J.J.G. VAN SOEST*, Y. DZIECHCIAREK, A.P. PHILIPSE

STARCH-BASED MICROPARTICLES: A PRELIMINARY STUDY OF THE STRUCTURE AND PROPERTIES

Abstract

Starch is a cheap and abundant polysaccharide, which is found in nature as water insoluble semicrystalline granules with sizes in the range of 0.5-70 µm. Although starch is easily gelatinised or dissolved in water, it is not possible to obtain stable suspensions or colloidal systems from native starches. This inherent disadvantage of starch has limited its applications.

In this study potato starches were processed to obtain fully biodegradable microparticles, which behave as microgels or colloids in aqueous suspensions. The synthesis process is based on the unique combination of gelatinization and cross-linking performed in water-oil emulsions. The obtained starches are very stable in water and show an interesting shear-thinning behaviour even at high solid contents. In particular, the rheological behaviour of the new starches is unique. The starches offer new possibilities for preparing starch colloids with a range of properties. A range of starch microparticles was obtained opening the door to numerous food and non-food markets (paints and coatings, inks and pigments, superabsorbent polymers, food additives, personal care products, pharmaceuticals, ceramics, paper additives, adhesives, thickeners, emulsifiers, ...).

In order to make a wide-scale industrial use of these new materials possible, it is necessary to acquire detailed knowledge about the structure and properties of the prepared particles. By a multidisciplinary approach, a start was made on their process-structure-property relationships. The final goal of this work is to establish the relationships between synthesis parameters and the structural, colloidal and rheological features.

Particles were prepared using epichlorohydrin and trisodium trimetaphosphate as cross-linkers. In this paper important reaction parameters, such as temperature, time and composition of the reactants (starch, cross-linker, hydroxide), which influence the structure of the microparticles during synthesis, were identified. Using Bohlin reometry the formation of the starch network structure was studied. Particle sizes of the microgels are in the range of 60 nm up to 10 µm. The synthesised particles were slightly negative (in the range of - 5 to - 45 mV). Features such as size and charge of the particles depended on the type and amount of cross-linker used. Descriptions of the rheological properties of starch-based microparticles in aqu-

---

*corresponding author.

*Agrotechnological Research Institute (ATO), P.O. Box 17, 6700 AA Wageningen, The Netherlands. E-mail: J.J.G.vanSoest@ato.wag-ur.nl[1]

A.P. Philipse, Van't hoff Laboratorium for Physical and Colloidal Chemistry, Padualaan 8, 3584 CH Utrecht, The Netherlands.

eous suspensions, both in dilute and concentrated systems, were given. The microgel-type particles showed a behaviour that is typical for (slightly) charged materials or polyelectrolytes.

Introduction

Fully biodegradable starch-based microparticles only became the subject of detailed studies over the last decades [1, 2]. Microparticles can be applied in a range of products. Typical applications are: copying paper, detergents, adhesives, cosmetics, foods, biomedical and pharmaceutical products, controlled release systems, ceramics, et cetera [3-6]. Microparticles give the opportunity to obtain unique colloidal and rheological properties when applied in suspensions or emulsions. The stability of suspensions is increased and mixing of immiscible ingredients is facilitated. Starch and most starch derivatives are favoured over synthetic polymer based materials because they are biodegradable and biocompatible [7].

Most native starches are composed of 20–30% amylose, an almost linear polysaccharide consisting of α-1,4-linked anhydro-glucose with a molecular mass of 10^3–10^6 g/mol, and of 70–80% amylopectin, a highly branched, high molecular mass (10^8 g/mol) polymer consisting α-1,4 linked anhydro-glucose and α-1,6 linked anhydro-glucose at the branching points [8]. Native starches are isolated from various sources like roots, tubers and seeds in the form of semicrystalline, cold-water insoluble granules. At present, the use of starches for many applications is limited. Native starches need to be heated before use to free the starch polymers. Gelatinized or melted starches show gelation, phase separation, retrogradation or crystallisation. Products from starch show problems with regard to their stability during storage or use. The properties of starch-containing materials are highly water and temperature sensitive. It is not possible to obtain highly stable aqueous starch suspensions from unmodified starches. Processing of starches is difficult because of the high viscosity and shear-thickening of starch already at low solid contents. The range of functional properties of the starches is limited even for chemical modified starches with high degree of substitution [9, 10].

Preparation of microparticles dates back to the fifties, where they were used in carbon-less copying paper [11]. Several manufacturing methods were developed since then using a broad range of monomeric as well as polymeric starting materials [12]. In particular, emulsion polymerisation and cross-linking (X-linking) methods are used and well understood for synthetic materials but also several biomaterials, such as agarose and cellulose [13-14].

There are several options available for preparing starch-based microparticles based on water-in-oil emulsion X-linking. In the first option, soluble starches are grafted with active groups, which can be X-linked. Usually this renders the materials nonbiodegradable or non-biocompatible. The second route makes use of chemically modified starches, such as carboxy methyl starch, containing groups, which can be internally X-linked by heat-treatment or complexation with salts. These materials are usually not very stable in aqueous environment. In the third route, the hydroxyl groups of the starch chains or other chemically introduced groups react directly with a X-linking agent in a water-in-oil emulsion. An extensive list of X-linkers is known for starches among which phosphoryl chloride, epichlorohydrin (ECH) and trisodium trimetaphosphate (TSTP) [15-17].

The X-linked starch microparticles are swellable in water and can be considered as microgels. Although no detailed studies have been performed on the influence of structural features, such as the influence of X-link density on rheological properties, some studies have been performed over the last decades for instance poly(methyl methacrylate) microgel systems [18-19].

In this paper, microgel particle materials will be prepared using emulsion X-linking of potato starches. A start was made in characterising and understanding these materials. The preliminary results will be presented. The influence of processing conditions will be discussed in relation to the structure and properties.

Materials and methods

Materials – Starches (Native *(Farina)* potato starch (PN), Paselli SA2 (Pa2) and Paselli SA6 (Pa6)) were supplied by Avebe (Foxhol, The Netherlands). The moisture contents of the starches were determined gravimetrically as 17.5, 15.0 and 15.0% for PN, Pa2 and Pa6, respectively.

Preparation of the particles – Two main types of X-linkers were used in this study, namely TSTP and ECH. The typical lay-out of the preparation of the various microparticles is shown in Figure 1.

Route one was typically used to prepare only TSTP X-linked materials. Starch (*x* g) was dispersed in 250 ml demiwater (deionized water).by stirring. Subsequently *x* g of TSTP was added as the X-linking agent. Cyclohexane (650 ml) was heated to 50°C after which 16.5 g Span-65 (sodium tristearate) was added. The hydrophobic and water phases were emulsified using a Branson emulsifier for 5 min. The white emulsion was stirred with a magnetic stirrer to prevent coagulation. During this *x* ml of 2 M sodium hydroxide (OH) was added. Reaction proceeded over night after which a stable slightly blue dispersion was obtained. Separation of the water phase from the hydrophobic phase was done with the addition of acetic or octanoic acid and shaking with excess water. The water phase was separated by centrifugation (Beckman centrifuge, Avanti J-251) at 10,000 rpm (3–4 times with intermediate washing with demiwater or sodium chloride solution). The purification was improved by adding ethanol. The white starch sediment was air-dried.

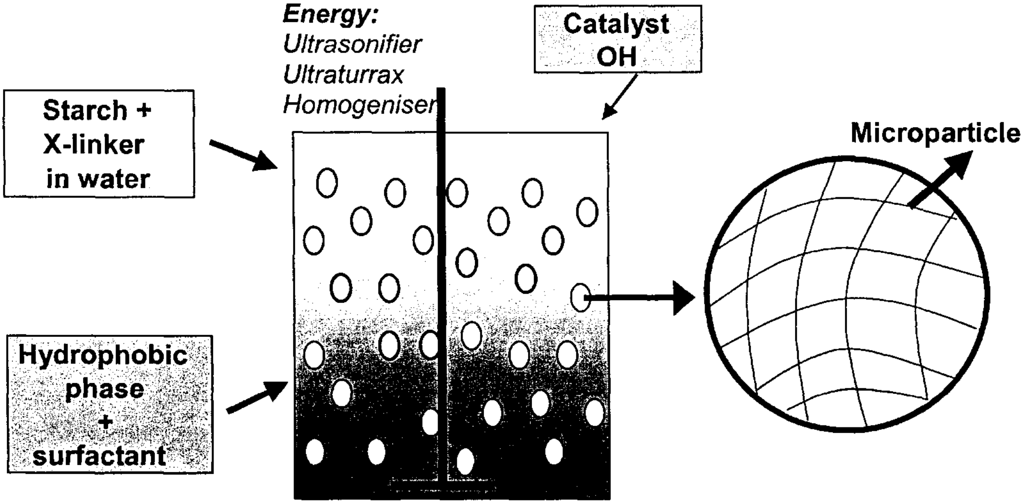

Fig. 1. General scheme of the synthesis route used for the preparation of microparticles.

ECH based (and some TSTP materials as comparison materials) particles were typically prepared by dispersing *x* g starch in 100 g OH (25 mM) aqueous solution. The mixture was stirred at 800 rpm until the starch was homogeneously distributed. ECH (*x* g) was added. After homogenisation, the water phase was poured into a 2-neck round-bottom flask containing ca. 5–7 g Symperonic NP5 (poly-oxyethylene nonphenyl ether) surfactant in 200 ml cyclohexane. An emulsion was obtained with an Ultra-Turrax (Janke & Junkel, IKA, type T25) initially operated at 13000 rpm. The speed was increased to 20000 rpm over a period of 10 min. The emulsion was cooled to ensure that the temperature was not higher than 45°C.

The reaction took place at room temperature under constant stirring (magnetic stirrer at 800 rpm). After 20 h, 250 ml demiwater/acetic acid was added to separate the cyclohexane phase from the water phase. The pH of the water phase was adjusted to 7. The aqueous milky suspension was centrifuged at 10000 rpm for 30 min. The particles were washed at least 3 times with demiwater. The washed materials were usually dialysed (membrane diameter 28.6 mm, MWCO 12–14 kDa, Fischer Scientific) at 4°C.

The exact compositions of the reactants are expressed in *x* grams of the various components used per 100 g water used in the emulsion during reaction. For example: the material referred to as **20Pa2-1ECH-1OH** means that 20 g of Paselli SA2, 1 g of ECH and 1 g NaOH was used during synthesis.

Structural analyses – Light microscopy was used to make rough estimates of the particle size and shape using a Zeiss axioplan MC1000 microscope. More detailed studies were performed using a transmission electron microscopy (TEM; Zeiss) and a cryo-scanning electron microscope (SEM; Philips type 5.15). An environmental-SEM (ESEM; Philips) was used to study the swelling of particles (containing 15% water) at various relative humidities (RH = 20–100%).

X-ray diffractometry (XRD) was performed on dry materials with a Philips PV-ARD diffractometer (model PW 3710) [20].

Dynamic light scattering (DLS) was performed at the University of Utrecht (van't Hoff Laboratory for Physical and Colloidal Chemistry) with an Ar laser at 541.1 nm (Spectra Physics Series 2000). Auto-correlation functions were measured with a Malvern multibit K7025 128 correlator at scattering angles between θ = 35–120°. Static light scattering (SLS) were also studied in Utrecht with a Fica-50 LS photometer using vertically polarised incident and detected light at a wavelength of 546 nm. The scattering angle was varied between 30–150°θ. The Guinier approximation was used to calculate the radius of gyration (R_g). The samples were filtered through a 10 μm millipore filter. All measurements were performed at 25°C.

Rheology – Dynamic oscillation viscosity measurements were performed with a Bohlin VOR Rheometer using a cylindrical geometry. The starch gels were covered with a thin layer of paraffin oil to prevent water from evaporating. By measuring the elasticity modulus (G') of the polymer solution versus time, during which the X-linking reaction reactions of TSTP and ECH with starch takes place, it is possible to estimate the number of X-links formed and the efficiency of the reaction [15-17].

High-shear rheological experiments were performed on concentrated suspensions (1–10% w/w) using a Contraves Rheomat 115 Viscometer at room temperature with a shear rate (γ) in the range of 1–500 s^{-1}.

Low shear viscosity measurements were carried out with a constant shear Contraves LS40 Rheometer in a MS 41/1S Couette geometry at a constant temperature (20.0 ± 0.1°C). The shear rate (γ) was in the range of 0.1–10 s^{-1}. Using a Finn pipette, 2 ml of the samples were put into the measuring device. The flow curves were measured successively for increasing and decreasing shear rates. The relative viscosity, η_r, was determined as the slope of a linear fit of the viscosity data for the dispersion, η_{disp}, and the solvent (*i.e.* water), η_{solv}:

$$\eta_r = \frac{\eta_{disp}}{\eta_{solv}} \tag{1}$$

Electrophoretic Light Scattering (ELS) – The Zeta-(ζ)-potential of the starch particles in salt-free and sodium or potassium chloride suspensions were measured with a Coulter Delsa 440 SX electrophoresis meter. From these measurements the ζ-potential was calculated using the Smoluchowski relation [21].

Results and discussion

X-linking reaction of starch

To be able to study the differences in the X-linking reaction as a function of starch and X-linker type and amounts, and the amount of hydroxide used, the G'-modulus was measured in time. This part of the research is important to understand the structural properties of the microparticles in terms of X-linking density and charge density of the hydrogel network. The assumption here is made that the particles can be visualised as microgels. In Figure 2 (top) some typical examples are shown of the increase in G'-modulus during reaction of starch (Pa-2) with ECH. The initial reaction rate is clearly dependent on hydroxide concentration. An increase in reaction rate is found with an increase in the OH concentration from 0.1 to 0.3 g/l. However at higher OH concentrations no further increase in the rate of the reaction is observed. The effect of starch and X-linker concentration and source shows a similar behaviour (data not shown) [16-17]. With increasing starch and X-linker concentration the initial reaction rate increases up to a certain starch concentration.

Usually a plateau or maximum in the G'-modulus is observed. The height of this maximum or plateau is determined by the composition of the reactants and temperature of the reaction. A decrease in the G'-modulus is observed at higher temperatures and reaction times and at high hydroxide concentrations. This is most likely due to molecular breakdown (hydrolysis) of the starch or X-links.

As a first approximation the Flory-Rhener swelling equilibrium theory is used to determine the X-linking efficiency [16-17]. According to this theory, the plateau G'-modulus is linear correlated with the number of elastically covalent entanglement points and inversely with the molecular mass between two entanglement points. Some preliminary results are shown in Figure 2 (bottom). It is shown that the effectiveness of the X-linking of starch with TSTP and ECH is low. The highest values obtained within this and other studies are 15% for ECH and 48% for TSTP, respectively [16-17]. The X-linking efficiency increases with increasing starch and X-linker concentration. An initial increase is found with increasing OH concentration. However, at high OH concentration the efficiency decreases. The effectiveness of TSTP seems to be higher than of ECH.

The low efficiency of the X-linking of starch is thought to be partly due competing hydrolysis of starch and the X-links and the formation of (short) free chain ends (*i.e.* single sided glycerol-linkages and phosphate mono-esters with different chain lengths). Part of the X-linker could have not reacted with starch at all. Furthermore the deviation from an ideal network attributes to imperfections in the gels, such as micro-heterogeneities, which can be the result of loops, loose ends (amylopectin outer branches), physical entanglements, and micro-aggregation of X-links.

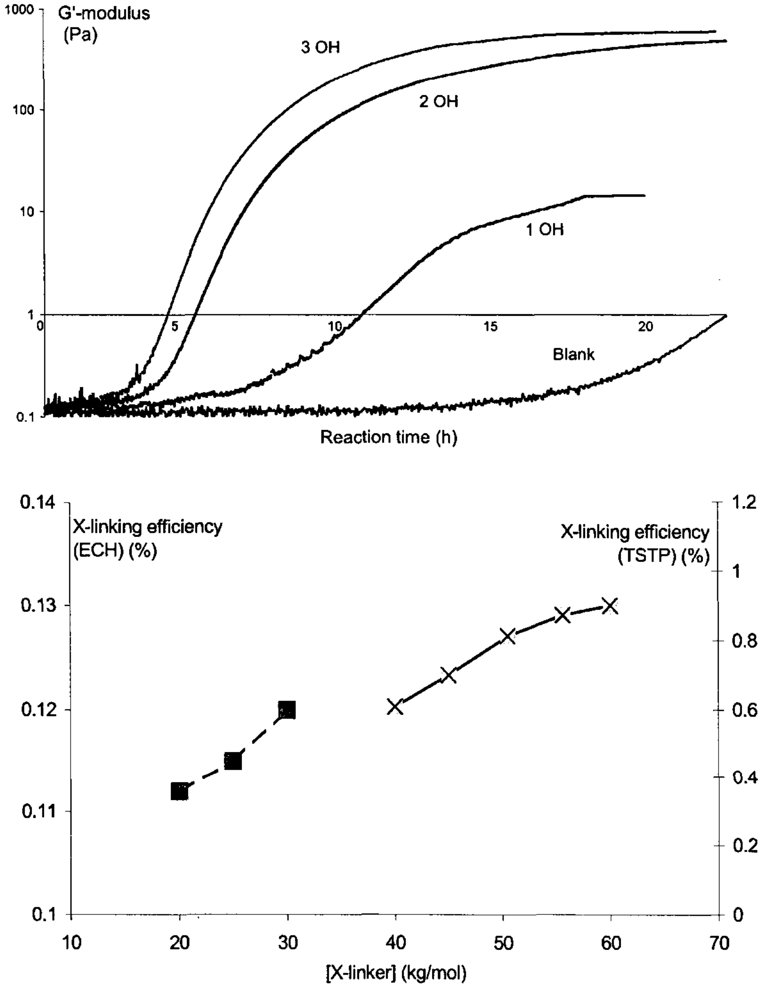

Fig. 2. Bohlin measurements. Top: The influence of hydroxide concentration (OH) on the G'-modulus during reaction. The blank is without the X-linker. The material composition used was: **20**Pa2-2ECH-*xOH*. Bottom: Estimation of the X-linking efficiency (expressed as the ratio of the number of calculated or effective X-links and the number of theoretical X-links times 100%) calculated on the basis of Bohlin reometry measurements using the Flory-Rhener approximation. A comparison of ECH and TSTP X-linked materials (**20**Pa2-**2**OH).

Structural characterisation

The starch-based microparticles were analysed by XRD. It was shown that the potato starch based materials were completely amorphous after purification and drying.

Apparently the X-linking reduces the retrogradation of the starch, which typical occurs during processing of gelatinized starches at high water contents.

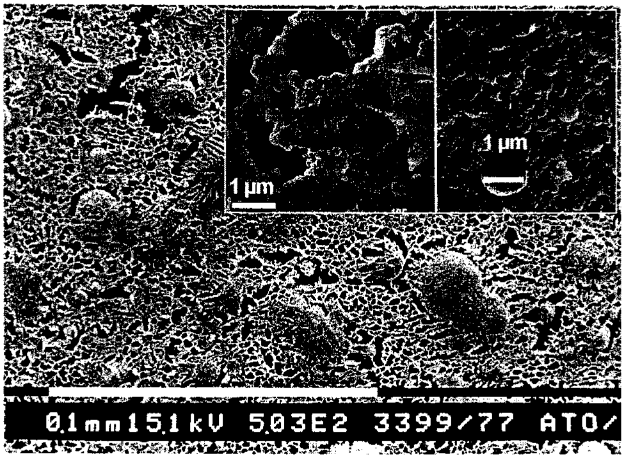

Fig. 3. EM photograph of TSTP-based microparticles after dispersing and swelling in demiwater. Inserts on the top right: EM photograph of TSTP-based microparticles after dispersing in water and freeze-drying the dispersion to about 12% water. The particle size is in the range of 150-800 nm.

Several microscopic techniques were used to study the size and shape of the microparticles. With light microscopy it was shown that the particles in suspension have a size below 10 µm. Figure 3 shows a typical example of a SEM study showing the appearance of a TSTP X-linked material after swelling in demiwater. Typical sizes are found for this material prepared with a low amount of X-linker in the range of 1–5 µm. A cryo-SEM is shown of more densely X-linked TSTP particles in Figure 3 (inserts on the right top side). More detailed studies showed that the particle size is depending on the starch/X-linker ratio. The particle size is clearly dependent on the degree of swelling in water, which was confirmed by ESEM at various RH. With increasing RH the microparticles increase in size as is seen in Figure 4. The studied ECH-based particles were spray-dried to obtain rather large and dented particles. The spray-dried material is shown in Figure 4 (Top left). The particles swell and form aggregates by increasing the humidity. By increasing the ECH concentration during synthesis (to 4.9 g/100 ml), it was shown that the swelling of dried particles was reduced significantly.

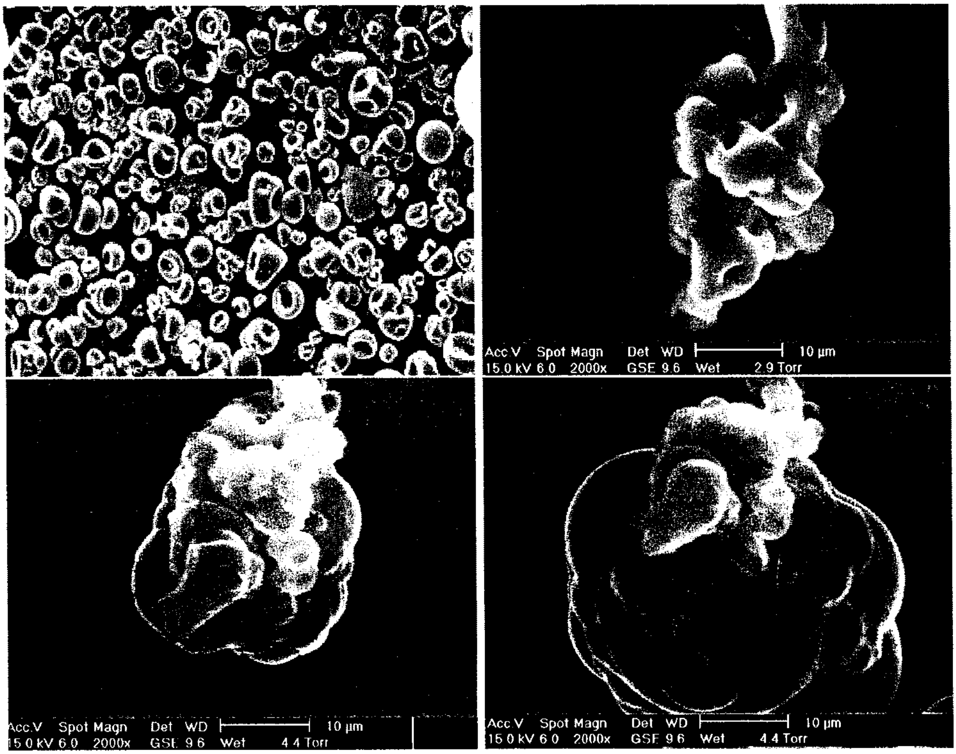

Fig. 4. (E)SEM photographs of the swelling behaviour of ECH X-linked microparticles (20Pa2-1ECH-2OH). Top left: SEM of spray-dried material. Top right, bottom left and bottom right: ESEM with increasing RH.

Table 1

The particle radius of two TSTP X-linked microparticles.

Composition	Average R_g (nm)	Polydispersity (%)
15Pa6-2TSTP-1OH	154	33
2.5PN-2TSTP-1OH	263	27

Using light scattering techniques typical particles sizes were measured in the range of 60–1000 nm. Two typical examples of the particle sizes measured with DLS of TSTP X-linked materials are given in Table 1. Characteristic SLS particle sizes of ECH X-linked materials as a function of amount of ECH are shown in Table 2. No linear relation is found. The X-linking efficiency of ECH with starch is low (as is shown in previous section) and not linearly related to the amount of reactants used over the whole range.

Table 2

The particle sizes of ECH X-linked materials (20Pa2-*x*ECH-2OH) as a function of ECH concentration during synthesis.

ECH (*x*)	ECH (weight fraction)	R_g at low angles (nm)	R_g at high angles (nm)	R_g ratio
0.5	0.004	90	204	0.44
0.5	0.004	76	140	0.55
1.0	0.008	70	129	0.54
2.0	0.016	69	140	0.49
4.0	0.032	62	189	0.33
4.9	0.039	84	192	0.44

It was shown that typically ECH X-linked microparticles had a ζ-potential in the range of -1 to -10 mV. While TSTP-based microparticles were in the range of -15 to -45 mV. The materials are thus slightly charged. Because of the presence of the phosphate groups in the TSTP-based materials, these materials are somewhat higher in charge density. Because of the inaccurate results obtained no clear relationships were found with the structural features of the various microparticles. The swelling of the particles in water is mainly due to the presence of the charges (osmotic pressure effect).

Rheological properties of starch microparticles

In Figure 5 (top) the viscosity as a function of concentration of TSTP-based microparticle (40Pa6-8TSTP-1OH) suspensions is shown for concentrated systems. The dispersions show shear-thinning behaviour at relatively high solid contents. The influence of salt concentration on the viscosity of ECH-based (22.2Pa2-1.9ECH-1.1OH) suspensions is shown in Figure 5 (bottom). The viscosity is significantly lower than the viscosity of the TSTP-based suspension shown in the Figure, which can be explained by the larger particle size and hydrodynamic volume of the TSTP-based microparticles. Visible is the effect of introducing low amounts of salt. The viscosity is reduced by a factor two at a shear rate of approximately 100 s^{-1}. Also the suspensions become less shear thinning. Because of the slightly negative particles, the hydrodynamic particle size is reduced by the addition of the salt. This is in agreement with the observed reduction of the swelling capacity of starch cross-linked hydrogels with increasing salt concentration [14-16]. This effect is even more pronounced for TSTP-based systems. The cross-linked microparticles show a characteristic behaviour of a ionic polymer.

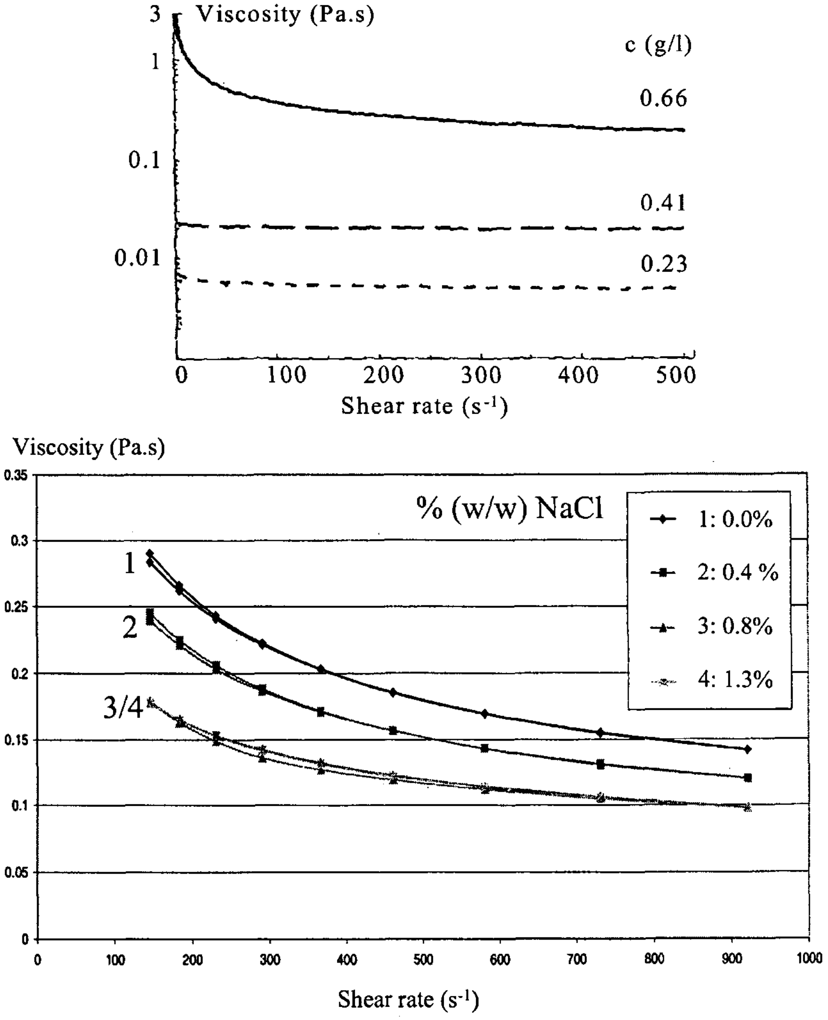

Fig. 5. Rheology of TSTP-based microparticle suspensions (40Pa6-8TSTP-1OH): Top: viscosity vs. shear rate. Bottom: Influence of sodium chloride concentration on the viscosity of a dialysed starch microparticles (22.2Pa2-1.9ECH-1.1OH) suspension (7.3 g/l). The amount of sodium chloride (%w/w NaCl) is indicated in the insert.

In Figure 6 an example of the relative viscosity (η_r) as a function of concentration is given obtained from low shear rheological experiments on dilute aqueous suspensions of ECH-based microparticles (with a size of circa 100 nm). For these samples a linear behaviour of the shear stress versus shear rate is obtained in the range of 0.1–50 s^{-1}. The specific viscosity shows a linear dependence on the concentration in dilute systems. The slope of is depending on the amount of X-linker used during synthesis of the microparticles as is seen in Figure 6 (insert).

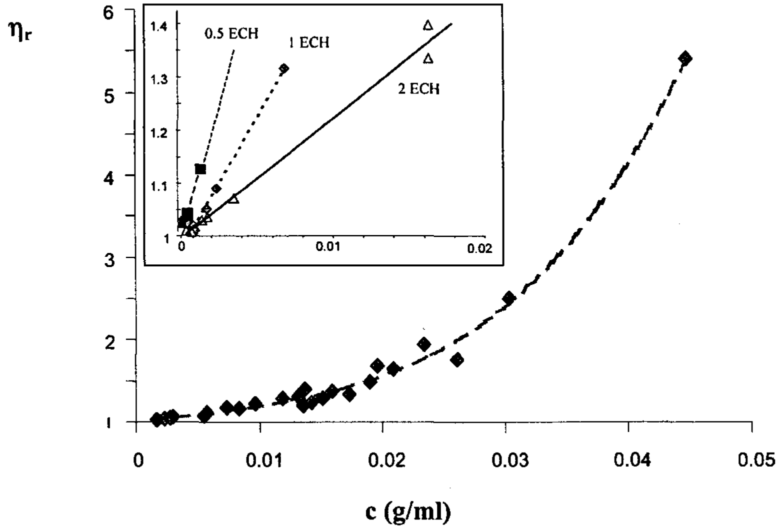

Fig. 6. Relative viscosity of ECH-based materials (20Pa2-1ECH-1OH) in dilute aqueous systems. The insert shows the dependence of ECH concentration during synthesis.

Conclusions

By emulsion X-linking of starches interesting microparticles are obtained. The particles can be considered as microgels. The (rheological) behaviour is characteristic of colloidal polyelectrolytes.

REFERENCES

[1] Jiugao Y. Jie L.: Starch, 46, 1994, 252.
[2] Soest J.J.G. van, Verduin H., Feil H.: Second World Congress on Emulsion, EDS, Paris 1997, 1-1-366/01-05.
[3] Soest J.J.G. van, Schijndel R.J.G. van, Gotlieb K.F.: PCT Pat. Appl. WO9901214A1, 1999.
[4] Velazquez J.M., Marmolejo-Rivas G., Riosdiaz R.G., Alvarez A.B., Marin-Carrillo E.M., Glogowski M.W.: PCT Pat. Appl. WO9955819, 1999.
[5] Gunther W., et al.: PCT Pat. Appl. WO9725073, 1997.
[6] Baensch J., Gumy D., Sievert D., Wursch P.: PCT Pat. Appl. WO 96/03057, 1996.

[7] Zee M. van der: Structure-biodegradability relationships of polymeric materials, P&L, Wageningen, 1997, 1-211.

[8] Galliard T., Bowler P.: Starch: Properties and potential, John Wiley, New York, 1987, 1-200.

[9] Ellis R.P. et al.: J. Sci. Food Agric., **77**, 1998, 289-311.

[10] Jane J.-L.: J. Macromol. Sci. Pure Appl. Chem., **4 (A32)**, 1995, 751.

[11] Green B.K., Schleicher L.: US Pat. 2800457.

[12] Arshady R.: Pol. Engin. Sci., **29**, 1989, 1746.

[13] Porath J., Johnson J.C., Láàs T.: J. Chrom, **60**, 1971, 167.

[14] Chitumbo K., Brown W.: J. Pol. Sci., **C36**, 1971, 297.

[15] Kulicke W.M., Nottelmann H.: Polym. Mater. Sci. Engin., **57**, 1987, 265.

[16] Kulicke W.M., Aggour Y.A.:, Starch, **41**, 1989, 140.

[17] Kulicke W.M., Aggour Y.A.: Starch, **42**, 1990, 134.

[18] Wolfe M.S., Scopazzi C.: J. Coll. Interface Sci., **133**, 1989, 265.

[19] Antonietti M., Briel A., Förster S.: J. Chem. Phys., **105**, 1996, 7795.

[20] Soest J.J.G. van, Tournois H., Wit D. de, Vliegenthart J.F.G.: Carboh. Res., **279**, 1995, 201.

[21] Lycklema J.: Fundamentals of Interface and Colloidal Science, Vol. II, Acad. Press, San Diego, Chpt. 2.

MIKROCZĄSTECZKI SKROBIOWE WSTĘPNE BADANIA BUDOWY I WŁAŚCIWOŚCI

Streszczenie

Skrobie poddano obróbce w celu otrzymania w pełni biodegradowalnych mikrocząsteczek, które w roztworze wodnym zachowywałyby się jak mikrożele lub koloid. W tym celu połączono kleikowanie z sieciowaniem w emulsji wodno olejowej. Takie skrobie były bardzo stabilne w wodzie, wykazując właściwości rozrzedzania ścinaniem nawet w roztworach o dużej zawartości frakcji stałej. Są to właściwości bardzo unikalne w przypadku skrobi. W ten sposób można otrzymać nowe koloidy o zróżnicowanych właściwościach. Skrobie takie mogą znaleźć zastosowanie do celów spożywczych i niespożywczych (farby i pokrycia, tusze i pigmenty, superabsorbenty, dodatki do żywności, środki higieny osobistej, farmaceutyki, ceramika, dodatki do papieru, zagęstniki, emulgatory i inne zastosowania).

Cząteczki otrzymano stosując jako czynniki sieciujące epichlorohydrynę z trimetafosforanem trisodowym. Badano wpływ istotnych parametrów reakcji na przebieg syntezy i właściwości produktów.