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Soy Protein Microparticles for Enhanced Oral Ibuprofen Delivery: Preparation, Characterization, and *In Vitro* Release Evaluation

Maria Antonieta Anaya Castro,^{1,2} Isabelle Alric,¹ Fabien Brouillet,² Jérôme Peydecastaing,¹ Sophie Girod Fullana,² and Vanessa Durrieu^{1,3,4}

Abstract. The objective of this work was to evaluate soy protein isolate (SPI) and acylated soy protein (SPA) as spray-drying encapsulation carriers for oral pharmaceutical applications. SPI acylation was performed by the Schotten–Baumann reaction. SPA, with an acylation rate of 41%, displayed a decrease in solubility in acidic conditions, whereas its solubility was unaffected by basic conditions. The drug encapsulation capacities of both SPI and SPA were tested with ibuprofen (IBU) as a model poorly soluble drug. IBU-SPI and IBU-SPA particles were obtained by spray-drying under eco-friendly conditions. Yields of 70 to 87% and microencapsulation efficiencies exceeding 80% were attained for an IBU content of 20 to 40% *w/w*, confirming the excellent microencapsulation properties of SPI and the suitability of the chemical modification. The *in vitro* release kinetics of IBU were studied in simulated gastrointestinal conditions (pH 1.2 and pH 6.8, 37°C). pH-sensitive release patterns were observed, with an optimized low rate of release in simulated gastric fluid for SPA formulations, and a rapid and complete release in simulated intestinal fluid for both formulations, due to the optimal pattern of pH-dependent solubility for SPA and the molecular dispersion of IBU in soy protein. These results demonstrate that SPI and SPA are relevant for the development of pH-sensitive drug delivery systems for the oral route.

KEY WORDS: vegetal protein; microencapsulation; oral route; modified release; pH sensitivity.

INTRODUCTION

The oral route is the predominant and preferred drug delivery route, due to several advantages relating to physiological factors, patient compliance and economical production methods (1). Research into innovative drug delivery systems for this route has increased over the last 20 years, largely driven by the growing need for novel drug delivery systems to deal with the many newly developed chemical entities with low solubility or poor permeation characteristics, and the need to improve the delivery of existing drugs (2,3). The development of pH-sensitive formulations is a particularly relevant strategy for protecting both the gastric mucosa and the active pharmaceutical ingredient (API), controlling the delivery of the API to the gastrointestinal tract and as an attractive alternative to coating (better safety

and fewer processing steps). Microencapsulation is one of the most effective methods for drug delivery systems, as it makes it possible to keep the API in an appropriate form in the desired therapeutic range of concentrations, and to optimize drug release (4). Spray-drying is a particularly appropriate technique for microencapsulation. It is the most widely used drying technology in the production of biopharmaceutical formulations, with excellent properties, and it can be used at the industrial scale, as a one-step microencapsulation process (5,6).

The development of effective green or environmentally friendly excipients is a key challenge in pharmaceutical development that has triggered considerable interest in natural polymers, due to their relative abundance, low cost, and biodegradable and eco-friendly profiles (7). Proteins are a biodegradable and biocompatible option with pertinent functional properties for use as encapsulating materials for APIs. Animal proteins have been investigated for several years and have been found to have good solubility characteristics, over a large pH range, and to be flexible; they therefore have good encapsulating properties (8), including for pharmaceutical applications (9,10).

However, given the increasing numbers of vegetarians and people avoiding dietary lactose, together with the need to prevent potential health problems due to contamination (*e.g.*,

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bovine spongiform encephalopathy), plant proteins appear to be a more appropriate alternative (11–13), although their potential for use in pharmaceutical applications has yet to be explored. Given its low cost, emulsifying properties, gelling capacity, nutritional and technological properties, soy protein isolate (SPI) is one of the most frequently studied plant proteins and is already widely used as a carrier for food and nutraceutical applications (14–18). However, only a few studies have focused on the use of SPI for API encapsulation and pharmaceutical applications, and even fewer have considered chemical modifications to the protein to modulate its functional properties (19).

In this study, we compared soy protein isolate (SPI) and chemically modified soy protein (acylated, SPA), as spray-drying encapsulation carriers for oral pharmaceutical applications. Acylation, by grafting fatty chains onto the free amino groups of the protein, should modulate its pH sensitivity, significantly decreasing its solubility in acid media, thereby reducing drug release in the stomach, and improving bioavailability and absorption in the intestine. Indeed, the attachment of hydrocarbon chains may modify the charge and structural properties of water-soluble proteins, increasing their hydrophobicity and improving protein affinity for hydrophobic drugs and microencapsulation efficiency (20).

Ibuprofen, a BCS (Biopharmaceutical Classification System) class II molecule, was chosen as the model hydrophobic drug for this study. Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug (NSAID) widely used as an analgesic, anti-inflammatory, and anti-pyretic agent, with poor solubility in water (21–23). The oral administration of IBU can lead to gastrointestinal side effects, such as gastric erosion, peptic ulcer, and bleeding (24,25). Therefore, there is a need for innovative formulations providing better gastric protection and more rapid release of ibuprofen in the intestine.

The first part of this study involved the acylation of SPI to modulate its pH sensitivity and the characterization of the acylated soy protein (SPA) generated. We then assessed the potential of SPI and SPA for drug encapsulation by spray-drying, for various ibuprofen/protein ratios. Spray-drying conditions were optimized so as to generate ibuprofen-soy protein dried particles in a single step. The microparticles obtained were characterized on the basis of their shape (SEM observations), size (laser granulometry), microencapsulation rate, and efficiency. The *in vitro* release kinetics of IBU were determined in simulated gastrointestinal conditions, at pH 1.2 and pH 6.8 and 37°C. The encapsulated IBU was studied by X-ray diffraction (XRD) and thermogravimetric analysis (TGA).

MATERIALS AND METHODS

Materials

SPI (90% pure) was purchased from Solae Belgium NV (Ieper, Belgium). Ibuprofen (100% pure) was purchased from BASF Corporation (USA). NaOH, 37% HCl, dodecanoyl chloride, acetonitrile (HPLC grade), and pepsin porcine gastric mucosa tested according to European Pharmacopeia 9.2 (Ph. Eur.) were purchased from Sigma (Saint-Quentin Fallavier, France).

SPI Characterization

The proximate composition of SPI was determined, including moisture, ash, protein, and lipid contents. Moisture and ash contents were obtained with the standard AOAC methods (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 by conventional Soxhlet extraction in cyclohexane for 6 h. Total carbohydrate content was determined by subtracting the sum of the moisture, ash, protein, and lipid contents from the total mass (27). Proximate analysis was carried out in triplicate.

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

Acylation of Soy Proteins

Acylated soy protein isolate (SPA) was prepared by the Schotten–Baumann reaction (Fig. 1), as described by Nesterenko *et al.* (28). Dodecanoyl chloride (C_{12}) chains were grafted onto the free terminal amine or lysine groups of SPI. This reaction was performed in water and was catalyzed by sodium hydroxide (pH 10), at 50°C for 180 min, with a NH_2/C_{12} molar ratio of 1/2. The product was lyophilized and stored at 4°C.

The acylation rate (%) was evaluated by determining the number of functional amine groups by the OPA method (29).

Protein Solubility Profile

Protein solubility profile was determined as described by Zheng *et al.* (30). Protein samples (SPI and SPA) were prepared in deionized 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, as appropriate. 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 (triplicate). Protein solubility (S) was calculated as follows:

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

Microencapsulation Process

Preparation of Protein/Ibuprofen Solutions

Various ratios of SPI or SPA to IBU were tested: 90/10, 80/20, and 60/40. The solutions were prepared as follows: the protein (acylated or not) was dissolved in deionized water (8% *w/w*) at room temperature, with magnetic stirring. The pH was then adjusted to 8 (with 2 M NaOH) to dissolve ibuprofen, with magnetic stirring for 30 min. The solutions were also stirred mechanically at 500 rpm for 30 min. High-pressure homogenization was carried out with an APV Systems homogenizer (Albertslund, Denmark), at

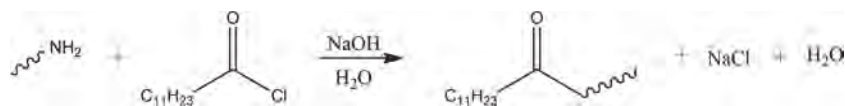


Fig. 1. Acylation *via* Schotten–Baumann reaction

50 MPa with double circulation, and to enhance both the stability of the solution and the encapsulation properties of the proteins (31).

Spray-Drying

A Mini Spray Drier B-290 (Büchi, Flawil, Switzerland) was used to spray 200 mL of solution under the following conditions: inlet air temperature $120 \pm 4^\circ\text{C}$ and outlet air temperature $74 \pm 4^\circ\text{C}$, 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:

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

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

HPLC Analysis

Ibuprofen concentration was measured with a HPLC system equipped with a reverse-phase column (Phenomenex Gemini® 5 μm C18 110 \AA , 250×4.6 mm) and a precolumn filled with the same phase. The system consists 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. UV detection was performed at 222 nm. The mobile phase was a 60:40 (*w/w*) mixture of 0.1 M sodium acetate (pH 7) and acetonitrile.

The microparticles (10 mg) were dissolved in 50 mL of mobile phase and sonicated for 10 min, then magnetically stirred for 20 min. All samples were passed through cellulose acetate (CA) filters with 0.45 μm pores and analyzed, in triplicate, by HPLC.

The amount of ibuprofen in the microparticles was determined from a calibration curve (ibuprofen concentration range 0.05–1 mg/mL, in the mobile phase). For the *in vitro* release study, two different ibuprofen calibration curves were used to determine the amount of ibuprofen, depending on pH: pH 1.2 with pepsin (ibuprofen concentration range 0.0005–0.02 mg/mL) and pH 6.8 (ibuprofen concentration range 0.003–0.2 mg/mL).

Microencapsulation Efficiency and Microencapsulation Rate

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

$$\text{MEE} = \text{IBU}_{\text{exp}} / \text{IBU}_{\text{theo}} \times 100$$

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

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

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

Microparticle Characterization

Microparticle Size Distribution

Microparticle volume diameters ($D_{4,3}$) were determined by laser diffractometry with a Sirocco 2000 apparatus (Malvern Instruments Ltd., Worcestershire, UK), using a refractive index of 1.52 and a dispersion air pressure of 4 bars.

Morphology

The morphology (internal and external) of the microparticles was analyzed by scanning electron microscopy (SEM) with a LEO435VP scanning electron microscope (LEO Electron Microscopy Ltd., Cambridge, UK) operating at 10 kV. The microparticles were deposited on conductive double-faced adhesive tape and sputter-coated with silver.

In Vitro Release Kinetics

Drug release experiments were conducted with models SPI/IBU (90/10) and SPA (90/10). The studies were performed under sink conditions, in flow-through cells (Sotax CE6), as recommended by the European Pharmacopeia 9.2 (Ph. Eur.). Simulated gastrointestinal fluids (SGF and SIF) were used in the closed system (300 or 100 mL of dissolution media, at pH 1.2 to simulate gastric conditions and pH 6.8 to simulate conditions in the intestine). The dissolution media were prepared as recommended by Ph. Eur.: simulated gastric fluid (SGF) at pH 1.2, 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 sampling 1 mL ($n=3$) of the medium, at predefined time intervals, for up to 2 h. After each sampling, 1 mL of pure medium was added in order to maintain sink conditions. The amount of drug released at each time point was determined by the HPLC-UV method described above.

X-ray Diffraction

The powder crystallinity of ibuprofen, SPI, SPA, 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 region of $5^\circ \leq 2\theta \leq 40^\circ$, with an angular increment of $0.02^\circ \cdot \text{s}^{-1}$ (32).

Thermogravimetric Analysis

TGA of ibuprofen, and ibuprofen/protein physical mixtures and microparticles was carried out with an ATG/ETD Q600 from TA Instruments (Newcastle, USA) at a linear heating rate of 10°C/min, under air flow, from 20 to 600°C.

Statistical Analysis

All analyses were carried out in triplicate and the data collected were analyzed by one-way ANOVA with a confidence level of 0.95 and Tukey-Kramer multiple mean comparisons in the MINITAB Release 17 statistical package (Minitab Inc., USA).

RESULTS AND DISCUSSION

Before chemical modification and evaluation, the composition of SPI was determined, to check that acylation was feasible.

SPI Composition

SPI was found to consist predominantly of protein (87.6%), but it also contained 0.4% lipids, 5.3% ash, 1.4% carbohydrates, and 5.4% moisture. Its amino acid composition (Fig. 2) was determined. The protein chains consisted essentially of 17 amino acids, with two amino acids, glutamic acid (17.8%), and aspartic acid (11.6%), predominating, a feature characteristic of plant proteins (15). The high levels of carboxyl groups in these plant proteins should confer good solubility in basic conditions. SPI also contained relatively high levels of lysine (6.3%), within the normal range for the proteins of leguminous plants (33). The amino group of lysine can take part in various chemical reactions involving the formation of amino bonds. SPI is, therefore, particularly suitable for chemical modifications, such as acylation.

SPI Acylation

SPI was acylated with dodecanoyl chloride, a cheap, biosourced reagent, which has been shown to improve the encapsulation properties of SPI more effectively than

octanoyl and hexadecanoyl chlorides (28). A $\text{NH}_2/\text{C}_{12}$ molar ratio of 1/2 was used to obtain a sufficiently high acylation ratio (41%).

The attachment of alkyl chains should increase the surface hydrophobicity of the protein chains, and greater amphiphilicity induced should have an impact on protein solubility and protein/drug interactions. The efficiency of this chemical modification was checked by comparing SPI and SPA solubility profiles.

SPI and SPA pH Solubility Profiles

The pH solubility profiles of SPI and SPA were compared (Fig. 3). The pH sensitivity of proteins is determined by the presence of acidic and basic groups in their amino acid chains, which groups can accept or release protons, depending on the pH of the medium (34). The solubility curve of SPI was U-shaped, like those of most plant proteins. Both proteins had a minimal solubility around pH 4–5, corresponding to their isoelectric point. At lower pH values, the protonation of amino groups increased SPI solubility, whereas acylation, which decreased the number of available lysine cationic ammonium groups, prevented this phenomenon (35). At pH values above 6, the carboxylic groups of glutamic and aspartic acids were in their sodium carboxylate form, increasing the solubility of both proteins. In basic conditions (pH 8–10), the solubility profiles of the two proteins were similar, with about 60% solubility, sufficient for effective microencapsulation (28), although SPA appeared to be slightly less soluble than SPI, probably due to the presence of lipophilic fatty chains.

These results demonstrate the efficacy of the chemical strategy developed: acylation greatly decreases protein solubility in acidic conditions, without significantly modifying the maximal solubility in basic conditions.

Both SPI and SPA have been tested as potential carriers for encapsulating IBU *via* spray-drying, in optimized experimental conditions (28).

Microencapsulation Results

The properties of loaded spray-dried microparticles are presented in Table I.

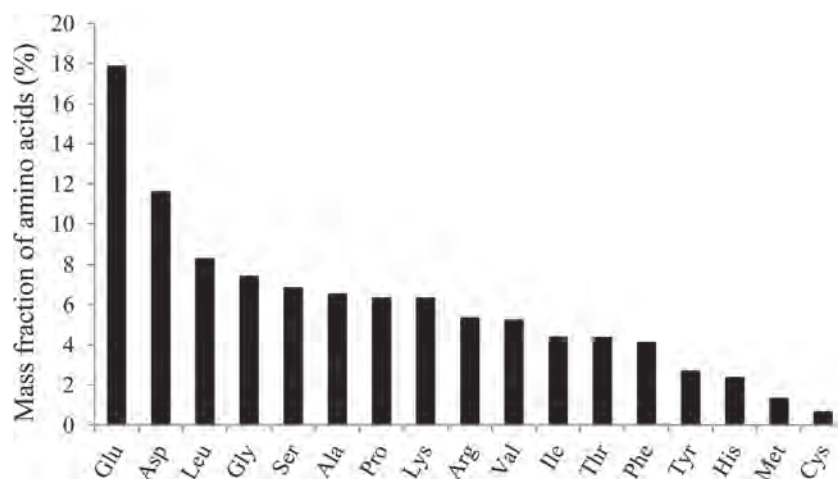


Fig. 2. Amino acid composition of soy protein isolate

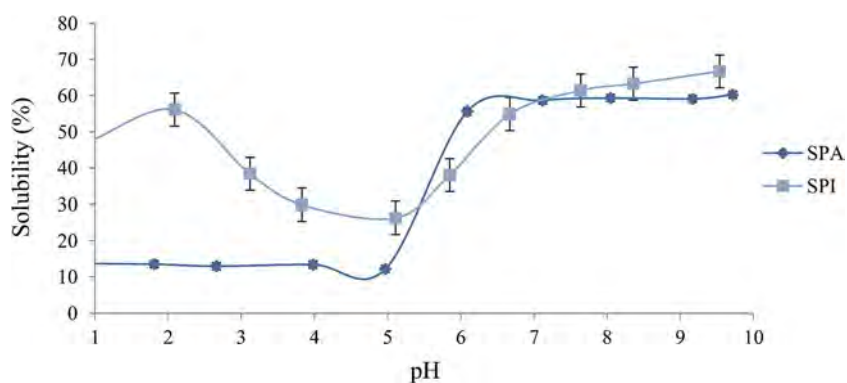


Fig. 3. Solubility profiles of SPI and SPA at 3% w/w and 37°C

Spray-drying yields of 70 to 87% were obtained. These values are higher than those previously reported for microencapsulation with plant proteins, ranging from 50 to 70% (28,36). The spray-drying yields obtained in this study, reaching a maximum of 80%, even for high ibuprofen ratios, are very promising in terms of the industrial feasibility of this process.

High MEE values (systematically greater than 80%) were obtained for both proteins and for all soy protein/IBU ratios, confirming the excellent microencapsulation properties of SPI and the suitability of the chemical modification based on acylation. However, SPI- and SPA-loaded microparticles behaved differently.

For SPI-loaded microparticles, an increase in the proportion of IBU (SPI/IBU ratio changing from 90/10 to 60/40) leads to a decrease in MEE from 92 to 82%. Similar results were reported in several previous studies on SPI (37,38), and on other natural polymers, such as modified starch (39). In these studies, the decrease in MEE was explained principally by an increase in total solid content. In this study, increase in the proportion of ibuprofen also resulted in a higher total solid content. However, the decrease in MEE may also be related to the hydrophobicity of ibuprofen, preventing effective protection by SPI when the protein/IBU ratio was too low (high amount of IBU).

By contrast, MEE increased with decreasing SPA/IBU ratio, reaching 94% for SPA microparticles with a SPA/IBU ratio of 60/40. The grafting of hydrophobic hydrocarbon

chains onto the protein probably enhanced the affinity of the acylated proteins for the hydrophobic core, even for high ibuprofen contents. Previous studies reported similar results (36,40), for various plant proteins, from soy, sunflower, and rapeseed, for example, confirming the suitability of acylation for improving the encapsulation properties of plant proteins.

Microparticle moisture content was between 1.4 and 3.7%, ensuring microbiological stability (41). The SPI-loaded microparticles had a higher moisture content than the SPA-loaded microparticles, due to the greater hygroscopy of unmodified proteins. Similarly, highly loaded microparticles had a lower moisture content, due their higher content of ibuprofen, a hydrophobic drug.

Microparticles were between 3.5 and 5.8 μm in diameter, except for the SPI 60/40 sample, for which some aggregates were observed, making it difficult to determine the size of the microparticles correctly. These values are consistent with those expected for spray-dried microspheres (31).

Morphology

The morphology of the microparticles was observed by SEM. Some representative micrographs are displayed in Fig. 4. The powder consisted of spherical particles with a smooth surface, without fissures or cracks, which protected the ibuprofen well. An uneven shrinkage of the microparticles was observed, and such shrinkage is generally

Table I. Properties of Loaded Spray-Dried Microparticles

Soy protein/ibuprofen (% w/w)	Spray-drying yield (%)	MEE (%)*	MER (%)*	Microparticle size ($D_{4,3}$) Mm	Moisture content of microparticles (%)
SPI	80	–	–	5.0 ± 0.1^{bc}	5.1 ± 0.1^a
SPA	ND	–	–	4.2 ± 0.1^{bc}	3.5 ± 0.3^b
SPI 90/10	82	92.6 ± 9.3^a	9.3 ± 0.9^d	3.5 ± 0.2^c	3.7 ± 0.1^b
SPI 80/20	87	84.1 ± 3.6^a	16.8 ± 0.7^c	4.4 ± 0.3^{bc}	2.3 ± 0.1^c
SPI 60/40	80	81.9 ± 2.8^a	32.6 ± 1.1^b	19.1 ± 2.0^a	3.2 ± 0.1^c
SPA 90/10	70	87.4 ± 3.7^a	8.7 ± 0.4^d	4.3 ± 0.1^{bc}	3.7 ± 0.1^b
SPA 80/20	81	86.6 ± 4.5^a	17.3 ± 0.9^c	4.5 ± 0.1^{bc}	2.8 ± 0.1^d
SPA 60/40	87	93.9 ± 10.2^a	37.6 ± 4.1^a	5.8 ± 0.1^b	1.4 ± 0.1^f

Superscripts with the same letters in the same column were not significantly different $P > 0.5$ (Tukey-Kramer multiple mean comparisons) ND not determined

*Determined by HPLC

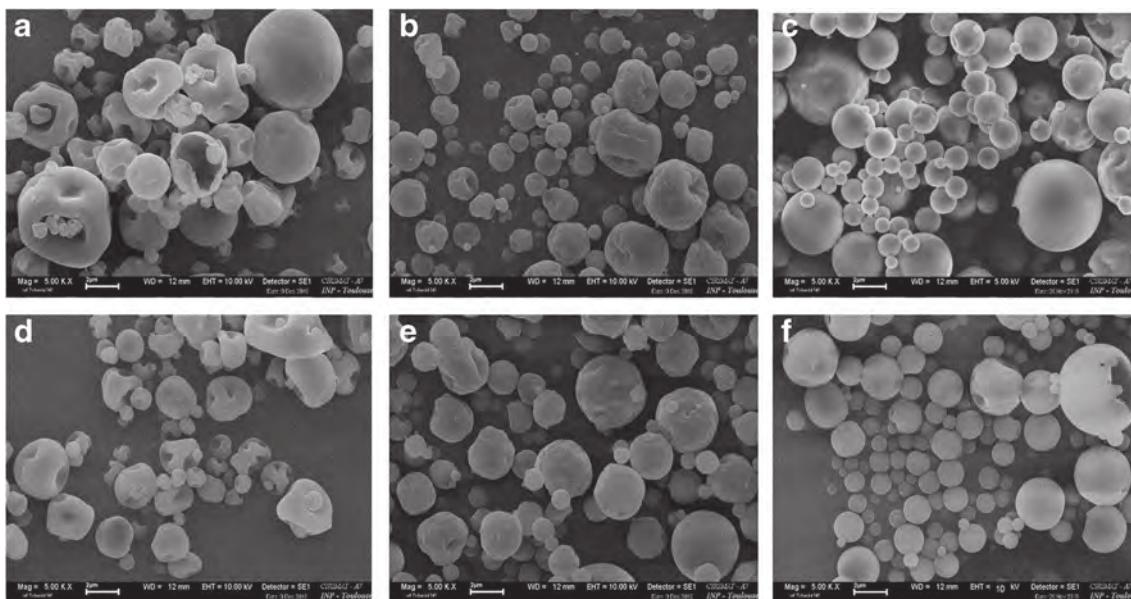


Fig. 4. Scanning electron micrographs of microparticles. **a** SPI microparticles. **b** SPI/IBU 80/20. **c** SPI/IBU 60/40. **d** SPA. **e** SPA/IBU 80/20. **f** SPA/IBU 60/40. Magnification of $\times 5000$, scale bars $2 \mu\text{m}$

associated with the drying and/or cooling step(s) (37). The external structure had wrinkled surface, typical of spray-dried microparticles with a high protein content (42), but no significant differences according to the protein or ibuprofen/protein ratio were observed. The internal structure revealed no evident porosity in the matrices, but thinner shells were observed for highly loaded microparticles (60/40 protein/IBU ratio). Moreover, no free drug crystals were observed even for high IBU encapsulation rates.

The size of the microspheres, from 1 to $9 \mu\text{m}$, confirmed the values obtained by laser granulometry.

In Vitro Release Studies

IBU, a BCS (Biopharmaceutical Classification System) class II molecule, was chosen as the model drug for encapsulation in SPI and SPA microparticles. The absorption of BCS class II drugs (characterized by low solubility in water and high permeation) can be significantly improved by optimizing the formulation so as to ensure that the drug

remains solubilized at the absorption site (43). This study investigated whether SPI and SPA could be used to improve the delivery of this poorly soluble drug with gastric side-effects.

The effect of encapsulation in SPI and SPA microparticles on the solubility of ibuprofen was assessed in dissolution studies performed *in vitro* in sink conditions, with SPI and SPA microparticles loaded with various amounts of IBU (from 10 to 40%) in USP buffers at pH 1.2 or 6.8 USP, for 2 h at 37°C . The dissolution profiles of IBU in two ibuprofen-loaded preparations, SPI 90/10 and SPA 90/10, are presented in Fig. 5. IBU release kinetics varied considerably with the pH of the medium.

In acidic conditions (*i.e.*, in simulated gastric fluid (SGF)) in the presence of a gastric enzyme (pepsin), a slow release profile was obtained for the IBU encapsulated in microparticles of SPA or SPI, with 28.0 ± 4.3 and $42.8 \pm 4.4\%$ IBU release, respectively, within the first hour. Significantly less IBU was released from the SPA 90/10 formulation than from the SPI 90/10 formulation ($p < 0.05$).

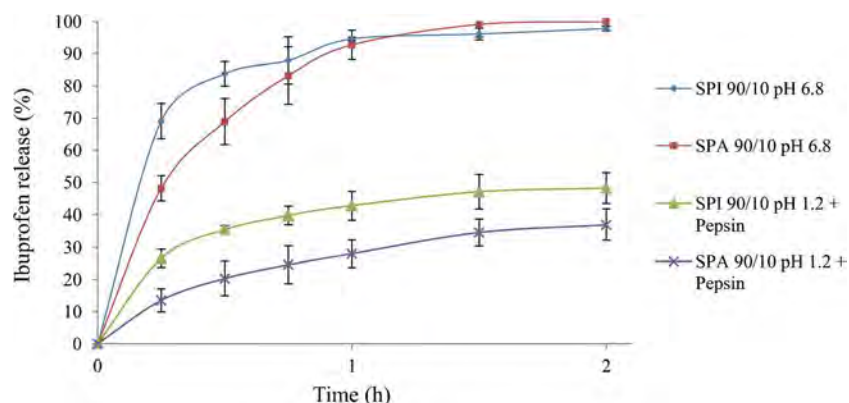


Fig. 5. Ibuprofen release kinetics with SPI and SPA (at 90/10 ratio) in simulated gastric fluid (SGF) at pH 1.2 with pepsin and simulated intestinal fluid at pH 6.8 (SIF)

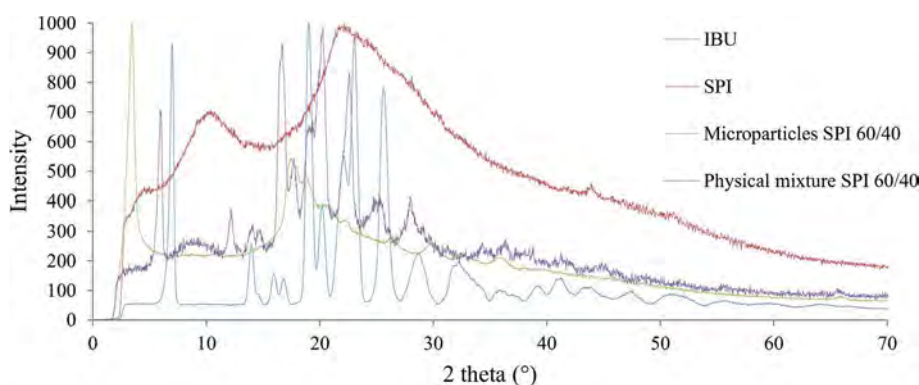


Fig. 6. DR-X patterns of ibuprofen, SPI, microparticles, and physical mixture of SPI/IBU 60/40

These results may be considered to reflect the pH sensitivity of SPI and SPA. Based on solubility data, pH had a significant effect on drug release from IBU-loaded SPI and SPA microspheres. Below the isoelectric point of SPI and SPA (pH=4.5–5), the conformation of the protein chain resulted in a compact, shrunken structure and low release rate. This effect was particularly marked for SPA, which insolubility in acidic conditions was enhanced by acylation. In such conditions, the observed release was probably due to drug diffusion, as already reported for whey proteins (44). However, the observed release levels were higher than expected in SGF, as release was evaluated in the presence of pepsin, a proteolytic enzyme. In this context, the release mechanism involved a combination of diffusion and erosion, for which the rate of release depends on protein content (data not shown). This phenomenon was limited in our case, as pepsin preferentially cleaves the peptide bonds of aromatic amino acids (histidine, phenylalanine, tryptophan, and tyrosine) (34), of which there are very few in SPI, as shown in Fig. 2. The pH sensitivity of SPI and SPA made it possible to obtain pH-sensitive profiles of IBU release kinetics compatible with the oral administration of this drug.

In neutral conditions (*i.e.*, in simulated intestinal fluid (SIF) without enzymes), the release rates were faster, with almost 100% of the IBU released within 90 min for both SPA and SPI (99.1 ± 3.9 and 96.1 ± 1.8%, respectively). At pH 6.8, the 90/10 ratio curves for SPA and SPI were similar, with a release of at least 50% of the encapsulated IBU within the

first 30 min. These results for SIF may be partly explained by the solubility of SPI and SPA, and, thus, the dissolution of particles at pH 6.8, but remain surprising for a poorly soluble crystalline drug as IBU.

We decided to explore the interactions of IBU with SPI and SPA, which may have a major influence on drug solubility or thermal stability, and, consequently, on drug delivery performance and bioavailability.

The possible interaction between the drug (IBU) and the carrier (SPI and SPA) was investigated, with a view to explaining the results for dissolution in SIF, by characterizing the prepared microparticles, ibuprofen alone, and physical mixtures, by XRD and TGA. An absence of characteristic peaks/transitions of the drug after processing or a shifting of those peaks, on the XRD pattern or the TGA thermogram, indicates changes in the characteristics of the drug or the possibility of drug-carrier interactions (45).

X-ray Diffraction

The X-ray diffraction patterns of IBU were compared with those of IBU-SPI or IBU-SPA physical mixtures and microparticles, for each protein/IBU ratio tested. The X-ray diffraction patterns of IBU, SPI, and of the 60/40 SPI/IBU physical mixture and microparticles are presented in Fig. 6. The powder X-ray diffractogram of IBU has sharp peaks at diffraction angles $2\theta = 6^\circ$, 16° , 17° , 19° , 20° , and 22° , showing a typical crystalline pattern (23). SPI powder was semicrystalline, with two major peaks (32).

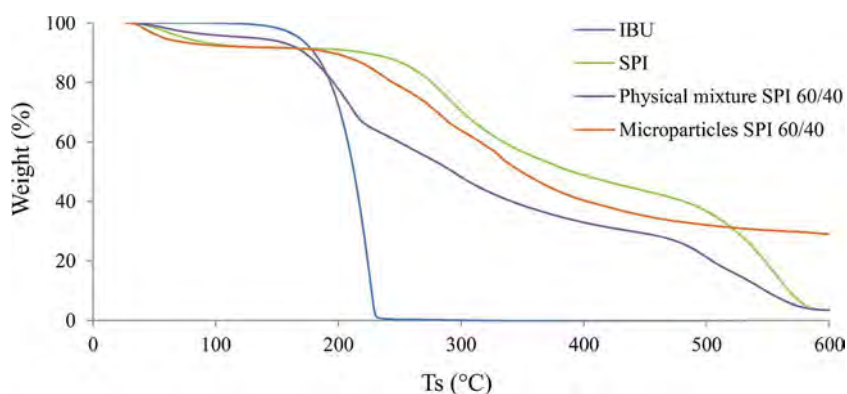


Fig. 7. TGA curves of IBU, SPI, physical mixture, and microparticles of SPI/IBU 60/40

All the major characteristic crystalline peaks of IBU were also observed in the physical mixture, but were barely detectable in microparticles, suggesting that the IBU was present in an amorphous state in the microparticles. Similar results were reported for IBU encapsulation with gelatin by spray-drying (32) or riboflavin encapsulation with soy/zein protein (15). Our results suggest that the combination of soy protein and spray-drying led to a change in the crystallinity state of IBU, from a crystalline to an amorphous form. Similar behavior was observed for SPA, confirming that acylation has no impact on the encapsulation properties of soy protein at neutral pH.

Spray-drying is one of the most widely used solvent evaporation procedures in the production of solid dispersions and it often produces amorphous materials (46,47). The amorphous solid state has the advantage of greater solubility and, therefore, faster dissolution rates than crystalline material, as no energy is required to break up the crystal lattice during the dissolution process (48). This, together with the solubility of SPI and SPA at neutral pH, may account for the better dissolution of encapsulated IBU in SIF. This change in crystalline state should increase the oral bioavailability of IBU, by facilitating rapid absorption in the intestine (45).

Thermal Analysis

In addition to XRD, we also performed TGA studies. TG mass loss curves were generated for IBU, SPI, and SPI/IBU physical mixtures and microparticles with a 60/40 ratio of protein to IBU (Fig. 7). Similar behavior was observed for both proteins and for higher protein/IBU ratios. Ibuprofen degradation occurred at about 170°C, with complete decomposition at 230°C. Protein degradation did not start until 210°C for SPA, and 250°C for SPI, with mass loss maximal (up to 70%) between 220 and 450°C, as generally observed for plant proteins (28,36).

The small loss of mass observed at around 100°C on all curves (other than that for IBU alone) can be attributed to the evaporation of the residual water molecules present in plant proteins (SPI and SPA).

A comparison of the curves for physical mixtures and microparticles demonstrated the efficacy of IBU protection by spray-drying encapsulation. Indeed, thermal degradation began at 170–175°C for physical mixtures, but not until 220°C for microparticles, confirming the improvement in the thermal stability of IBU conferred by this microencapsulation process.

CONCLUSION

The results presented here demonstrate the suitability of SPI and SPA as encapsulating materials for API by spray-drying, particularly for delayed release oral applications. Their advantages include pH sensitivity, the possibility of incorporating high proportions of drug (up to 40%), and excellent encapsulation efficiencies (greater than 80%). The acetylation of SPI considerably decreases its sensitivity to enzymes and acidity and, thus, greatly decreases drug release in acidic conditions (simulated gastric fluid). Furthermore, an amorphous molecular dispersion of IBU was obtained, improving the thermal stability of the drug and resulting in its rapid dissolution at neutral pH (simulated intestinal fluid).

Thus, SPI and SPA are promising excipients for the development of hydrophobic drug delivery systems for the oral route.

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