm resulted in significant decrease in the coating extent ($p = 0.0015$). The high speed of rotation might have caused rapid evaporation of the solvent without uniform deposition of the polymer onto the core.

Results also show that increasing the concentration of plasticizer (PEG 400) resulted in a significant increase in the extent of coating of the microcapsules ($p = 0.0211$). Plasticizers are used in polymeric coating dispersions to optimize the properties of the film. Such properties include permeability, solubility, adhesiveness, and mechanical properties such as flexibility, and brittleness (Okarter and Singla, 2000). Incorporation of a plasticizer is recom-

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**Table 3** Regression results of the measured responses (coded values).

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>$X_1$</th>
<th>$X_2$</th>
<th>$X_3$</th>
<th>$X_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$</td>
<td>+9.57</td>
<td>+5.3</td>
<td>+31.14</td>
<td>+42.13</td>
</tr>
<tr>
<td>$b_1$</td>
<td>+4.07</td>
<td>−3.28</td>
<td>−15.82</td>
<td>−18.06</td>
</tr>
<tr>
<td>$b_2$</td>
<td>+0.18</td>
<td>+0.34</td>
<td>−0.70</td>
<td>−1.43</td>
</tr>
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</tr>
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<tr>
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<td>+1.02</td>
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**Fig. 4.** Scanning electron micrographs of BHCl–resin microcapsules prepared by o/w technique: (a) X85 and (b) X120.
mended for polymer coating formulations due to the high glass transition temperatures of polymers. Zelko et al. (2002) reported that addition of plasticizer to Eudragit® dispersions increases the free volume in the polymeric film, allowing more space for molecular movements, and thus molecular groups and side chains possess larger mobility. This leads to reduction in the glass transition temperature, and enables film formation at a lower temperature. In another study, Gupta et al. (2001) claimed that Eudragit® RS is a hard polymer with low elongation at break and hence requires the addition of plasticizer to form elastic and flexible films.

On the other hand, changing the coating percentage has a non-significant effect on the extent of coating of the microcapsules ($p = 0.8685$). This result was confirmed by the lower value of coefficient of this term in the polynomial equations for $Y_1$ (Table 3).

Fig. 6 shows the response surface plot of the effect of the Eudragit® RS 100 concentration ($X_1$), and speed of rotation ($X_3$) on the extent of coating. Increasing the speed of rotation significantly decreased the extent of coating of drug resinate particles coated with high Eudragit® RS 100 concentration than those coated with low Eudragit® RS 100 concentration. This may be attributed to the rapid solvent evaporation caused by the high speed of rotation, which may have caused the deposition of most of the polymer of the concentrated Eudragit solution on the walls of the container during the microencapsulation process.

Fig. 7 shows the response surface plot of effect of the coating level ($X_2$), and concentration of plasticizer ($X_4$) on the extent of coating. The fact that the bars for $X_2X_4$ extend after the reference line and the relatively larger coefficient for this term in the polynomial equations for $Y_1$ (Table 3) indicated that there is an interaction between the variables $X_2$ and $X_4$. The addition of plasticizer resulted in an increase in the extent of coating at high coating ratio whereas it caused nearly the same extent of coating on the microcapsules at low coating level. The inability of plasticizer to increase coating extent may be due to insufficient amount of coating polymer at low coating percentage.

In order to investigate the effect of plasticizer on the formed Eudragit® film coat, scanning electron microgram was done to examine the surface of the prepared Eudragit® RS 100 BHCl–resin
microcapsules. Fig. 8a–d shows the scanning electron micrographs of two microcapsule formulations, one without plasticizer (R14) (Fig. 8a and b), and the other for a formulation with the same composition in addition to 10% PEG 400 (R5) (Fig. 8c and d). It is clear that the surface of formulation R14 appears irregular, and cracked, and shows uneven polymer deposition. On the other hand, the surface of formulation R5 appears smooth and regular, which shows that addition of plasticizer was beneficial in the preparation of Eudragit® RS 100 BHCl–resin microcapsules.

3.4.2. The effect of formulation and process variables on the in vitro release of BHCl from BHCl loaded microcapsules using a full factorial design (2^4)

Fig. 5b–d shows that the bars for the concentration of Eudragit® RS 100 in the coating solution, and the speed of rotation extend after the reference line in the Pareto chart for the percentage BHCl released after 1 h (Y2), after 6 h (Y3), and 12 h (Y4) indicating that these variables have a significant effect on the release rate of BHCL from the microcapsules.

A significant impact of the Eudragit® RS 100 concentration on the drug release was demonstrated. Increasing the Eudragit® RS 100 concentration from 5% to 20% resulted in significant decrease in the percentage BHCl released after 1, 6, and 12 h (p < 0.0001). The increase in Eudragit® RS 100 concentration results in the formation of longer diffusion path, and so the release is retarded. This is attributed to the low permeability of Eudragit® RS 100 which resulted in more retardation of drug release. These results correlate well with the results of the extent of coating of the microcapsules, where increasing the concentration of Eudragit® RS 100 resulted in increasing the extent of coating on the microcapsules, and hence more retardation in the drug release. Similar results were obtained by Kim and Kim (1994) in the preparation and evaluation of terbutaline microspheres using Eudragit® RS 100.

The speed of rotation also demonstrated a significant influence on the drug release. Increasing the speed of rotation from 500 to
1500 rpm resulted in significant increase in the percentage BHCl released after 1, 6, and 12 h ($p < 0.0001$). This may be due to non uniform polymer film formation on the resinate beads. Again, these results correlate well with the results of the extent of coating of the microcapsules, where increasing the speed of rotation resulted in decreasing the extent of coating of the microcapsules, and hence increase in the percentage of BHCl released. This result is in agreement with the results obtained by Haznedar and Dortunc (2004) in a study on the preparation of Eudragit® microspheres containing acetazolamide. They reported that the increasing speed of rotation of the system during preparation resulted in increasing the release rate of the drug from the microspheres.

The fact that the bar for the concentration of PEG 400 appears after the reference line only in the Pareto chart for $Y_4$ (Fig. 5d) indicates that the concentration of PEG 400 is only important in controlling BHCl release during the late stage of drug release and that increasing the concentration of plasticizer (PEG 400) has no significant effect on the percentage drug released after 1, and 6 h ($p = 0.2507$, and $p = 0.4898$, respectively). Addition of plasticizer resulted in a significant increase in the percentage BHCl released after 12 h ($p = 0.0366$). After 12 h exposure to the dissolution medium, PEG 400, being a hydrophilic plasticizer was leached out of the polymeric film resulting in increasing the film permeability, and consequently, increasing the drug release. It was reported that the addition of adjuvants changes the physicochemical properties of monolithic films such as their polymer tortuosity and porosity which can influence drug diffusion. For example, the addition of PEG 400 enhanced drug release from the Eudragit RSPM films, whereas PEG 8000 decreased the rate of drug release from the Eudragit RSPM films (Jenquin et al., 1992).

Pareto charts (Fig. 5) also show that changing the coating percentage has no significant effect on the percentage BHCl released from different microcapsules after 1, 6, and 12 h ($p = 0.4331$, $p = 0.6779$, and $p = 0.4331$, respectively). Again these results correlate well with the results of the extent of coating where changing the coating level had no significant effect on the extent of coating of the microcapsules. Similar results were obtained by Akhgarai et al. (2005) in a study on the preparation of coated indomethacin pellets with pH – dependent methacrylic polymers for colonic delivery. They reported that the coating level of Eudragit® did not significantly affect the percentage drug released after 5 h (Akhgari et al., 2005). Also Marvola et al. (1999) reported that coating levels above a critical value did not retard the drug release to a more extent.

Figs. 9 and 10 show the response surface plots of the effect of Eudragit® RS 100 concentration ($X_1$) and speed of rotation ($X_3$) on the percentage BHCl released after 1, and 6 h, respectively. Increasing the speed of rotation increases with greater extent drug release for resin particles coated with small polymer concentration than that coated with high polymer concentration. This could be explained on the basis that the high speed of rotation and the small polymer concentration have a synergistic effect on the reduction of coat thickness and the consequent increase in drug release. However, at high polymer concentration, the effect of increasing speed of rotation on the release rate was counterbalanced with the increase in polymer concentration.

Fig. 11 shows the response surface plots of the effect of the Eudragit® RS 100 concentration ($X_1$) and concentration of plasticizer ($X_4$) on the percentage BHCl released after 1 h. It is obvious that the highest extent of drug release occurs on using small polymer concentration with no plasticizer which may be due to cracks present in the coat allowing water penetration and drug release. It is obvious that addition of plasticizer decreases the drug release at small polymer concentration due to the formation of coherent film of the polymer having no cracks. However, addition of plasticizer slightly increases the release at high polymer concentration due to the increase in the amount of the hydrophilic plasticizer which leaches out the coat, forming channels or pores in the coat, allowing penetration of the dissolution medium (Okarter and Singla, 2000).

Figs. 12 and 13 show the response surface plots of the effect of the coating percentage ($X_2$) and concentration of plasticizer ($X_4$) on the percentage BHCl released after 6, and 12 h, respectively. It is clear that the most retardant effect of these formulation variables
on the in vitro release of BHCl from microcapsules occurs when both variables were used together at their most low or high values. This observation confirms that the formation of coherent retardant films is facilitated at these conditions.

3.5. Kinetic analysis of in-vitro release of BHCl from different microcapsules

In many experimental situations, the mechanism of drug diffusion deviates from the Fickian equation and follows a non-Fickian (anomalous) behavior. In these cases, to analyze the release mechanism of the drug from these matrices, the release data obtained were fit to a simple power equation (Peppas, 1985):

\[
\frac{M_t}{M_\infty} = Kt^n
\]

where \(\frac{M_t}{M_\infty}\) is the fraction of drug released at time \(t\) and \(K\) denotes the constant incorporating structural and geometrical characteristics of the drug/polymer system and the \(n\) is the diffusion exponent related to the mechanism of the drug release. For non-Fickian (anomalous) release from spheres, the \(n\) value falls between 0.43 and 0.85 (where release is controlled by a combination of diffusion and polymer relaxation) while for Fickian (Case I) diffusion, \(n \leq 0.43\) (\(t^{1/2}\) dependence) and for zero-order release (Case II transport), \(n = 0.85\) where the drug release rate is independent of time and involves polymer relaxation and chain disentanglement (Harland et al., 1988). It is important to note that for determination of the exponent \(n\), only the initial portion of the release curve (\(M_t/M_\infty < 0.6\)) must be used (Peppas, 1985).

The value of \(K\) and \(n\) were estimated by linear regression of \(\log\left(\frac{M_t}{M_\infty}\right)\) on \(\log(t)\) where \(\log K\) is the intercept and \(n\) is the slope of the straight line.

\[
\log \frac{M_t}{M_\infty} = \log K + n \log t
\]

The kinetic analysis of the in vitro release data of BHCl from BHCl–resin microcapsules are presented in Table 4. According to determination coefficient (\(R^2\)), the in vitro release data were in favor of Koresmeyer–peppas release kinetics (for formulae 3, 6, 7, 8, 10, 13, 14, and 16), diffusion-order release kinetics (for formulae 1, 2, 5, 11, and 12), and zero-order kinetics (for formulae 4, 9 and 15). The values of \(n\) were >0.43 and <0.85 indicating non-Fickian (anomalous) transport for most formulae (except for formulae 6, 8, 9, 12, and 15).

3.6. Optimization BHCl–resin microcapsules

The aim of the optimization of pharmaceutical dosage formulations is generally to determine the levels of variables from which a robust product with high quality characteristics may be produced. The coating formulation was optimized for the responses \(Y_2, Y_3, Y_4\). The desirable range of these responses was restricted to

- \(5\% \leq Y_2 \leq 11\%\),
- \(27.5\% \leq Y_3 \leq 42.5\%\), and
- \(60\% \leq Y_4 \leq 75\%\).

The target ranges of these responses were determined based on the target release model deduced from zero-order dissolution profile of BHCl for once-daily administration. The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert software and based on the criterion of desirability (Basalious et al., 2010). Therefore, two optimized formulations of BHCl–resin microcapsules with the predicted levels of formulation and process variables were prepared to confirm the validity of the optimization procedure. The two optimized BHCl–resin microcapsules equivalent to 32 mg drug were filled into hard gelatin capsules and subjected to the in vitro release test. The composition and predicted and observed responses of the two optimized formulations of BHCl–resin microcapsules are presented in Table 5. Results show that the observed values of the two new batches were mostly similar with predicted values. Fig. 14 shows the release profiles...
Kinetic analysis of the in vitro release data of BHCl from BHCl–resin microcapsules.

Table 4

<table>
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<tr>
<th>Formulae</th>
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A novel multiple unit controlled release formulation of BHCl with satisfactory release characteristics was successfully prepared. A mathematical model was developed connecting the important formulation and process variables with the measured responses. The model provides predictions of coat compositions, and process variables based on an aimed drug-release profile. The release profiles of the optimized BHCl–resin microcapsules are presently investigated.

References


4. Conclusion

A novel multiple unit controlled release formulation of BHCl with satisfactory release characteristics was successfully prepared. A mathematical model was developed connecting the important formulation and process variables with the measured responses. The model provides predictions of coat compositions, and process variables based on an aimed drug-release profile. The release profile of the optimized BHCl–resin beads, microencapsulated using 14.42% Eudragit solution, 50.63% coating level and 9.94% PEG 400 at approximately 1500 rpm, was comparable to that of the target release profile deduced from zero-order dissolution profile of BHCl for once-daily administration. Further studies for in vivo evaluation of controlled release solid dosage forms of the optimized BHCl–resinate microcapsules are presently investigated.


