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Physico-chemical state influences *in vitro* release profile of curcumin from pectin beads

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ABSTRACT

Curcumin is a polyphenolic compound with diverse effects interesting to develop health benefit products but its formulation in functional foods or in food supplement is hampered by its poor water solubility and susceptibility to alkaline conditions, light, oxidation and heat. Encapsulation of curcumin could be a mean to overcome these difficulties. In this paper, curcumin was encapsulated by ionotropic gelation method in low methoxyl pectin beads associated with different surfactants: Solutol®, Transcutol® and sodium caseinate. After encapsulation, physico-chemical properties of encapsulated curcumin such as its solubility, physical state, tautomeric forms and encapsulation efficiency as well as encapsulation yield were characterized. *In vitro* dissolution of curcumin from beads displayed different kinetic profiles according to bead composition due to different matrix network. As Solutol® was a good solvent for curcumin, the drug was present into amorphous form in these beads inducing a rapid release of curcumin in the simulated digestive fluids. In contrast, drug release was slower from sodium caseinate beads since curcumin was not totally dissolved during the manufacturing process. Moreover, the FLIM studies showed that a part of curcumin was encapsulated in caseinate micelles and that 34% of this drug was in keto form which may delay the curcumin release. The Transcutol beads showed also a slow drug release because of the low curcumin solubility and the high density of the matrix.

1. Introduction

Curcumin is a yellow-orange polyphenol found in the rhizomes of *Curcuma longa*, commonly known as turmeric, used as a spice, food colouring and in traditional Indian medicine for centuries [1]. It is a lipophilic fluorescent molecule with phenolic groups and conjugated double bonds [2]. Curcumin belongs to the group of β-diketones and exhibits tautomerism between enol and keto structures, respectively. It has a low intrinsic toxicity but a wide range of activities including antioxidant, anti-inflammatory, antimicrobial, anti-amyloid and antitumor properties [3,4]. Preclinical studies on curcumin have demonstrated its ability to inhibit carcinogenesis in a variety of cell lines including breast, colon, gastric, liver, leukaemia, oral epithelial, ovarian, pancreatic and prostate cancers [5–7]. So curcumin can be very interesting to develop health benefit products in functional foods or in food supplements [8,9].

However, there are major barriers for the use of curcumin in food industry that are its poor water solubility, its sensitivity to alkaline conditions, its photodegradation, or poor stability upon oxidation or heat which also limits its efficacy [10,11]. Encapsulation has been used to overcome most of these drawbacks since it can immobilize the entrapped molecule, protect it against degradation and control its release control [12]. Encapsulation of curcumin does not only help to protect it from adverse environmental factors or transformation processes induced by light and heat but also increases its solubility improving therefore its bioavailability [11]. Moreover, the release of curcumin in complex biological systems or in food products can be controlled by its encapsulation [13]. Curcumin can be also considered as a bioactive model and the results can be applied to other compounds of interest with the same problem as piperine or quercetin.

Polysaccharides such as alginate, chitosan, modified starch or pectin are used as food encapsulation matrix because they

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are degradable, food-grade and low cost [8,12,14,15]. The active substance can be encapsulated in polysaccharides by different techniques such as spray-drying [8], cryotropic [15] and ionotropic gelation [14].

Pectin is a naturally occurring water-soluble polysaccharide which is found in the cell wall of most plants. It contains linear chains of α-(1→4)-D-galacturonic acid residues. These uronic acids have carboxyl groups, some of which are naturally present as methyl esters and others are reacted with ammonia to produce carboxamide groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are important means to classify pectin and adjust their properties [16]. The pectin with low DE can be used as a gelling agent by binding with divalent cations (calcium ions). Indeed, calcium binding reduces pectin solubility, inducing non-covalent associations of carbohydrate chains through "egg-box" complexes [17]. This is the reason why calcium pectinate has been investigated as an insoluble hydrophilic matrix for sustained-release delivery by an interfacial complexation process [18,19].

In this study, the encapsulation of curcumin was obtained by the ionotropic gelation method in order to obtain the calcium pectinate gel beads with different surfactants (Solutol® HS 15, Transcutol® HP and sodium caseinate) [19,20]. These curcumin beads were characterized for their chemical properties (curcumin solubility, encapsulation efficiency) and their physical properties (curcumin physical state by differential scanning calorimetry (DSC) and tautomeric forms were evidenced by Fluorescence lifetime imaging microscopy (FLIM) [21]). Furthermore, the release of the curcumin beads was studied *in vitro* to investigate the influence of the physico-chemical properties on the kinetic profile.

2. Materials and methods

2.1. Materials

Curcumin (purity > 65%) was purchased from Sigma–Aldrich, Germany. Amidated low-methoxy (LM) pectin (Unipectin 305C, DE ≈ 22–28% and DA ≈ 20–24%) was obtained from Cargill (France). Solutol® (Macrogol 15 Hydroxystearate, melting point: 30 °C, hydrophilic–lipophilic balance ≈ 14–16) was provided by BASF, Germany. It is a non-iononic surfactant commonly used in the pharmaceutical field for manufacturing aqueous parenteral preparations with vitamins A, D, E and K and a number of other lipophilic pharmaceutical active agents such as propanidid, nifedipine, miconazole, *etc.* This product is considered as relatively safe (rat oral LD_{50} is approximately 20 g/kg) [22]. Transcutol® HP was a gift from Gattefossé, France. This is a highly purified diethylene glycol monoethyl ether EP/NF which is used in self-emulsifying lipid formulations as a co-solvent [23]. Transcutol® is a powerful solubilizing agent used in several dosage forms due to its ability to solubilize many drugs [24,25]. The LD_{50} of Transcutol® administered orally is 8.69 g/kg in rat [26]. Sodium caseinate (moisture ≈ 5.5% (w/w), protein ≈ 91% (w/w) powder) was obtained from Armor Proteins, France. Sodium caseinate from milk proteins is obtained by precipitation in the presence of sodium cations. It is used in the food industry as an emulsifier and a stabilizer. Calcium chloride ($CaCl_2$) was purchased from Sigma–Aldrich. All other chemicals used were of reagent grade.

2.2. Encapsulation of curcumin in calcium pectinate gel beads

The encapsulation of curcumin was prepared by the ionotropic gelation method described by Chambin et al. [27]. Pectin solutions were prepared by dissolving 1.6 g of pectin in 20 mL of deionised water (pH = 6.8) with magnetic stirring at high speed (700 rpm) for 1 h. Then surfactants were added and dissolved in the pectin solution by stirring at room temperature. The surfactants used were either 10 g of Solutol® HS 15 or 5 mL of Transcutol® HP or 2 g of sodium caseinate. Their amounts used were selected so that they do not increase the viscosity of the pectinate solution in order to prepare beads. These mixtures were then adjusted to 40 mL with deionised water to obtain pectin solution (4%, w/v). 500 mg of curcumin were added to this solution and dispersed by stirring (30 min) and sonication (15 min, 37 Hz), before the cross-linking. Dispersions were pumped and poured dropwise into a solution of $CaCl_2$ 10% (w/v) (pH = 4.5–5) using an IPC Ismatec pump at 10 rpm with a 1.5 mm needle in diameter. The cross-linking was instantaneous. Beads were cured 5 min in $CaCl_2$ solution before washing with 200 mL of deionised water. They were then collected and dried in an oven at 37 °C during 48 h and stored at 4 °C prior to use. The obtained beads were called: Pectin bead, Solutol bead, Transcutol bead, Caseinate bead, respectively when no surfactant was used or when Solutol®, Transcutol® or caseinate were introduced in the formulation. The size and the shape of these beads were characterized by optical microscopy (stereomicroscope CETI). The water content was determined using an infrared moisture analyzer at 160 °C until bead weight stabilization was achieved (OHAUS MB35, Switzerland).

2.3. Chemical characterization

2.3.1. Solubility studies

The solubility of curcumin in surfactant solutions was evaluated at room temperature. Surfactant solutions (5%, w/v) were prepared by dissolving 1 g of each surfactant in 20 mL of deionised water. An excess amount of curcumin (20 mg) was added and dispersed in these solutions. After homogenization by stirring (30 min) and sonication (15 min), these dispersions were kept overnight for decanting insoluble curcumin which was then removed. After 12 h, 2 mL of each supernatant were taken and mixed with 2 mL of ethanol (EtOH) 96% (v/v). The mixture was finally, diluted with EtOH 96% (v/v) before measuring the absorbance at 429 nm. The standard curve of curcumin in EtOH 96% (v/v) was obtained to calculate the amount of curcumin (concentration range from 1 mg/L to 20 mg/L). All experiments were performed in triplicate.

2.3.2. Encapsulation efficiency and encapsulation yield (EE and EY)

For each type of bead, 100 mg were introduced in 200 mL of phosphate buffered solution (pH = 6.4), until their total degradation. 5 mL of dispersion were removed and mixed with 2 mL of Solutol®. The mixture was finally diluted with deionised water and the absorbance was measured at 426 nm. A standard curve of curcumin in Solutol® 5 g/L was obtained to calculate the amount of curcumin (concentration range from 1 mg/L to 20 mg/L). All experiments were performed in triplicate.

The EE and EY of curcumin in calcium pectinate gel beads were determined by the Eq. (1):

$$EE(\%) = \frac{Q_E}{Q_T} \times 100; \quad EY(\%) = \frac{Q_E}{Q_M} \times 100 \tag{1}$$

where Q_E was the amount of curcumin encapsulated, Q_T was the total of curcumin used; Q_M was the total amount of beads.

2.4. Physical characterization

2.4.1. DSC study

DSC analysis was applied to study the physical forms of encapsulated curcumin within the different beads. A Perkin Elmer DSC 8000 was used with Indium for enthalpy calibration. Curcumin (2 mg or