Enzymatic Degradation of Epichlorohydrin Crosslinked Starch Microspheres by α-Amylase

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Purpose. The influence of chemical parameters on the sensitivity to enzymatic degradation by α-amylase of starch microspheres cross-linked by epichlorohydrin was studied.

Methods. Starch microspheres were prepared using epichlorohydrin as a crosslinking agent. Their swelling degree, reflecting the number of glycerol diether bridges in the polymeric network, and the number of non-crosslinking monoglycerol ether groups corresponding to a side-reaction of epichlorohydrin with starch were determined. Degradation rates of the microspheres in presence of porcine α-amylase were determined by a microvolumetric method.

Results. Degradation by α-amylase was surface-controlled and could be modulated by the introduction in the polymeric network of: (i) non-hydrolysable α-1,6 bonds related to the presence of amylopectin in the raw starch, (ii) glycerol diether and, (iii) monoether groups, all of these being likely to block the activity of α-amylase. In the case of highly cross-linked microspheres, the number of glycerol monoether pendent chains had a predominant effect on the degradation rate which ranged between 10^{-2} and 10^{-5} min^{-1}.

Conclusions. It was possible to modulate simultaneously the swelling degree and the enzymatic degradability of starch microspheres by adjusting the chemical parameters during the crosslinking reaction.

KEY WORDS: starch microspheres; epichlorohydrin; crosslinking ratio; glycerol monoether pendent groups; porcine α-amylase; degradation rate.

INTRODUCTION

Microspheres as controlled-release drug delivery systems have seen an extensive development during the past 20 years. However, their formulation can be sometimes delicate because many pharmaceutical applications necessitate conferring simultaneously a series of features to the particles, including size distribution, high drug entrapments, ability to control drug release and biodegradability. Microspheres can be made either of swellable or non swellable polymers. Swellable microspheres are attractive for many applications because the swelling of the polymeric network constituting the microspheres can be used to control the release of an active drug (1–6) and/or to ensure specific properties, such as mucosal bioadhesivity (3).

Swellable microspheres are commonly manufactured by crosslinking different types of polymers, including proteins (4), polysaccharides (1,3,5,6) and other hydrosoluble polymers (2) under dispersion conditions. The crosslinking reaction creates a new polymeric structure by introducing bridges between polymeric chains. The conditions of this chemical reaction are likely to affect considerably the swelling properties of the microspheres in biological media and their biodegradability. Various studies have shown the polymer concentration (4–6), the nature of the crosslinking agent (7), and obviously the crosslinking density were likely to modulate the degradation rates, the swelling (rate and degree) and the drug release mechanism.

Starch microspheres have been proposed for embolization (8), parenteral administration (9), and nasal administration (3). Starch microspheres can be conveniently prepared by the action of epichlorohydrin on starch under alkaline conditions. However, as for many cross-linking agents, the reaction of epichlorohydrin with starch is complex in nature and depends on the experimental conditions (10–15). Therefore, the aim of this work was to study the influence of chemical parameters on the swelling properties and the sensitivity to enzymatic degradation of starch microspheres cross-linked by the action of epichlorohydrin. For this purpose, microspheres were prepared by an inverse emulsion technique and attempts were made to correlate the reaction parameters with the structural characteristics of the hydrogel network constituting the microspheres and the degradation pattern of the microspheres by α-amylase.

MATERIALS AND METHODS

Materials

Soluble maize starch (Glucidex 6®) was supplied by Roquette Frères (Lille, France). The corresponding dextrose equivalent number was comprised between 5 and 9, as indicated by the manufacturer. Starch molarities were expressed as anhydro glucose units (AGU, Mw = 180). Epichlorohydrin (ECH, Mw = 92.53), solvents, and reagents were purchased from Prolabo (Paris, France) and were analytical grade. Sorbitane monooleate was supplied by ICI. Phosphate buffer (pH = 7.4) supplemented with calcium (0.132 g/l) was obtained from GIBCO (Eragny, France). Porcine pancreatic α-amylase with Mw = 56 000 Da, formaldehyde solution (37% in water), sulphuric acid, disodium 4, 5 dihydroxy-naphathalene disulfonate, sodium arsenite ($NaAsO_2$) were supplied from Sigma (St Quentin-Fallavier, France).

Preparation of Starch Microspheres

Cross-linked starch microspheres were prepared according to an inverse emulsion technique (16). An aqueous phase was prepared by dissolving a suitable amount of soluble starch (Glucidex 6) in a 2 N sodium hydroxide water solution under mechanical stirring. The aqueous phase was emulsified in 100 mL of a cyclohexane-chloroform mixture (4.1:v/v) containing 2% (v/v) of sorbitane monooleate. This emulsion was homogenized by high speed mechanical stirring (9500 rpm) for 3 minutes (Ultra Turrax T25, Janke et Hunkel). Further, a suitable amount of epichlorohydrin (ECH) was added under magnetic stirring at 600 rpm. The stirring was maintained for 18 hours at 40°C. Microspheres were then isolated by centrifugation, washed, and freeze dried.

Different types of microspheres were prepared by adding different ECH concentrations ranging from 1.6 to 10% v/v of organic phase. The crosslinking ratio [ECH/AGU] was expressed as the number of epichlorohydrin molecules (ECH)

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to the number of anhydro glucose unit (AGU). This ratio ranged from 0.24 to 3.73

Equilibrium Swelling Degree

The equilibrium swelling degree (ESD) of the starch microspheres was determined by swelling a suitable volume of dried crosslinked starch microspheres in 5 mL PBS, overnight in a measuring glass. The ESD (mL/g) was expressed as the ratio of the swollen volume V_s to the mass of dried starch microspheres m_d.

Chromotropic Acid Reagent

A chromotropic acid reagent was prepared by adding 150 mL of concentrated sulphuric acid to 100 mL of distilled water under stirring in a flask immersed in an ice bath. 1.13 g of disodium 4,5 dihydroxy-naphthalenedisulfonate were dissolved in 100 mL of distilled water and further added to the acidic phase. The reagent was further filtrated.

Determination of Starch Glycerol Monoether Groups

The number of glycerol monoether groups formed during the reaction process was determined by periodate oxidation of cross-linked starch and quantitative determination of the formaldehyde produced during the oxidation of the diol end groups by a chromotropic method adapted from (17).

Briefly, 0.03 g of starch microspheres were treated with 5 mL of 0.1 M sodium metaperiodate. This solution was kept in the dark at 50°C for one hour, until complete oxidation. Then, the solution was acidified with 1 mL of 5 M sulfuric acid and added with 5 mL of 1 M sodium arsenite. The solution was finally diluted to 50 mL with distilled water. The cross-linked gel oxidized residues were separated from the liquid by filtration of the reaction mixture. Further, aliquots were rapidly mixed with 10 mL of the chromotropic acidic reagent. The mixture was heated in a boiling water-bath for 30 min. The solution was removed and cooled at room temperature, and the absorbance was read at 570 nm in a spectrophotometer.

In Vitro Degradation Studies

Degradation studies were performed as described previously (16). Briefly, a suspension of microspheres (800 μg in 100 mL of phosphate buffer saline supplemented with calcium and 100 IU/L streptomycin) was agitated and placed in the stand of a Coulter counter (Multisizer, Coultronics, Margency, France) equipped with a 50 μm orifice tube. Measurements were performed in manometer mode by analysing 100 μl of the particles suspension. Countings were regularly performed resulting in the determination of the volume size distributions before and after addition of 50 IU/L of α-amylase. The total volume of the particles before degradation, V_0, and the remaining volume during degradation, V_t, were determined.

Scanning Electron Microscopy

Scanning electron microphotographs of microspheres were obtained after stopping their degradation by addition of EDTA at preset times (palladium/gold coating, Jeol instrument, JSM-840A).

RESULTS

Influence of the Crosslinking Ratio and Starch Concentration on the Equilibrium Swelling Degree

Three series of microspheres corresponding to three starch concentrations (2, 4, and 9 mole AGU/L) were prepared by varying the amount of epichlorohydrin (ECH) added to the organic phase (1.6, 2, 5 to 10% ECH/organic phase (v/v). The concentrations of ECH were expressed as molar ratios between ECH and starch which was expressed as anhydro glucose units (AGU). The different molar ratios are tabulated in Table I.

Microspheres were obtained as non-aggregated white powders after freeze-drying. Observations by light microscopy gave evidence that freeze-dried microspheres were intact. Scanning electron microphotographs indicated that microspheres were perfectly spherical for any preparative conditions. Relatively narrow size distributions were obtained and the mean size ranged from 13 to 34 μm for the different batches. After water swelling the microspheres were transparent and remained spherical.

The extent of crosslinking was estimated by measurement of the equilibrium swelling degree (ESD) by a volumetric method. Swelling degrees ranged from 3.9 to 22 mL/g. As shown in Fig. 1, an increase in the crosslinking ratio caused a decrease in the equilibrium swelling degree for each starch concentration. When the crosslinking ratio was raised, the ESD of the microspheres decreased due to an increase in the number of bridges of the glycerol diether groups between the polymer chains. For high starch concentrations (4 and 9 mol AGU/L), the effect of ECH on the ESD was very pronounced when the crosslinking ratio was less than 1. On the contrary, when the starch concentration was 2 mol/L, an increase in the concentration of ECH had less influence on the ESD.

For a given starch concentration, it could be assumed the ESD reflected the amount of cross-links formed during the reaction of ECH with starch. From Fig. 1, it was obvious the ESD was governed by the different interdependent molar ratios set during the crosslinking reaction. From a practical point of view, it could be concluded it was possible to produce microspheres of a given equilibrium swelling degree by adjusting at the same time the initial starch concentration and the ECH/AGU ratio. Comparable results have been reported by Kuniak *et al.* (13) for mass polymerization experiments conducted under similar conditions. Additionally, the present results showed that the data gained in the chemistry of starch and epichlorohydrin under homogeneous reaction conditions could be transposed to reactions occurring in disperse (heterogeneous) conditions.

Influence of the Crosslinking Ratio and Starch Concentration on the Formation of Monoglycerol Ether Units

The reaction of ECH on starch may result not only in the cross-linking of starch by glycerol diether groups but also in the monoetherification of starch by ECH. In this case, the formation of cross-linking glycerol diether groups and mono-substituent glycerol monoether (GME) groups is the result of a competition between starch and water (12). As depicted in Fig. 2, for a given [ECH/AGU] ratio, the formation of glycerol monoether groups was favoured by an increase in the water/starch ratio. As expected when the water/starch ratio maintained

Table I. Influence of the Reaction Conditions on the Formation of Glycerol Monoether Units (GME) and on the Enzymatic Degradation by α-Amylase

AGU mol/L	ECH added %	ECH/AGU mol/mol	H_2O/AGU mol/mol	Size μm	Polydispersity μm	Degradation rate × 10^{-2} (min^{-1})	AGU/GME mol/mol
2	1.6	0.6		16 ± 5	13 ± 5	0.773	183
	2.5	0.93	27.45	13 ± 3	8 ± 4	0.044	158
	5	1.86		14 ± 4	10 ± 5	0.002	131
	10	3.73		11 ± 2	11 ± 2	0.008	123
4	1.6	0.37		24 ± 3	20 ± 4	0.773	356
	2.5	0.58	13.7	23 ± 2	19 ± 3	0.658	317
	5	1.16		20 ± 3	14 ± 5	0.141	262
	10	2.33		15 ± 5	14 ± 5	0.025	241
9	1.6	0.24		34 ± 3	13 ± 3	1.200	807
	2.5	0.39	11.43	32 ± 5	14 ± 4	1.055	639
	5	0.77		29 ± 5	12 ± 6	0.827	586
	10	1.55		27 ± 3	9 ± 3	0.702	356

Note: ECH, epichlorohydrin; AGU, anhydroglucose units; GME, glycerol monoether units. (n = 3 replicates.)

constant, it was also favoured by an increase in the epichlorohydrin/starch ratio [ECH/AGU]. However, this effect was sensitive only when the concentration of starch in the water phase of the droplets was low.

Effect of the Crosslinking Ratio and Starch Concentration on the Enzymatic Degradation Properties

Degradation of the microspheres by α-amylase was assessed by a method based on microvolume measurements during the time-course of enzymatic degradation by a Coulter counter method as described previously (16). This method consisted in the measurement at different times of the volume of aliquots of a suspension of microspheres in presence of α-amylase. The degradation process could be described by the ratio of (V_t/V_0) as a function of time, where V_t stands for the volume of the particles at time t and V_0 for the total volume of the particles at initial time (before degradation).

As expected, the volume decreased progressively with time (Fig. 3a, 3b, 3c). The volume decrease was markedly affected by the cross-linking conditions. The degradation was almost completed for the microspheres prepared with high starch concentrations (9 mole AGU/L) whatever the amount of epichlorohydrin. However, for batches prepared with lower starch concentrations (4 and 2 mole AGU/L), the degradation was slowed down when the concentration of epichlorohydrin was increased.

Degradation rates were obtained by linearizing the volume degradation profiles (16) by using equation (2) which assumes a surface degradation mechanism:

$$3\sqrt{\frac{V_t}{V_0}} = 1 - bt \qquad (1)$$

where t stands for the degradation time (min) and b for the degradation rate (min^{-1}). Whatever the preparation parameters, the volume degradation profiles could be linearized, indicating as discussed earlier (16) that the degradation of the microspheres by α-amylase was controlled by a surface erosion mechanism

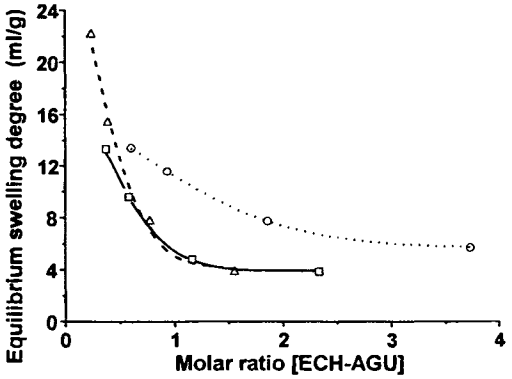

Fig. 1. Influence of the molar ratio between starch and epichlorohydrin [ECH/AGU] on the equilibrium swelling volume of the microspheres for three different starch concentrations: (○) 2 mole AGU/L, (□) 4 mole AGU/L, (△) 9 mole AGU/L.

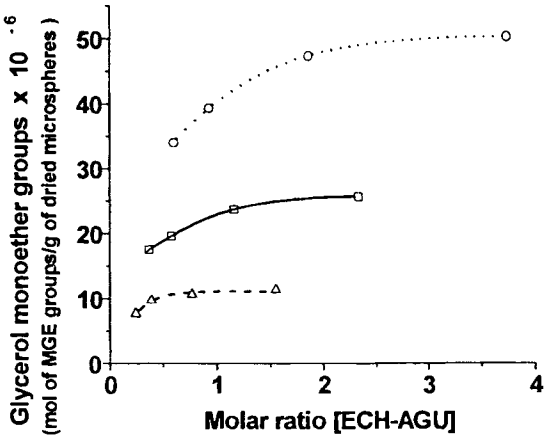

Fig. 2. Influence of the molar ratio [ECH/AGU] on the formation of glycerol monoether groups in the microspheres for three different starch concentrations: (○) 2 mole AGU/L, (□) 4 mole AGU/L, (△) 9 mole AGU/L.

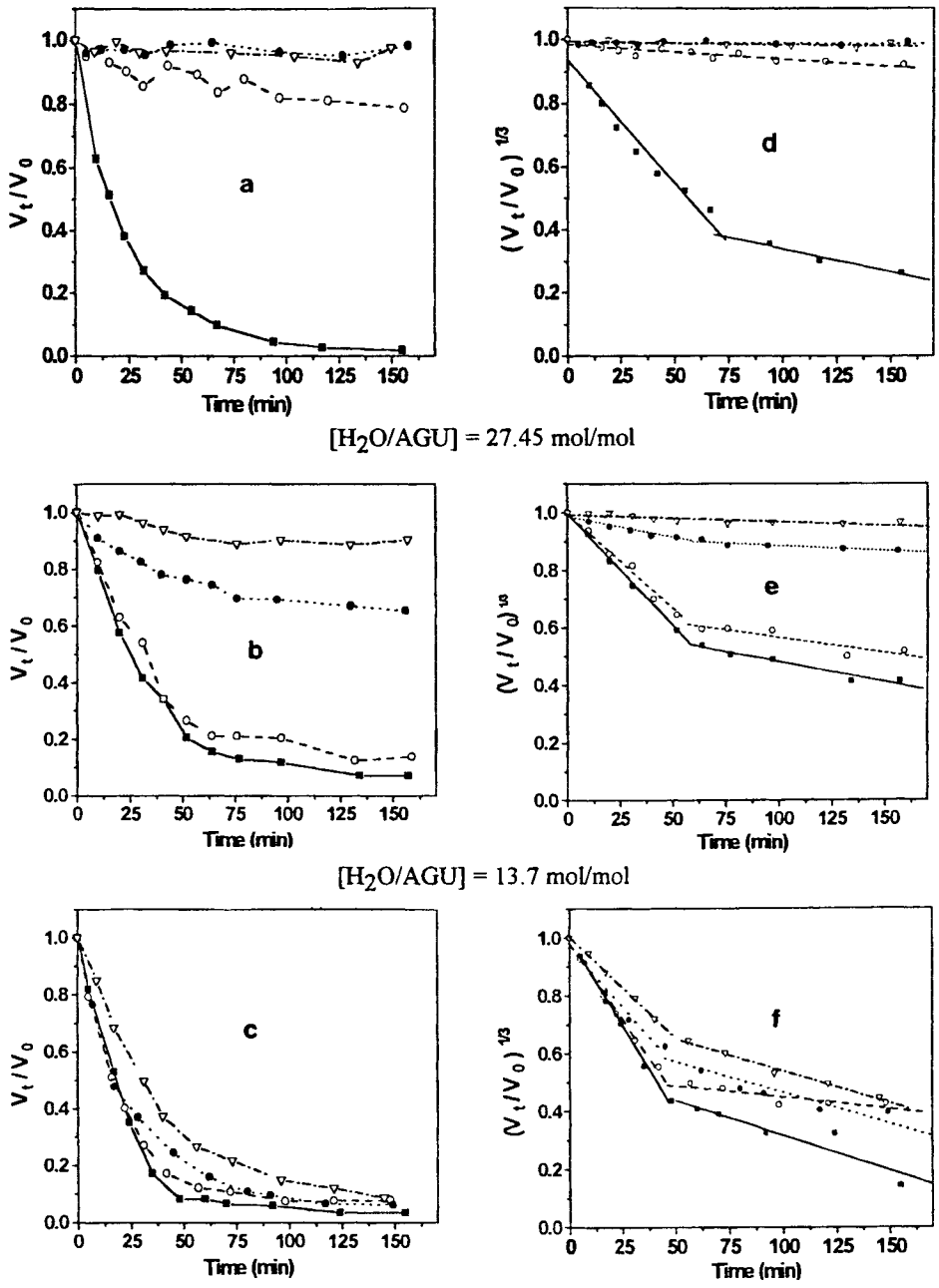

Fig. 3. Volumetric degradation profiles (a, b, c) and linearized degradation profiles (d, e, f) of starch microspheres crosslinked with (■) 1.6 % ECH, (○) 2.5% ECH, (●) 5% ECH, (▽) 10% ECH in the presence of 50 IU/L porcine α-amylase as a function of time at different starch concentration.

(Fig. 3d, 3e, 3f). As stressed by Lindberg (8), it could be expected the degradation was simultaneously under the control of the size and the chemical structure of the microspheres. In the present case, as can be seen from Table I, the chemical structure of the microspheres had a very predominant effect on the degradation compared to the effect induced by size variation. The size distributions have been maintained as close as possible from one batch to the other in order to minimize any surface-related effect. Moreover, as an estimate, it can be calculated that the specific surface of the different batches varied 3 fold, which should induce a similar variation in the degradation rate if the microspheres structure was kept constant. Simultaneously, it can be observed that the degradation rates varied 600 fold, suggesting the degradation was mainly driven by the chemical structure of the microspheres.

Except for slowly degrading microspheres, the linearized degradation profiles were biphasic (Fig. 3d, 3e, 3f). As discussed earlier, this effect was attributed to the polydispersity

in the initial size distribution of the microspheres (16). Degradation rates were obtained from the slopes of the initial linear portions of the degradation profiles and were used further for characterizing the degradation properties of the microspheres. This was justified by the following considerations. Firstly, it was preferable to use the initial degradation rate, in order to avoid any possible inhibition of the α-amylase activity by the degradation products. Secondly, the initial portion of the profiles was representative of the major part of the initial volume of the microspheres (70–90%).

The hypothesis of surface erosion of the microspheres has been confirmed by microphotographs taken at different degradation times (Fig. 4). At initial time, the samples were constituted of spherical and polydisperse particles (dried size

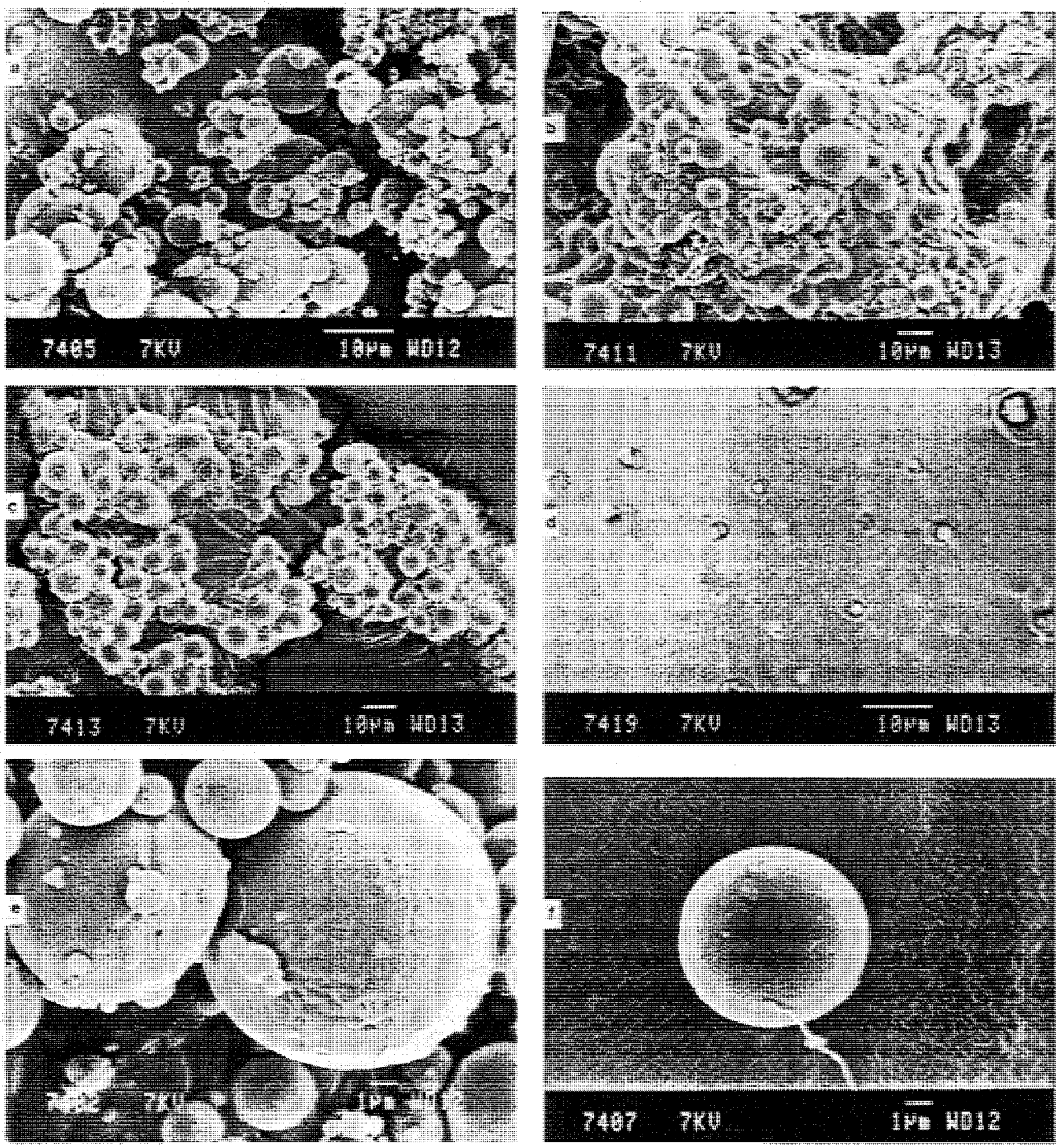

Fig. 4. Scanning electron microphotographs of starch microspheres (starch concentration of 4 mole AGU/L and 2.5% ECH) during their degradation by 50 IU/L of α-amylase, at different times: (a, e) before the degradation; (b, f) at 15 min; (c, g) at 60 min; (d, h) at 150 min.

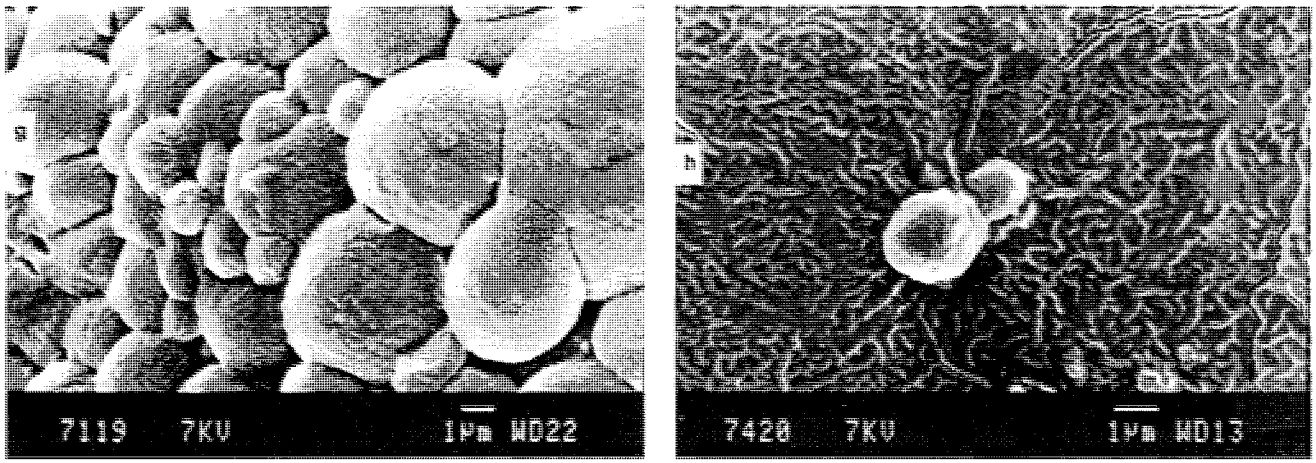

Fig. 4. Continued.

ranging from 0.3 μm to 12 μm)(Fig. 4a, 4e). A size decrease during degradation was obvious from the first column (Fig. 4b, 4c 4d) while no topographical changes were observed in the surface (Fig. 4f, 4g, 4h). After 60 min (Fig. 4c, 4g), the small microspheres disappeared and no holes could be noticed on the surface. At the end of the degradation, the number of microspheres which were apparent on the observation fields was decreased. No fracture of the microspheres was observed during the degradation process, supporting the finding that degradation was controlled by surface erosion rather than enzyme diffusion into the network of the microspheres

Influence of the Crosslinking Degree and the Glycerol Monoether Pendant Chains on Enzymatic Degradability

Figure 5 presents the relationship between the swelling degree, which reflects the cross-linking degree, and the degradation rate of the different starch microspheres. As expected, the degradation of the microspheres in presence of α-amylase was the faster as the swelling degree was increased. However, the swelling degree was not the only parameter governing the degradation. Microspheres prepared with high starch concentrations (9 and 4 moles AGU/L) were degradable whatever the swelling

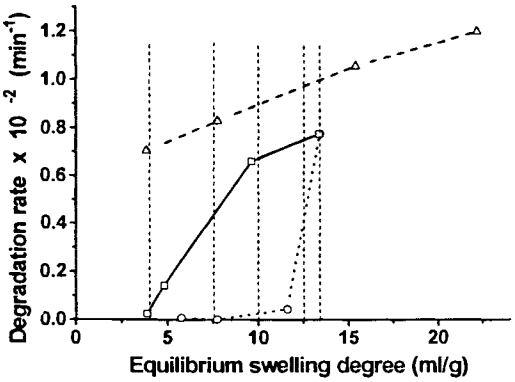

Fig. 5. Dependence of the degradation rate on the equilibrium swelling degree for three different starch concentrations: (○) 2 mole AGU/L, (□) 4 mole AGU/L, (△) 9 mole AGU/L.

degree. On the contrary, when prepared with a low starch concentration (2 moles AGU/L) the degradation rate was almost nil, excepted for high ESD. From a practical point of view, this result clearly demonstrated the possibility of a modulation of the degradation rate of the microspheres exhibiting a given swelling degree, by simply varying the chemical parameters of the cross-linking reaction. As an example, for a swelling degree value of 5 mL/g, three different types of microspheres could be obtained, which possessed degradation rates ranging between 0.008 10^{-2} and 0.773 10^{-2} min^{-1}, respectively.

As shown in Table I, an increase in [ECH/AGU] ratio resulted in an increase in the amount of glycerol monoether groups. Because GME groups could contribute to the blocking the degradation by α-amylase, the degradation rate was plotted as a function of GME groups (Fig. 6, insert graph). It can be seen that for a given starch concentration, the degradation rate dropped sharply suggesting the creation of pendant chains on the starch backbone slowed down the degradation. However, it was not the only parameter governing the degradability of the microspheres. It should be remembered the production of GME groups was due to an increase in the [ECH/AGU] ratio, leading at the same time, to an increase in the number of cross-links in the hydrogel network.

If one assumes the ESD reflects the number of cross-links in the microsphere networks, it should be possible to evaluate the specific role of GME groups on the degradation rate by plotting the degradation rate as a function of GME groups for different microspheres of given cross-linking degree. A verification has been attempted in Fig. 6 (main graph) by tracing the curves corresponding to iso-equilibrium swelling degrees. Because it was time-consuming to obtain experimentally microspheres characterized by the same ESD but prepared under different cross-linking conditions (by varying at the same time the [ECH/AGU] ratio and the starch concentration), in order to increase the number of pendant GME groups, degradation rate values were interpolated for different given ESD values in Fig. 5 and reported in Fig. 6 as a function of GME groups. When examining the different iso-equilibrium swelling degrees, it appears the role of the pendant GME groups depended on the level of the ESD, that is to say on the importance of the number of cross-links. For high ESD (e.g., 12), the number of cross-links

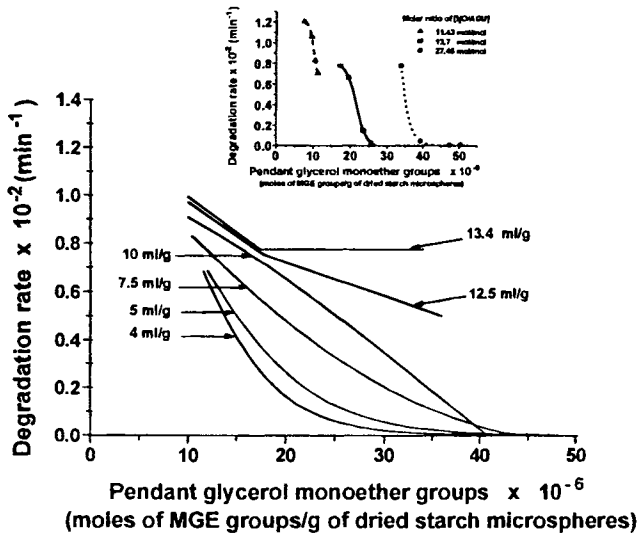

Fig. 6. Dependence of the degradation rate on the amount of pendant glycerol monoether groups MGE for iso-swelling microspheres (data extrapolated from Fig. 5). Insert: Influence of monoglycerol ether groups on the degradation rate for three series of microspheres prepared with three different starch concentrations: (●) 2 mole AGU/L, (■) 4 mole AGU/L, (▲) 9 mole AGU/L. This apparent effect corresponds to the simultaneous introduction of pendant glycerol monoether and glycerol diether groups in the polymeric network of the microspheres.

which could act as blocking groups for α-amylase was probably low. Therefore, the degradation rates were relatively high. Under these conditions, the grafting of GME pendant groups on the starch chains, which could also block the enzyme activity, had little effect on the degradation rate. On the contrary, low levels of ESD (e.g., 5) corresponded to the introduction of numerous cross-links in the hydrogel structure. In this case, the introduction of additional GME blocking groups in the structure had a drastic effect on the degradability of the microspheres, leading to very low degradation rates for high levels of GME groups. Therefore, it could be concluded the degradability of the microspheres depended not only on the introduction of cross-links in the hydrogel network, but also on the grafting of GME groups. The

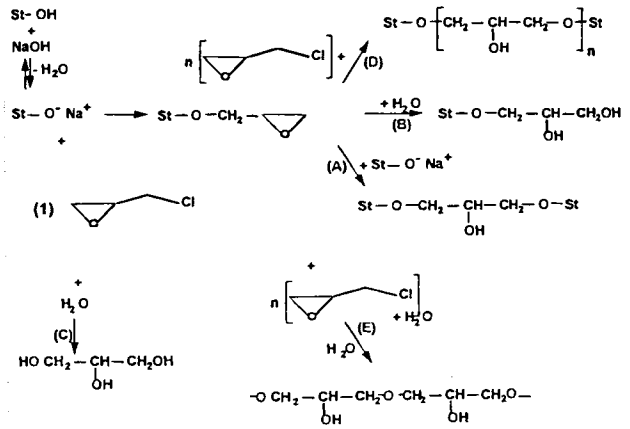

Fig. 7. The different reactions of epichlorohydrin with starch: (A) crosslinking, (B) formation of glycerol monoether, (C) hydrolysis of ECH, (D) crosslinking with more than one ECH unit, (E) polymerization of ECH. (St-OH = starch, (1) = epichlorohydrin).

relative importance of these factors depended on the chemical parameters adopted during the cross-linking reactions.

DISCUSSION

Reaction of Epichlorohydrin with Starch Under Disperse Conditions and Resulting Structural Characteristics of the Microspheres

Epichlorohydrin is a widely used bifunctional cross-linking reagent from the family of oxiranes which reacts very easily with hydroxyl groups. The reaction of epichlorohydrin with starch has been extensively studied, but mainly under homogeneous conditions (13–15). The reaction is very complex, due to the possibility for epichlorohydrin to react with structurally similar hydroxyle on the same substrate molecule (single substitution), on two different substrates molecules (cross-linking), or on itself (polymerization). Additionally, epichlorohydrin can react with water hydroxyl groups (hydrolysis) resulting in the production of glycerol.

Theoretically, the cross-linking reaction of epichlorohydrin with the hydroxyl groups of starch contains three steps (15). In the first step, starch in alkaline solution reacts as a week acid and forms rapidly a starch anion. In a second step, an ECH molecule is subjected to the nucleophilic attack of the anion starch. The epoxide ring opens up resulting in the formation of a new epoxide starch macromolecule with the release of the sodium chloride molecule (NaCl). In the third step, another molecule of sodium hydroxide reacts in a similar manner as in the first step giving rise to crosslinked starch. The ECH molecule forms either a bridge (A) between two starch macromolecules, or a glycerol monoether (B) (Fig. 7). In some cases di- or tri-glycerol diethers can be formed (D) (18).

Due to the complexity of the reactional medium it has not been possible in the present study to determine the overall amount of ECH which had reacted during the reaction process. However, under homogeneous reaction conditions and for similar molar ratios (or heterogeneous conditions in aqueous phase), Kartha *et al.* (14), found 80–100% of the initial ECH was involved in the different reactions depicted above. Similarly, the amount of ECH hydrolyzed remained less than a few percent whatever the experimental conditions.

Glycerol diether cross-linking groups and pendant monoether chains were formed simultaneously during the reaction process. As expected, the balance between the formation of the glycerol diether and glycerol monoether was the result of a competition between the nucleophilic anion StO^- and HO^-/H_2O, as shown by the influence of the water/starch ratio on the ESD and the formation of GME groups. It has been pointed out that which both of the two will be formed depends upon the abundance as well as the nucleophilicity of these species (11–14). Additionally, as observed for many polymers (1,2), an increase in the cross-linking ratio ECH/AGU and in the starch/water ratio resulted in less swellable systems because of an increase in the number of diether bridges. As a side reaction, it favoured also the formation of GME groups.

Influence of the Structural Characteristics of the Microspheres on the Enzymatic Degradability

The enzymatic degradation of the microspheres by α-amylase was strongly affected by the structural characteristics

of the hydrogel formed during the cross-linking process. Enzymatic degradation comprises three steps: (i) fixation of the enzyme on the substrate starch chain, (ii) catalytic hydrolysis and (iii) release of the hydrolysis products.

The porcine α-amylase is an enzyme (MW = 56 000 Da) catalyzing the hydrolysis of internal α-1, 4 glycosidic bonds in glucose polymers. It has been postulated from X-ray crystallographic studies that the active site of α-amylase is composed of 5 to 11 subsites (5 in the case of the porcine α-amylase) corresponding to identified loops in the protein chain which are able to bind each to a glucose unit in a α-1, 4 glucopyranose polymer (20). The fixation of the porcine α-amylase on the substrate necessitates the presence of at least 5 glucose units linked by α-1, 4 bonds. However, it results only in the hydrolysis of one α-1, 4 glycosidic bond. In order to continue the hydrolytic process, the enzyme can either slide along the substrate macromolecular chain without dissociation (multiple attack) or dissociate from the substrate directly after the hydrolysis (preferential attack) (21). Whatever the mechanism, the hydrolytic activity is stopped when the enzyme encounters a modification in the α-1, 6 glycosidic chain, such as an α-1, 6 bond in amylopectin (19) or an other chemical modification.

The starch used in the present study was a partially hydrolyzed starch containing amylopectin which is the main constituent of starch granules and also dextrins. Amylopectin is a branched molecule containing 5% of α-1, 6 glucopyranose clustering a large amount of short α-1, 4 chains (average degree of polymerization 20) (19). Due to a partial hydrolysis of the starch during the manufacturing process, the global degree of polymerization of the α-1, 4 linked segments was probably low. In such conditions, the hydrolysis of the microspheres was likely to result in the production of limit dextrins, which have been shown to be competitive inhibitors for the adsorption of α-amylase of *B. licheniformis* and *B. subtilis* (22). However, as shown by experiments with highly degradable microspheres, the amount of limit dextrins or degradation oligomers produced during degradation studies was unable to block the activity of α-amylase.

More likely, the degradability of the microspheres depended on the structure of the hydrogel network in the swollen microspheres. Different chemical modifications or groups were able to block the degradation process. The introduction of additional groups in the structure during the manufacturing process was likely to block completely the enzymatic activity of α-amylase. As observed in the degradation studies, the glycerol mono- and diether groups introduced during the cross-linking process were able to block also the hydrolytic process. The number of these groups which were introduced in the macromolecular chains was rather low (e.g., the number of glucose for one monoglycerol ether group ranged between 800 and 120). However, it can be concluded this amount was critical to modulate the degradability of the microspheres.

CONCLUSIONS

The results presented in this study demonstrate it was possible to induce structural modifications in the hydrogel structure of starch microspheres, leading to modifications in the cross-linking and introduction of monoglycerol ether groups in the starch chains. Interestingly, these internal changes could be obtained without varying the equilibrium swelling degree of the particles, which is a major parameter for many practical applications of those microspheres. Moreover, it has been shown the degradation of the starch microspheres by α-amylase was surface controlled. The rate of degradation depended on the number of blocking groups introduced in the hydrogel network, including cross-links and pendant glycerol monoether groups. As a result, the degradation rates of the microspheres could be easily modulated by varying the cross-linking conditions without varying their overall swelling properties, which make these particles convenient tools for drug delivery.

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