DOI: 10.1208/s12249-018-1250-6

Spray-Dried Succinylated Soy Protein Microparticles for Oral Ibuprofen Delivery

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Abstract. The potential value of succinylated soy protein (SPS) as a wall material for the encapsulation of ibuprofen (IBU), a model hydrophobic drug, by spray-drying was investigated. A succinylation rate of 93% was obtained for soy protein isolate, with a molar ratio of 1/1.5 (NH_2/succinic anhydride). The solubility profile at 37°C showed that this chemical modification decreased the solubility of the protein below its isoelectric point, whereas solubility increased in alkaline conditions. Various SPS/IBU ratios (90/10, 80/20, and 60/40) were studied and compared with the same ratio of soy protein isolate (SPI/IBU). High encapsulation efficiency was achieved (91–95%). Microparticles were spherical and between 4 and 8 μm in diameter. The spray-drying of protein/IBU solutions appeared to be beneficial, as it resulted in an amorphous solid dispersion of IBU within the microparticles, coupled with an increase in the thermal stability of IBU. *In vitro* release was evaluated in acidic (pH 1.2 in the presence of pepsin) and neutral (pH 6.8) conditions similar to those in the gastrointestinal (GI) tract. IBU was released significantly more slowly at pH 1.2, for both proteins. However, this slowing was particularly marked for SPS, for which rapid (within 2 h) and complete release was observed at pH 6.8. These results validate the hypothesis that SPS is suitable for use as a coating material for hydrophobic active pharmaceutical ingredients (APIs) due to its pH sensitivity, which should delay IBU release in the gastrointestinal tract.

KEY WORDS: plant protein; succinylation; green excipient; microencapsulation; oral route; modified release; pH sensitivity.

INTRODUCTION

In recent decades, the pharmaceutical industry has developed several strategies for the controlled delivery of active pharmaceutical ingredients (APIs), for oral administration in particular, as this is the favoured route and most widely used in patients. Microencapsulation, in particular, has been widely studied, as this method can be used to generate different dosage forms, such as powders, tablets, and capsules (1,2).

There is currently very strong demand for new drug delivery systems (DDSs) suitable for the chosen administration route and able to deliver APIs to particular sites, to limit adverse reactions and side effects, and to improve patient comfort (1). Microencapsulation is a technological strategy that seems to perform well, particularly not only for APIs with a short half-life that are rapidly hydrolysed or broken down by enzymes *in vivo* but also for APIs with low solubility or poor permeation characteristics (3,4).

Spray-drying is a well-established method that is already widely used in the pharmaceutical industry to convert liquid formulations into dry powders, in cheap, fast, easy, and scalable one-step processes (5). This technology can also be used to encapsulate sensitive APIs, to generate relatively uniform and stable particles with high encapsulation efficiencies and without the need for an organic solvent (6).

The choice of encapsulation carrier is one of the key parameters in the design of delivery systems for APIs based on spray-drying. Various natural and synthetic polymers are currently being explored, but the persistent demand for environmentally friendly excipients has led to intensive research into natural polymers, such as polysaccharides and proteins. Plant proteins are cheap and readily available. They also seem to be highly suitable for medical applications, thanks to their bio- and cytocompatibility and their biodegradability (7,8). Moreover, their surfactant and film-forming properties make them attractive for microencapsulation applications. Finally, the presence of amino and carboxyl

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groups on protein chains renders them pH responsive, a feature that is particularly useful for APIs sensitive to gastric conditions, for which bioavailability may be decreased by passage through the gut (9), or for those known to have gastric side effects (10,11).

Soy, wheat, and maize proteins are the most abundant plant proteins and have been shown to be potentially useful for medical applications (8). Microspheres of the maize protein zein have even been formulated as carriers for anticancer drugs (12).

However, there have been few studies investigating the potential of plant protein microparticles for oral API delivery systems and even fewer evaluating the utility of chemical modifications to improve their pH sensitivity. Conversely, the potential of chemical modifications, such as acylation or succinylation, to expand the range of functions of plant proteins has driven research activities, particularly for research into microencapsulation applications (9,13–16).

Succinylation is the addition of succinic acid to protein amino groups, resulting in the replacement of mostly positively charged lysine ε-amino groups with carboxyl groups, which are negatively charged (for a pH range of about 4 to 10). This modification leads to chain unfolding and a lower isoelectric point (pI), accompanied by a decrease in solubility below the pI and an increase in solubility above it (17). This chemical modification is used to enhance certain functional properties of proteins, such as solubility, emulsification, and foaming capacity (18). Succinylation is, thus, a possible method for modifying the pH sensitivity of soy protein and controlling the release of active ingredients in gastrointestinal conditions. Furthermore, succinylation is a "soft" modification, and the use of succinic anhydride is approved by the Codex Alimentarius (19) and this molecule is considered to be GRAS (generally recognised as safe) by the FDA (Food and Drug Administration) (20).

The potentialities of succinylated soy proteins under microparticulate form for oral applications have never been explored. Succinylation of proteins has demonstrated its interest in food research to increase food protein emulsifying and foaming properties (21). Succinylated proteins, namely soy protein (9) and β-lactoglobulin (22,23), have also been proposed as excipients to design enteric tablets by direct compression of mixed proteins and active pharmaceutical ingredient (API) powders. More recently, their use for enteric coating of capsules has been studied (24). However, the influence of succinylation on (soy) protein encapsulation properties has never been explored and is the subject of this study. Encapsulation could permit innovative formulations, obtained with alternative industrialisable process (spray-drying), which may be of great interest for pharmaceutical applications. In a previous study, we have evaluated the influence of soy proteins acylation on their encapsulation and release properties for oral purposes (16). We here used the same methodology to evaluate succinylation influence, with the goal to compare both chemical modifications.

The aim of this study was to investigate the microencapsulating properties of succinylated soy protein (SPS), comparing them with those of soy protein isolate (SPI), and to determine its suitability for use as a multiparticulate drug delivery system.

Ibuprofen (IBU) was chosen as the model drug for this study. Its poor solubility in water and the gastric adverse effects reported following its oral administration (25) make this drug a clear candidate for encapsulation and delayed release.

In the first part of the study, SPI was succinylated and the impact of this modification on soy protein solubility was analysed. Modified and native proteins were then used for IBU microencapsulation by spray-drying, with various protein/IBU ratios. The effects of protein succinylation on process yield, IBU encapsulation efficiency, microparticle morphology, and size distribution were examined. IBU encapsulation state was assessed by DRX and thermogravimetric analysis (TGA). Finally, *in vitro* IBU release kinetics were studied in simulated gastrointestinal conditions (pH 1.2 with pepsin and 6.8, 37°C).

MATERIALS AND METHODS

Materials

SPI (90% pure) was purchased from Solae Belgium NV (Ieper, Belgium). Ibuprofen (100% pure) was purchased from BASF Corporation (USA). All other chemicals for protein modification were of analytical grade. NaOH, 37% HCl, succinic anhydride, acetonitrile (HPLC grade), and pepsin from porcine gastric mucosa tested according to European Pharmacopoeia 9.5 (Ph. Eur.) were purchased from Sigma (Saint-Quentin Fallavier, France).

SPI Characterisation

SPI proximate composition analysis included determinations of moisture, ash, protein, and lipid contents. Moisture and ash contents were determined according to the standard methods of the AOAC (26). Ash content was determined gravimetrically, by incineration at 550°C, and moisture content was determined by drying samples to a constant weight at 105°C. Protein content was determined by the Kjeldahl method ($N \times 6.25$) (27) and lipid content was determined by conventional Soxhlet extraction with cyclohexane for 6 h. Total carbohydrate content was obtained by subtracting the sum of moisture, ash, protein, and lipid contents from the total mass (27). Proximate analysis was carried out three times for each sample.

The amino acid profile of SPI was determined with a Biochrom 30-amino acid analyser (Serlabo Technologies, Entraigues sur la Sorgue, France).

Succinylation of Soy Proteins

The succinylation reaction (Fig. 1) involves the replacement of positively charged lysine ε-amino groups with negatively charged carboxyl groups through the addition of succinic anhydride.

Succinylated soy protein isolate (SPS) was prepared as described by Shilpashree *et al.* for the succinylation of milk protein (20). A 200-g 5% *w*/*w* protein solution in deionised water was prepared in a three-necked flask equipped with a mechanical stirrer and a pH meter. The pH was adjusted to 8–8.5 with 2 M NaOH. Succinic anhydride was added in three

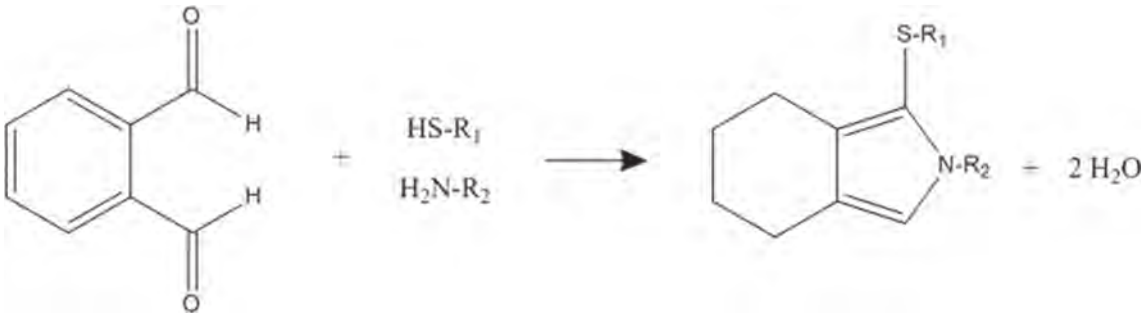

Fig. 1. Protein succinylation reactions

stages, over a 30-min period, with mechanical stirring (500 rpm) at room temperature. The molar ratio of NH_2-proteins/succinic anhydride was 1/1.5. The reaction mixture was then heated at 37°C for 1 h, and pH was maintained at 8–8.5 by adding 2 M NaOH solution. The solution was then cooled at room temperature and the pH was lowered to 3–4 by adding 2 M HCl, leading to protein precipitation. The reaction mixture was centrifuged at 5000×*g* at 20°C for 20 min in a 6-16K Sigma Centrifuge (Osteode, Germany). The supernatant was removed and the precipitate was dissolved in 100 mL of water. The pH was adjusted to 7 (with 2 M NaOH solution). The reaction product was lyophilised (Lyophiliser Alpha 2-4, Christ Martin) and stored at 4°C.

The succinylation rate (SR) was calculated by determining the number of amine functional groups by the OPA method (28).

$$\mathrm{SR}(\%) = [(N_0 - N_s)/N_0] \times 100$$

where N_0 is the number of mmol of amine groups in the native protein and N_s is the number of mmol of amine groups in the protein after succinylation.

A succinylation rate of 93% was obtained.

Protein Solubility Profile

Protein solubility profile was determined according as described by Zheng *et al.* (29). Protein samples (SPI and SPS) were mixed with deionised water (3% *w*/*w*) and the pH of the mixture was adjusted to 1.0–10.0 with 4 M NaOH or 4 M HCl. Each sample was stirred for 1 h at 37°C and centrifuged at 10000×*g* for 15 min (Sigma Laborzentrifugen, Osterode, Germany). The soluble protein content in the supernatant was determined by the Kjeldahl method (in triplicate). Protein solubility (S) was calculated with the following formula:

$$S(\%) = 100 \times \text{protein weight in the supernatant/total protein weight in solution}$$

Microencapsulation Process

Preparation of Protein/Ibuprofen Solutions

Protein/IBU solutions were prepared with different ratios of SPI/IBU or SPS/IBU: 90/10, 80/20, and 60/40, as previously described (16). Protein (succinylated or not) was dissolved in deionised water (8% *w*/*w*) at room temperature, with magnetic stirring. The pH was then adjusted to 8 and the IBU was gradually added, with magnetic stirring, over a period of 30 min. The solutions were mechanically stirred at 500 rpm for 30 min and homogenised at high pressure (50 MPa) with double circulation (APV Systems, Albertslund, Denmark).

Viscosity of Protein/Ibuprofen Solutions Prior to Spray-Drying

The analysis of apparent viscosity of all solutions was determined at 25°C with shear stress of 0 and 1 N/m^2 for 2 min, using a Rheometer Anton Paar MCR-302 (United Kingdom) with cone-plate geometry of 6 mm diameter and 0.035 rad angle (30).

Spray-Drying

The solutions (200 mL) were spray-dried in a Mini Spray dryer B-290 (Büchi, Flawil, Switzerland) under the following process conditions: inlet air temperature at 120 ± 4°C and outlet temperature at 74 ± 4°C, drying air flow rate of 470 L/h, liquid feed flow rate of 0.33 L/h, and 100% aspiration. Microparticles were collected and stored at 4°C.

Spray-drying yield (%) was calculated as follows:

$$\text{Spray-drying yield}(\%) = M_p/M_{SP+IBU} \times 100$$

where M_p is the mass of the powder collected and M_{SP+IBU} is the initial mass of solid content in the solution, including soy protein (succinylated or not) and IBU.

HPLC Analysis

IBU concentration was determined in an HPLC system equipped with a reverse-phase column (Phenomenex Gemini® 5 µm C18 110 Å, 250 × 4.6 mm) and a precolumn filled with the same phase. This system consisted of a Dionex P680 quaternary pump, a Dionex ASI-100 autosampler, an UltiMate 3000 thermostat-controlled column compartment, and a Thermo Scientific Ultimate 3000 UV-DAD detector. A wavelength of 222 mm was used for UV detection. The mobile phase was a 60:40 (*w*/*w*) mixture of 0.1 M sodium acetate (pH 7) and acetonitrile.

Microparticles (10 mg) were dissolved in 50 mL of the mobile phase and sonicated for 10 min and were then stirred magnetically for 20 min. All samples were filtered through

CA filters with 0.45 μm pores and then analysed, in triplicate, by HPLC.

The amount of IBU in microparticles and for the *in vitro* study was determined from ibuprofen calibration curves, as previously described (16).

Microencapsulation Efficiency and Microencapsulation Rate

Microencapsulation efficiency (MEE) and microencapsulation rate (MER) were calculated as follows:

$$MEE = IBU_{exp}/IBU_{theo} \times 100$$

where IBU_{exp} is the amount of ibuprofen in microparticles, as determined by HPLC, and IBU_{theo} is the amount of ibuprofen theoretically introduced in the solutions.

$$MER = m_{IBU}/m_m \times 100$$

where m_{IBU} is the estimated mass of ibuprofen in microparticles, and m_m is the mass of the microparticle sample analysed.

Microparticle Characterisation

Moisture Content

The moisture content of the microparticles was determined gravimetrically, by oven-drying at 105°C to constant weight.

Microparticle Size Distribution

The size distribution of the microparticles was determined by laser diffractometry with a Sirocco 2000 machine (Malvern Instruments, Worcestershire, UK). A refractive index of 1.52 was used, with an air dispersion pressure of 4 bars. The volume-based particle diameters ($D_{4,3}$) and surface-based particle diameters ($D_{3,2}$) were calculated as the mean of three measurements per sample.

Morphology

Microparticles were observed by scanning electron microscopy (SEM) with a LEO435VP scanning electron microscope (LEO Electron Microscopy Ltd., Cambridge, UK) operating at 10 kV. They were deposited on a conductive double-faced adhesive tape and sputter-coated with silver.

X-Ray Diffraction

The powder crystallinity of ibuprofen, SPI, SPS, microparticles, and physical mixtures was assessed by X-ray powder diffraction (EQUINOX 1000, France) at room temperature with a Co target at 30 mA and 30 kV in the $5° \leq 2\theta \leq 40°$ region, with an angular increment of $0.02° \ s^{-1}$ (31).

Thermogravimetric Analysis

TGA was carried out with an ATG/ETD Q600 machine from TA Instruments (New Castle, USA), at a linear heating rate of 10°C/min under air flow, from 20 to 600°C. The thermal stabilities of ibuprofen, ibuprofen/protein physical mixtures, and microparticles are presented on TG curves.

In Vitro Release Kinetics

Drug release experiments were performed under sink conditions in flow-through cells (Sotax EC6), as recommended by the European Pharmacopoeia 9.5 (Ph. Eur.), with SPI/IBU and SPS/IBU (90/10) microparticles. Simulated gastrointestinal fluid (SGIF) was used in a closed system (300 or 100 mL of dissolution medium at pH 1.2 and pH 6.8, respectively). The dissolution medium was as recommended by Ph. Eur. The release medium was simulated gastric fluid (SGF) at pH 1.2 supplemented with 0.32% pepsin (*w*/*v*) and simulated intestinal fluid (SIF) at pH 6.8. The cells were maintained at 37°C, under a constant flow rate of 8 mL/min. Drug release was followed by taking 1 mL samples ($n = 3$) at predefined time intervals for 2 h. The amount of drug released at each time point was determined by the previously described HPLC-UV method.

Statistical Analysis

The experimental data were subjected to statistical analysis with the MINITAB Release 17 statistical package (Minitab Inc., USA). A one-way analysis of variance (ANOVA) with a confidence level of 0.95 and Tukey-Kramer tests for multiple comparisons were performed.

RESULTS AND DISCUSSION

SPI Composition

As previously reported (16), the SPI used not only consisted essentially of protein (87.6%) but also contained 0.4% lipids, 5.3% ash, 1.4% carbohydrates, and 5.4% water.

An analysis of SPI amino acid composition revealed a predominance of two amino acids: glutamic acid (17.8%) and aspartic acid (11.6%), accounting for the low isoelectric point of the protein (pI = 5). Lysine levels were also relatively high (6.3%), and the free amino groups of this amino acid could be used for chemical modifications, such as succinylation.

Zhao *et al.* (2018) reported very similar results for a SPI containing 18.37–17.59% glutamic acid, 10.45–11.47% aspartic acid, and 5.16–5.62% lysine (32).

Impact of Succinylation on Soy Protein Solubility

The effect of succinylation on soy protein solubility is shown in Fig. 2. SPI has a typical bell-shape pH-solubility profile, with minimum solubility at the isoelectric point. Succinylation led to a significant decrease in solubility at pH values below the pI. This effect can be explained by the removal of ammonium groups from lysine residues by succinylation, leading to the presence of too few hydrophilic cationic groups to counterbalance hydrophobic protein-

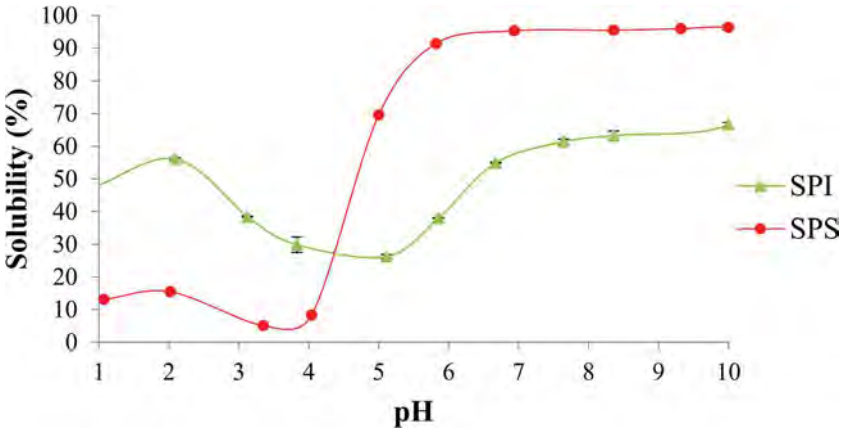

Fig. 2. Solubility profile of SPI and SPS as a function of the pH of the medium

protein interactions and, thus, protein-protein aggregation (17). Conversely, at pH values above 6 (*i.e.* above the pKa), the carboxyl groups of both glutamic and aspartic acids, and those induced by succinylation, were in their hydrophilic sodium carboxylate forms, enhancing interactions between the protein and water and, thus, protein chain unfolding and solubility (20). As it can be seen on Table I, for similar protein/IBU ratio, the SPS solutions demonstrated a significant higher viscosity than those with SPI ($p < 0.05$), traducing higher solubility and protein chain unfolding.

These solubility profiles highlight the relevance of the chosen chemical modification, as succinylation greatly decreased protein solubility in acidic conditions and maximised solubility (almost 100%) at pH values above 6.

Protein/IBU solution spray-drying experiments were performed at pH 8, to maximise the solubility of IBU and proteins and ensure effective encapsulation.

Impact of Succinylation on Microencapsulation

Process yield, microencapsulation efficiency (MEE) and rate (MER), and microparticle size (Table II) and morphology (Fig. 3) were determined for spray-dried microparticles of unloaded SPI and SPS and for SPI/IBU and SPS/IBU microparticles loaded with various amounts of IBU (from 90/10 to 60/40 ratios, % *w*/*w*).

Microparticles loaded with protein and IBU were successfully prepared by the spray-drying technique. Spray-drying yield ranged from 72 to 87% and was higher than published values for the use of plant proteins for spray-drying encapsulation (13,15,33,34) and IBU encapsulation (5,35).

MEE values were highly satisfactory, exceeding 80% for all experiments. However, several significant differences were observed. For a given protein/IBU ratio, MEE was higher for SPS than for SPI, particularly for high IBU contents (91.4% and 81.6%, respectively), confirming the relevance of the chosen chemical modification for optimising IBU encapsulation. This higher encapsulation efficiency can be attributed to the higher solubility of SPS (at pH 8), enhancing protein chain unfolding, thereby improving the encapsulation of the IBU molecules.

Conversely, MEE decreased for at high levels of IBU loading, for both proteins, but this decrease was significantly smaller for SPS microparticles. With SPS, it was possible to load the microparticles with large amounts of IBU (MER greater than 36%) with a very high MEE, greater than 91%.

IBU-loaded microparticle moisture content was slightly lower than reported in many previous studies (30) due to the hydrophobicity of IBU. It ranged from 2.3 to 3.8%, ensuring microbiological stability (36).

SEM micrographs (Fig. 3) showed that spray-dried microparticles had similar morphologies, regardless of the protein used and the protein/IBU ratio. They were round, with no fissures or disruption, ensuring that the API was well-embedded (37). A wrinkled surface may occur according to primary drying kinetics of spray-drying process and feeding solution composition and solid/liquid ratio (38). Microparticles volume diameter, as measured by laser granulometry, ranged from 3.5 to 7.6 μm (Table II), consistent with SEM observations. The aggregates of SPI microparticles observed on SEM may account for the large diameters obtained for these microparticles (19.1 μm). In

Table I. Viscosity of Protein/Ibuprofen Solutions Prior to Spray-Drying

Soy protein/ibuprofen (% *w*/*w*)	SPI	SPI 90/10	SPI 80/20	SPI 60/40	SPS	SPS 90/10	SPS 80/20	SPS 60/40
Viscosity mPa s	3.2 ± 0.1^{g}	3.7 ± 0.0^{f}	4.2 ± 0.0^{e}	7.2 ± 0.1^{c}	7.4 ± 0.1^{bc}	7.5 ± 0.2^{b}	6.6 ± 0.0^{d}	11.4 ± 0.1^{a}

Superscripts with the same letters (a–g) in the same line were not significantly different $p > 0.05$ (Tukey-Kramer multiple mean comparisons)
SPI soy protein isolate, *SPS* succinylated soy protein

Table II. Properties of Loaded Spray-Dried Microparticles

Soy protein/ibuprofen (% *w/w*)	Spray-drying yield (%)	MEE (%)*	MER (%)*	Microparticle size		Moisture content of microparticles (%)
				($D_{4,3}$) μm	($D_{3,2}$) μm	
SPI	80	–	–	5.0 ± 0.1^{c}	2.15 ± 0.1	5.1 ± 0.1^{a}
SPS	77	–	–	5.3 ± 0.1^{c}	2.01 ± 0.1	3.5 ± 0.4^{bc}
SPI 90/10	82	92.6 ± 9.3^{ab}	9.3 ± 0.9^{e}	3.5 ± 0.2^{c}	1.68 ± 0.1	3.7 ± 0.1^{b}
SPI 80/20	87	84.1 ± 3.6^{bc}	16.8 ± 0.7^{d}	4.4 ± 0.3^{c}	1.71 ± 0.0	2.3 ± 0.1^{d}
SPI 60/40	80	81.6 ± 2.8^{c}	32.6 ± 1.1^{b}	19.1 ± 2.0^{a}	2.89 ± 0.1	3.2 ± 0.1^{bc}
SPS 90/10	78	95.4 ± 0.6^{a}	9.5 ± 0.1^{e}	4.7 ± 0.1^{c}	1.88 ± 0.0	3.8 ± 0.1^{b}
SPS 80/20	76	92.4 ± 1.0^{ab}	18.5 ± 0.2^{c}	5.0 ± 0.1^{c}	1.95 ± 0.0	3.2 ± 0.1^{bc}
SPS 60/40	72	91.4 ± 0.7^{abc}	36.6 ± 0.3^{a}	7.6 ± 0.1^{b}	2.79 ± 0.1	2.8 ± 0.2^{cd}

Superscripts with the same letters (a–c) in the same column were not significantly different $p > 0.05$ (Tukey-Kramer multiple mean comparisons)
MEE microencapsulation efficiency, *MER* microencapsulation rate, $D_{4,3}$ volume-based particle diameters, $D_{3,2}$ surface-based particle diameters, *SPI* soy protein isolate, *SPS* succinylated soy protein
*Determined by HPLC

addition, the microparticles had a trimodal size distribution (results not shown), with a majority population at about 5 μm. Microparticle size was unaffected by formulation. The initial solutions had a low viscosity, and the particle sizes obtained were those expected for a B-290 spray-dryer (13).

Crystalline State of Encapsulated IBU

The crystalline state of encapsulated IBU was assessed by X-ray powder diffraction (XRD). The XRD patterns of IBU were compared with those for physical mixtures and

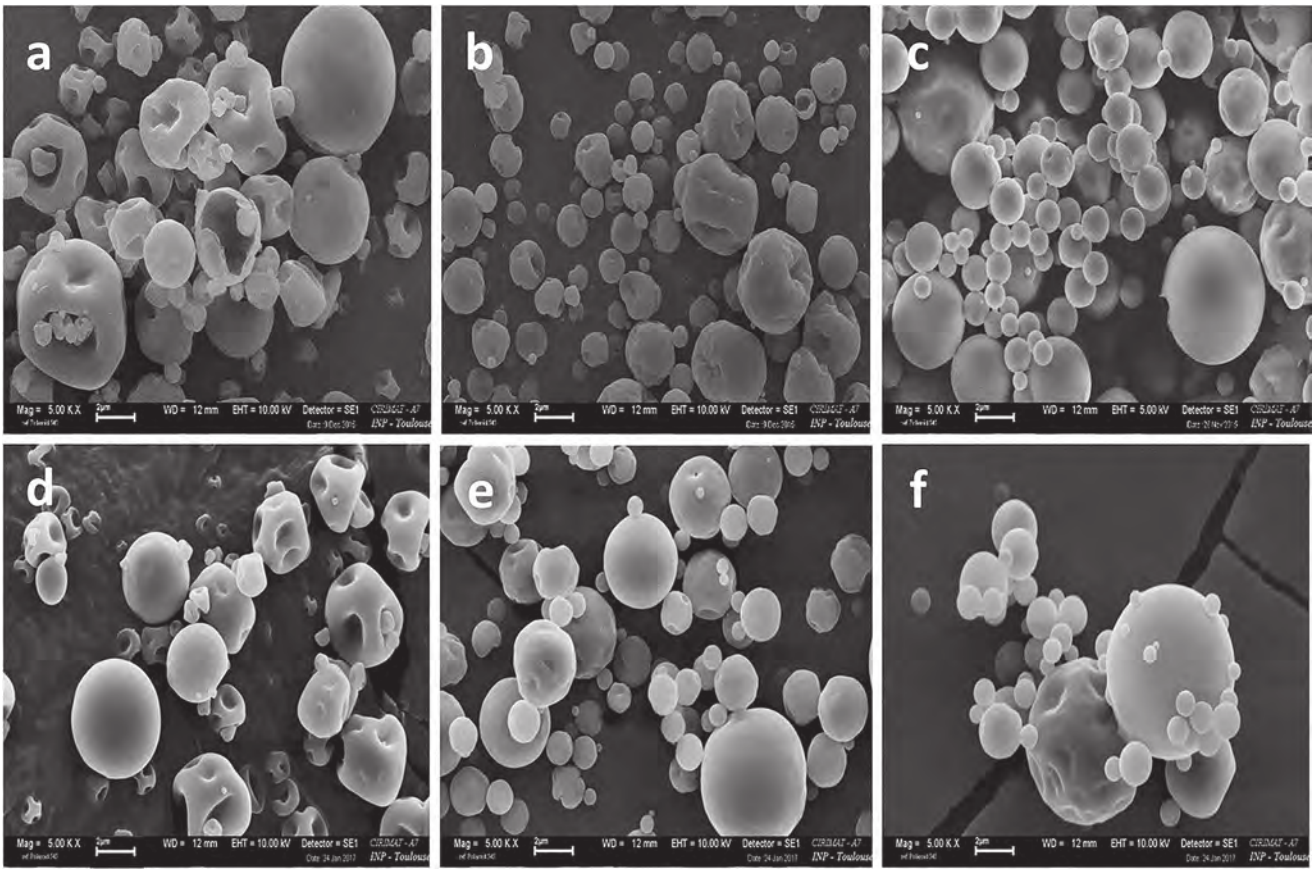

Fig. 3. Scanning electron micrographs of microparticles: **a** SPI microparticles, **b** SPI 80/20, **c** SPI 60/40, **d** SPS, **e** SPS 80/20, and **f** SPS 60/40. X5000 scale bar, 2 μm

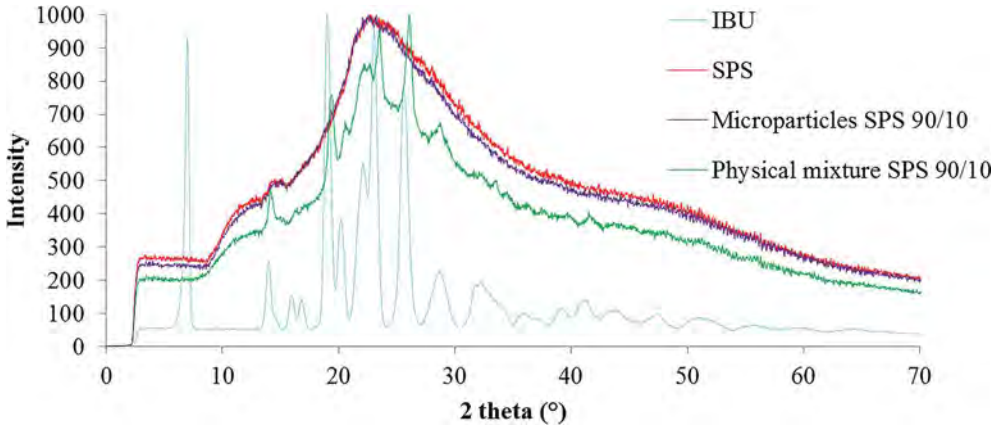

Fig. 4. XRD patterns of ibuprofen, SPS, and the SPS/IBU 90/10 physical mixture and microparticles

microparticles of SPI or SPS with IBU, for each protein/IBU ratio tested. The XRD patterns of IBU, SPS, and a 90/10 SPS/IBU physical mixture and microparticles are presented in Fig. 4. The powder X-ray diffractogram of IBU had sharp peaks at diffraction angles $2\theta = 6°$, 16°, 17°, 19°, 20°, and 22°, demonstrating a typical crystalline pattern (39). SPS powder was semicrystalline, with only one main peak, whereas SPI was characterised by two major peaks (40). This change in XRD pattern suggests that the chemical modification led to a change in conformation. All the characteristic major crystalline peaks of IBU were also observed in the physical mixture but were barely detectable in microparticles, suggesting that IBU was in an amorphous state in the microparticles. Spray-drying is known to trigger the formation of amorphous solid dispersions (41,42), a phenomenon that has been reported for IBU encapsulation with gelatin (31), acylated soy protein (16), or HPMCP-HP55 and Kollidon VA 64 (5). This change in crystalline form would be expected to increase solubility and, thus, the bioavailability of drugs poorly soluble in water (43).

Thermal Properties of Encapsulated Ibuprofen

The thermal properties of encapsulated IBU were studied by thermogravimetric analysis (TGA). The TG mass loss curves of IBU, SPS, and SPS/IBU 60/40 physical mixtures and microparticles are presented in Fig. 5. Similar behaviour was observed for the two proteins and for other protein/IBU ratios. Ibuprofen degradation occurred at about 170°C, with complete decomposition at 240°C, due to the evaporation of IBU (44,45). Protein degradation did not begin until 230°C for SPI and 250°C for SPS, with maximal mass loss occurring between 250 and 450°C, as generally observed for plant proteins (30,34).

The small mass loss observed at about 100°C, for all curves (except that for IBU), can be attributed to the evaporation of residual water molecules from the plant protein (SPS).

A comparison of the curves for physical mixtures and microparticles demonstrated that encapsulation in SPS by spray-drying effectively protected the IBU. Indeed, the thermal degradation of physical mixtures began at 170–175°C, whereas no degradation was observed until 250°C for SPS/IBU microparticles, confirming that this microencapsulation process prevented the thermal degradation of IBU. To ensure that thermal treatment did not induce non-volatile degradation products, we performed HPLC analysis on ibuprofen loaded microparticles exposed to 200°C for 1 h: the chromatograms remained unchanged (data not shown), confirming the IBU protection against thermal degradation

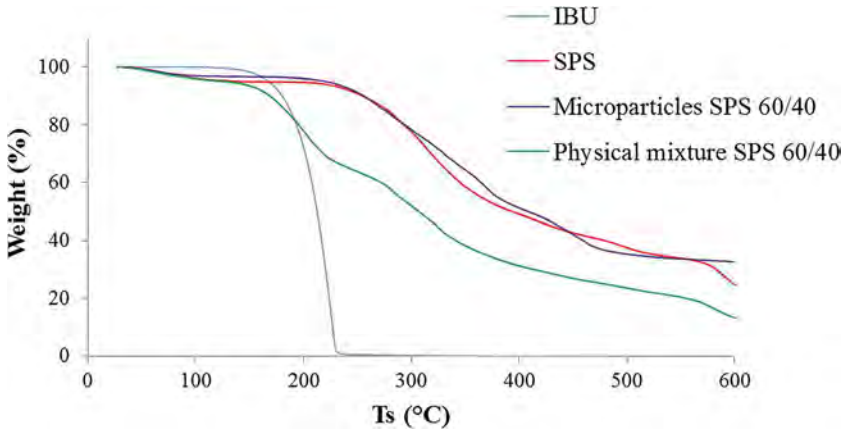

Fig. 5. TGA curves of IBU, SPS, and the SPS/IBU 60/40 physical mixture and microparticles

after encapsulation by spray-drying within soy protein particles.

Impact of Succinylation on the Release of IBU *In Vitro*

The kinetics of IBU release *in vitro* were studied in simulated gastrointestinal fluids, SGF and SIF, in sink conditions, for 2 h at 37°C. IBU was chosen as a model BCS (Biopharmaceutical Classification System) class II drug for this study. Indeed, the absorption of BCS class II drugs (characterised by low solubility and high permeability) can be significantly improved by optimising the formulation so as to maintain the class II drug in a soluble state at the absorption site (46). Moreover, IBU is a good candidate for delayed release because it is known to have gastric side effects.

We showed, in a previous study, that the pH sensitivity of SPI can be used to decrease IBU release in acidic conditions (16). In this study, we investigated whether succinylation could be used to optimise control over this advantageous property.

In vitro dissolution studies were performed on IBU-loaded SPI and SPS microparticles, with IBU contents of 10 to 40% *w/w*. The dissolution profiles of IBU from two IBU-loaded preparations, SPI 90/10 and SPS 90/10, are presented in Fig. 6.

In both cases, IBU release kinetics differed considerably between pH conditions, due to the change in soy protein solubility with pH. Below the isoelectric point, the conformation of the protein chains resulted in a dense structure responsible for lower levels of IBU release in SGF. In these conditions, the mechanism of IBU release in SGF may involve a combination of (i) rapid release into the medium of IBU molecules located at the surface of the microparticles (burst but limited release); (ii) the diffusion of encapsulated IBU out of the hydrated microparticles; and (iii) the action of pepsin, a proteolytic enzyme, accelerating IBU release in a protein content-dependent manner, by triggering microparticle erosion (9). However, this effect is probably very limited with soy protein, as pepsin preferentially cleaves the peptide bonds of aromatic amino acids (histidine, phenylalanine, tryptophan, and tyrosine) (47), of which there are very few in SPI. Above the isoelectric point (*i.e.* in SIF), the protein microparticles rapidly dissolved, releasing their content.

Soy protein succinylation amplified this phenomenon, particularly in acidic conditions. Under simulated gastric conditions, the release profile of IBU differed significantly ($p < 0.05$) between SPI and SPS microparticles. A statistical difference between IBU release from SPI and SPS formulations exists at each time point of the kinetics in acidic pH, except for the first one ($t = 0.25$ h). IBU release was slower for SPS, reaching 34% in 2 h, whereas about 50% of the total IBU content was released over the same time period for SPI. These findings are consistent with the solubility profiles of the two proteins (Fig. 2), as the solubility of the modified protein is much lower than that of the native protein in acidic conditions. A difference in particle size may also contribute to a difference in dissolution performance. Nevertheless, regarding the $D(2, 3)$ values (Table II), the surface exposed was barely affected and this difference not sufficient to explain the difference in ibuprofen release.

The replacement of positively charged lysine residues with negatively charged carboxyl groups decreased both the isoelectric point of the protein (pI from 4.5 to about 3.5) and its charge density (48), resulting in a decrease in electrostatic repulsion between proteins at pH 1.2 and protein unfolding (23). Consequently, forces acting over short distances, such as van de Waals attraction and hydrophobic effects, dominated (9), potentially leading to IBU–SPS interactions, slowing IBU release. At acidic pH, SPS is practically uncharged (the succinylation rate is of 93%) and so does IBU whose pKa is 4.54. As a consequence, van der Waals and hydrophobic interactions are the only potential interactions that can occur in the dissolution medium and may explain slower IBU release.

Under simulated intestinal conditions, at pH 6.8, IBU release was rapid for both formulations, with more than 70% of the IBU released after 15 min and complete IBU release after 1 h. The rate of IBU release from SPS was slightly higher than that from SPI, but this difference was not statistically significant. The amorphisation of encapsulated IBU, which increased solubility, leading to the rapid degradation of protein microparticles in the release medium, did not reveal an effect of greater SPS solubility at neutral pH. However, IBU release from SPS microparticles appeared to be more even, with a lower standard deviation during the first hour. SPS appears to be a valuable candidate for controlling and delaying the release of hydrophobic APIs, given the

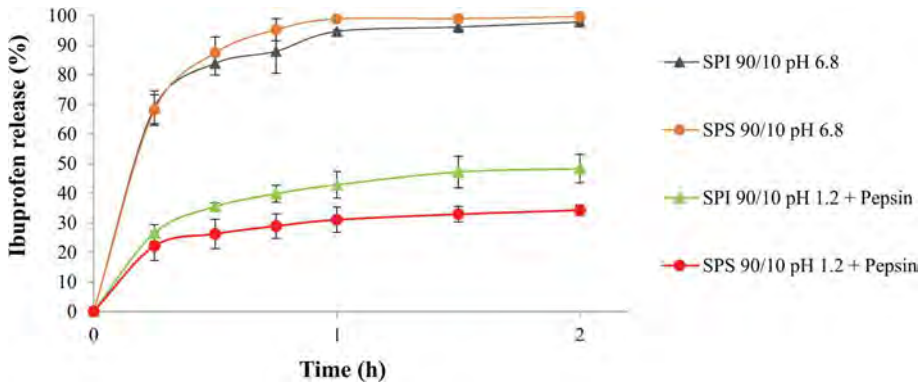

Fig. 6. Ibuprofen release kinetics with SPI and SPS (at a 90/10 ratio) in simulated gastric fluid (SGF) at pH 1.2 with pepsin and simulated intestinal fluid at pH 6.8 (SIF)

lower levels of IBU release observed in acidic conditions and the rapid release of this API at neutral pH.

CONCLUSION

The results presented demonstrate the suitability of SPS for the encapsulation of hydrophobic APIs by spray-drying, particularly for delayed-release oral applications. Succinylation greatly decreased protein solubility at acidic pH while maximising solubility (almost 100%) at pH values above 6. Higher encapsulation efficiencies were thus obtained for IBU (higher than 90%), a model hydrophobic drug. Individual microparticles were observed, even at high IBU contents (protein/IBU ratio of 60/40). *In vitro* release kinetics reflected the optimised pH sensitivity of SPS, resulting in lower levels of IBU release in acidic conditions than were observed with SPI microparticles. The chemical modification of soy protein did not affect the change in IBU crystalline state upon spray-drying. An amorphous molecular dispersion of IBU was obtained, which improved the thermal stability of the drug (by comparison with IBU alone and with IBU encapsulated in SPI) and resulted in rapid dissolution at neutral pH (simulated intestinal fluid), for both proteins. SPS therefore appears to be a promising material for the encapsulation of hydrophobic API in a multiparticulate form for delayed delivery. Such microparticles could be administered as a powder or in a capsule form, *via* the oral route.

ACKNOWLEDGEMENTS

The authors would like to thank the Mexican Council of Science CONACyT for providing financial support, Yannick Thébault and Cédric Charvillat from CIRIMAT for SEM and XRD analyses, and Christine Rey-Rouch from LGC for TGA analyses.

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