Carbohydrate Polymers 108 (2014) 257–265

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Nasal polysaccharides-glucose regulator microparticles: Optimization, tolerability and antidiabetic activity in rats

Enas Elmowafy, Rihab Osman*, Abd El-Hameed A. El-Shamy, Gehanne A.S. Awad

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Egypt

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 18 February 2014
Accepted 19 February 2014
Available online 28 February 2014

Keywords:
Microparticles
Polysaccharides
Antidiabetic
Spray drying
Nasal powder
Insufflator

ABSTRACT

The aim of the present study was to load the post-prandial glucose regulator, repaglinide (REP), on spray dried mucoadhesive microparticles (MPs) comprising anionic polysaccharides. The formulation parameters of the polysaccharides-REP spray dried powders (SDP) namely, polysaccharide type and drug to polymer (D/P) ratio, were optimized for % release after 5 min ($R\%_{5\,min}$) and time required for 80% release ($T_{80\%}$). The suitability of the selected formulae for nasal application was evaluated by *ex vivo* mucoadhesion, *in vitro* cytocompatability and tolerability studies. A pharmacodynamic study in diabetic rats was conducted. Results showed that both polysaccharide type and amount greatly influenced the chosen responses. REP was highly incorporated in mucoadhesive MPs with proven safety on the rat nasal mucosa. The selected REP loaded powders exhibited a significant two to threefold increase in total decrease in blood glucose compared to the nasal and intravenous solutions.

1. Introduction

Promising physicochemical features along with biodegradability, biocompatibility and absence of toxicity attracted widespread interest in natural polysaccharides as drug carriers especially for nasally administered drugs (Illum, 2012; Mahajan & Gattini, 2009). Their mucoadhesive potential, with the ability to withdraw water from the nasal cavity acting as a penetration enhancer (Pereswetoff-Morath & Edman, 1995), make them tolerable safe nasal carriers. Among the available carbohydrates, the choice of a particular one does not depend merely on the route and dosage form but also on the drug properties and therapeutic activity.

Repaglinide (REP), a post-prandial insulinotropic drug, is a carbamoyl methyl benzoic acid derivative acting as a short-acting potent post-prandial glucose regulator, structure – Fig. 1S, supplement (Lund et al., 2007; Makino, Sakai, & Yabuki, 2010). REP benefit stems from its preventive treatment of endothelial dysfunction in type 2 diabetes, due to specific targeting of post-prandial hyperglycemia (Manzella, Abbatecola, Grella, & Paolisso, 2005). In addition, lower risk of existing antidiabetics adverse effects such as hypoglycemia, secondary failure and cardiovascular complications were claimed (Blickle, 2006; Makino et al., 2010). Although REP is rapidly absorbed after oral administration owing to its lipophilicity (log *P*, 3.97), it has a short plasma half-life (<1 h) with low variable bioavailability (BAV), 50%, resulting from low aqueous solubility (34 μg/ml at 37 °C) and extensive first pass metabolism (Jain & Saraf, 2009; Mandić & Gabelica, 2006). Intranasal (IN) delivery of REP can provide quick onset of therapeutic effect, reduce absorption variability (Plucinski & Rezaizn-Yazdi, 2009), avoid first-pass effect, in addition to its non-invasiveness and ease of administration even in emergency settings (Upadhyay, Parikh, Joshi, Upadhyay, & Chotai, 2011). To tackle its solubility problem, uniform dispersion of REP in various hydrophilic polysaccharides using the spray drying (SD) technique was planned in this work.

It has been postulated that polyanionic polysaccharides possess better mucoadhesive properties compared to polycationic or non-ionic mucoadhesive ones *via* formation of hydrogen bonds with mucin hydroxyl groups (Gombotz & Wee, 1998). In this work, pectin, gellan gum and dextran were chosen for formulating IN microparticulate dry powders. These polyanionic heterogeneous polysaccharides are made up of various backbone sugars, see structures 2S. While pectin has a plant origin with gelation properties dependent on the degree of esterification, GG and DXT are produced by bacterial fermentation. They have been widely used in medical and drug delivery fields (Anitha et al., 2011; Cao et al., 2009; Illum, 2012; Watts & Smith, 2009). Their non-diabetogenic nature and the expected ability to synchronize the drug release with MPs nasal residence time were other attributes for their selection as REP carriers (Vidgren & Kublik, 1998).

* Corresponding author at: Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, African Organization Unity Street, P.O. Box 11566, Cairo, Egypt. Tel.: +20 1221022566; fax: +20 224051107.
E-mail address: rihabosman@pharma.asu.edu.eg (R. Osman).

http://dx.doi.org/10.1016/j.carbpol.2014.02.064
0144-8617/

Currently, nasal insufflators provide dose accuracy, efficient delivery and distribution pattern (Patil & Sawant, 2011). This accurate nasal delivery must be preceded by powder made of particles having optimal fundamental and derived properties, such as shape, size and flow properties (Buttini, Colombo, Rossi, Sonvico, & Colombo, 2012).

The goal of this study was to prepare IN-REP polysaccharides powders by the spray drying method. The formulation variables were changed according to a statistically designed experiment. Physico-chemical and morphological characterization tools were used to investigate underlying control mechanisms. Mucoadhesivity, biocompatibility and potential *in vivo* application in diabetic rats were tested.

2. Materials and methods

2.1. Materials

Low methoxy pectin (PT), degree of esterification 28%, kindly supplied by: CP Kelco, Denmark. Dextran sulfate (DXT), Mw 500,000: Fisher Scientific. REP: kindly provided by EIPICO, Cairo, Egypt. Leucine (LEU): Fluka, Switzerland. Gellan gum (GG), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), HEPES buffer, fetal calf serum, gentamycin, dimethyl sulfoxide (DMSO), sodium lauryl sulfate (SLS) and streptozotocin (STZ): Sigma Chemical Co., USA. Minimum Essential Medium Eagle (MEM medium) was purchased from LONZA Co., Belgium. L-Glutamine from GIBCO®, Invitrogen Co., USA. All other materials and reagents were of analytical grade.

2.2. Preparation of spray dried REP loaded polysaccharides powders

Different amounts of each of the selected polymers (PT, GG and DXT) were dissolved in 100 mL of 0.01 N sodium hydroxide followed by REP addition to obtain drug to polymer ratios (D/P) of 1:9, 1:6 and 1:3. The solutions were magnetically stirred for 15 min followed by addition of 0.3% (w/v) of LEU. The solutions, with a total solid concentration of 1% (w/v), were spray dried using a Buchi spray dryer at air flow rate of 400 L/h. The inlet temperature was set at 120 °C, aspiration: 92% and pump rate: 4 mL/min. The outlet temperature ranged from 76 to 79 °C. Spray dried powders (SDP) were collected and stored in a vacuum desiccator on silica gel for further analysis.

2.3. Experimental design

A 3-level full factorial experimental design was built up to evaluate the main effects and interactions of two variables: polysaccharide type (factor A) and drug to polymer ratio (D/P: factor B). The complete setup of the 3^2 full factorial design is shown in Table 1. The studied responses were: % release after 5 min ($R\%_{5\,min}$) and time required for 80% release ($T_{80\%}$).

2.4. Powder characterization

The spray drying process yield values were calculated by gravimetry. The fixed height cone method was adopted for calculating the angle of repose (Staniforth, 2002). Water content was determined by thermogravimetric analysis (TGA), using a TGA 7 (Perkin Elmer, Waltham, Massachusetts, USA). The particle size (PS), expressed as volume mean diameter (VMD), was calculated using a laser diffraction particle size analyzer (Mastersizer X, Malvern Instruments Ltd., Worcestershire, UK) after suspending 2–3 mg of each SDP in isopropanol (Jalalipour, Gilani, Tajerzadeh, Najafabadi, & Barghi, 2008). The thermal properties of REP, polysaccharides, REP-polysaccharides physical mixtures, blank and REP-loaded MPs were investigated using differential scanning calorimetry (DSC) (DC-60, Shimadzu, Kyoto, Japan). Fourier transform Infrared (FTIR) spectra were recorded with the FTIR spectrometer (Nicolet 6700 FTIR; Thermal Scientific; Class 1 laser product; USA) using KBr disc method. A Philips PW 3710 X-ray powder diffractometer (XRPD), running at 45 kV, 30 mA, and scanning from angles of 5–45° was used to detect powder crystallinity. MPs morphological characteristics were examined by scanning electron microscopy (SEM) (Zeiss, Germany).

2.5. Drug incorporation efficiency percent (IE%)

Drug content was determined by UV spectrophotometry (UV-1601 PC, Shimadzu, Kyoto, Japan) at 283 nm (Arama, Nicolescu, Nedelcu, & Monciu, 2011) after dissolving 10 mg of the sample in 0.01 N NaOH and IE% was calculated as follows:

$$\text{IE\%} = \left[\frac{\text{actual REP amount}}{\text{theoritical REP amount}}\right] \times 100 \quad (1)$$

2.6. In vitro REP release

An incubator adjusted at 37 ± 0.5 °C, 30 rpm, in which tubes containing 10 mL of phosphate buffer solution (PBS, pH 6.8) were placed, was used (Gavini, Rassu, Muzzarelli, Cossu, & Giunchedi, 2008). An amount of each SDP, containing 1 mg REP, was added in each test tube. At predetermined time intervals, aliquots were removed from dissolution medium and replaced with fresh PBS. After centrifugation, the samples were diluted if necessary and analyzed spectrophotometrically at 283 nm. *In vitro* release profiles were analyzed using different kinetic models (zero order, first order and Higuchi's model) (Liu, Desai, Tang, & Chen, 2006).

2.7. In vitro swelling studies

MPs were suspended in PBS (pH 6.8), and then put on a magnetic stirrer. At specified time intervals (0–15–30–60–120–180–240 min), PS of swelled REP-loaded MPs was measured using laser diffraction (Gavini et al., 2008) and the swelling index (SI) was calculated as follows:

$$\text{SI} = \frac{d_2 - d_1}{d_1} \quad (2)$$

where d_1 is the mean diameter measured in isopropanol (where no swelling was expected) and d_2 is the mean diameter measured after specified time in buffer solution.

2.8. Mucoadhesion studies

Mucoadhesion was carried out on freshly excised sheep nasal mucosa obtained from the local slaughter house using a texture analyzer (TAXT plus, Stable Micro Systems, UK) as previously explained elsewhere (Sensoy et al., 2009). Peak detachment force (N) was calculated using Texture Exponent software.

2.9. Insufflator dose reproducibility

A quantitative assay was used to test the dose reproducibility of the monodose nasal insufflator (MIAT, Italy) at two doses (5 and 10 mg). Accurately weighed amounts of selected SDP were filled into no. 3 capsules and placed in the insufflator. The rubber bulb was squeezed to allow the SDP to be sprayed. After each puff, for a total of three puffs, the amount of delivered SDP was determined using weight difference by weighing the capsule before and after each actuation (Patil & Sawant, 2011) and the fraction of delivered dose was calculated.

Table 1
Characterization of REP-loaded MPs prepared according to the experimental design.

Formula code (D/P)	Design matrix*		REP-loaded MPs characterization								Drug release mechanism
	A	B	Y^a	θ^b	M^c	$D[4,3]^d$	S^e	IE (%)[f]	$R\%_{5\,min}$ [g]	$T_{80\%}$ [h]	
PT1 (1:9)	−1	−1	64.70 ± 3.65	40.90 ± 0.60	3.76	2.24 ± 0.00	0.77	98.53 ± 2.07	18.23 ± 4.31	186 ± 1.79	Higuchi
PT2 (1:6)	−1	0	60.06 ± 5.79	37.24 ± 1.58	1.95	4.65 ± 0.02	1.46	80.71 ± 1.90	25.04 ± 0.59	39 ± 1.41	Higuchi
PT3 (1:3)	−1	1	69.15 ± 7.12	40.57 ± 1.38	1.10	4.42 ± 0.04	1.78	75.66 ± 3.56	26.07 ± 3.10	38 ± 2.08	Higuchi
GG1 (1:9)	0	−1	56.49 ± 2.63	39.49 ± 0.66	9.03	2.67 ± 0.11	2.32	94.94 ± 4.55	32.02 ± 2.02	13.5 ± 2.12	1st order
GG2 (1:6)	0	0	37.92 ± 1.74	37.28 ± 0.32	3.19	2.76 ± 0.02	1.43	84.95 ± 1.78	39.34 ± 4.65	15 ± 1.41	1st order
GG3 (1:3)	0	1	36.38 ± 2.30	39.66 ± 1.48	3.53	4.45 ± 0.49	1.46	77.92 ± 0.36	51.03 ± 1.42	21 ± 0.2	1st order
DXT1 (1:9)	1	−1	51.87 ± 6.17	40.86 ± 0.51	3.8	1.83 ± 0.11	2.03	86.62 ± 2.96	19.06 ± 0.43	178 ± 2.82	Higuchi
DXT2 (1:6)	1	0	45.27 ± 6.25	40.72 ± 1.54	1.63	3.20 ± 0.03	2.32	78.29 ± 3.94	37.83 ± 5.03	60 ± 5.65	Higuchi
DXT3 (1:3)	1	1	68.65 ± 1.66	40.36 ± 0.68	1.24	3.35 ± 0.03	2.06	74.92 ± 4.48	37.40 ± 1.12	92 ± 5.66	Higuchi

* −1 denotes low level, 0 denotes middle level and +1 denote high level.
[a] Yield.
[b] Angle of repose.
[c] Moisture content.
[d] Volume mean diameter.
[e] Span.
[f] Incorporation efficiency.
[g] % Release after 5 min.
[h] Time required for 80% release.

2.10. Tolerability studies

The tolerability, both on an *in vitro* cellular and *in vivo* tissue levels, of selected SDP was evaluated using MTT assay and histopathological examinations of rat nasal tissue after drug administration as follows.

2.10.1. MTT assay

Cytotoxicity was determined by MTT assay on Calu-3 cells as previously explained elsewhere (Nasr et al., 2011). Briefly, Calu-3 cells were grown on MEM medium supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, HEPES buffer, and 50 μg/mL gentamycin. All cells are maintained at 37 °C in a humidified atmosphere (95% RH) with 5% (v/v) CO_2 (Humid CO_2 incubator, SHEL LAB model 2406, USA). The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 μl of the growth medium. Different concentrations of dry powders from 0.03125 to 2 mg/mL were prepared in 100 μl of the growth medium and added to the cell monolayers with incubation for 24 h. Twenty μl of MTT solution (5 mg/mL in PBS, pH 7.4) were added to the cells followed by incubation at 37 °C for 4 h. The medium was then removed and the formazan crystals formed were solubilized with 100 μl dimethyl sulfoxide (DMSO). The absorbance was read at 570 nm on a microplate reader (Tecan Sunrise®, Switzerland). Sodium lauryl sulfate (SLS), REP powder and blanks of the selected formulae were used as controls. The cell viability percentage was calculated according to the following equation:

$$\text{Viability\%} = \left[\frac{A\ (\text{test})}{A\ (\text{control})}\right] \times 100 \quad (3)$$

where A (test) is the absorbance obtained for each of the concentrations of the test substance, and A (control) is the absorbance obtained for the untreated cells (incubated with medium only). The latter reading was assumed to correspond to 100% cell viability. The IC_{50}, which is the concentration of particles causing decrease in cell viability by 50%, was calculated from dilution data.

2.10.2. Histopathological evaluation

The experimental procedures conformed to the Ethical Committee of Faculty of Pharmacy, Ain-Shams University on the use of animals. Male Wister albino rats, weighing 180–220 g, received once daily nasal administration of one of the selected spray dried formulae for 7 days using the monodose insufflator. A control group receiving IN saline using a micropipette was included in the study. The nasal mucosa from the bottom of inferior meatus was dissected after scarifying the animal; the tissues were separated, fixed in 10% formaldehyde for 24 h, decalcified, washed with tap water and then dehydrated by ethyl alcohol. Specimens were cleared in xylene embedded in paraffin at 56 °C in a hot air oven for 24 h. Tissue blocks of paraffin beeswax were then prepared for sectioning at 5 mm thickness with the microtone (Rotary Leica RM2245, USA). The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains. The sections were examined and photographed using light microscope (Axiostar Plus, Zeiss, USA) (Bshara, Osman, Mansour, & El-Shamy, 2014).

2.11. Antidiabetic activity in rats

2.11.1. Animal handling

Sixty male Wister albino rats weighing between 180 and 220 g were divided into ten groups, each consisting of six. For acclimatization, rats were kept for seven days at constant temperature of 25 °C. Meanwhile, they received normal diet and water *ad libitum.*

2.11.2. Drug administration

Before drug administration, diabetes was induced in animals of all groups except group I (normal control receiving IN saline) by intraperitoneal administration of STZ solutions (Abu Abeeleh et al., 2009). A glucometer (One Touch®, Select®, Life Scan) was used 72 h post-administration of STZ to confirm diabetes in animals. Only animals with BG levels >250 mg/dL were included in the study (Pachisia & Agrawal, 2012). REP was then administered IN in a dose of 0.1 mg/kg (Jain & Saraf, 2009) to the following animal groups: 4, 5, 6 and 7, where group 4 received drug solution and groups 5–7 received formulae PT3, GG2 and DXT2 respectively. Groups 8–10 received the blanks of the previous formulae, respectively. Group 2 represented diabetic control receiving saline IN and group3 received drug solution IV *via* the tail vein of the rats using an insulin syringe. IN administration of saline and drug solution was done by the use of a micropipette attached to a polyethylene tube. A volume of 30 μl of saline or REP solutions was used. For IN powder administration, the insufflator connected to a short polyethylene tube was placed in rat nostril. A pump allows SDP, each previously filled in no. 3 capsule, to be aerosolized. REP solution for IV and IN administrations was prepared by dissolving REP powder in a small amount of 0.1 M sodium hydroxide solution which was diluted to the desired

volume with normal saline solution. The pH was adjusted to 7.4 with 0.1 M hydrochloric acid (Mark & Grell, 1997).

2.11.3. Blood sampling

Blood was obtained from the retro-orbital venous plexus at fixed time points up to 6 h. Samples were immediately placed on the Glucostrips and BG levels were determined. The antihyperglycemic activity of different REP formulations in terms of percentage change from baseline BG (obtained at zero time) and percentage reduction of BG level at different time points post-treatment were calculated.

The following parameters were then calculated:

C_{min}: minimum BG level as % of initial and was taken directly from BG levels *versus* time profiles. T_{min}: time for minimum BG level in hours.

AUEC: area under the effect *versus* time curve. The linear trapezoidal method was used for calculating AUEC values of different groups from t_0 to $t_{6\,h}$.

D%: the total decrease in plasma glucose level calculated as follow (Rathnam, Balasubramani, & Saravanakumar, 2011):

$$D\% = \left[\frac{AUEC_d - AUEC_f}{AUEC_d}\right] \times 100 \quad (4)$$

where $AUEC_d$ and $AUEC_f$ are AUEC after administration of normal saline (IN) and REP formulations (IV solution, IN solution and IN SDP) respectively to diabetic rats.

2.12. Statistical analysis

Data are expressed as mean of 3 or 6 determinations ± standard deviation (SD) or standard error (SE). The factorial design responses data were analyzed using Design-Expert® v.8.0.7.1 (Stat-Ease, Inc., Minneapolis, MN, USA) by which ANOVA was performed. AUC values were calculated using Graph Pad Prism software program (version 5.01; Graph Pad software Incorporated, La Jolla, CA). Comparison of the mean values was performed using either Student's *t* test or ANOVA using Graph Pad Instat software program. Statistical significance was set at *p*-value ≤ 0.05.

3. Results and discussion

3.1. MPs characterization

According to the set factorial design, nine formulae were prepared. Table 1 shows that the yield of the recovered powders varied considerably and was dependent on formulae composition. The recorded ranges were: 60–69, 36–56 and 45–68 for PT, GG and DXT loaded MPs, respectively. In contrast with the other two polymers, the yield variation with the change in D/P ratio was the least in case of PT-MPs. Furthermore, higher yield values were obtained with PT-MPs and DXT-MPs in 1:3 D/P ratio. GG-MPs exhibited the lowest yield value. Hence, the polymer nature, viscosity and concentration are the key factors for yield variation. Conversely, due to the same leucine content, narrow variations were found in powders flowability (angle of repose values from 37.24 to 40.90).

PT and DXT-MPs exhibited close moisture content ranging from 1.24 to 3.76%. GG loaded REP powders showed the highest amounts of residual water with an exceptionally high value (9%) for GG1prepared at (1:9 D/P ratio). This was probably due to the higher polymer viscosity which contributed to its low yield as reported also in a previous work (Sensoy et al., 2009). Except for GG1, moisture content values were in line with other studies where up to 7.5% (w/w) of water was found in SDP (Chew, Tang, Chan, & Raper, 2005).

MPs size, expressed as VMD, ranged from 1.83 to 4.65 μm with low span values (0.772–2.71) indicating narrow size distributions. A significant increase in VMD was observed by increasing REP loading from 7 to 10% in PT and DXT-MPs which was not altered by further increase in drug loading up to 17% (1:3 D/P ratio). On the other side, similar VMD was obtained with GG formulae prepared with 7 and 10% REP loading, but the size doubled by increasing REP loading to 17%. The variation in PS depended on D/P ratio as well as polysaccharides viscosities and nature, all with suitable size for nasal application (Alhalaweh, Andersson, & Velaga, 2009; Chen, Di Sabatino, Albertini, Passerini, & Kett, 2013). Moreover, the swelling and mucoadhesive properties of the polysaccharides would favor powder localization in the nose, preventing its inhalation.

Taking into account the upper limit of nasally deliverable powders (25 mg/dose) (Kushwaha, Keshari, & Rai, 2011), it was important to achieve a high REP content in the polysaccharides. Actually, REP was highly associated in the SDP with IE exceeding 75% whatever the polysaccharide type, a matter that could be attributed to the similarity in their polyanionic nature, in addition to structural–function relationship such as molecular arrangement, chemical composition and functional groups of the chosen polymers. Increased IE% with increasing the polymer amount was also noted.

Fast onset of action and prolonged release during MPs residence in nasal mucosa were our targets especially when trying to control BG level. Thus, the responses chosen for the factorial analysis were: % release after 5 min ($R\%_{5\,min}$) and time required for 80% release ($T_{80\%}$).

3.2. Drug release and data analysis

One of the targets of this study was to ensure a high drug release during nasal residence time (4–6 h). *In vitro* release profiles of REP from the MPs were studied, see Fig. 3S(a–c). REP powder, as received, showed very slow dissolution rate with only 15.2% being dissolved in 4 h. Spray drying REP from a sodium hydroxide solution (0.01 N) doubled this amount (39%), while its co-spray drying in polymer solutions led to a great improvement in its release achieving 100% over 90–240 min depending on the polymer type and concentration. This might be due to the uniform molecular dispersion in the hydrophilic polysaccharides polymeric network during the spray drying as previously outlined (Gavini et al., 2008). Modulation of the rate and extent of release of this poorly soluble drug will affect both onset and duration of therapeutic activity, a matter of significant importance for drugs, administered nasally, and of special importance for the control of hyperglycemia. It is obvious from Fig. 3S that REP was rapidly released from PT2 and PT3-MPs achieving 100% release in 90 and 120 min, respectively. Meanwhile, with all DXT concentrations and the highest PT concentration, the drug release was maintained for 4 h or more.

From the results of Table 1, it can be seen that Higuchi model is the most suitable model describing the kinetics of REP release from PT and DXT-MPs. This model indicates that the diffusion through matrices is the main factor controlling REP release. On the other hand, GG-MPs displayed first order release kinetics depending mainly on drug concentration.

Statistical analysis for the 3^2 full factorial design responses data followed by ANOVA revealed that the fitted model for the three dependent variables was found to be the two-factor interaction (2FI) model ($p < 0.0001$). The sum of squares and *F*-values of polysaccharide type, D/P ratio, their interactions and the comparative significance of these effects are shown in Table 2. High model accuracy values (R^2) of 0.9679 and 0.9934 were found with $R\%_{5\,min}$ and $T_{80\%}$ respectively.

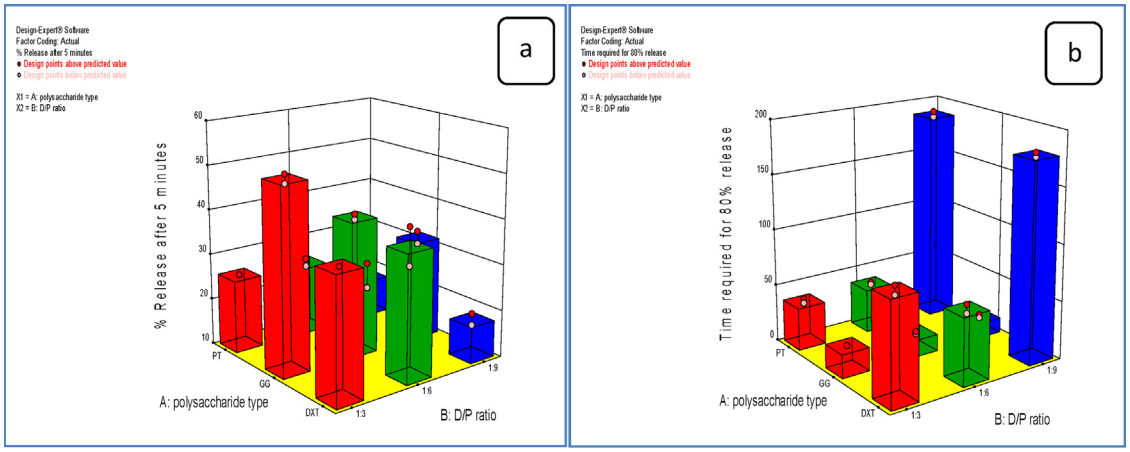

Fig. 1. 3D response surface plots for (a) $R\%_{5\,min}$ and (b) $T_{80\%}$ analysis.

Table 2
Sum of squares and *F*-value for the measured responses.

Model term	Sum of squares		*F*-value	
	$R\%_{5\,min}$	$T_{80\%}$	$R\%_{5\,min}$	$T_{80\%}$
Model	1853.84	72,579.78	33.93	169.93
A = Polymer type	955.80	28,611.44	69.98[s]	267.95[s]
B = D/P ratio	735.40	27,134.11	53.85[s]	254.12[s]
AB	162.64	16,834.22	5.95[s]	78.83[s]

[s] Significant at $p < 0.001$.

3.2.1. $R\%_{5\,min}$

Table 2 reveals that both polysaccharide type and D/P ratio had significant effects on $R\%_{5\,min}$ ($p < 0.0001$). In comparison with the other polymers, GG-MPs exhibited the highest $R\%_{5\,min}$ at all respective ratios especially at 1:3 D/P ratio showing that the drug located at the MPs periphery matrices was released quickly. The presence of the significant AB interaction highlighted the influence of combination between polysaccharide type and D/P ratio on the individual effects, as shown in Fig. 1a. The following equations are describing $R\%_{5\,min}$ for the used polymers, confirming the highest coefficient for the D/P ratio in case of GG:

$$\text{For PT}: R\%_{5\,min} = +17.59 + 27.83 * \text{D/P ratio}$$
$$\text{For GG}: R\%_{5\,min} = +24.80 + 80.98 * \text{D/P ratio}$$
$$\text{For DXT}: R\%_{5\,min} = +19.14 + 61.42 * \text{D/P ratio}$$

3.2.2. $T_{80\%}$

Likewise their influence on $R\%_{5\,min}$, both polysaccharide type and D/P ratio had similar significant effects on $T_{80\%}$ ($p < 0.0001$). For GG (Fig. 1b), small variation in $T_{80\%}$ was found by modifying D/P ratios with extremely lower values for this response. On the other hand, DXT lowest $T_{80\%}$ value was found at a D/P ratio of 1:6 denoting an optimum D/P association with the polymer and amorphization of the drug was achieved at this ratio. At higher polymer concentration with both DXT and PT, the drug was retained in the polymer matrix. The difference in speed and degree of swelling and visible expansion of the prepared formulae upon contact with the dissolution medium might also explain the differences in the drug release rate. The used polysaccharides were of different nature and carbohydrate composition exposing different thickness of diffusion layer followed by variable erosion and drug release (Corrigan, Corrigan, & Healy, 2006). For more in-depth investigation, swelling studies, DSC and FT-IR characterization tools were performed.

3.3. In vitro swelling studies

The dimensional changes of REP-MPs resulting from swelling were evaluated using laser diffraction and were expressed as swelling index (SI). It could be seen from Fig. 4S that the SI showed a similar ranking to the MPs polysaccharide content where higher SI were found with formulations prepared with higher polysaccharide amounts. This was attributed to the hydration of the hydrophilic polymer, which enhanced the water transport rate and extent. It can also be seen that SI increased with time, then declined probably due to the relative dissolution of polymer matrix over time, depending on the degree of polysaccharide hydrophilicity.

3.4. DSC

Thermograms of 1:1 physical polysaccharide:REP mixtures (Figs. 5 and 7S) were simple superpositions of the peaks of individual components, with slight endothermic peak shift, not exceeding 2 °C, due to dilution effect. The typical melting point of REP, seen in Fig. 5S(a), was absent in the thermograms of SDP, at all prepared D/P ratios (Figs. 5–7S), indicating that the drug was molecularly dispersed within the polymer matrix. An obvious retardation of polymer degradation was also seen with DXT-MPs, see Fig. 6S. Fig. 8S shows a sample of X-ray diffraction assay confirming drug amorphization within the polysaccharide matrices. This finding is an added reason for improvement of drug dissolution besides the hydrophilic nature of the used polysaccharides, a matter of special importance for better drug rearrangement within the MPs to ensure their optimum mucoadhesion.

3.5. FT-IR

Several characteristic absorbance peaks can be identified in the FT-IR of REP, LEU and pure polysaccharides (supplement, Figs. 9–11S). They were in accordance with those reported in previous works (Arama et al., 2011; Elmowafy, Awad, Mansour, & El-Shamy, 2008; Mishra, Anisb, Mondalb, Dutta, & Banthiab, 2009; Osman et al., 2013). The spectra of physical mixtures corresponded to superposition of the IR spectra of each polysaccharide and LEU with REP. In the spectrum of blank MPs, the polymer peaks were shifted to lower wavenumbers after spray drying. Moreover, the polymer hydroxyl group showed some variations where it became broader in case of PT, converted into a singlet at 3390.9 cm^{-1} with GG, or shifted to lower wavenumber (3486.6 cm^{-1}) for DXT. By loading REP into the MPs, the O—H stretching peak was shifted to a higher wavenumber in the range of +11–24 cm^{-1} and +8–11 cm^{-1}

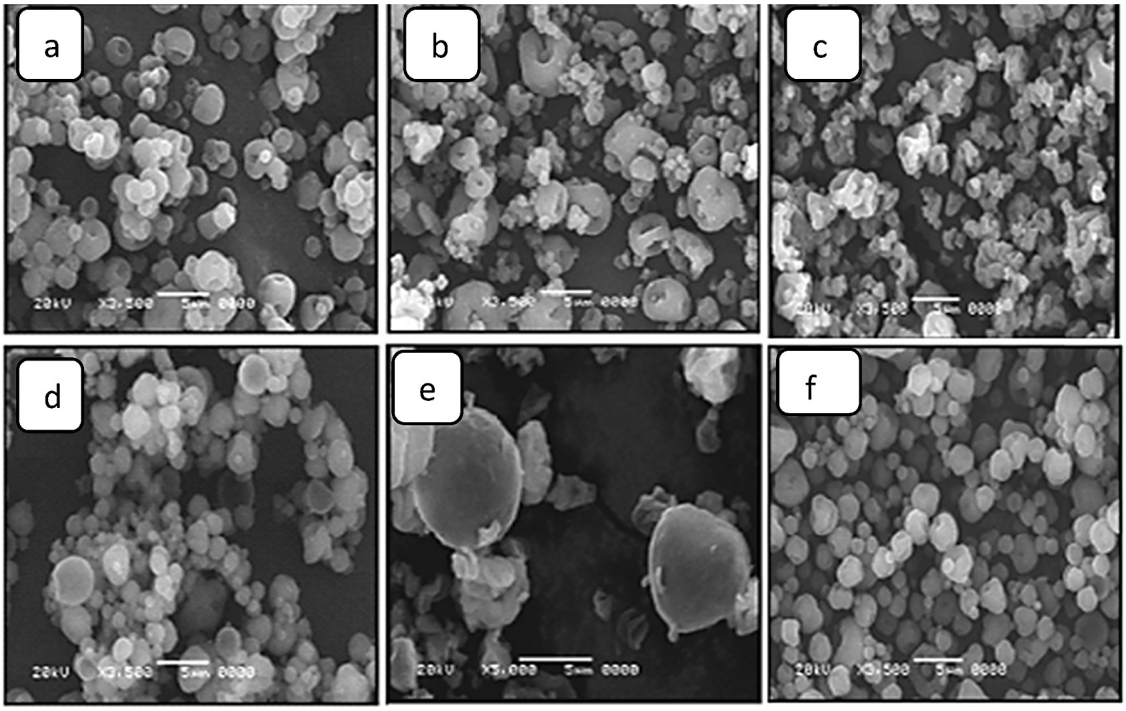

Fig. 2. SEM images of blank MPs of (a) PT3, (b) GG2, (c) DXT2 and REP loaded MPs formulae of (d) PT3, (e) GG2 and (f) DXT2.

in case of GG and DXT-MPs respectively, whatever REP/polymer ratio. This could predict the possible formation of intermolecular hydrogen bonding between hydroxyl groups of the polymer (GG or DXT) and REP carboxylic group. The lower OH shift noticed in PT-MPs denoted weaker interaction with REP justifying 100% drug release especially at 1:3 and 1:6 D/P ratios. The higher PT content enhanced the matrix forming ability of the polysaccharide overcoming the weak drug polymer interaction. Further inspection of REP loaded MPs spectrum revealed that the characteristic N—H stretching vibration peak of REP at 3307.5 cm^{-1} completely disappeared, indicating a possible chemical interaction between the carboxylic groups of polysaccharides and NH of the drug in all MPs matrices.

From the previous results, one formula from each polymer (PT3, GG2 and DXT2) was selected for further investigations. Inclusion criteria were the lowest $T_{80\%}$ at the lower polymer content with IE% exceeding 75%. These formulae were further examined by SEM, *ex vivo* mucoadhesion and cytocompatability studies.

3.6. Morphological examination

SEM micrographs, shown in Fig. 2, indicate that all dry powders were nearly spherical, non-fused with a diameter less than 10 μm, confirming the results of laser diffraction. No crystalline particles could be seen in the micrographs of REP loaded MPs, confirming the drug amorphization within the polymeric matrices (Learoyd, Burrows, French, & Seville, 2008). The type of polysaccharide used influenced the particle shape and morphology. Blank PT and GG-MPs, Fig. 2(a and b), displayed doughnut shape, whereas blank DXT-MPs were dimpled and wrinkled (Fig. 2(c)). After REP loading, the particles (Fig. 2(d–f)) became more spherical and less wrinkled, indicating strengthened matrices. While uniform MPs were obtained with PT and DXT, Fig. 2(d and f), GG-MPs showed size variation as evidenced by appearance of small and large-sized populations, confirming the bimodal size distribution obtained during PS analysis (Fig. 2(e)).

3.7. Mucoadhesion studies

Mucoadhesion peak detachment forces of selected formulae are presented in Table 3. No significant change in mucoadhesive strength was seen after drug loading which might be due to the amorphous nature of the drug previously confirmed by X ray studies. According to the mucoadhesion detachment force, the loaded formulae could be arranged as follows: GG2 > DXT2 > PT3. Statistical analysis revealed that mucoadhesive properties of GG2 were significantly higher ($p < 0.05$) than PT3 or DXT2 with no statistical significance between the last two ($p > 0.05$). A similar ranking was found with SI results. The strong swelling ability of GG was seen after immersion in phosphate buffer pH 6.8 for 5 min. Fast taking up of water and swelling accompanied by erosion led eventually to overall fast release.

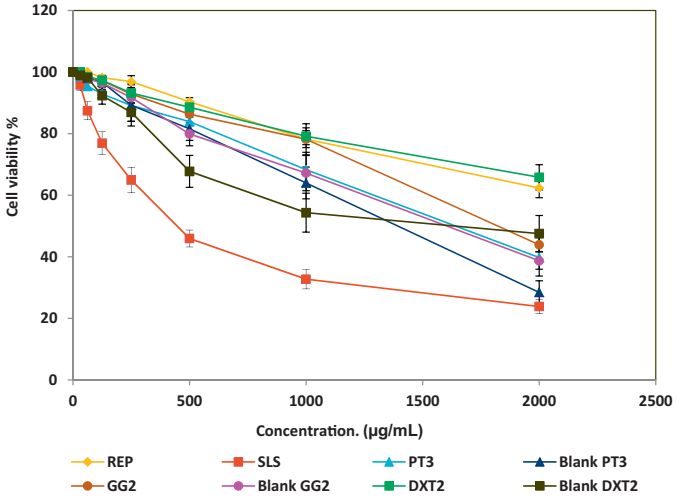

Fig. 3. Viability of Calu-3 cells by MTT assay after incubation with various concentrations of MPs at 37 °C for 24 h.

Table 3
Insufflation, mucoadhesion and pharmacodynamic parameters of REP loaded spray dried polysaccharides powders.

Formulations	% Emitted dose[g] from insufflator after			Peak detachment force[h] (N)	C_{min} (%)	T_{min} (h)	D%
	1st puff	2nd puff	3rd puff				
IV[a] REP solution	NA[c]	NA	NA	NA	47.24 ± 1.85	2	24.09
IN[b] REP solution	NA	NA	NA	NA	49.28 ± 5.29	1	26.89
PT3[d]	90.34 ± 0.20	96.78 ± 0.37	98.01 ± 1.02	4.58 ± 0.32	31.54 ± 1.32	4	61.20
GG2[e]	91.90 ± 0.78	96.47 ± 1.09	98.56 ± 0.96	6.57 ± 0.67	35.64 ± 3.08	2	58.70
DXT2[f]	93.05 ± 0.98	98.89 ± 0.20	98.89 ± 0.57	4.70 ± 0.85	30.51 ± 3.01	6	49.00

[a] Intravenous.
[b] Intranasal.
[c] Not applicable.
[d] Spray dried powder containing pectin in D/P 1:3.
[e] Spray dried powder containing gellan gum in D/P 1:6.
[f] Spray dried powder containing dextran in D/P 1:6.
[g] Calculated for a 10 mg dose.
[h] Values of blank formulae were 4.81 ± 0.43 for blank PT3, 7.04 ± 1.23 for blank GG2 and 4.35 ± 1.84 for blank DXT2.

3.8. Insufflator dose reproducibility

The relationship between the loaded dose and the fraction emitted dose expressed as % was examined in capsules containing increasing amounts of SDP (5 and 10 mg). It was found that more than 98% of the dose was delivered from the device with no significant difference in percentage of dose delivered after three puffs for 5 (data not shown) and 10 mg loadings (Table 3).

3.9. Tolerability studies

3.9.1. MTT assay

Fig. 3 shows that SLS, the positive control, easily damaged the bioactivity of the cells, resulting in a significant decrease in their viability. ANOVA revealed that cell viabilities obtained with REP and the MPs formulae were significantly higher than those obtained by the positive control (SLS) at $p < 0.05$ at all tested concentrations. Cell viability values (90.36–100%), on Calu-3 cells, were found for REP up to 500 μg/mL concentration. REP loaded MPs showed high cell viability >90% after exposure to concentrations up to 125 μg/mL for PT-MPs and 250 μg/mL for GG and DXT-MPs after which the cell viability started to show concentration dependent decrease. The viability did not vary greatly among the three medicated formulae except at 2 mg/mL where the cytotoxicity of PT and GG was significantly higher than that of DXT. All tested formulae could be considered safe on the cell up to a concentration of 1000 μg/mL. Blank MPs gave lower cell viabilities compared to their corresponding medicated particles, probably due to their higher polymer content (Osman et al., 2013). The higher toxicity seen at the high polymer concentration could result from the high concentration of the particles, which after swelling, interfered with the cell viability in the small wells. For further investigation of the safety of the particles, histopathological examinations of the nasal tissues after nasal administration to rats were done.

3.9.2. Histopathological evaluation

Normal histological structure of the nasal mucosa and underlying cartilage were seen in the photomicrographs (Fig. 4(a–d)). The nasal mucosa of rats receiving either normal saline or selected formulae (blank and medicated) revealed no signs of necrosis or hemorrhage. Besides, sloughing of epithelial cells was absent.

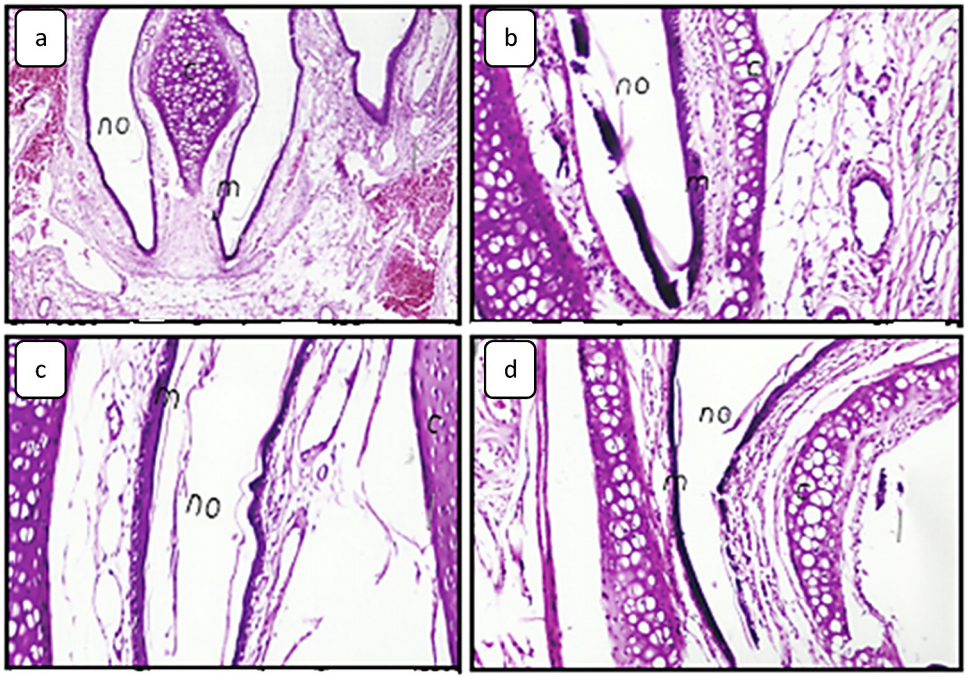

Fig. 4. Light photomicrograph of rat epithelium treated with (a) normal saline and microparticle formulae of (b) PT3, (c) GG2 and (d) DXT2 (magnification size 64×).

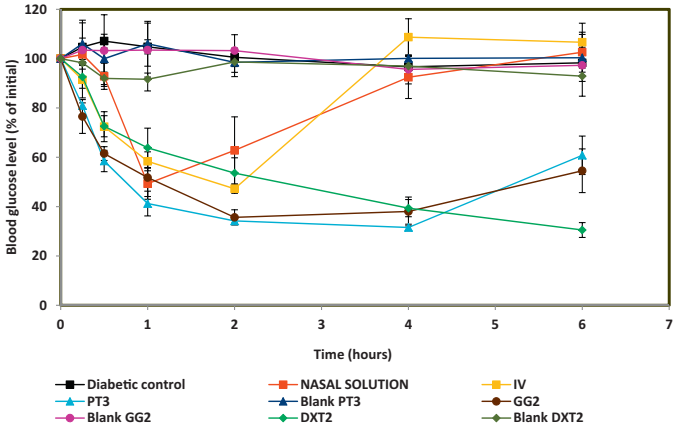

Fig. 5. Percentage change of blood glucose levels after IV and nasal administration of REP solutions and selected blank and medicated MPs powders.

Hence, the use of IN-REP dry powders made of polysaccharides could be considered as safe.

3.10. Antidiabetic activity in rats

Percentage changes of BG from their basal levels were presented in Fig. 5 and pharmacodynamic analysis data were listed in Table 3. The hypoglycemic effect was considered significant by achieving 25% reduction in BG level (Yadav, Kumar, Prajapati, & Shafaat, 2011). Absence of stress during the experiment was proved by normal BG levels in animals of group 1. Poor diabetic control in group 2 was evident by non-significant reducing effect of rat BG levels. The BG levels of animals in groups 8–10 did not increase after administration of the blank MPs, proving the non-diabetogenic nature of the selected carbohydrate polymers confirming their suitability as REP carriers for the BG control.

For intravenously treated animals, BG levels gradually decreased reaching a significant reduction within 30 min with a C_{min} value of 47.24% after 2 h. IN REP solution started its antidiabetic effect after 15 min with the C_{min} value of 49.28% after 1 h. Only animals of group 6, receiving formula GG2, showed significant reductions in BG level after 15 min indicating an almost immediate antidiabetic effect. Furthermore, the C_{min} values of the three selected polysaccharide formulae were nearly similar with respective values of 31.54, 35.65 and 30.51%. However, these formulae exhibited different onset for maximum reduction (T_{min}) with respective times of 4, 2 and 6 h post-administration. The earlier and faster pharmacological response seen with GG2 might be due to its higher $\%R_{5\,min}$ and faster overall *in vitro* release pattern as compared to other formulae added to its higher mucoadhesive properties.

The total decrease in BG level ($D\%$) showed also different patterns among the tested formulae. Lower values in case of REP solutions (24.09 and 26.89% for IV REP and IN REP, respectively) which can be attributed to the high accessibility of dissolved REP molecules for systemic absorption giving rapid and short lasting pharmacological efficacy. Significant rises in $D\%$ reaching two to threefold increase were seen when using polysaccharides formulae with, respective $D\%$ values of 61.20%, 58.70% and 49.00% for PT3, GG2 and DXT2. The significant nasal absorption and hence, hypoglycemic enhancing effect of MPs, when administered in dry powder form, might have arisen from the hydrogel nature of these polysaccharides allowing the absorption of water from the nasal mucosa, thus resulting in a temporary dehydration of the epithelium with subsequent the tight junction opening. Therefore an increased permeability through the paracellular pathway could have been promoted. In addition, the better swelling and mucoadhesive properties might have also contributed to an overall improved hypoglycemic activity. A prolonged hypoglycemic effect over 6 h was of great importance for normalization of meal time glucose excursion (Vijayan, Ravindra Reddy, Sakthivel, & Swetha, 2013), especially in case of moderate and severe diabetes (Makino et al., 2010).

4. Conclusion

Physicochemical characteristics, morphology, drug release, swelling and mucoadhesion properties of dry powder MPs were greatly affected by anionic polysaccharide types and amounts. Amorphization of the drug in the three polysaccharides was important, so as the mucoadhesive properties of the polymers was not affected. The selected non-diabetogenic polysaccharides successfully improved the dissolution of REP giving 100% of drug release after 90–240 min. The selected REP loaded powders were safe and well tolerated on nasal mucosa. The nasal administration of the formulae showed two to three fold increase in total BG decrease compared to the nasal and IV solutions. The provided fast antihyperglycaemic effect was maintained for 6 h.

Conflict of interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.02.064.

References

Abu Abeeleh, M., Ismail, Z. B., Alzaben, K. R., Abu-Halaweh, S. A., Al-Essa, M. K., Abuabeeleh, J., et al. (2009). Induction of diabetes mellitus in rats using intraperitoneal streptozotocin: A comparison between 2 strains of rats. *European Journal of Scientific Research*, *32*(3), 398–402.

Alhalaweh, A., Andersson, S., & Velaga, S. P. (2009). Preparation of zolmitriptan–chitosan microparticles by spray drying for nasal delivery. *European Journal of Pharmaceutical Sciences*, *38*(3), 206–214.

Anitha, V. G., Deepagan, V. V., Divya Rani, Deepthy Menon, S. V., Nair, R., & Jayakumar. (2011). Preparation, characterization, in vitro drug release and biological studies of curcumin loaded dextran sulphate–chitosan nanoparticles. *Carbohydrate Polymers*, *84*, 1158–1211.

Arama, C., Nicolescu, C., Nedelcu, A., & Monciu, C.-M. (2011). Synthesis and characterization of the inclusion complex between repaglinide and sulfobutylether-b-cyclodextrin (Captisol). *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *70*(3–4), 421–428.

Blickle, J. F. (2006). Meglitinide analogues: A review of clinical data focused on recent trials. *Diabetes Metabolism*, *32*, 113–120.

Bshara, H., Osman, R., Mansour, S., & El-Shamy, A. (2014). Chitosan and cyclodextrin in intranasal microemulsion for improved brain buspirone hydrochloride pharmacokinetics in rats. *Carbohydrate Polymers*, *99*, 297.

Buttini, F., Colombo, P., Rossi, A., Sonvico, F., & Colombo, G. (2012). Particles and powders: Tools of innovation for non-invasive drug administration. *Journal of Controlled Release*, *161*, 693–702.

Cao, S., Rena, X., Zhanga, Q., Chena, E., Xua, F., Chena, J., et al. (2009). *In situ* gel based on gellan gum as new carrier for nasal administration of mometasone furoate. *International Journal of Pharmaceutics*, *365*, 109–115.

Chen, K. H., Di Sabatino, M., Albertini, B., Passerini, N., & Kett, V. L. (2013). The effect of polymer coatings on physicochemical properties of spray-dried liposomes for nasal delivery of BSA. *European Journal of Pharmaceutical Sciences*, *50*, 312–322.

Chew, N. Y., Tang, P., Chan, H. K., & Raper, J. A. (2005). How much particle surface corrugation is sufficient to improve aerosol performance of powders? *Pharmaceutical Research*, *22*, 148–152.

Corrigan, D. O., Corrigan, O. I., & Healy, A. M. (2006). Physicochemical and in vitro deposition properties of salbutamol sulphate/ipratropium bromide and salbutamol sulphate/excipient spray dried mixtures for use in dry powder inhalers. *International Journal of Pharmaceutics*, *322*, 22–30.

Elmowafy, E. M., Awad, G. A., Mansour, S., & El-Shamy, A. (2008). Release mechanisms behind polysaccharides-based famotidine controlled release matrix tablets. *AAPS PharmSciTech*, *9*(4), 1230–1239.

Gavini, E., Rassu, G., Muzzarelli, C., Cossu, M., & Giunchedi, P. (2008). Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for nasal

administration of metoclopramide. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*, 245–252.
Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews*, *31*, 267–285.
Illum, L. (2012). Nasal drug delivery – Recent developments and future prospects. *Journal of Controlled Release*, *161*, 254–263.
Jain, S., & Saraf, S. (2009). Repaglinide-loaded long-circulating biodegradable nanoparticles: Rational approach for the management of type 2 diabetes mellitus. *Journal of Diabetes*, *1*(1), 29–35.
Jalalipour, M., Gilani, K., Tajerzadeh, H., Najafabadi, A. R., & Barghi, M. (2008). Characterization and aerodynamic evaluation of spray dried recombinant human growth hormone using protein stabilizing agents. *International Journal of Pharmaceutics*, *352*, 209–216.
Kushwaha, S. K. S., Keshari, R. K., & Rai, A. K. (2011). Advances in nasal trans-mucosal drug delivery. *Journal of Applied Pharmaceutical Science*, *1*(7), 21–28.
Learoyd, T. P., Burrows, J. L., French, E., & Seville, P. C. (2008). Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*, 224–234.
Liu, C., Desai, K. G. H., Tang, X., & Chen, X. (2006). Drug release kinetics of spray-dried chitosan microspheres. *Drying Technology*, *24*, 769–776.
Lund, S. S., Tarnow, L., Stehouwer, C. D. A., Schalkwijk, C. G., Frandsen, M., Smidt, U. M., et al. (2007). Targeting hyperglycaemia with either metformin or repaglinide in non-obese patients with type 2 diabetes: Results from a randomized crossover trial. *Diabetes, Obesity and Metabolism*, *9*(3), 394–407.
Mahajan, H. S., & Gattini, S. G. (2009). Gellan gum based microparticles of metoclopromide hydrochloride for intranasal delivery: Development and evaluation. *Chemical and Pharmaceutical Bulletin*, *57*(4), 388–392.
Makino, C., Sakai, H., & Yabuki, A. (2010). Nateglinide controlled release tablet containing compressionable enteric coated granules. *Chemical and Pharmaceutical Bulletin*, *58*(9), 1136–1141.
Mandić, Z., & Gabelica, V. (2006). Ionization, lipophilicity and solubility properties of repaglinide. *Journal of Pharmaceutical and Biomedical Analysis*, *41*(3), 866–871.
Manzella, D., Abbatecola, A. M., Grella, R., & Paolisso, G. (2005). Repaglinide administration improves brachial reactivity in type 2 diabetic patients. *Diabetes Care*, *28*(2), 366–371.
Mark, M., & Grell, w. (1997). Hypoglycaemic effects of the novel antidiabetic agent repaglinide in rats and dogs. *British Journal of Pharmacology*, *121*, 1597–1604.
Mishra, R. K., Anisb, A., Mondalb, S., Dutta, M., & Banthiab, A. K. (2009). Preparation and characterization of amidated pectin based polymer electrolyte membranes. *Chinese Journal of Polymer Science*, *27*(5), 639–646.
Nasr, M., Awad, G. A. S., Mansour, S., Taha, I., Elshamy, A., & Mortada, N. D. (2011). Different modalities of NaCl osmogen in biodegradable microspheres for bone deposition of risedronate sodium by alveolar targeting. *European Journal of Pharmaceutics and Biopharmaceutics*, *79*(3), 601–611.
Osman, R., Kan, P. L., Awad, G. A. S., Mortada, N., EL-Shamy, A., & Alpar, O. (2013). Spray dried inhalable ciprofloxacin powder with improved aerosolisation and antimicrobial activity. *International Journal of Pharmaceutics*, *449*, 44–58.
Pachisia, N., & Agrawal, S. S. (2012). Formulation, development and evaluation of transdermal drug delivery system of glimepiride. *International Journal of Pharmacy and Pharmaceutical Science Research*, *2*(1), 1–8.
Patil, S. B., & Sawant, K. K. (2011). Chitosan microspheres as a delivery system for nasal insufflation. *Colloids and Surfaces B: Biointerfaces*, *84*, 384–389.
Pereswetoff-Morath, L., & Edman, P. (1995). Dextran microspheres as a potential nasal drug delivery system for insulin – In vitro and in vivo properties. *International Journal of Pharmaceutics*, *124*, 37–44.
Plucinski, G., & Rezaizn-Yazdi, M. (2009). Pharmaceutical compositions comprising a hypoglycemic agent and methods of using same. US Patent 0318502, 1–9.
Rathnam, G., Balasubramani, P., & Saravanakumar, A. (2011). Nasal drug delivery of anti-diabetic drug repaglinide using biodegradable starch microspheres. *International Journal of Pharmaceutical Sciences and Research*, *2*(4), 940–947.
Sensoy, D., Cevher, E., Sarıcı, A., Yılmaz, M., Ozdamar, A., & Bergisadi, N. (2009). Bioadhesive sulfacetamide sodium microspheres: Evaluation of their effectiveness in the treatment of bacterial keratitis caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a rabbit model. *European Journal of Pharmaceutics and Biopharmaceutics*, *72*(3), 487–495.
Staniforth, J.N. (2002). Powders comprising antiadherant materials for use in dry powder inhalers. USA 6,475,523.
Upadhyay, S., Parikh, A., Joshi, P., Upadhyay, U. M., & Chotai, N. P. (2011). Intranasal drug delivery system – A glimpse to become maestro. *Journal of Applied Pharmaceutical Science*, *1*(3), 34–44.
Vidgren, M. T., & Kublik, H. (1998). Nasal delivery systems and their effect on deposition and absorption. *Advanced Drug Delivery Reviews*, *29*, 157–177.
Vijayan, V., Ravindra Reddy, K., Sakthivel, S., & Swetha, C. (2013). Optimization and characterization of repaglinide biodegradable polymeric nanoparticle loaded transdermal patches: In vitro and in vivo studies. *Colloids and Surfaces B: Biointerfaces*, *111*, 150–155.
Watts, P. J., & Smith, A. (2009). PecSys: *In situ* gelling system for optimised nasal drug delivery. *Expert Opinion in Drug Delivery*, *6*, 543–552.
Yadav, V. K., Kumar, B., Prajapati, S. K., & Shafaat, K. (2011). Design and evaluation of mucoadhesive microspheres of repaglinide for oral controlled release. *International Journal of Drug Delivery*, *3*, 357–370.