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Research Article

Probucol Release from Novel Multicompartmental Microcapsules for the Oral Targeted Delivery in Type 2 Diabetes

Armin Mooranian,[1] Rebecca Negrulj,[1] Hesham S. Al-Sallami,[2] Zhongxiang Fang,[3] Momir Mikov,[4,5] Svetlana Golocorbin-Kon,[4,5] Marc Fakhoury,[6] Gerald F. Watts,[7] Vance Matthews,[8] Frank Arfuso,[9] Amanda Lambros,[10] and Hani Al-Salami[1,11,12]

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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-dependent 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 and 37°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 multiphasic 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.

KEY WORDS: anti-inflammatory; antioxidant; artificial-cell microencapsulation; diabetes mellitus; probucol; type 2 diabetes.

INTRODUCTION

Diabetes mellitus is a disease characterised 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 desensitisation 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 minimising variations between peaks and troughs of blood glucose levels in diabetic patients (3). Common antidiabetic drugs include the following: sulfonylureas, such as gliclazide that enhances insulin production, pancreatic β-cell functionality and

[1] Biotechnology and Drug Development Research Laboratory, School of Pharmacy, Curtin Health Innovation Research Institute, Biosciences Research Precinct, Curtin University, Perth, WA, Australia.
[2] School of Pharmacy, University of Otago, Dunedin, New Zealand.
[3] School of Public Health, Curtin University, Perth, WA, Australia.
[4] Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia.
[5] Department of Pharmacy, Faculty of Medicine, University of Montenegro, Podgorica, Montenegro.
[6] Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.
[7] School of Medicine and Pharmacology, Royal Perth Hospital, University of Western Australia, Perth, WA, Australia.
[8] Laboratory for Metabolic Dysfunction, UWA Centre for Medical Research, Harry Perkins Institute of Medical Research, Nedlands, WA, Australia.
[9] School of Biomedical Science, Curtin Health Innovation Research Institute, Biosciences Research Precinct, Curtin University, Perth, WA, Australia.
[10] Faculty of Health Sciences, School of Occupational Therapy and Social Work, Curtin University, Perth, WA, Australia.
[11] School of Pharmacy, Curtin University, Bently, WA, Australia.
[12] To whom correspondence should be addressed. (e-mail: hani.al-salami@curtin.edu.au), URL: http://healthsciences.curtin.edu.au/teaching/pharmacy_people.cfm/Hani.Al-Salami

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 neutralising 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 multicompartmental 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% Ultrasonic water-soluble gel in 100-mL 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 h, stored in the refrigerator and used within 48 h 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 1,000–1,500 Hz 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, three 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.

Characterisation of PB-Loaded Microcapsules

Drug Content, Production Yield, Microencapsulation Efficiency and Stability Studies

Drug Content, Production Yield and Microencapsulation Efficiency. One gram 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 h. Two milliliters of the solution was then transferred to a 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 s) 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 a UV spectrophotometer (Shimadzu 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.

$$\%\,\text{Drug Content} = \frac{\text{Calculated amount of PB in the microcapsules}}{\text{Total weight of microcapsules}} \times 100 \quad (1)$$

$$\%\,\text{Production Yield} = \frac{\text{Total weight of the microcapsules}}{\text{Total weight of the polymer} + \text{drug solution}} \times 100 \quad (2)$$

$$\%\,\text{Encapsulation Efficiency} = \frac{\text{Drug content}}{\text{Theoretical content}} \times 100 \quad (3)$$

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 deionised 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 utilising a Nikon YS2-H mounted with a ToupTek photonics FMA050 fixed calibrated microscope adaptor (Japan). Sample analysis was carried out in triplicate. Briefly, predetermined 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 characterisation 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 and 37°C) for 6 h. 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):

$$\text{Swelling Index} = \frac{\text{Final weight}}{\text{Initial weight}} \tag{4}$$

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 h, 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 min using a Hewlett-Packard-based time-controlled UV-spec mounted with a close-loop flow system under sink conditions (Fig. 1). All analyses were carried out in triplicate (n=3). Additionally, unloaded microcapsules (containing no drug) were analysed spectrophotometrically at λ_{Max}=242 nm 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.

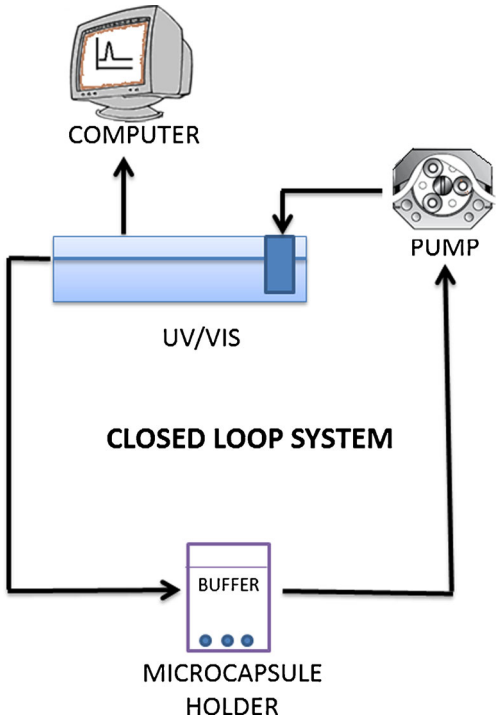

Fig. 1. Closed loop flow system for microcapsule-drug release

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, 5, 25 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 analysed 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, the first 20 mL was 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 a 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 0.01–4 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 *t* 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 I. The results of the drug content and encapsulation efficiency showed minimum variation amongst 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.

Microcapsule Size Analysis and Zeta Potential Determination

Analysis of the size of the microcapsules obtained from each formulation, as demonstrated in Table I, 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 a 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 −66 mV (SA) and −72.9 mV (PB-SA), the dispersion of microcapsules suggested a stable system (22), with the PB-SA formulation being more charged (Table I). 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 Fig. 2, the mean diameter of SA microcapsules (Fig. 2a) (average±SD) was 800±20 μm, whilst that of PB-SA microcapsules (Fig. 2b) was 850±50 μm. Results obtained also include the horizontal diameter (L1), the vertical diameter (L2) and the microcapsule width (L3).

Swelling Studies

Figures 3 and 4 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 (Fig. 5), 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 emphasise 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).

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 h 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

Table I. Drug Content, Production Yield, Encapsulation Efficiency, Zeta Potential and Mean Particle Size of SA and PB-SA Microencapsulation Formulations

Formulation code	Formula composition	Drug content (%)±SD	Production yield (%)±SD	Encapsulation efficiency (%)±SD	Zeta potential (mV)±SD	Mean particle size (μm)±SD
SA	Low-viscosity sodium alginate microcapsules	N/A	N/A	N/A	−66±1.6	1033±33
PB-SA	Probucol low-viscosity sodium alginate microcapsules	2.6±0.25	85±5	92±2	−72.9±1.6	1094±50

N/A not applicable

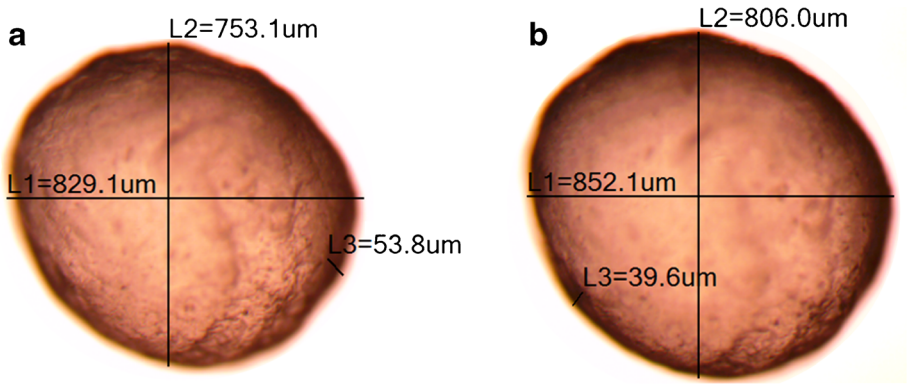

Fig. 2. SA microcapsule **a** and PB-SA microcapsule **b**. *L1* is the horizontal diameter, *L2* is the vertical diameter, and *L3* is the microcapsule width

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 Fig. 5, 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 (Fig. 5). 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 multiphasic 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 multiphasic drug release pattern depicted in PB-SA dissolution data (Fig. 5) 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.

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, 5, 25 and 40°C and at a relative humidity of 35%.

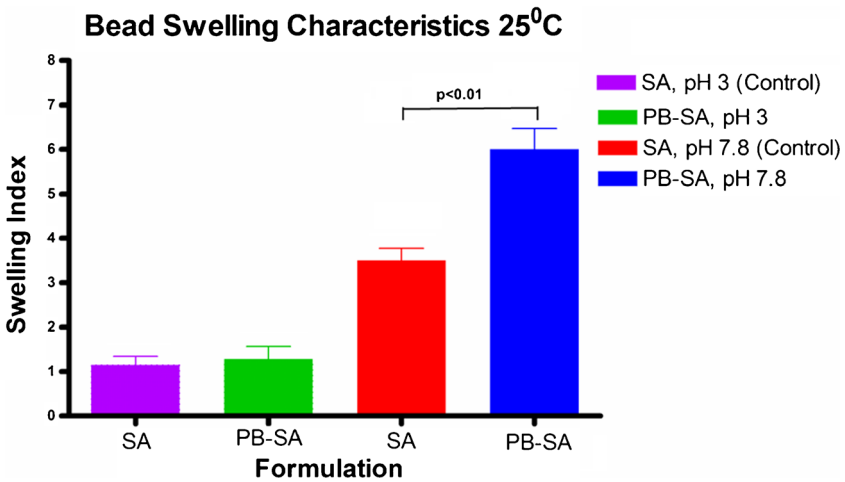

Fig. 3. Swelling characteristics of PB-SA and SA microcapsules (pH 3 and 7.8) at 25°C

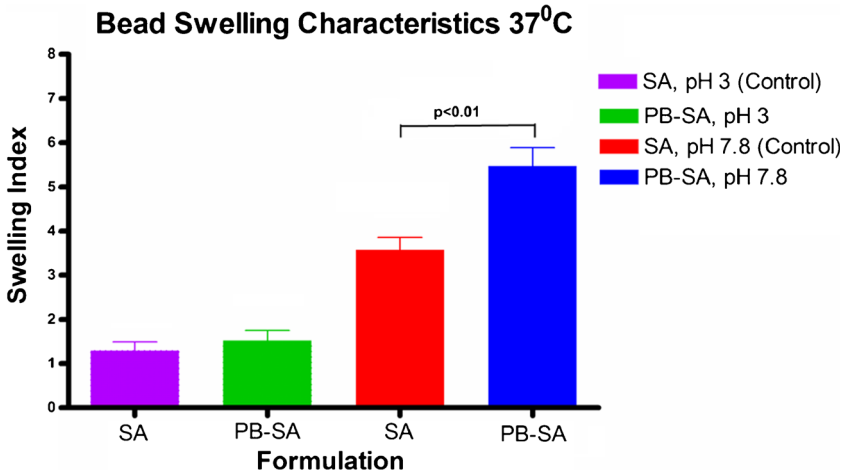

Fig. 4. Swelling characteristics of PB-SA and SA microcapsules (pH 3 and 7.8) at 37°C

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, whilst 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 white (5 and 25°C) to 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 3 days of accelerated stability testing revealed an average percent 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 a 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

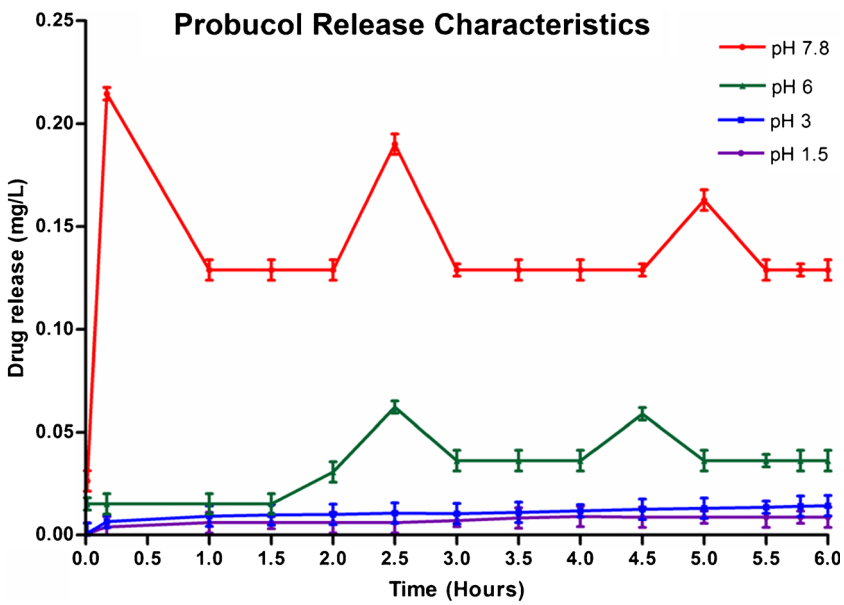

Fig. 5. Probucol release from PB-SA microcapsule over time across various pH values

stability and uniformity. However, the multiphasic 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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Conflict of Interest The authors declare no conflict of interest.

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