An optimized probucol microencapsulated formulation integrating a secondary bile acid (deoxycholic acid) as a permeation enhancer

Abstract: The authors have previously designed, developed, and characterized a novel microencapsulated formulation as a platform for the targeted delivery of therapeutics in an animal model of type 2 diabetes, using the drug probucol (PB). The aim of this study was to optimize PB microcapsules by incorporating the bile acid deoxycholic acid (DCA), which has good permeation-enhancing properties, and to examine its effect on microcapsules’ morphology, rheology, structural and surface characteristics, and excipients’ chemical and thermal compatibilities. Microencapsulation was carried out using a BÜCHI-based microencapsulating system established in the authors’ laboratory. Using the polymer sodium alginate (SA), two microencapsulated formulations were prepared: PB-SA (control) and PB-DCA-SA (test) at a constant ratio (1:30 and 1:3:30, respectively). Complete characterization of the microcapsules was carried out. The incorporation of DCA resulted in better structural and surface characteristics, uniform morphology, and stable chemical and thermal profiles, while size and rheological parameters remained similar to control. In addition, PB-DCA-SA microcapsules showed good excipients’ compatibilities, which were supported by data from differential scanning calorimetry, Fourier transform infrared spectroscopy, scanning electron microscopy, and energy dispersive X-ray studies, suggesting microcapsule stability. Hence, PB-DCA-SA microcapsules have good rheological and compatibility characteristics and may be suitable for the oral delivery of PB in type 2 diabetes.

Keywords: artificial cell microencapsulation, diabetes, bile acids, probucol, antioxidant, anti-inflammatory, BÜCHI B390

Introduction

Diabetes mellitus is a metabolic disorder classified as type 1 diabetes or type 2 diabetes (T2D). Type 1 diabetes 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 levels. T2D develops due to genetic and environmental factors that lead to tissue desensitization to insulin. Current therapies are aimed at resolving the persistent hyperglycemia by increasing available insulin or improving tissue sensitivity. However, damage of pancreatic β-cells and the build-up of free radicals and toxins, together with persistent inflammation, remain detrimental in diabetes treatment and long-term prognosis.

There is growing evidence for the use of antioxidants as adjuncts in the treatment of T2D. Probulcol (PB) is a drug that was initially developed to treat hyperlipidemia, but its strong anti-inflammatory and antioxidant properties have made it a potential therapeutic agent in the treatment of T2D. In addition, PB has shown a protective effect on pancreatic β-cells. It is highly lipophilic and accumulates extensively...
in adipose tissues. PB has very low bioavailability but its pharmacokinetic and pharmacodynamic parameters show great variation between individuals. Thus, the low and variable oral bioavailability and the non-linear distribution and clearance of PB contribute to the variability in its efficacy as well as its side effects. So, despite the huge potential of PB in T2D, its variable and poor kinetics remain major obstacles to its clinical use. Designing a novel and stable formulation with good rheological parameters and permeation-enhancing properties is anticipated to overcome these obstacles. This can be achieved by incorporating a bile acid with permeation-enhancing characteristics, such as deoxycholic acid (DCA), in a novel delivery system using artificial cell microencapsulation technology.

Artificial cell microencapsulation is commonly used to improve the delivery of lipophilic drugs that exhibit low bioavailability and poor dissolution and absorption kinetics. It encapsulates a drug using a biodegradable polymer such as sodium alginate (SA), which protects the drug and provides a pH-sensitive targeted delivery after oral administration. The choice of polymer has a significant impact on the formulation properties and efficacy as well as on the drug’s chemical and thermal characteristics. Previous work by the authors’ research group on the formulation of the antidiabetic drug gliclazide, alone or combined with bile acids (in vitro and in vivo), and their recently designed and formulated microcapsule platform have demonstrated improved targeted delivery when using an SA-based formulation. Thus, this study aimed to examine the potential of these newly developed microcapsules in producing a novel and stable PB formulation suitable for oral delivery in T2D through the incorporation of the bile acid DCA.

Materials and methods

Materials

PB (98%), low-viscosity SA (99%), and DCA (98%) were purchased from Sigma-Aldrich Co., (St Louis, MO, USA). Calcium chloride dihydrate (CaCl2·2H2O, 98%) was obtained from Scharlab S.L. (Sentmenat, Spain). All solvents and reagents were supplied by Merck KGaA (Darmstadt, Germany) and were of high-performance liquid chromatography grade and used without further purification.

Drug preparation

Stock suspensions of PB (20 mg/mL) and DCA (1 mg/mL) were prepared, as previously described, by adding the powder to 10% ultrapure water-soluble gel. The CaCl2 stock solution (2%) was prepared by adding CaCl2 powder to high-performance liquid chromatography 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

Microcapsules of PB-loaded low-viscosity SA were prepared using a BÜCHI-based microencapsulating system (BÜCHI Labortechnik AG, Flawil, Switzerland). Polymer solutions containing SA and PB with or without DCA were made up to a final concentration (of PB-DCA-SA) in a ratio of 1:3:30, respectively. This ratio was based on the authors’ published work and was found to exhibit maximum consistency and best morphology. Parameters used include: a frequency range of 1,000–1,500 Hz, air flow of 4 mL/minute, and a constant air pressure of 300 mbar. Microcapsules were collected from the microencapsulating system, and for each formulation, three independent batches were prepared and tested separately (n=3). All microcapsules (PB-SA-loaded and PB-DCA-SA-loaded) were prepared and treated in the exact same way. Microcapsules were dried by using stability chambers (ACS Environmental and Climatic Test Chamber; Angelantoni Test Technologies Srl, Massa Martana, Italy).

Characterization of loaded microcapsules: morphology, size analysis, and chemical characterization of microcapsules

All microcapsules were freshly made, stored in the refrigerator, and used within 48 hours of preparation. The appearance and size of microcapsules were examined using light microscopy followed by scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS). The particle size distribution and mean particle size diameter were calculated using the software provided.

Optical microscopy

Morphological characteristics and particle size analysis were determined with optical microscopy utilizing a YS2-H microscope (Nikon Corporation, Tokyo Japan) mounted with an FMA050 fixed calibrated microscope adaptor (ToupTek Photonics Co., Ltd., Hangzhou, People’s Republic of China). Sample analysis was carried out in triplicate as previously described. Briefly, predetermined quantities (ten microcapsules from each formulation) of freshly prepared microcapsules were loaded onto a glass slide mounted to a calibrated scale. Optical microscopy software (ToupTek; ProSciTech, Australia) capable of particle size analysis, microcapsule
characterization, and morphological assessments was utilized
to determine basic characteristics of the microcapsules to
complement the SEM studies.

**SEM and EDS**
The surface morphology of the microcapsules was examined
using SEM (Neon 40 EsB; Carl Zeiss Meditec AG, Jena,
Germany) with 0.8 nm calibrated resolution. The chemical
characterization of the microcapsules was examined using
EDS (INCA X-ray; Oxford Instruments plc, Abingdon,
UK). The samples were mounted on a glass stub with
double-sided adhesive tape and coated under vacuum with
platinum (5 nm) in an argon atmosphere prior to examina-
tion. Micrographs at different magnifications were recorded
to study the morphological and surface characteristics of the
microcapsules.

**Determination of dispersing media viscosity**
Viscosities of both preparations (PB-SA-loaded and PB-
DCA-SA-loaded microcapsules) were measured for freshly
prepared mixtures, using 15 mL aliquots (n=3) at room
temperature using a viscometer (Bohlin Visco 88; Malvern
Instruments, Malvern, UK).

**Differential scanning calorimetric (DSC) analysis**
DSC thermograms of PB, DCA, and low-viscosity SA pow-
ders, their physical mixture, and their microencapsulated
formulations were carried out in a DSC instrument (DSC
8000; PerkinElmer Inc., Waltham, MA, USA) as previously
described. Briefly, 5 mg samples were placed in sealed
aluminum pans and heated at 20°C/minute under a nitrogen
atmosphere (flow rate 30 mL/minute) in the 35°C–240°C
range. An empty aluminum pan was used as a reference.
The equipment was calibrated for baseline and temperature
with zinc metal.

**Fourier transform infrared (FTIR) spectral studies**
FTIR spectra of pure components, their physical mixture,
and the microcapsules were recorded using an attenuated
total reflectance FTIR spectrometer (Spectrum TWO™;
PerkinElmer), and infrared measurements were performed
in transmission in the scanning range of 450–4,000 cm⁻¹
at room temperature.

**Statistical analysis**
Values are expressed as mean ± standard deviation. Size
measurements were assessed using Student’s t-test. Statisti-
cal analysis was done using a two-way analysis of variance,
with Tukey’s honest significant difference post hoc compari-
son of means used only when the associated main effect or
interaction was statistically significant. The best fit model
was derived using Prism® version 6.0 software (GraphPad
Software, Inc., La Jolla, CA, USA). Statistical significance
was set at P<0.05.

**Results and discussion**
**Morphology, size analysis, and chemical
characterization of microcapsules**
Microcapsules were obtained using a low-viscosity SA
polymer, PB, and DCA at a constant ratio of 30:1:3, respec-
tively. Using the microencapsulation system, all microcap-
sules were of similar size. The mean diameters ranged from
2,000–2,300 μm for all batches of both formulations, which
suggest they may also be considered as macrocapsules. The
mean particle size was not significantly affected by the pre-
seance of DCA.

**Optical microscopy**
Microcapsules obtained from optical microscopy revealed
uniform consistency and spherical shaped microcapsules
with similar sizes, as determined via a calibrated scale
mounted onto a glass slide. Ten microcapsules were selected
for particle size and morphological analysis from each
formulation and repeated in triplicate. The mean diameter
of PB microcapsules (average ± standard deviation) was
2,150±150 μm, while that of PB-DCA-SA microcapsules was
2,350±90 μm. Figure 1A shows PB-SA microcapsules and
Figure 1B shows PB-DCA-SA microcapsules. The mean
microcapsule membrane thickness, L2 shows the horizontal
diameter, and L3 shows the vertical diameter. The calibrated
optical microscopy size analysis revealed very similar sizes
and morphological characteristics between both microen-
capsulated formulations, and thus the addition of DCA to
PB microcapsules did not adversely affect the size, shape,
or characteristics of the microcapsules.

**SEM**
The results from optical microscopy were further comple-
mented by SEM data of a PB-SA microcapsule (Figure 2) and
PB-DCA-SA microcapsule (Figure 3), which were randomly
selected from a few batches. SEM results showed consistently
uniform microcapsules with well-defined spherical shapes,
and reflect the optical microscopy findings. The microcap-
sule size difference between different formulations was not
significant, meaning the addition of DCA did not adversely
affect the size, morphology, uniformity, and sphericity of
Figure 1 Optical microscopy of PB-sa and PB-Dca-sa microcapsules.
Notes: (A) PB-sa microcapsules. (B) PB-Dca-sa microcapsules.
Abbreviations: DCA, deoxycholic acid; L1, outer membrane thickness; L2, horizontal diameter; L3, vertical diameter; PB, probucol; SA, sodium alginate.

Figure 2 Scanning electron micrographs of a PB-sa microcapsule.
Notes: (A) 200 μm scale. (B) Surface morphology at 10 μm scale. (C) 200 nm scale. (D) 1 μm scale.
Abbreviations: PB, probucol; SA, sodium alginate.
the microcapsules. The microcapsule surfaces were rough but consistent from one microcapsule to another. For PB-SA microcapsules, small crystals were distributed throughout the microcapsule surface (Figure 2C), either as large clumps or smaller groups. These crystals coating the microcapsule surface were believed to be PB, but this was not observed with the PB-DCA-SA microcapsules (Figure 3). They were confirmed to be PB by EDS studies (Figures 4 and 5).

**EDS**

The surface composition of both PB-SA and PB-DCA-SA microcapsules were analyzed through EDS. This form of analysis allows identification of surface elemental composition, as each element from the periodic table displays unique EDS patterns.\(^3\) Surface crystal depositions and microcapsule surface composition and characteristics were also evident from this analysis. Figures 4 and 5 show an example of both PB-SA and PB-DCA-SA microcapsule surfaces with the corresponding surface sites where EDS analysis was conducted. Figures 4A–B and 5A–B show PB deposition on the surface of the microcapsules, while Figures 4C–D and 5C–D show general surface characteristics of the microcapsules.

EDS examination of crystal depositions on the microcapsule surfaces (Figures 4 and 5) showed high levels of sulfur atoms, evident as small crystals on the surface of PB-SA (Figures 4A and B) and, to a lesser extent, of PB-DCA-SA (Figures 5A and B) microcapsules. The crystals were from PB deposition as no other excipient contains sulfur.\(^3\)–\(^5\) The SEM (Figures 2 and 3) and EDS (Figures 4 and 5) results suggest that PB preferentially coats the microcapsule surface, forming scattered clumps of drug agglomerates distributed around the microcapsule surface. Interestingly, and in line with the authors’ previous studies, DCA seems to reduce the extent of PB surface deposition and the overall porosity, thus providing membrane reinforcement and improving microcapsule integrity.\(^36\)

**Viscosity of the microencapsulated formulation**

Table 1 shows the viscosity, shear rate, shear stress, and torque for both microencapsulated formulations under various
**Figure 4** Energy dispersive X-ray spectra of PB-SA microcapsules.

Notes: (A and B) PB deposition (indicated by 1) with corresponding analysis. (C and D) Surface composition (indicated by 2) with corresponding analysis.

Abbreviations: PB, probucol; SA, sodium alginate.

**Figure 5** Energy dispersive X-ray spectra of PB-DCA-SA microcapsules.

Notes: (A and B) PB deposition (indicated by 1) with corresponding analysis. (C and D) Surface composition (indicated by 2) with corresponding analysis.

Abbreviations: DCA, deoxycholic acid; PB, probucol; SA, sodium alginate.
Table 1  Viscosities and related parameters of both microencapsulated formulations

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Set speed</th>
<th>rpm</th>
<th>Viscosity (mPa s)</th>
<th>Shear rate (s⁻¹)</th>
<th>Torque (mNm)</th>
<th>Shear stress (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-SA</td>
<td>1</td>
<td>20</td>
<td>UD</td>
<td>23.9±1</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>35</td>
<td>UD</td>
<td>42.2±2</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>61</td>
<td>UD</td>
<td>74.5±2</td>
<td>0.08±0.02</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>107</td>
<td>25±0.2</td>
<td>125.1±2</td>
<td>0.11±0.01</td>
<td>3.2±0.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>187</td>
<td>20±0.1</td>
<td>222.2±3</td>
<td>0.17±0.03</td>
<td>4.5±0.01</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>327</td>
<td>15±0.1</td>
<td>385.7±4</td>
<td>0.21±0.01</td>
<td>5.7±0.03</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>572</td>
<td>10±0.3</td>
<td>681.8±8</td>
<td>0.26±0.03</td>
<td>6.9±0.02</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1,000</td>
<td>9±0.1</td>
<td>1,194±10</td>
<td>0.40±0.01</td>
<td>10±0.01</td>
</tr>
<tr>
<td>PB-DCA-SA</td>
<td>1</td>
<td>20</td>
<td>UD</td>
<td>28±1</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>35</td>
<td>UD</td>
<td>42.1±2</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>61</td>
<td>UD</td>
<td>74.4±3</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>107</td>
<td>UD</td>
<td>124.9±5</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>187</td>
<td>UD</td>
<td>221.9±7</td>
<td>0.11±0.01</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>327</td>
<td>12±0.2</td>
<td>385.0±5</td>
<td>0.16±0.03</td>
<td>4.5±0.03</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>572</td>
<td>11±0.1</td>
<td>681.0±10</td>
<td>0.27±0.02</td>
<td>7.2±0.01</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1,000</td>
<td>10±0.2</td>
<td>1,195±12</td>
<td>0.43±0.01</td>
<td>11.7±0.02</td>
</tr>
</tbody>
</table>

Note:  n=3. Data are described as mean ± SD.

Abbreviations: DCA, deoxycholic acid; PB, probucol; SA, sodium alginate; SD, standard deviation; UD, undetected (below the instrument limit of detection).

speeds (20, 35, 61, 107, 187, 327, 572, and 1,000 rpm). The PB-SA formulation was more viscous, and both formulations behaved as non-Newtonian fluids under shear stress. Both formulations behaved as thixotropic fluids under increasing stress, as evidenced by parallel reductions in their apparent viscosity. Increasing speeds resulted in the solutions forming rapid circular motions away from the origin of the centrifugal force, suggesting that both formulations also behaved in a non-Weissenberg fashion.

DSC

DSC is commonly used for the thermal characterization of various materials. In PB microencapsulation, DSC measures how physical properties of PB molecules change, along with temperature, against time. This occurs through determining the temperature and heat flow (35°C–240°C) associated with PB transitions as a function of time. DSC spectra were analyzed for PB powder (Figure 6A), DCA powder (Figure 6B), SA powder (Figure 6C), PB-DCA-SA physical powder mixture (Figure 6D), PB-SA microcapsules (Figure 6E), and PB-DCA microcapsules (Figure 6F).

PB powder analysis (Figure 6A) showed a large peak at 128°C, which corresponds to the melting point of PB. DCA powder analysis (Figure 6B) showed a small peak at 178°C, which corresponds to the melting point of DCA. SA powder (Figure 6C) showed a large peak at 200°C, which corresponds to the melting point of SA. PB-DCA-SA physical powder mixture (Figure 6D) showed only two distinct peaks, one peak at 130°C, which corresponds to PB, and one at 200°C, which corresponds to SA. The slight shift to the right in both peaks suggests an increase in the melting point of the combined mixture, which reflects findings from the authors’ previous work. PB-DCA-SA powder analysis only showed two peaks, PB and SA, while the peak that corresponds to DCA was missing, possibly due to a shift in the thermal capacity within the 35°C–240°C range or due to an overlap between DCA and SA peaks. The PB-SA microcapsule (Figure 6E) showed two peaks – a small peak at 128°C, which corresponds to the melting point of PB, and a large peak at 202°C, which corresponds to the melting point of SA – with a slight shift to the right possibly due to ionic interaction between PB and SA post-microencapsulation. The PB-DCA-SA microcapsule (Figure 6F) showed two peaks, similar to the PB-DCA-SA powder mixture (Figure 6D). Overall, DSC analysis showed a transparent and interference-free integration of two prominent peaks – one corresponding to PB and the other to SA, with the possibility of overlap between DCA and SA. There was a noticeable slight shift pre- and post-microencapsulation, which could represent potential chemical interactions between SA and DCA in the microcapsule matrix, alterations in the crystallinity, polymorphism, and plasticization of SA, or a possible combination of factors leading to an endothermic shift. Most importantly, PB did not seem to significantly participate in a crosslinking reaction, and retained its chemical integrity during the microencapsulation process, which is also supported by the FTIR results (Figure 7).
FTIR spectral studies
The FTIR method is widely used to stimulate vibrational levels of known chemical groups in a molecule, and was used to confirm the chemical compatibility of PB with the SA polymer and DCA in the microencapsulation formulation. In line with the DSC analysis (Figure 5), FTIR spectra were analyzed for PB powder (Figure 7A), DCA powder (Figure 7B), SA powder (Figure 7C), PB-DCA-SA powder (Figure 7D), PB-SA microcapsules (Figure 7E), and PB-DCA-SA microcapsules (Figure 7F). FTIR spectral analysis of individual powders, mixed powders containing all the ingredients, and final microcapsules attained was necessary to ensure the chemical compatibility of PB pre- and post-microencapsulation.

FTIR PB powder analysis (Figure 7A) showed characteristic peaks at 2,959, 1,422, and 1,310 cm⁻¹. DCA and SA individual powder analysis (Figures 7B and C) showed similar characteristics to the authors’ published work. The PB-DCA-SA physical powder mixture (Figure 7D) showed peaks that correspond to PB, DCA, and SA with no interference, dilution, or alterations. This confirms compatibility of all the ingredients in the powder form pre-microencapsulation.

PB-SA microcapsule analysis (Figure 7E) showed five distinct and interference-free peaks, which correspond to PB and SA analysis, at 3,350, 1,426, 1,309, 1,601, and 1,025 cm⁻¹. Similar peaks were noticed after DCA addition, which is in line with the DSC findings (Figure 6), suggesting an interaction between DCA and SA. PB-DCA-SA microcapsule analysis (Figure 7F) showed three PB peaks (at 3,350, 1,426, and 1,309 cm⁻¹) and two SA peaks (at 1,601 and 1,025 cm⁻¹), which is in line with the DSC analysis (Figure 6F).

Overall, FTIR analysis showed compatibility of the formulation pre- and post-microencapsulation. The absence of DCA peaks is in line with the DSC analysis (Figure 6) and the authors’ previously published work, and suggests an

Figure 6 Differential scanning calorimetric thermograms of the individual powders, mixed powder containing all the ingredients, and microcapsules.
Notes: (A) PB powder. (B) DCA powder. (C) SA powder. (D) PB-DCA-SA powder. (E) PB-SA microcapsules. (F) PB-DCA-SA microcapsules.
Abbreviations: DCA, deoxycholic acid; PB, probucol; SA, sodium alginate.
interaction between DCA and the polymer SA. However, this interaction did not interfere with PB peaks as shown by the chemical (FTIR) and thermal (DSC) analyses. This suggests PB stability pre- and post-microencapsulation, as its chemical composition and structural integrity were maintained when microencapsulated with DCA.

This is a comprehensive study with in-depth in vitro analysis of novel bile acid–PB microcapsules with good potential in the treatment of T2D. A major limitation, and thus future work, is in vivo analysis carried out by administering these microcapsules to an animal model of T2D and elucidating efficacy and safety profiles.

**Conclusion**

PB-DCA-SA microcapsules showed good morphology and stability and may be suitable for optimized oral delivery of PB in T2D. A future study may investigate the release kinetics and pH-targeted delivery of the microcapsules at various pH and temperature values.

**Acknowledgments**

The authors acknowledge the Curtin Health Innovation Research Institute at Curtin University, and the Curtin-seeding grant for the support, and also acknowledge the use of equipment and scientific and technical assistance of the Curtin University Electron Microscope Facility, which has been partially funded by the University, State, and Commonwealth Governments.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


