Microcapsules prepared from starch derivatives

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In this work, vinyl groups were introduced on soluble starch by reaction with 2-vinyl-4,4-dimethyl-2-oxazolin-5-one. The polysaccharides obtained were characterized by ^{1}H-NMR and DSC. The ^{1}H-NMR spectra showed high degrees of substitution and the DSC thermograms suggest a low crystallinity in the modified starch. The modified starch was used to obtain microcapsules prepared through interfacial crosslinking with DPGDA (dipropyleneglycol diacrylate) by a water-in-oil emulsion polymerization.

1. Introduction

In the last few years, the preparation of supports for controlled drug delivery systems has been the subject of many publications. The need for using biocompatible and biodegradable carriers for this purpose leads to the use of natural polymers as the materials for the preparation of drug delivery systems. As reported by some authors [1–3], polysaccharides such as starch or dextran have been introduced as the base materials to obtain microparticles.

From the literature, one can conclude that there are various methods of microparticles preparation. However, not all of them are suitable to obtain supports with the right chemical and physical characteristics. Some authors advocate the use of a crosslinking agent in their preparation to improve the stability and characteristics of the microparticles.

Rothman *et al.* [4] used epichlorohydrin as the crosslinking agent for starch microspheres and Lévy and Andry [3] used terephthaloyl chloride for hydroxyethylstarch or carboxymethylstarch microspheres. N,N-methylenebisacrylamide is one of the most widely employed crosslinking chemicals, and is used for the crosslinking of starch and dextran [1, 2]. In order to obtain substrates with vinyl end groups that could polymerise in a water/oil emulsion, starch and dextran had previously been modified chemically either with acrylic acid glycidylester [1], or with acryloylchloride [5]. These methods have some inherent disadvantages: the reactions with acrylic acid glycidylester requires a long time and gives rise to products with a degree of limited substitution. On the other hand, the reaction with acryloyl chloride is a pH-controlled reaction. In order to make starch suitable for the preparation of microcapsules, vinyl groups were introduced on soluble starch by reacting the polysaccharide with 2-vinyl-4,4-dimethyl-2-oxazolin-5-one. The systems obtained were characterized by ^{1}H-nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC). The modified starch was used to obtain microcapsules prepared through interfacial crosslinking with DPGDA (dipropyleneglycol diacrylate) by a water-in-oil emulsion polymerization.

2. Materials and methods

2.1. Materials

The soluble starch was obtained from Merck and was dried over phosphorous pentoxide before use. The 2-vinyl-4,4-dimethyl-2-oxazolin-5-one (azolactone) was purified prior to use by vacuum distilation. The 4-dimethylaminopyridine (DMAP) (Jansen Chemical), dipropyleneglycol diacrylate (DPGDA) (UCB Chemicals, Belgium), N,N,N′,N′-Thetramethylethylenediamine (TEMED) (Merck) and potassium persulphate (Merck) were used as obtained. Dimethylsulfoxide (DMSO) was dried over calcium hydride and distilled before use.

2.2. Methods

2.2.1 Preparation of starch-(acryloyl-2-methyl) propionate

The starch (2.75 g) was dissolved in 40 cm^3 of DMSO at room temperature with stirring. The DMAP (0.1 g) and the azolactone were added and the reaction mixture was magnetically stirred for 3 days at room temperature. The product was isolated by precipitation either in 500 cm^3 of methanol : ether (1:1) or 500 cm^3 of acetone, depending on the degree of substitution. The precipitate was dissolved in distilled water, purified by dialysing for 60 h and freeze dried. The degree of substitution (DS) was dependent on the amount of the azolactone added.

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2.2.2. Characterization of the modified starch

The relation vinyl end groups/glucose residues, referred to as the degree of substitution (DS) was determined by ^{1}H-NMR. The ^{1}H-NMR spectra were registered in D_2O in a Varian Unity-500 Spectrometer. The modified starch was characterized by differential scanning calorimetry (DSC) using a PL-DSC plus V apparatus.

2.2.3. Enzymatic degradation of starch in vitro

The degradation of the modified starch was evaluated by incubating the samples (150 mg) in a solution containing 40 mg of amyloglucosidase in 9 cm^3 of 0.1 M citrate buffer at room temperature with stirring. After 24 h, the reaction was stopped by adding 1 cm^3 of trichloroacetic acid (10% w/v). The samples were centrifuged and the supernatant analysed for glucose content using the 3,5-dinitrosalicylic acid test (DNS test).

2.2.4. Determination of glucose using 3,5-dinitrosalicylic acid test

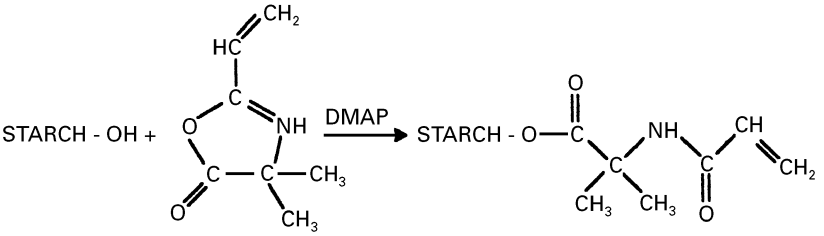
The samples (1 cm^3) were dissolved in a mixture of 2 cm^3 of distilled water and 1 cm^3 of DNS reagent (10 g of 3,5-dinitrosalicylic acid in 200 cm^3 of NaOH 2M was added to a 500 cm^3 solution of sodium potassium tartarate (300 g) and the volume was adjusted to 1l) and boiled for 5 min in a water bath. Distilled water (8.5 cm^3) was added to each sample and, after mixing, the absorbance was read in a Jasco 7800 UV/VIS spectrophotometer at 540 nm. Standard curves were obtained with glucose solutions.

2.2.5. Preparation of starch microcapsules

The microcapsules preparation method was a modification of the one described by Artursson *et al.* [2]. The modified starch was dissolved in 0.1 M phosphate buffer pH = 7.0 containing potassium persulphate (54 mg) and the DPGDA. This solution (2.5 cm^3) was added to 150 cm^3 of chloroform:toluene (1:4) with PluronicF68 (0.5 g) and stirred magnetically to produce a water-in-oil emulsion. The reaction was accelerated by adding TEMED to the emulsion. During the reaction time the temperature was kept at 50 °C and nitrogen was bubbled through the system. The microcapsules were separated by centrifugation and washed several times with 0.9% NaCl solution.

3. Results and discussion

In the derivatization reaction of starch with azolactone (I), vinyl end groups were chemically introduced in the chains of the polysaccharide as indicated below.



This modification enables polymerization and the consequent preparation of microparticles.

In order to determine the yield of vinyl groups in the modified starch, this polymer was characterized by ^{1}H-NMR. As an example, Fig. 1 shows the modified starch when 18.8 mmol of azolactone was used. The chemical shifts of the CH_3 groups to the azolactone are at 1.5 ppm, while the anomeric H of the starch is at 5.3 ppm. The percentage of substitution was determined by integration of ^{1}H-NMR resonances of those chemical groups. From these calculations, we obtained a value of 38.6% substitution.

The results are very promising, showing that this method gives higher substitution degrees (DS) than those previously reported [1, 2], where the starch was modified by using either glycidyl methacrylate or acryloyl chloride.

The starch, modified starch and microcapsules were characterised by DSC. The results, shown in Table I, suggest that starch crystallinity decreases with sample modification. It is also seen that the melting temperature is higher when microspheres are analysed. This could be due to the crosslinking reaction.

As was stated earlier, microspheres from this modified starch were prepared and analysed by optical microscopy. Fig. 2 shows that the particles obtained are transparent, and spherical in shape with a mean particle size of 150 μm.

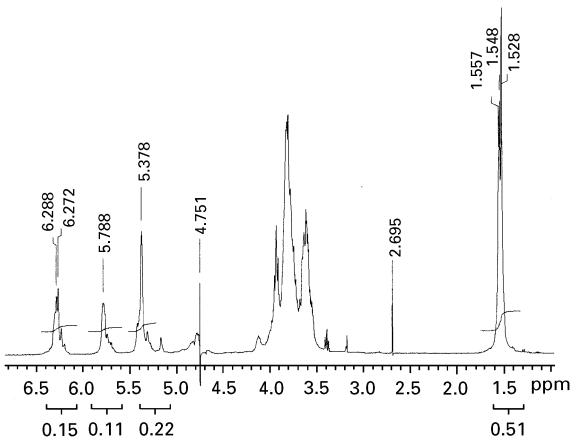


Figure 1 ^{1}H-NMR spectra of modified starch obtained with 18.8 mmol of azolactone.

TABLE I DSC characterization of starch, modified starch and microspheres.

Sample	Enthalpy (cal/g)	Fusion (°C)
Soluble starch	81.22	136.40
Starch (40% DS)	73.08	117.02
Starch (76% DS)	83.98	112.62
Microcapsules	107.27	123.84

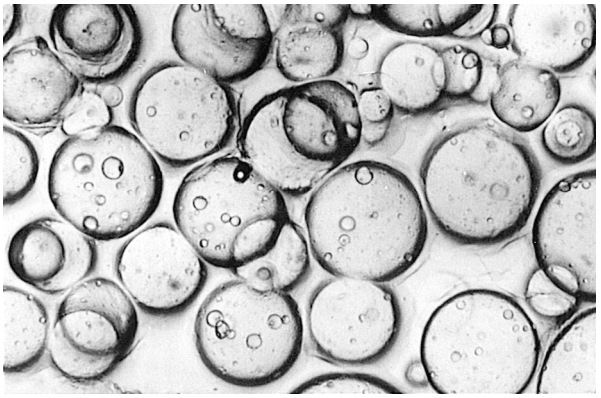


Figure 2 Microspheres from the modified starch (30% substitution). ($\times 100$)

4. Conclusions

From the results obtained it is suggested that this new material is very suitable for the preparation of microcapsules, and that these systems could be used as drug delivery systems.

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