Chitosan lactate wafer as a platform for the buccal delivery of tizanidine HCl: In vitro and in vivo performance

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ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 25 March 2014
Accepted 25 March 2014
Available online 26 March 2014

Keywords:
Chitosan lactate
Na tripolyphosphate
Buccoadhesive Wafer containing beads
Central composite face-centered design

ABSTRACT

Tizanidine HCl is a skeletal muscle relaxant that suffers from extensive hepatic metabolism resulting in 34–40% oral bioavailability. It also suffers from short half-life (2.1–4.2 h) that necessitates frequent administration thus reducing patient compliance. In addition, tizanidine HCl is water soluble, so it is a challenging candidate for controlled drug delivery. In our study, tizanidine was encapsulated in chitosan lactate beads cross-linked with sodium tripolyphosphate. The beads were further incorporated into chitosan lactate wafer to be easily applied to buccal mucosa, aiming to bypass the hepatic metabolism. A central composite face-centered design was applied to statistically optimize the formulation variables; tripolyphosphate concentration, chitosan lactate concentration and polymer/drug ratio. The optimized formula suggested by the software composed of: 3.03% tripolyphosphate, 4.92% chitosan lactate and 2.13% polymer/drug ratio. It provided encapsulation efficiency of 56.5% and controlled tizanidine release over 8 h. It is also characterized by being mucoadhesive and nonirritant. Pharmacokinetic parameters of tizanidine from the optimized formula were compared to those of the immediate release tablet, Sirdalud®, as reference in human volunteers using a randomized crossover design. Significant increase was observed for $T_{max}$ and $AUC_{(0–→)}$. The increase in relative bioavailability of TIZ from the optimized formula was 2.27 fold.

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1. Introduction

Tizanidine HCl (TIZ) is a myotonolytic agent in patients with spasticity (Wagstaff and Bryson, 1997). It suffers from rapid and extensive metabolism in the liver during the first pass which results in poor bioavailability (34–40%) after oral administration and exhibits a short elimination half-life of 2.5 h (Pendekal and Tegginamat, 2012). The maximum recommended daily dose of TIZ is 36 mg daily in divided doses (Lawson, 1998). Owing to its short half-life, frequent administration of TIZ is required which may result in reduced patient compliance.

Buccoadhesive buccal drug delivery offers several advantages. It is easily accessible allowing ease of both application and removal of the delivery device (Bruschi and de Freitas, 2005). In addition the buccal mucosa is a well-vascularized tissue with a rich blood supply and it is relatively permeable, robust and has lower enzymatic activity in comparison with other mucosal tissues (Salamat-Miller et al., 2005; Scholz et al., 2008). Also, drugs absorbed through buccal mucosa enter the systemic circulation through the external jugular vein, avoiding the hepatic first pass metabolism and leading to higher bioavailability than if orally administered, thus reduction in the dose and the associated side effects (Shinkar et al., 2012). It is obvious that TIZ is a good candidate for controlled release buccoadhesive buccal dosage forms.

Encapsulating drugs in chitosan beads can be used as a manoeuvre for controlled drug delivery. Beads are discrete spherical microcapsules that act as the solid substrate with the drug encapsulated in the core or coated on the surface (Patil et al., 2010). The general method used for preparation of beads is ‘ionotropic gelation’ which is based on the ability of counterionos cross-link polyelectrolytes forming hydrogel beads (Patil et al., 2012). Chitosan is the N-deacetylated derivative of chitin. It has received considerable attention owing to its safety and mucoadhesive properties (Takka and Gurel, 2010). Also it has been known for its permeation enhancing effect (Aranaz et al., 2009). It is a weak base with a pKa value of ~6.2 to 7; therefore, it is insoluble at neutral and alkaline pH values, but it is soluble in dilute acids such as acetic and formic acids (Ravi Kumar, 2000; Weecharangsan et al., 2006). Water soluble derivatives of chitosan are

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http://dx.doi.org/10.1016/j.ijpharm.2014.03.049
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commercially available such as chitosan lactate (Ch-Lac) (Papineau et al., 1991). Sodium tripolyphosphate (TPP) is a commonly used polymer in provoking the ionotropic gelation of chitosan. Many authors have described this method for preparing chitosan beads (Bodmeier et al., 1989; Govender et al., 2005; Shu and Zhu 2000; Win et al., 2003).

In order to introduce beads into the buccal cavity in a feasible way so that it retains there and stays adhering for hours without being swallowed, releasing the drug in a controlled manner, they need to be incorporated into a single-unit mucoadhesive solid dosage form. Compression of beads pellets into tablets is a challenging task that necessitates the optimization of various process and formulation variables. The beads must be hard enough in order to withstand the compression force, yet they must be soft enough to get slightly deformed without brittle fracture on compression (Murthy Dwibhashyam and Ratna, 2008). Our novelty is to incorporate beads into lyophilized wafers as an alternative to direct compression tablets.

Wafers are relatively novel formulations prepared by lyophilization (freeze-drying) of polymeric gels or solutions yielding solid porous cakes that are easily applied to buccal mucosa (Ayensu et al., 2012). Freeze-dried wafers show many advantages over ordinary compressed tablets. Being prepared by lyophilization, they are lighter, which would increase the patient’s compliance. Furthermore, the sponge-like nature ensures fast hydration and gelation of these wafers and thus alleviating the foreign body sensation (Refai and Tag, 2011), when applied to the buccal mucosa. Wafers, can maintain their swollen gel structure for a longer period and therefore longer residence time than semi solid polymer gels which flow easily after application, this allows more effective drug absorption. They have a higher drug loading capacity compared to the thin and continuous solvent cast films, due to their higher surface area and porous structure (Ayensu et al., 2012). They have been used in different drug delivery systems including fast dissolving wafers and wound healing dressings (Matthews et al., 2005, 2006, 2008), as well as controlled release wafers (Ayensu et al., 2012).

The aim of this study was to control the release of TIZ by encapsulating it in Ch-Lac beads cross linked with TPP. The prepared beads were further incorporated into Ch-Lac wafer to provide a buccal, biodegradable and mucoadhesive dosage form that could be well tolerated by the patient, aiming to bypass the extensive first pass effect, thus enhancing the bioavailability of TIZ. To study the effect of formulation variables and to reach an optimum formula a central composite face-centered design (CCFD) was applied because it is a systematic and efficient method for this purpose (Talkka and Curel, 2010). In vivo pharmacokinetic study was carried out to compare the optimized formula with the market product.

2. Materials and methods

2.1. Materials

The following materials were used: chitosan oligosaccharide lactate (Ch-Lac), average Mn 5000, deacetylation degree >90% and sodium tripolyphosphate (TPP) were both purchased from Sigma-Aldrich. Tizanidine HCl (TIZ) was kindly gifted by Hi Pharm for Manufacturing Drugs and Chemicals, Cairo, Egypt. Cyanoacrylate adhesive, Alteco Chemical PTE LTD, Japan. All other ingredients used were of analytical grade.

2.2. Experimental design

A three-level three-factor central composite face-centered design (CCFD) was employed to statistically optimize the formulation variables of TIZ beads preparation, in order to obtain high encapsulation efficiency and to achieve controlled drug release from the wafer after incorporating the beads. CCFD requires much fewer experiments than a full-factorial design. Generation and evaluation of the experimental design was carried out using the Design Expert® software (Version 7, Stat-Ease Inc., Minneapolis, MN). The design consisted of 8 factorial points, 6 axial points and 3 center points, giving a total of 17 formulas. The factorial points help in estimating the linear terms and two factor interactions, the axial points help in estimating the quadratic terms, and the center points were repeated three times to help estimate the pure experimental uncertainty at the factor levels (Aboelwafa and Makhlouf, 2012). The independent variables were; TIP concentration (X1), Ch-Lac concentration (X2) and polymer/drug ratio (X3). The levels of each factor were designated as (−1, 0, +1). The corresponding actual values of each variable are shown in Table 1. The compositions of the 3² CCFD for the TIZ Ch-Lac/TPP beads are shown in Table 2. Analysis of variance (ANOVA) was carried out to estimate the significance of model and term. Probability p-values (p < 0.05) denoted significance.

2.3. Preparation of beads by ionotropic gelation

TIZ was dissolved in Ch-Lac solution in distilled water. The bubble-free polymeric-drug solution (4 ml) was extruded drop wise through a syringe needle (22 G) at a rate of 0.4 ml/min into a gently agitated solution of TPP (10 ml). The droplets gelled instantaneously upon contact with the TPP solution forming discrete beads that were left to cure in the solution for 20 min then were filtered and washed with distilled water. The prepared beads were left to dry in air till a constant weight was reached (Bodmeier et al., 1989; Srinatha and Pandit, 2008).

<p>| Table 1 | Variables in the 3² CCFD experimental design for TIZ beads preparation and constraints for formulation optimization. |</p>
<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1: tripolyphosphate concentration (%w/v)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>X2: chitosan lactate concentration (%w/v)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>X3: polymer/Drug ratio</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Constraints</td>
<td></td>
<td></td>
</tr>
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</table>

Table 2
Composition of the $3^3$ CCD for the TIZ Ch-Lac/TPP beads.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Factors levels in actual values</th>
<th>$X_1$</th>
<th>$X_2$</th>
<th>$X_3$</th>
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<tbody>
<tr>
<td>Factorial points</td>
<td></td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
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<td>5</td>
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<td>3</td>
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<td>4</td>
<td>2</td>
</tr>
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<td>17</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

2.4. Incorporation of the prepared TIZ beads into buccoadhesive chitosan lactate wafer

A 5% Ch-Lac solution in distilled water was prepared and left till became bubble free. The polymer solution (0.3 ml) was then cast into plastic cylindrical moulds (diameter: 1 cm, thickness: 0.2 cm), then a weighed amount of beads (each of the 17 formulae individually) equivalent to 4.58 mg TIZ was added to the polymer solution in each individual mould and mixed to become homogenously distributed in it then frozen for 24 h. The frozen solutions were then placed in the lyophilizer (Savant Instruments, Novalyphen NL 500, Holbrook, NY) for 24 h (Portero et al., 2007). The resultant wafers were stored in desiccator until use.

Beads-free wafer was prepared by dissolving TIZ (4 mg) directly in 5% Ch-Lac solution, casted and treated as above to prepare a wafer with non-encapsulated TIZ intended for comparison with the wafers containing beads.

2.5. Beads characterization

2.5.1. Encapsulation efficiency

Fifty milligrams beads were digested in 100 ml 0.1 N hydrochloric acid (pH 1.2) in a volumetric flask for 24 h to make sure the drug completely dissolved. The solution was filtered through Whatman filter paper and an aliquot was used to assay for drug content spectrophotometrically at the predetermined $A_{max}$ of TIZ (319 nm). The encapsulation efficiency was then calculated by expressing the percentage ratio of the actual amount of drug entrapped to the initial amount of drug added (Srinatha and Pandit, 2008). Experiments were carried out in triplicates.

2.5.2. Bead size

The diameter of the prepared beads was measured using optical lens and micrometer (Malik et al., 2013). Measurements were carried out for 50 beads to determine the average bead size.

2.5.3. In vitro drug release of TIZ beads

The release of TIZ from the prepared beads was performed using USP Dissolution tester, rotating basket (Apparatus I). A weighed amount of beads equivalent to 4.58 mg TIZ, was placed in each basket and rotated at 50 rpm in 250 ml simulated saliva (pH 6.8) (Mashru et al., 2005), maintained at 37°C ± 0.5°C (Chakraborty et al., 2013; Ishak et al., 2007). Aliquots each of 5 ml from the dissolution medium were withdrawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h time intervals and replaced by fresh medium. The withdrawn samples were analyzed for TIZ content spectrophotometrically at 319 nm. All release studies were done in triplicates.

2.6. In vitro release of TIZ from chitosan lactate wafers containing beads

The 17 prepared wafer formulae were evaluated for their in vitro release. The release of TIZ was performed in USP Dissolution tester, Apparatus I. The flat side of the wafer was adhered with cyanoacrylate to the bottom flat end of the stirring shaft instead of the basket fixture (Perioli et al., 2007). The vessel was filled with 250 ml simulated saliva fluid and stirred at a rotation speed of 50 rpm at a temperature of 37 ± 0.5°C (Chakraborty et al., 2013). Aliquots each of 5 ml from the dissolution medium were withdrawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h time intervals. The amount of TIZ released was measured spectrophotometrically at 319 nm. All release studies were done in triplicates.

2.7. Formulation optimization

The optimized formula was obtained using the Design Expert® software by applying constraints on encapsulation efficiency of the beads and percentage drug released from the wafer after 1, 5 and 8 h, as shown in Table 1, with the aim to obtain a target profile of zero order release over 10 h. The suggested optimized formula was then prepared and evaluated in triplicate to check the validity of the calculated optimal formulation factors and predicted responses given by the software.

2.8. Characterization of the optimized formula

2.8.1. Surface pH

The surface pH of the wafer was determined by allowing it to swell in contact with 5 ml simulated saliva fluid (pH 6.8) for 2 h at room temperature then the electrode of the pH-meter (Jenway
3510, Barloworld Scientific, UK) was brought in contact with wafer surface and left to equilibrate for 1 min (Thombre and Gaikwad, 2013). The surface pH was determined in triplicate.

2.8.2. In vivo mucociliation performance

After approval of the Research Ethics Committee, Faculty of Pharmacy, Cairo University, five healthy volunteers applied the wafer to their right or left gum under the upper lip and they were asked to assess residence time and possible irritations. The wafer was applied by pressing lightly with the fingertip for 20 s (Perioli et al., 2008). The study lasted for 8 h and the volunteers were allowed to drink during this time, while food was not allowed.

2.9. Scanning electron microscopy (SEM)

The surface morphology and cross-sections of the selected optimum beads and after its incorporation into the Ch-Lac wafer were sputter layered with gold under argon atmosphere using Edwards Sputter coater, to achieve a film of 150 Å thickness. The samples were then examined using JEOL (JXA-840A, Japan) electron probe microanalyzer.

2.10. In vivo pharmacokinetic study in healthy human volunteers

2.10.1. Study design and sample collection

The study was carried out to compare the pharmacokinetics of TIZ from the optimized wafer containing beads formula to the marketed immediate-release tablet (Sirdalud®, Novartis, Egypt, Batch no.: Y0057) following administration of single 4 mg dose of each, using an open label, two-treatment, two-period, randomized, crossover design (4.58 mg TIZ salt in both preparations was equivalent to 4 mg tizanidine base).

Six healthy male volunteers participated in this comparative study. Their age ranged from 25 to 35 years, mean body weight was 70.4 ± 7.2 kg and mean height was 172.5 ± 4.5 cm. The biochemical examination of the volunteers revealed normal kidney and liver function. The nature and the purpose of the study were fully explained to them. The volunteers were informed to withhold taking medicines one week before the participation in the study to the end of the experiment. All subjects fasted for at least 10 h before the study day (U.S. Department of Health and Human Services, 2002). An informed written consent was obtained from each volunteer and the study was approved by the Cairo University Ethics Committee and the protocol complies with the declarations of Helsinki for humans.

The volunteers were randomly assigned to one of two groups of equal size. The study was performed on two periods: period I, volunteers of group 1 received the optimized wafer containing beads formula (treatment A) and volunteers of group 2 received the conventional commercial tablet Sirdalud®, treatment (B) which is considered as a reference standard. The test treatment was applied to the upper gum above the canine tooth without moistening before application by applying light force for 30 s using finger tip, and it was removed after 8 h. The standard treatment was ingested with 200 ml of water. Food and drinks (other than water, which was allowed after 2 h) were not allowed until 8 h after dosing and then a standard lunch and dinner were served to all volunteers according to a time schedule. A wash-out period of one week separated the periods. On period II, group 1 received treatment B and group 2 received treatment A.

A physician supervised the study and was also responsible for the volunteers’ safety and collection of samples. Venous blood samples (5 ml) were collected into heparinized tubes at the following set points: 0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, and 12 h after administration of each treatment. Plasma was separated by centrifugation at 3500 rpm for 10 min at 4 °C (Centurion Scientific Ltd., West Sussex, UK). The plasma was pipetted directly into 5 ml plastic tubes and stored frozen at −20 °C pending drug analysis.

2.10.2. Sample preparation

Fifty microliters of sildenafil, as internal standard (IS) (from a stock solution of concentration 1000 ng/ml) and 5 ml of diethyl ether: ethyl acetate 50:50 (v/v) were added to each sample (0.5 ml plasma), vortexed for 1 min and centrifuged for 10 min at 4000 rpm (cooling centrifuge, Sigma, 2-16PK). The organic layer was transferred to another tube filtered through 0.22 μm Millipore filter, then evaporated to dryness using vacuum concentrator (Eppendorf Vacufuge plus, Germany). Dry residues were reconstituted with 200 μl mobile phase (10 mM formic acid:methanol: acetonitrile [30:30:40] (v/v/v)), and finally 3 μl were injected on the column for analysis.

2.10.3. LC-MS/MS assay of TIZ

Plasma concentrations of TIZ were analyzed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. LC system (Agilent 1260®, Germany) coupled with triple quadrupole MS/MS detector (Agilent 6410®, Germany) was used. The chromatographic separation was carried out on a C18 Inertsil® ODS-3, 5 μm, 150 × 4.6 mm, (Japan, C/N 5020-01731 – S/N 2E185313). The mobile phase was composed of 10 mM formic acid:methanol:acetonitrile [30:30:40] (v/v/v). The flow rate was set as 0.6 ml/min. The analysis was operated at the MRM (multiple reaction monitoring) mode, and its MS parameters are shown in Table 3.

2.10.4. Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed by non-compartmental pharmacokinetic models using computer program, WinNonlin®. The maximum drug concentration (Cmax, ng/ml) and the time to reach Cmax (Tmax, h) were obtained from the individual plasma concentration–time curves. The area under the curve from zero to 12 h (AUC(0–12), ng h/ml) and to infinity (AUC(0–∞), ng h/ml), were calculated using the trapezoidal rule method. Results are expressed as mean values of 6 volunteers ±SD. The pharmacokinetic parameters, Cmax, t1/2, AUC(0–12), and AUC(0–∞) were compared between treatments A and B with ANOVA test for the untransformed data using the software SPSS 17.0 (SPSS Inc., Chicago, IL). The nonparametric signed ranks test (Wilcoxon) was used to compare the medians of Tmax and MRT for treatments A and B. A p-value of ≤0.05 was considered statistically significant.

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor ion (Da)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Sildenafil</td>
</tr>
<tr>
<td>Tizanidine</td>
</tr>
</tbody>
</table>
2.10.5. Establishment if in vitro/in vivo correlation (IVIVC)

Four levels of IVIVC (level A, B, C and multiple level C) have been described by the FDA guidance (Shah et al., 2009). Level A represents a point to point correlation between the in vitro property of an extended release dosage form (as extent or rate of drug release) and the in vivo response (as amount of drug absorbed or plasma concentrations). Level A is the highest level of correlations and the most informative, thus it was selected for our study (Shah et al., 2009). To estimate a level A correlation, a deconvolution procedure was done on the mean TIZ plasma concentration vs. time profile of the optimized wafer containing beads formula using Wagner–Nelson method (Sankalia et al., 2008). The fraction drug absorbed (FRA) obtained by deconvolution was then plotted against the fraction of drug dissolved (FRD) at the same time and the correlation coefficient ($r^2$) was determined.

3. Results and discussion

3.1. Preparation of beads by ionotropic gelation

In preliminary studies (data are not shown), high molecular weight chitosan was investigated to encapsulate TIZ but these trials resulted in insignificant encapsulation efficiencies (maximum of 8% w/w). Chitosan is only soluble in dilute acids such as 1% v/v acetic acid, where the amino groups of chitosan become protonated at pH < 6.5 leading to solubilization of the polymer (Abreu and Campaña-Filho, 2005). The solubility of TIZ was found to be 41.55 mg/ml in distilled water and increased to 56.65 mg/ml in 1% v/v acetic acid, which indicates that TIZ has high water solubility and being an ionizable drug its solubility increases in acidic medium. The previous findings explain the low encapsulation of TIZ, into high molecular weight chitosan, where during beads preparation, TPP rapidly diffuses into the chitosan droplets and at the same time acetic acid within the chitosan droplets diffuses to the external phase, and in the case of an ionizable drug such as TIZ whose solubility increases at low pH, the drug was almost completely lost to the external phase with acetic acid (Bodmeier et al., 1989). Attempts were done to increase pH of TPP solution so that to minimize TIZ solubility in it, but unfortunately the diffusion of acetic acid gradually decreased pH of the external medium, resulting in diffusion of TIZ outside the beads as well as its precipitation in the medium and on the beads surface and encapsulation of TIZ in the beads was still insignificant.

These preliminary studies proved that the encapsulation of TIZ was highly challenging, thus in an attempt to overcome these difficulties, the use of Ch-Lac, a water soluble derivative of chitosan (Papineau et al., 1991), was investigated. Ch-Lac does not need a weak acid for its dissolution; it is easily dissolved in water which means that during the beads formation, water, instead of acetic acid, diffuses to the external phase. Although TIZ is water soluble but its water solubility is less than that in acetic acid as aforementioned, so its diffusion to the external phase was substantially decreased after the use of Ch-Lac, as was demonstrated by the increase in encapsulation efficiencies.

3.2. Beads characterization

The Ch-Lac beads were not completely spherical, with a rough irregular surface and a firm texture and they were yellow in color, as illustrated in Fig. 1. The spherical shape of the beads at the wet state collapsed to this irregular surface due to quick drying (Angadi et al., 2012). The bead size ranged between 1.2 and 2.15 mm.

The encapsulation efficiencies (%EE) of the beads formulae ranged from 15.77 to 81% as represented in Table 4. ANOVA test for the observed %EE indicates that the quadratic regression model was significant and fitting for the data ($R^2 = 0.8335$). The resulting equation, after omitting the non-significant model terms, was as follows:

$$\%\text{EE} = 56.93 + 17.68 \times X_3 - 21.46 \times X_1^2$$

The positive coefficient of the term, $X_1$, indicates that increasing TPP concentration was associated with significant increase in %EE ($p = 0.0053$). TPP is a polyfunctional cross-linking agent that reacts with the protonated amino groups of chitosan, so at a higher TPP concentration cross-linking becomes more efficient resulting in more efficient encapsulation of the drug (Liu and Gao, 2009; Rao et al., 2010). The equation also reveals significant decrease in %EE ($p = 0.0497$) with increasing polymer/drug ratio ($X_3$) and this could be explained by the decrease in drug concentration associated with high polymer/drug ratio which resulted in lower amount of drug available for entrapment (Angadi et al., 2010; Sharma et al., 2010).

The response surface plot for the effect of $X_1$ and $X_3$ on %EE is illustrated in Fig. 2.

The in vitro release of TIZ from the beads was characterized by a high initial burst represented by $Q_{0.5h}$ which ranged from 22.7 to 92.4%. The burst release could be explained by the migration and diffusion of the drug molecules, by convection with water, to the beads surface during the drying process (Huang and Brazel, 2001). This resulted in heterogeneous drug distribution across the beads, with higher concentrations towards the surface. Taking in consideration the high solubility of TIZ, rapid dissolution of the surface embedded drug molecules in the beads occurred when they came in contact with the dissolution medium, resulting in the burst effect (Bansal et al., 2011). In addition, the porous, cracky structure of the beads might have facilitated the rapid diffusion of the drug (Huang and Brazel, 2001). Drug released after 2 h ($Q_{2h}$) ranged from 60 to 100%. Fig. 3 represents the in vitro release profiles of TIZ from the Ch-Lac beads, $Q_{0.5h}$ and $Q_{2h}$ were chosen for analysis of TIZ release from the Ch-Lac beads formulae, and their results are shown in Table 4. Statistical analysis revealed that both responses followed the quadratic regression model ($R^2 = 0.748$ and $R^2 = 0.854$, for $Q_{0.5h}$ and $Q_{2h}$, respectively). The reduced equations (after omitting the non-significant terms) describing each response in terms of coded variables are as follows:

$$Q_{0.5h} = 61.22 - 13.52 \times X_1 - 23.97 \times X_1^2$$

$$Q_{2h} = 596.54 + 7.32 \times X_1 - 10.88 \times X_1^2$$

It is obvious that for both responses the significant terms were $X_1$ and $X_1^2$ ($X_1$: TPP concentration and $X_1$: polymer/drug ratio). ANOVA test indicates their significance with the following $p$-values; for $Q_{0.5h}$ ($p = 0.0301$ and $p = 0.0416$ for $X_1$ and $X_1^2$, respectively), and for $Q_{2h}$ ($p = 0.008$ and $p = 0.0257$ for $X_1$ and $X_1^2$, respectively). The negative coefficients for $X_1$ indicate that TPP
Table 4
Observed responses for TIZ beads and wafer containing beads formulae.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Beads %EE</th>
<th>$Q_{0.5}$</th>
<th>$Q_{2}$</th>
<th>Wafer containing beads $Q_{0.5}$</th>
<th>$Q_{2}$</th>
<th>$Q_{8}$</th>
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</thead>
<tbody>
<tr>
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<td>67.02</td>
<td>92.59</td>
<td>42.20</td>
<td>87.42</td>
<td>96.65</td>
</tr>
<tr>
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<td>81.00</td>
<td>22.71</td>
<td>60.10</td>
<td>12.39</td>
<td>40.06</td>
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<td>74.01</td>
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<td>62.56</td>
<td>97.92</td>
<td>8.33</td>
<td>45.11</td>
<td>65.08</td>
</tr>
</tbody>
</table>

%EE: encapsulation efficiencies; $Q_{0.5}$, $Q_{2}$, $Q_{8}$ are the % of TIZ released at 0.5, 1, 2, 5 and 8 h, respectively.

Fig. 2. Response surface plot showing the effect of $X_1$ and $X_2$ on encapsulation efficiency of TIZ Ch-lac beads.

Concentration has a retardant effect on TIZ release from the Ch-Lac beads formulae at the different release intervals, which means as TPP concentration increased, TIZ release decreased. The negative coefficients for $X_2^2$ indicate that as polymer/drug ratio increased, TIZ release increased to a maximum after which it decreased. The effect of TPP could be attributed to the increased cross-linking efficiency with higher concentrations of TPP. The efficiently cross-linked chitosan beads have lower water uptake and less swelling ability than the loosely cross-linked beads, resulting in a slower release rate (Madgulkar et al., 2009). The ultimate decrease in TIZ release associated with increase in polymer/drug ratio was expected, where increase in polymer/drug ratio meant more polymeric content in the beads, so the hydrated polymeric matrix would be more compact and decrease drug diffusion (Murtaza et al., 2010; Pillay et al., 1998). Dinarvand et al. (Dinarvand et al., 2003) reported that the less drug loading associated with high polymer/drug ratio, created less pores within the polymeric matrix, thus drug diffused at a lower rate. The response surface plots showing the effect of $X_1$ and $X_2$ on in vitro release of TIZ from the Ch-Lac beads after 0.5 and 2 h are represented in Fig. 4.

Ch-Lac concentration did not have any significant effect on either the encapsulation efficiency of the beads or the drug release;
this could be attributed to the small difference between the levels adopted. It is noteworthy that in the preliminary studies Ch-Lac concentrations less than 3% did not form discrete beads when dropped into the TPP solution owing to the relatively low viscosity. On the other hand, concentrations above 5% were too viscous to be dropped via a syringe, so the levels chosen for Ch-Lac concentration were limited to 3, 4 and 5%.

Generally, it could be concluded that the use of Ch-Lac beads was inefficient in controlling the drug release for more than 2 h. But further incorporation of the prepared beads into the buccoadhesive wafer was expected to exert more control on the drug release.

3.3. Wafer characterization

The lyophilized wafers had smooth surface with spongy texture and the beads were totally embedded inside, as shown in Fig. 5a. After incorporating the prepared TIZ Ch-Lac beads into the wafer, a significant decrease in drug release was achieved, where the drug continued to be released for 8 h and $Q_{8\text{h}}$ ranged from 34.6 to 98%. In addition, no burst release was observed, as shown by Fig. 6. By the end of drug release, Ch-Lac wafer containing beads show the polymeric matrix swollen and eroded and the beads became exposed (Fig. 5b).

For analysis of the release data, $Q_{1\text{h}}$, $Q_{5\text{h}}$ and $Q_{8\text{h}}$, were selected to compare the different wafer formulae, and their results are shown in Table 4. ANOVA test showed that the quadratic regression model was significant for $Q_{3\text{h}}$, ($R^2 = 0.8555$), while $Q_{5\text{h}}$ and $Q_{8\text{h}}$ followed the linear regression model ($R^2 = 0.5998$ and $R^2 = 0.6063$, respectively). The reduced equations (after omitting the non-significant model terms) describing each response are as follows:

$$Q_{1\text{h}} = 9.88 - 13.99 \times X_1$$

$$Q_{5\text{h}} = 60.49 - 19.64 \times X_1$$

$$Q_{8\text{h}} = 75.04 - 17.02 \times X_1$$

For the three responses the only significant model term was the TPP concentration ($X_1$) ($p = 0.0018$, $p = 0.0011$ and $p = 0.001$, respectively), where it had a retardant effect on the drug release extent at different time intervals, which means by increasing TPP concentration, the drug release from the wafer decreased (Malik et al., 2013), as represented by the response surface plots (Fig. 7). It is worth mentioning that the wafer matrix composition is fixed for all formulae, and they differ only in the composition of the beads embedded. As previously discussed, TPP concentration and polymer/drug ratio significantly affected the drug release rate from the Ch-Lac beads. But, the only significant factor affecting drug release from the wafer formulae was the TPP concentration. It could be suggested that the matrix effect of the wafer dominated and nullified the effect of polymer/drug ratio. This could also be explained by the need of drug molecules to diffuse through same path length through the wafer matrix to the surface, on the contrary to the beads where the drug at the beads surface was exposed to the release medium. So the wafer size, which was kept constant during preparation by using the same mould, neglected the effect of polymer/drug ratio.

In order to examine the necessity of incorporating beads into the wafer, a bead-free wafer was prepared with the drug directly dispersed in the matrix. The drug release profile showed initial burst release of 60% within 30 min, and reached 80% release at 3 h, as shown in Fig. 8. This indicates that encapsulating the drug into Ch-Lac beads before incorporating into the wafer was essential to
3.4. Formulation optimization

After applying constraints (Table 1) on %EE and $Q_{A1c}$, $Q_{A1b}$, and $Q_{A1h}$ for the wafer, to optimize encapsulation of TIZ into the Ch-Lac beads and obtain a controlled release from the wafer after incorporating this optimized beads formula, the Design Expert® software suggested one formula to be prepared. The overall desirability of the optimized formula was 1. After the suggested formula was prepared and evaluated, the residual between the predicted and observed responses was calculated. The residual showed to be small demonstrating the validity of the optimization process used to predict the formulation variables. Values for the optimized formula are shown in Table 5. Fig. 9 represents the in vitro release of TIZ from the optimized formula.

3.5. Characterization of the optimized formula

No difference in mucoadhesion and surface pH was expected between the wafers formulae, as they all had the same composition and differed only in the beads embedded inside, so these tests were performed only for the optimized formula.

3.5.1. Surface pH

It is favorable to keep the surface pH of the wafer close to neutral, as an acidic or alkaline pH may irritate the buccal mucosa. Surface pH of the wafer was found to be 6.36 ± 0.155, which indicates that it will not produce any local irritation upon contact with the buccal mucosa, as it is in the range of salivary pH (5.5–7.0) (Darwish and Elmeshad, 2009).

3.5.2. In vivo mucoadhesion performance

Fig. 10 shows the adherence of the wafer to the buccal mucosa and it is clear that the wafer adhered immediately when applied to the gums, and it remained adhering for 8 h without causing any irritation.

3.6. Scanning electron microscopy (SEM)

SEM micrographs of the optimized beads and after incorporating into the wafer are illustrated in Fig. 11 and Fig. 12. The surface topography of the beads showed rough irregular surface with large pores (Fig. 11a and c), and the cross-sectional view shows the accumulation of drug towards the beads surface (Fig. 11e). Both observations could justify the initial burst as well as the non-controlled release from the beads formulae before incorporation into the wafer. SEM micrographs of the beads after release of the drug, show the collapsed and shrunk surface of the beads (Fig. 11b and d), and the cross-sectional view shows the absence of drug leading to hollow cavities inside the collapsed beads (Fig. 11f). This indicates the complete diffusion of drug outside the beads at the end of release.

Surface topography of the Ch-Lac wafer, shows porous structure (Fig. 12a). Cross-section through the wafer shows a stretchy porous structure (Fig. 12b), this was probably caused by the lyophilization process (Phaechamud and Charoenteeraboon, 2008). Diameter of pores ranged from 20 to 68 µm and they were well interconnected through the wafer, which explains the controlled release from the wafer. Fig. 12c shows the beads embedded in the wafer matrix.
Fig. 8. In vitro release of TIZ from the bead-free chitosan lactate wafer.

Table 5
The predicted and observed values for optimized formula.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Optimized level</th>
</tr>
</thead>
<tbody>
<tr>
<td>( X_1 ): triopolyphosphate concentration</td>
<td>3.03</td>
</tr>
<tr>
<td>( X_2 ): chitosan lactate concentration</td>
<td>4.92</td>
</tr>
<tr>
<td>( X_3 ): polymer/drug ratio</td>
<td>2.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response</th>
<th>Expected</th>
<th>Observed</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y_1 ): encapsulation efficiency (%EE)</td>
<td>53.321</td>
<td>56.526</td>
<td>3.206</td>
</tr>
<tr>
<td>( Y_2 ): ( Q_{1h} ) from the wafer</td>
<td>11.021</td>
<td>9.475</td>
<td>-1.546</td>
</tr>
<tr>
<td>( Y_3 ): ( Q_{5h} ) from the wafer</td>
<td>58.523</td>
<td>45.048</td>
<td>-13.475</td>
</tr>
<tr>
<td>( Y_4 ): ( Q_{8h} ) from the wafer</td>
<td>72.971</td>
<td>65.841</td>
<td>-7.130</td>
</tr>
</tbody>
</table>

Residual = expected value – observed value.

Fig. 9. In vitro release profile of TIZ from the optimized formula in comparison with target release profile.

Fig. 10. Wafer mucoadhesion to the upper gum: (a) directly after application, and (b) after 8 h of adhesion.
3.7. In vivo pharmacokinetic study in healthy human volunteers

Fig. 13 illustrates the average plasma concentration vs. time profiles of TIZ obtained after single oral administration of both the optimized wafer containing beads formula and the marketed immediate release tablet. The estimates of the mean pharmacokinetic parameters obtained by non-compartmental fitting of the concentration–time data of TIZ are given in Table 6. Statistically insignificant differences ($p > 0.05$) were found between the two treatments for $C_{\text{max}}$, $AUC(0–12)$ and $t_{1/2}$. Statistically significant ($p < 0.05$) differences between the two treatments for $t_{\text{max}}$ and $AUC(0–\infty)$ were obtained. Additionally, a statistically significant ($p < 0.05$) prolongation in the MRT was obtained with the optimized formula. Compared to commercially available TIZ tablet,
Fig. 12. SEM micrographs of the wafer containing beads: (a) surface morphology (magnification of ×25) (b) cross-sectional view (magnification of ×200) and (c) cross-sectional view showing the interface between the beads and wafer matrix (magnification of ×25).

Fig. 13. Average (±SD) plasma TIZ cementsations following administration of 4 mg TIZ in optimized wafer containing beads formula and marketed Sirdalud® immediate release tablet (reference) in six subjects.

Table 6
Mean (±SD) pharmacokinetic parameters of TIZ after oral administration of the optimized wafer containing beads formula and the Marketed immediate release tablet (Sirdalud®) to six healthy human volunteers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimized wafer containing beads formula</th>
<th>Sirdalud®</th>
<th>Statistical test (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>0.887 ± 0.240</td>
<td>0.670 ± 0.219</td>
<td>0.187</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>6</td>
<td>2</td>
<td>0.041</td>
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<tr>
<td>AUC&lt;sub&gt;0–12&lt;/sub&gt; (ng h/ml)</td>
<td>5.273 ± 1.633</td>
<td>2.828 ± 0.747</td>
<td>0.056</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (ng h/ml)</td>
<td>7.973 ± 2.770</td>
<td>3.056 ± 0.761</td>
<td>0.004</td>
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<tr>
<td>MRT&lt;sup&gt;a&lt;/sup&gt; (h)</td>
<td>9.719</td>
<td>4.819</td>
<td>0.028</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>5.627 ± 3.986</td>
<td>3.246 ± 0.991</td>
<td>0.203</td>
</tr>
</tbody>
</table>

<sup>a</sup> Median.

Fig. 14. Level A IVIVC linear plot for the optimized wafer containing beads formula.
the relative bioavailability judged from the AUC_{(0→∞)} of the average profile was found to be 227.24%. The significant increase in T_{max} of the optimized formula indicated retardation in the absorption rate of TIZ which was expected from its in vitro release profile that was prolonged to 8 h. The significant increase in AUC_{(0→∞)} and thus in the relative bioavailability of the optimized formula in comparison to Sirdalud® indicates improvement in extent of TIZ absorption, attributed to the buccal route of administration that bypasses the extensive first pass metabolism. Also, this increase might be partly due to the permeation enhancing effect of Ch-Lac.

3.8. Establishment of in vitro/in vivo correlation (IVIVC)

The evaluation of IVIVC is important to display the ability of in vitro dissolution characteristics to predict the in vivo performance of a drug product and thus it can be useful as a surrogate for human bioequivalence studies (Sankalia et al., 2008). Fig. 14 represents the linear correlation plot of FRA vs. FRD. A central composite face-centered design was applied to optimize the formulation variables, namely; TPP and Ch-Lac concentrations and polymer/drug ratio. The optimized formulawas composed of; 3.03% TPP, 4.92% Ch-Lac and 2.13 as the polymer/drug ratio. It showed a relatively high EE and TIZ release from the wafer was controlled over 8 h with no initial burst. It also showed mucoadhesion for 8 h to the buccal mucosa without irritation. The pharmacokinetic study confirmed that the optimized formula controlled drug release in vivo with a median T_{max} of 6 h, it also showed increased bioavailability of TIZZ 2.72 folds when compared to the immediate release Sirdalud®. Thus, the developed formula could be considered as a promising buccoadhesive system that could control the release of TIZ and bypass its extensive first pass metabolism, enhancing its bioavailability and reducing frequency of administration, thus improving patient compliance. A level A IVIVC was developed for the optimized formula and it showed good correlation (r^2 = 0.9589). Such IVIVC can guide a new product development.

Acknowledgment

The authors would like to thank PharmaSolutions Research Center, Cairo, Egypt for performing the in vivo study part.

References


