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Probucol release from novel multicompartmental microcapsules for the oral targeted delivery in Type 2 Diabetes

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Abstract

In previous studies, we developed and characterised multicompartmental microcapsules as a platform for the targeted oral delivery of lipophilic drugs in Type 2 diabetes (T2D). We also designed a new microencapsulated formulation of Probucol-Sodium Alginate (PB-SA), with good structural properties and excipient compatibility. The aim of this study was to examine the stability and pH-dependant targeted release of the microcapsules at various pH values and different temperatures. Microencapsulation was carried out using a Büchi-based microencapsulating system developed in our laboratory. Using SA polymer, two formulations were prepared: empty SA microcapsules (SA, control) and loaded SA microcapsules (PB-SA, test), at a constant ratio (1:30) respectively. Microcapsules were examined for drug content, Zeta-potential, size, morphology and swelling characteristics, and PB release characteristics at pH 1.5, 3, 6, and 7.8. The production yield and microencapsulation efficiency were also determined. PB-SA microcapsules had 2.6 ± 0.25% PB content, and Zeta-potential of -66 ± 1.6%, suggesting good stability. They showed spherical and uniform morphology and significantly higher swelling at pH 7.8 at both 25 ^{o}C and 37^{o}C ($p<0.05$). The microcapsules showed multiphasic release properties at pH 7.8. The production yield and microencapsulation efficiency were high (85 ± 5 and 92 ± 2 %, respectively). The PB-SA microcapsules exhibited distal gastrointestinal tract targeted delivery with a multi-phasic release pattern, and with good stability and uniformity. However, the release of PB from the microcapsules was not controlled, suggesting uneven distribution of the drug within the microcapsules.

Keywords: Probucol, artificial-cell microencapsulation, diabetes mellitus, anti-inflammatory, antioxidant, Type 2 Diabetes

Introduction

Diabetes mellitus is a disease characterized by hyperglycaemia and metabolic disorders. It is classified as Type 1 diabetes (T1D) or Type 2 diabetes (T2D). T1D is an autoimmune disease marked by the destruction of β-cells of the pancreas resulting in a partial or complete lack of insulin production and the inability of the body to control glucose homeostasis [1]. T2D develops due to genetic and environmental factors that lead to tissue desensitization to insulin [2]. Despite strict glycaemic control and the fact that new and more effective antidiabetic drugs are continuously appearing onto the market, diabetic patients still suffer from the disease and its complications [3]. Antidiabetic drugs are effective in minimizing variations between peaks and troughs of blood glucose levels in diabetic patients [3]. Common antidiabetic drugs include: sulfonylureas, such as Gliclazide that enhances insulin production, pancreatic β-cell functionality and improves insulin sensitivity; and the biguanide Metformin, which reduces glucose production in the liver [3]. However, the risks of hypoglycaemia, free radical and toxin build up remain major issues associated with T2D [4, 5]. Thus, there is an urgent need for new and more efficacious medications for diabetes that are capable of exerting a stronger protection of β-cells and have considerable anti-free radical and antioxidant effects. An advantage is optimising the formulations of drugs that have already shown desirable antidiabetic effects such as lowering of blood cholesterol and reducing the formation of atherosclerotic plaques.

Probucol (PB) is a highly lipophilic drug that has been shown to protect β-cells of the pancreas through its strong anti-free radical and antioxidant effects, and thereby neutralizing reactive oxygen species and alleviating oxidative stress [6, 7]. PB was developed as an antihyperlipidemic drug, but was withdrawn in some countries owing to high interindividual variation in absorption and potential adverse effects [8]. PB has high affinity for adipose tissues and has huge inter- and intra-individual variations in absorption after an oral dose [9]. The variations in absorption and efficacy are predicted to contribute significantly to its adverse effects, and compromise its potential clinical benefits in T2D [10]. Thus, developing a novel and stable formulation with high uniformity, efficient targeted delivery, and consistent release kinetics is anticipated to overcome these variations and maximise its potential use in T2D.

In a recent study carried out in our laboratory (manuscript currently under review), we designed novel multi-compartmental microcapsules of PB that displayed uniform and homogenous characteristics and exhibited pseudoplastic-thixotropic properties. These newly designed PB microcapsules showed good compatibility and structural properties. Accordingly, in this study, we aimed at describing further the targeted delivery, stability, and release properties of these PB microcapsules.

Materials and methods

Materials

Probucol (PB, 99%) and low viscosity sodium alginate (LVSA, 99 %) were purchased from Sigma Chemical Co, USA. Calcium chloride dihydrate ($CaCl_2$. $2H_20$, 98%) was obtained from Scharlab S.L, Australia. All solvents and reagents were supplied by Merck (Australia) and were of HPLC grade and used without further purification.

Drugs preparations

Due to PB being highly insoluble [11] in aqueous media, it was dissolved in 10% freshly prepared Ultrasonic suspension prior to carrying out of experiments. Stock suspensions of PB (20 mg/mL) were prepared by adding the powder to 10% Ultrasonice water-soluble gel in 100mL HPLC water. The $CaCl_2$ stock solution (2%) was prepared by adding $CaCl_2$ powder to HPLC water. All preparations were mixed thoroughly at room temperature for 4 hours, stored in the refrigerator, and used within 48 hours of preparation.

Preparation of microcapsules

Vibrational-jet flow microencapsulation of PB-loaded LVSA was prepared using a Büchi-390 based-microencapsulating system (BÜCHI Labortechnik, Swizerland). Polymer solutions containing SA with and without PB were made up to a final concentration of PB-SA in a ratio of 1:30 respectively. Parameters were set in a frequency range of 1000-1500Hz and a flow rate of 4 mL/min under a consistent air pressure of 300 mbar. Vibrational-jet flow prepared microcapsules were collected from the microencapsulating system and, for each formulation, 3 independent batches were prepared and tested separately (n=3). All microcapsules (unloaded and PB-loaded) were prepared and treated in the exact same way. Furthermore, the microcapsules were dried using stability chambers (Angelantoni Environmental and Climatic Test Chamber, Italy). The weight of the recovered dry particles was then recorded and the PB contents, production yield, microencapsulation efficiency, zeta potentials, and mean particle size of each preparation were all measured and compared, as described below.

Characterization of PB-loaded microcapsules

Drug content, production yield, microencapsulation efficiency, and stability studies:

Drug content, production yield, and microencapsulation efficiency: 1 g of microcapsules was carefully weighed, ground, and dissolved in 200 mL of phosphate buffer (pH 7.8) and the suspension was stirred with a magnetic stirrer for 6 hours. 2 mL of the solution were then transferred to 100 mL flask and diluted with phosphate buffer (vehicle) to 100 mL. Aliquots of the dissolution medium (2 mL) were withdrawn at predetermined time points (every 200 seconds) and filtered through a 0.22 µm Millipore filter. The amount of dissolved drug was determined spectrophotometrically at λ_{Max} = 242 nm against the buffer as blank [12, 13]. The measurements were performed under sink conditions, and average values were calculated. Absorbance was measured using an UV spectrophotometer (Shimatzu UV-Vis spectrophotometer 1240, Japan). PB concentrations were calculated from the calibration curve. All analyses were carried out in triplicate (n=3). Drug contents, production yield, and microencapsulation efficiency were calculated from the following equations.

1. $$\%\boldsymbol{Drug\ Content} = \frac{\boldsymbol{Calculated\ amount\ of\ PB\ in\ the\ microcapsules}}{\boldsymbol{Total\ weight\ of\ microcapsules}}\ \boldsymbol{x\ 100}$$

2. $$\%\boldsymbol{Production\ Yield} = \frac{\boldsymbol{Total\ weight\ of\ the\ microcapsules}}{\boldsymbol{Total\ weight\ of\ the\ polymer + drug\ solution}}\ \boldsymbol{x\ 100}$$

3. $$\%\ \boldsymbol{Encapsulation\ Efficiency} = \frac{\boldsymbol{Drug\ content}}{\boldsymbol{Theoretical\ content}}\ \boldsymbol{x\ 100}$$

Zeta-potential and size analysis: To determine the electrokinetic stability and size uniformity of the microcapsules in the colloidal system, zeta potential and size distribution for the microencapsulated formulation of SA and PB-SA were measured by photon correlation spectroscopy using a Zetasizer 3000HS (Malvern Instruments, Malvern, UK), and by the Mie and Fraunhofer scattering technique using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). The measurements were performed at 25°C with a detection angle of 90°, and the raw data were subsequently correlated to Z average mean size using a cumulative analysis via an OmniSEC-Zetasizer software package. Each sample was measured 10 times. All analyses were performed on samples appropriately diluted with filtered deionized water. All determinations were performed in triplicate and results were reported as mean ± SD.

Optical microscopy (OM): Morphological characteristics and particle size analysis were determined utilizing a Nikon YS2-H mounted with a ToupTek photonics FMA050 fixed calibrated microscope

adaptor (Japan). Sample analysis was carried out in triplicates. Briefly, pre-determined quantities (10 microcapsules from each formulation) of freshly prepared microcapsules were loaded onto a glass slide mounted to a calibrated scale. OM software (ToupTek Digital, Japan) capable of particle size analysis, microcapsule characterization, and morphological assessments was used to determine the basic characteristics of the microcapsules that are needed to complement the scanning electron microscopy (SEM) studies.

Swelling Studies: To determine the swelling properties of the microcapsules (SA and PB-SA), 50 mg dry microcapsules were weighed and placed in 20 mL of two pH values (3 and 7.8) and two temperatures (25°C and 37°C) for 6 hours. The selection of the two temperatures, pH values, and study duration was based on our previously published work [14, 15]. The swollen microcapsules were then removed at periodically predetermined intervals (hourly). The wet weight of the swollen microcapsules was determined by blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing on an electronic balance. All experiments were done in triplicate (n=3). The swelling index of the microcapsules was calculated from the following formula **[16, 17]**:

4. $$\textbf{\textit{Swelling Index}} = \frac{\textbf{\textit{Final weight}}}{\textbf{\textit{Initial weight}}}$$

Drug release studies (in-vitro dissolution test): A weighed sample (2 g) of PB loaded microcapsules was suspended in 200 mL of phosphate buffer solution at pH values of 1.5, 3, 6, and 7.8 for 6 hours, as appropriate. The dissolution medium was stirred at 200 rpm. Sink conditions were maintained throughout the assay period [18, 19]. All the experiments were carried out at 25°C. The absorbances of the solutions were measured every 30 minutes using a Hewlett Packard-based time controlled UV-spec mounted with a close-loop flow system under sink conditions (Figure 1). All analyses were carried out in triplicate (n=3). Additionally, unloaded microcapsules (containing no drug) were analysed spectrophotometrically at λ_{Max} = 242nm using phosphate buffer at all four pH values (temperature maintained at 25°C) in order to exclude any interference in the analytical data and to ensure that only PB was being measured at that particular wavelength and experimental condition.

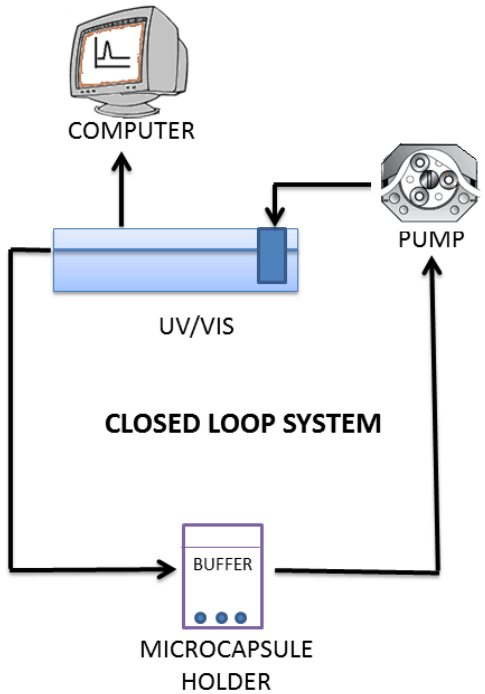

Figure 1: Closed-loop flow system for microcapsule-drug release measurements.

Physical and chemical stability:

The stability test was carried out by placing predetermined amounts of freshly prepared microcapsules onto sterile petri dishes (30 microcapsules in each) and storing them in thermostatically controlled ovens at -20°C, 5°C, 25°C, and 40°C respectively, with relative humidity set at 35% for 3 days. The experiment was conducted using a stability chamber (Angelantoni environmental and climatic test chamber, Italy). A temperature and humidity regulator was used to ensure constant experimental conditions. At the end of the experiment, the microcapsules were analyzed for any changes in appearance and morphology, and for the determination of the amount of drug remaining in each formula, using a validated UV-Vis stability-indicating method [20, 21]. Briefly, the dosage forms were crushed and dissolved in a 200 mL phosphate buffer at pH 7.8. The solution was filtered and the first 20 mL were removed; and 10 mL of the filtrate were diluted to 100 mL in a volumetric flask. Then, 1 mL aliquot of the prepared solution was transferred to 10 mL volumetric flask, and the volume was completed with the buffer. A calibration curve was constructed for PB in phosphate buffer across the concentration range of 0.01 mg to 4 mg/ mL with R^2=0.99 (data not shown). Physical stability data (morphology and appearance) were recorded for both microencapsulated formulations (SA and PB-SA), and chemical stability (drug content remaining) was recorded for the PB-SA formulation.

Statistical analysis

Values are expressed as means ± SD. Drug content, production yield, and microencapsulation efficiency were assessed using Student's t-test. Swelling index and drug dissolution comparison for the different formulations were also assessed and compared using Student's test. The best fit model was derived using GraphPad Prism software (V6; GraphPad Software, Inc., USA). Statistical significance was set at $p < 0.05$ and all statistical analyses were performed using GraphPad Prism software.

Results and Discussion

Drug content, production yield, and microencapsulation efficiency:

Significant levels of PB-loading (microencapsulation) efficiency were achieved for all microcapsules as shown in

Table 1. The results of the drug content and encapsulation efficiency showed minimum variation among repeated samples, which confirms the reproducibility of our developed microencapsulation method. Additionally, high production yield with low variability in drug content and good drug loading efficiency were achieved. Neither any peaks for a biodegradable polymer nor any alteration of the chromatographic pattern of PB was observed, which is in line with our published work.

Table 1: Drug content, production yield, encapsulation efficiency, zeta potential, and mean particle size of SA and PB-SA microencapsulation formulations.

Microcapsule size analysis and Zeta potential determination:

Analysis of the size of the microcapsules obtained from each formulation, as demonstrated in

Table 1, revealed uniform and consistent particle size distribution, and thus the addition of the drug did not alter the size of the microcapsules, ensuring effective encapsulation without adverse effects on size. SA and PB-SA microcapsules had an range in size of 0.8-1 mm. The significant uniformity in particle size distribution of the microcapsules ensures reproducibility.

As depicted by Zeta potential values of -66mV (SA) and -72.9mV (PB-SA), the dispersion of microcapsules suggested a stable system [22], with the PB-SA formulation being more charged (

Table 1). This is assuming the higher charge (>25 mM) indicates stronger surface electrical charge of the suspended drug particles. Additionally, the PB-SA formulation would be more stable, given the greater repulsion created within the suspension system [23].

Optical Microscopy:

From both formulations, SA and PB-SA, ten microcapsules were randomly selected for particle size and morphological analysis. Results show an overall consistent and uniform shape as determined via a calibrated scale mounted onto a glass slide. As evident in Figure 2, the mean diameter of SA microcapsules (Figure -a) (average ± SD) was 800 ± 20 µm, while that of PB-SA microcapsules (Figure -b) was 850 ± 50 µm. Results obtained also include the horizontal diameter (L1), the vertical diameter (L2), and the microcapsule width (L3).

Figure 2: SA microcapsule (a) and PB-SA microcapsule (b). L1 is the horizontal diameter, L2 is the vertical diameter, and L3 is the microcapsule width.

Swelling studies:

Figure and Figure show that the formulation type, the pH of the medium, and the temperature do have an effect on the swelling characteristics of the microcapsules. Evidently, the higher the temperature and pH, the more swelling of microcapsules in both formulations.

In line with PB-SA *in-vitro* dissolution data (Figure), the swelling index corresponds to degree of drug release. The greater the swelling, the higher the amount of drug that diffuses into the dissolution media. This is due to water uptake, expansion, and subsequent erosion of the alginate matrix, resulting in the loss of microcapsule structural integrity and release of both surface bound and encapsulated PB [24]. The swelling index is heavily influenced by pH and temperature, and, by considering physiological parameters, it is evident that at pH 7.8 and 37°C, the greatest swelling and thus the most extensive drug release occurs from PB-SA microcapsules. PB-containing microcapsules swell more than empty SA microcapsules. This could be due to the fact that the surface of PB-SA microcapsules contains dry crystal agglomerates compromising the surface integrity, causing weak links in the alginate matrix and easy expansion and rupture upon contact with water.

By considering the swelling and dissolution data, it seems logical to emphasize the importance of the alginate matrix structural integrity and stability in order to ensure controlled drug release, particularly in physiological conditions. An important formulation excipient that should be considered for future work is a bile acid (BA), which has the potential to provide stability and matrix reinforcement [14]. Additionally, BAs have also been shown to be more hydrophobic than their corresponding salts, ensuring greater protection from water penetration as well as being very good tissue permeation enhancers in diabetes [25, 26].

Figure 3: Swelling characteristics of PB-SA and SA microcapsules (pH 3 and 7.8) at 25°C.

Figure 4: Swelling characteristics of PB-SA and SA microcapsules (pH 3 and 7.8) at 37°C.

Drug release studies and in-vitro dissolution:

Probucol release from the PB-SA microcapsules was studied in triplicates across four pH values (1.5, 3, 6, and 7.8) at 25°C for a period of 6 hours each. The selection of these four pH values was based on our previous studies examining the best sites of potential antidiabetic drug absorption in the gastrointestinal tract (GIT) [27-33]. However, the use of a gradient-pH system may have also provided good prediction of *in vivo* results.

The release of PB was dependant on temperature and pH, which is in line with previous studies using SA-drug formulations [34, 35]. As shown in Figure , PB release was slow and minimal but in a relatively controlled manner at low acidic pH values (1.5 and 3). As pH values were increased, the release of PB was also increased, in particular, at pH 7.8, which is expected [24, 36]. PB release from PB-SA microcapsules at pH 6 and 7.8 was biphasic and multiphasic, respectively (Figure). This has important implications in diabetes therapy as work in our laboratory has confirmed the distal GIT to be the site of intended drug delivery due to an abundance of efflux transporters, which have been associated with PB absorption after oral administration, such as the transporter ABCA1 [37, 38]. However, the

exact impact of such release patterns in PB oral absorption, efficacy, and safety profiles remains difficult to predict [39-41].

A possible explanation of the multi-phasic release of PB from the PB-SA microcapsules is that PB is unevenly distributed within the microcapsules, with some of it on the outside, as well as inside of the microcapsules. Thus, the multi-phasic drug release pattern depicted in PB-SA dissolution data (Figure) could be the preferential binding of PB to the microcapsule surface, and by coating the microcapsule surface, the drug would be quickly liberated following swelling and erosion of the alginate matrix.

Preferential deposition of encapsulated drugs onto microcapsule surfaces has been extensively studied, and may occur due to several factors such as the hydrophilic-lipophilic balance of the surface (HLB), the molecular weight, solubility, and degree of ionisation of the drug, as well as the surface charge of the microcapsule and the physicochemical properties and proportions of the excipients used [42]. It is also possible that the rapid release of PB from the microcapsule surface is attributed to its very low solubility in the release medium (creating thermodynamic instability); as such, release mechanisms often stem from drugs that are very lipophilic and their release patterns are characterised by short "burst" times followed by much slower release concentrations [43].

It appears that microencapsulation of PB using only the sodium alginate polymer results in drug coating of the surface by the drug, with some being distributed within the core of the microcapsule.

Figure 5: Probucol release from PB-SA microcapsule over time across various pH values

Accelerated stability studies (environmental chamber):

Accelerated stability studies were carried out over a 3 day period, testing both formulations (SA and PB-SA) at -20°C, 5°C, 25°C, and 40°C and at a relative humidity of 35%.

Both formulations (SA and PB-SA) appeared to retain their original morphological characteristics throughout the study. However, there were some changes in the colour, overall size, and quality of the microcapsule surfaces across the temperatures. In detail, at -20°C, some PB-SA microcapsules formed agglomerates that were easily re-dispersed, while others retained their original shape. The appearance of PB-SA at this temperature was white and spherical following the 3 day period, with the original quality (soft microcapsules) maintained. Similarly, SA microcapsules were also soft, spherical, and flexible but were much lighter in colour (opaque in appearance). At the higher temperatures, microcapsules appeared to change colour from a white (5°C and 25°C) to a light brown (at 40°C), most likely due to oxidation of the alginate, whilst retaining their spherical shapes and even homogenous particle size distribution. In terms of size, it was evident that an increase of the temperature resulted in greater shrinkage (by up to 50%), of the microcapsules, with the biggest effect seen at a temperature of 40°C. This may be explained in terms of loss of moisture content, reducing the overall surface area and volume of each microcapsule. In addition, the microcapsules at all temperatures (except at -20°C) had become harder and more brittle owing to loss of moisture within the microcapsules and reduction in their elasticity.

UV analysis of the microcapsules after three days of accelerated stability testing revealed an average % drug content of 2.6 ± 0.3 for PB-SA microcapsules, illustrating that various accelerated environmental conditions did not compromise drug content nor did it result in loss of drug structure. This complemented the visual characterisation of the microcapsules following accelerated stability testing and confirmed uniformity of drug contents.

Conclusion

Our vibrational-jet flow microencapsulation method of PB is effective in producing microcapsules with good stability and uniformity. However, the multi-phasic release characteristics may not result in optimised oral absorption. Thus, an interesting future investigation will be to incorporate BA as a formulation excipient, which may provide reinforcement to the alginate polymer matrix and enhance the controlled release of the drug, and perhaps optimise its potentials in T2D.

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References

1. Barbeau, W.E., J. Bassaganya-Riera, and R. Hontecillas, *Putting the pieces of the puzzle together - a series of hypotheses on the etiology and pathogenesis of type 1 diabetes.* Med Hypotheses, 2007. **68**(3): p. 607-619.
2. Moore, P.A., J.C. Zgibor, and A.P. Dasanayake, *Diabetes: a growing epidemic of all ages.* J Am Dent Assoc, 2003. **134 Spec No**: p. 11S-15S.
3. Negrulj R, M.A., Al-Salami H, *Potentials and Limitations of Bile Acids in Type 2 Diabetes: Applicatons of Microencapsulation as a Novel Oral Delivery System.* Journal of Endocrinology and Diabetes Mellitus, 2013. **1**: p. 1-11.
4. Cani, P.D., et al., *Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia.* Diabetologia, 2007. **50**(11): p. 2374-2383.
5. Goldfine, A.B., V. Fonseca, and S.E. Shoelson, *Therapeutic approaches to target inflammation in type 2 diabetes.* Clin Chem, 2011. **57**(2): p. 162-7.
6. Wu, R., et al., *Probucol ameliorates the development of nonalcoholic steatohepatitis in rats fed high-fat diets.* Digestive diseases and sciences, 2013. **58**(1): p. 163-71.
7. Yamashita, S. and Y. Matsuzawa, *Where are we with probucol: a new life for an old drug?* Atherosclerosis, 2009. **207**(1): p. 16-23.
8. Shimizu, H., et al., *Probucol attenuated hyperglycemia in multiple low-dose streptozotocin-induced diabetic mice.* Life Sci, 1991. **49**(18): p. 1331-8.
9. Russell, J.C., et al., *Cardioprotective effect of probucol in the atherosclerosis-prone JCR:LA-cp rat.* Eur J Pharmacol, 1998. **350**(2-3): p. 203-10.
10. Zimetbaum, P., H. Eder, and W. Frishman, *Probucol: pharmacology and clinical application.* J Clin Pharmacol, 1990. **30**(1): p. 3-9.
11. Vedantham, K., et al., *Development of a probucol-releasing antithrombogenic drug eluting stent.* J Biomed Mater Res B Appl Biomater, 2012. **100**(4): p. 1068-77.
12. Nourooz-Zadeh, J., et al., *Measurement of plasma probucol levels by high-performance liquid chromatography.* J Chromatogr B Biomed Appl, 1994. **654**(1): p. 55-60.
13. Ajun, W., et al., *Preparation of aspirin and probucol in combination loaded chitosan nanoparticles and in vitro release study.* Carbohydrate Polymers, 2009. **75**(4): p. 566-574.
14. Mooranian A, N.R., Martinez J, Mathavan S, Sciarretta J, Chen-Tan N, Mukkur TK, Mikov M, Lalic-Popovic M, Stojančević M, Golocorbin-Kon S, Al-Salami H, *Stability and release kinetics of an advanced gliclazide-cholic acid formulation: the use of artificial-cell microencapsulation in slow release targeted oral delivery of antidiabetics.* Journal of Pharmaceutical Innovation, 2014 (in press).
15. Mooranian A, N.R., Martinez J, Mathavan S, Sciarretta J, Chen-Tan N, Mukkur TK, Mikov M, Lalic-Popovic M, Stojančević M, Golocorbin-Kon S, Al-Salami H, *A complex microencapsulated system: a platform for optimised oral delivery of antidiabetic drug-bile acid formulations.* Pharmaceutical Development and Technology, 2014 (in press).
16. Pal, D. and A.K. Nayak, *Novel tamarind seed polysaccharide-alginate mucoadhesive microspheres for oral gliclazide delivery: in vitro-in vivo evaluation.* Drug Delivery, 2012. **19**(3): p. 123-131.
17. Awasthi, R. and G.T. Kulkarni, *Development of novel gastroretentive drug delivery system of gliclazide: hollow beads.* Drug development and industrial pharmacy, 2013(0): p. 1-11.
18. Mooranian, A., et al., *Stability and Release Kinetics of an Advanced Gliclazide-Cholic Acid Formulation: The Use of Artificial-Cell Microencapsulation in Slow Release Targeted Oral Delivery of Antidiabetics.* Journal of Pharmaceutical Innovation, 2014: p. 1-8.
19. Mooraniana, A., et al., *Novel artificial cell microencapsulation of a complex gliclazide-deoxycholic bile acid formulation: A Characterisation Study.* Drug Design, Development and Therapy, 2014 (in press).

20. Mladenovska, K., et al., *5-ASA loaded chitosan–Ca–alginate microparticles: Preparation and physicochemical characterization.* International journal of pharmaceutics, 2007. **345**(1): p. 59-69.
21. Abdelbary, A., N.A. El-Gendy, and A. Hosny, *Microencapsulation Approach for Orally Extended Delivery of Glipizide: In vitro and in vivo Evaluation.* Indian J Pharm Sci, 2012. **74**(4): p. 319-30.
22. Duro, R., et al., *The adsorption of cellulose ethers in aqueous suspensions of pyrantel pamoate: effects on zeta potential and stability.* Eur J Pharm Biopharm, 1998. **45**(2): p. 181-8.
23. Xie, H.G., et al., *Effect of surface wettability and charge on protein adsorption onto implantable alginate-chitosan-alginate microcapsule surfaces.* Journal of Biomedical Materials Research Part A, 2010. **92**(4): p. 1357-1365.
24. George, M. and T.E. Abraham, *Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan--a review.* J Control Release, 2006. **114**(1): p. 1-14.
25. Yang, L., et al., *Physicochemical and biological characterization of monoketocholic acid, a novel permeability enhancer.* Molecular pharmaceutics, 2009. **6**(2): p. 448-456.
26. Mikov, M., et al., *Pharmacology of bile acids and their derivatives: absorption promoters and therapeutic agents.* Eur J Drug Metab Pharmacokinet, 2006. **31**(3): p. 237-251.
27. Mikov, M., et al., *Pharmacokinetics and hypoglycaemic effect of 3 alpha, 7 alpha-dihydroxy-12-oxo-5beta-cholanate (MKC) in diabetic rat.* Febs Journal, 2006. **273**: p. 210-210.
28. Al-Salami, H., et al., *Probiotic treatment proceeded by a single dose of bile acid and gliclazide exert the most hypoglycemic effect in Type 1 diabetic rats.* Medical Hypothesis Research, 2008. **4**(2): p. 93-101.
29. Mikov, M., et al., *The influence of 3alpha,7alpha-dihydroxy-12-keto-5beta-cholanate on gliclazide pharmacokinetics and glucose levels in a rat model of diabetes.* Eur J Drug Metab Pharmacokinet, 2008. **33**(3): p. 137-42.
30. Al-Salami, H., et al., *Influence of the semisynthetic bile acid (MKC) on the ileal permeation of gliclazide in healthy and diabetic rats.* Pharmacol Rep, 2008. **60**(4): p. 532-41.
31. Al-Salami, H., et al., *Probiotics decreased the bioavailability of the bile acid analog, monoketocholic acid, when coadministered with gliclazide, in healthy but not diabetic rats.* Eur J Drug Metab Pharmacokinet, 2012. **37**(2): p. 99-108.
32. Al-Salami, H., et al., *Gliclazide reduces MKC intestinal transport in healthy but not diabetic rats.* Eur J Drug Metab Pharmacokinet, 2009. **34**(1): p. 43-50.
33. Al-Salami, H., et al., *Probiotic treatment reduces blood glucose levels and increases systemic absorption of gliclazide in diabetic rats.* Eur J Drug Metab Pharmacokinet, 2008. **33**(2): p. 101-6.
34. Al-Kassas, R.S., O.M. Al-Gohary, and M.M. Al-Faadhel, *Controlling of systemic absorption of gliclazide through incorporation into alginate beads.* Int J Pharm, 2007. **341**(1-2): p. 230-7.
35. Efentakis, M. and G. Buckton, *The effect of erosion and swelling on the dissolution of theophylline from low and high viscosity sodium alginate matrices.* Pharm Dev Technol, 2002. **7**(1): p. 69-77.
36. Silva, C.M., et al., *Insulin encapsulation in reinforced alginate microspheres prepared by internal gelation.* european journal of pharmaceutical sciences, 2006. **29**(2): p. 148-159.
37. Andersen, E., G. Karlaganis, and J. Sjovall, *Altered bile acid profiles in duodenal bile and urine in diabetic subjects.* Eur J Clin Invest, 1988. **18**(2): p. 166-172.
38. Cook, M.T., et al., *Microencapsulation of probiotics for gastrointestinal delivery.* J Control Release, 2012. **162**(1): p. 56-67.
39. Duboc, H., et al., *Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases.* Gut, 2013. **62**(4): p. 531-9.
40. Caesar, R., F. Fak, and F. Backhed, *Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism.* J Intern Med, 2010. **268**(4): p. 320-8.

41. Cani, P.D., et al., *Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability.* Gut, 2009. **58**(8): p. 1091-1103.
42. Huang, X. and C.S. Brazel, *On the importance and mechanisms of burst release in matrix-controlled drug delivery systems.* Journal of Controlled Release, 2001. **73**(2): p. 121-136.
43. Narasimhan, B. and R. Langer, *Zero-order release of micro-and macromolecules from polymeric devices: the role of the burst effect.* Journal of controlled release, 1997. **47**(1): p. 13-20.