



Faculty of Resource Science and Technology

**SYNTHESIS AND CHARACTERIZATION OF STARCH - BASED
NANOPARTICLES FROM NATIVE SAGO AND POTATO
STARCHES**

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SYNTHESIS AND CHARACTERIZATION OF STARCH-BASED NANOPARTICLES
FROM NATIVE SAGO AND POTATO STARCHES

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Declaration

No portion of work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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Abstract

Cross-linked sago and potato starch samples were prepared in the water-in-oil emulsion using epichlorohydrin (ECH) and trisodium trimetaphosphate (TSTP) as cross-linking agents. Effects of various synthesis parameters including the surfactant concentrations, types of cross-linker, cross-linker to starch ratio, emulsifying stirring speed, and organic to aqueous phase ratio on the resulting cross-linked starch samples were investigated. All samples were characterized with scanning electron microscope (SEM), total organic carbon analyzer (TOC), Fourier Transformed Infrared spectroscopy (FTIR). And Rapid Visco Analyzer (RVA) and equilibrium swelling study. SEM micrographs show the presence of nano-sized starch particles in the well-dispersed and less-dispersed cross-linked samples. Lyophilized cross-linked starch samples were observed to form densely packed and spongy porous aggregates or gel-like solid forms, all of which were not readily dispersible in aqueous media. The limited magnifying capability of the SEM used in this study did not allow quantitative determination of the particle size distribution, and to elucidate the effects of various synthesis parameters on the particle size of the cross-linked starch samples. Results from TOC, FTIR, RVA, and ESD have provided further supporting evidences on the formation of cross-linked starches, which were observed to have different physical and chemical characteristics from the native starches.

Key Words: starch-based nanoparticles, cross-linked starch, epichlorohydrin, trisodium trimetaphosphate, water-in-oil emulsion.

Abstrak

Kanji taut-silang sagu dan ubi kentang telah disediakan melalui taut-silang pengemulsian secara air-dalam-minyak dengan menggunakan epiklorohidrin (ECH) dan trinitium trimetfosfat (TSTP) sebagai agen taut-silang. Kesan-kesan pelbagai parameter sintesis termasuk kepekatan surfaktan, jenis agen taut-silang, nisbah kemolaran taut-silang kepada kanji, kala mengacau semasa pengemulsion dan nisbah fasa organik kepada fasa aques telah dikaji terhadap hasil sampel taut-silang. Kesemua sampel telah dicirikan dengan menggunakan Mikroskop Pengimbasan Elektron (SEM), Alat Analisis Jumlah Organik Karbon (TOC), Viskometer (RVA), Spektroskopi Transformasi Infra-merah (FTIR) dan kajian Keseimbangan Pengembangan (ESD). Mikrograf SEM menunjukkan kehadiran nano-saiz partikel kanji dalam sampel taut-silang melerai dengan baik dan melerai dengan kurang baik. Sampel-sampel taut-silang yang kering secara pembekuan telah diperhatikan terdapat kelompok yang padat dan berporos span atau bentuk pepejal seperti gel. Kuasa pembesaran terhad bagi SEM yang digunakan membataskan pengukuran kuantitatif terhadap penyebaran partikel saiz dan penjelasan kesan-kesan pelbagai parameter sintesis ke atas partikel saiz bagi sampel taut-silang. Data analisis , TOC, FTIR, ESD dan RVA telah memberikan bukti-bukti sokaongan berlanjut bagi pembentukan kanji taut-silang yang dioerhatikan menunjukkan ciri-ciri fizikal dan kimia yang berbeza daripada kanji semula jadi.

Kata kunci: Nanopartikel yang berasaskan kanji, kanji taut-silang, epiklorohidrin, trinitium trimetfosfat, pengemulsian secara air-dalam-minyak.

1. Introduction

1.1 Backgrounds

Starch is a natural occurring polymer which consists of amylose and amylopectin polysaccharide. Amylose is a linear (1→4)-link- α -D-glucan while amylopectin is a branched polysaccharide consists (1→4)-link- α -D-glucan and (1→6)- α -linked branches (Fasihuddin *et al.*, 1999). The normal ratio of amylose to amylopectin in the sago starch is 20%:80% (Orford *et al.*, 1987; Fasihuddin *et al.*, 1999). Several native starches are being produced in Malaysia, such as potato, sago, tapioca and corn starches.

Starch is a cheap and convenient food ingredient that can be applied in many foods such as puddings, ice cream, snack and low fat foods. Other type of foods include baby food where starch is used as a nutrient with low fermentability; noodles where starch improve consistency and mouth feel; sauces where the cross linking of starch with acid food resistance and allow retorting; meat products where starch retain and firm the final texture for prolonged periods. There are also products which starch is used such as soft drinks, beer, candy, confectionery and high boil sweet. Starch can also be used in non-food applications such as paper where starch is used as sizing and coating; textile where starch is used to improve abrasion resistance in fast looms; plastic and packaging where starch is used to improve the biodegradability of plastic; adhesive, tablets and gypsum plaster where starch is used as binder; detergent where starch is used as a redeposition inhibitor of dirt, agricultural use, stain remover and glue (International Starch Institute).

Sago starch is being extracted from the sago palm (*Metroxylon* spp.). Sago starch contains 10.6 - 20.0 % moisture content, 0.06 - 0.13 % ash, 0.10 - 0.13 % crude fat, 0.26 - 0.32 % fiber, 0.19 - 0.25 % crude protein and 24 - 31 % amylose (Fasihuddin *et al.*, 1999; Dayang, 2002; Paula, 2002). It has a C-type diffraction pattern (Fasihuddin *et al.*, 1999).

Potato starch is being isolated from the tuber potato (*Solanum tuberosam*). The moisture, ash, crude fat and amylose content in potato starch are 13.1 %, 0.25 %, 0.12 % and 28.1 % respectively. Potato starch showed a B-type diffraction pattern (Gunaratne *et al.*, 2002).

According to Fassihuddin *et al.* (1999), the properties of sago and potato starch are very similar. Though starch is easily gelatinized, it is difficult to obtain stable aqueous colloidal systems from native starch due to its high viscosity and shear thickening at low solid contents (Soest, *et al.*, 2002; Soest, *et al.*, 2003). The functional properties of the starch are therefore limited even for chemical modified starch with high degree of substitution. This permanent feature of the starch has limited its applications especially for non-food applications such as paper, coating and paint. Thus, chemical derivatisation has been studied to overcome the limitation of starch-based materials caused by the hydrophilic nature of starch and to produce water resistant materials (Peltonen *et al.*, 1996; Fang *et al.*, 2002).

According to Gunaratne *et al.* (2002), the shape of native starch granules ranged from oval to round to spherical to polygonal with characteristics dimension range of 3-110 μm . They have studied tuber and root starches which include potato, new cocoyam, cassava, taro and true yam. New cocoyam showed a very large surface area per unit weight as compared to other starches. The granule surface of all starches appeared to be smooth and showed no evidence of pin hole. The size or shape of the starches granules did not alter with heat-moisture treatments. The study has shown that the average degree of polymerization of isolated amylose followed the order of potato > new cocoyam > cassava > taro > true yam. The average chain length of isolated amylopectins followed the order of potato ~ true yam > taro ~ new cocoyam ~ cassava. The swelling factor and amylose leaching of native starch increased with increase in temperature. The swelling factor of native starches followed the order of potato > cassava > taro > true yam > new cocoyam. Whereas, amylose leaching

followed the order of potato ~ taro > true yam > cassava > new cocoyam. The gelatinization temperature of native starches followed the order of taro > true yam > new cocoyam > cassava > potato.

1.2 Literature Reviews

Two routes that have been developed to prepare biodegradable microparticles are emulsion processing and extrusion. Emulsion processing is a synthesis route based on the unique combination of gelatinization and cross-linking performed in water-in-oil emulsions. Extrusion is a preparation route based on combining starch melting with cross-linking at high shear and temperature (Soest *et al.*, 2002).

Previous studies have shown that starch-based microparticles that behave like microgels were successfully produced through cross-linking (Soest *et al.*, 2002; Hamdi *et al.*, 2001). The microgels were negatively charged, spherical and amorphous. The particle size of the microgels ranged from 180 nm up to 5 μm with a large size distribution. The particle sizes of microgels prepared from native starches depend on the type and amount of cross-linker used. The dry particle mass densities of the starch microgels were in the range of 1.36-1.70 g/ml. The mass densities of starch microgels decreased with increasing cross-linker concentration. According to Soest *et al.* (2002), ECH cross-linker tends to give smaller particles size compared to TSTP cross-linker due to differences in densities.

Cross-linked starch microparticles have been observed to swell in water. The starch microparticles swelled and formed aggregates by increasing the humidity (Soest *et al.*, 2000; Soest *et al.*, 2002). Soest *et al.*, (2002) reported that at 60 % r.h., the aggregates started to swell whereas at 70 % r.h. the particles were swollen up to ten times their dry volume. The particles were saturated with water and collapsed after 20 seconds. Almost no swelling or

conformational changes of more tightly cross-linked microgels took place upon increasing the moisture content (Soest *et al.*, 2002). By increasing the ECH concentration during synthesis (to 4.9 g/100 ml), it was shown that swelling of dried particles was reduced significantly. ECH cross-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 (Soest *et al.*, 2000).

The starch-based microparticles are stable in water with adjustable shear-thinning behavior at relative high solid contents (Soest *et al.*, 2002; Soest *et al.*, 2003). The viscosity of ECH-based system was significantly lower than the viscosity of TSTP-based microgels. This made the microgels suitable as thickeners in applications like paints (Soest *et al.*, 2002). Due to the reduced viscosity increase with decreasing particle concentration (Dziechciarek *et al.*, 2001; Soest *et al.*, 2002), starch-based nanoparticles are expected to be more stable in water compared to microparticles.

According to Hamdi *et al.* (2001), an increase in the stirring rate during the emulsification step would decrease the mean diameter and narrow the size distribution. The size of microparticles decreased when the organic-to-aqueous phase ratio was increased. The effect of the organic-to-aqueous phase ratio was less sensitive when the ratio was higher than 5/1. An increase in the surfactant concentration only contributed a slight decrease in the mean diameter of the microparticles. The epichlorohydrin to starch molar ratio did not affect the size of swollen starch microparticles. An increase of the epichlorohydrin to starch molar ratio reduced the ability of the microparticles to swell and the swelling degree decreased.

Besides cross-linking, various types of starch derivatives were prepared by reacting starch with mono- or dimethylated compounds such as urea, thiourea, and melamine. Three factors that affect the preparation reactions include curing duration, catalyst, and resin concentrations. The nitrogen content of the starch derivatives increased with increasing

duration of curing up to 20 minutes and decreased with longer curing times. The nitrogen content of starch derivatives increased with increasing catalyst concentration to reach a maximum value and decreased on using higher concentration. The maximum occurs at different concentrations depending on the resin type. The nitrogen content of starch derivatives increased with increasing resin to starch molar ratio (Khalil *et al.*, 2000).

It is also found that the properties of modified starch varied extensively due to the nature of substituents and the degree of substitution (Röper, 1996; Fang *et al.*, 2002). When the DS is high enough, esterification with organic acids will result in thermoplastic and hydrophobic materials. Undamaged starch granules can absorb water reversibly and are not soluble in cold water. They swell slightly but return to the original size on drying. The solubility properties of starch alter when hydrophobic acyl group is added into molecular structure of starch. This alteration in solubility of starch depends on the extent of acylation, the degree of degradation of the acylated molecule, the degree of polymerization of the ester, the fractionation of the starch or the derivative, the nature of the acyl substitute, the type of starch, and the solvent and temperature. It is noticed that with increase in degree of substitution and side-chain length increase the solubility in organic solvents and hydrophobicity (Fang *et al.*, 2002).

Various methods for removing heavy metal ions from solution have been developed (Wing, 1974; Wing *et al.*, 1978; Cavaseno, 1980; Kim *et al.*, 1999). Chemical precipitation, activated carbon, reverse osmosis and ion exchange by specific resins are among common methods used for heavy metal ions removal. Ion exchange by specific resins is used when the other methods are not successful, or when metal recovery is needed (Kim *et al.*, 1999). However, the recovery cost of the resins is quite high and the activity of the recovered resin is

generally not satisfactory. Therefore, in many cases, these removal or recovery methods for heavy metal ions are combined to increase their effectiveness. (Wing, 1974; Kim *et al.*, 1999)

A starch derivative has been developed as an effective metal scavenger at USDA in Peoria, Illinois, USA (Wing, 1974; Wing *et al.*, 1976; Kim *et al.*, 1999). Kim *et al.* (1999) have also shown that corn starch derivative is effective in removing heavy metal ions from solutions. There is a possibility that starch-based nanoparticles are more effective in heavy metal ions removal. Besides, starch is cheap and the cost of starch derivative is relatively less as compared to resins method.

Different neutral starch derivatives prepared via reacting starch with various mono- and dimethylol derivatives of urea, thiourea, and melamine were also used for heavy metals removal. The adsorption values depend on the metal ion on various derivatives. The adsorption values on starch derivatives follow the order of $\text{Hg}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+}$ (Irving *et al.*, 1948; Irving *et al.*, 1956; Khalil *et al.*, 1998; Khalil *et al.*, 2000).

Besides, some starch microspheres have been used for the controlled-delivery of drugs or for embolization (Björk *et al.*, 1988; Illum *et al.* 1990; Edman *et al.* 1992; Lindberg *et al.* 1984; Hamdi *et al.*, 2001). Pharmaceutical applications of starch microsphere necessitate controlled particle size and narrow size distributions because the localization and distribution of the particles in the body depend on these parameters. There is an optimum particle size and distribution for each route of administration. Therefore the effect of formulation and processing parameters such as surfactant concentration, type of cross-linker, organic phase volume, emulsification process and stirring rate is important (Hamdi *et al.*, 2001). In many applications, volume and conformation changes of starches are important because they controlled the determination of the rheology of the suspension (Soest *et al.*, 2002). Fully biodegradable starch nanoparticles are expected to be very stable in water and exhibit

interesting unique rheological and colloidal behaviors. They offer new possibilities for preparing starch colloids with a wide range of properties and henceforth numerous potential food and non-food applications.

In this study, both native sago and potato starches were processed in order to obtain fully biodegradable nanoparticles that behaved as microgels or colloids in aqueous suspensions. The focus was on establishing the relationship between synthesis parameters and the structural, rheological, and colloidal properties of starch-based nanoparticles in aqueous colloidal suspensions.

1.3 Objectives of Study

Among objectives of this study are:

1. to prepare fully biodegradable nanoparticles from native sago and potato starches, and
2. to determine the physical and chemical characteristics of these starch-based nanoparticles.

2. Materials and Methods

2.1 Materials

Native sago and potato starches used in this study were bought from the local supermarket. Trisodium trimetaphosphate (TSTP) and epichlorohydrin (ECH) were used as the cross-linking agents. Sorbitan mono-oleate (Span 80) was used as the emulsifier. The cyclohexane and chloroform mixture (4:1 v/v) was used as the organic phase.

2.2 Methods

2.2.1 Preparation of the Starch-based Nanoparticles

Starch-based nanoparticles were prepared according to a water-in-oil emulsion technique using epichlorohydrin and trisodium trimetaphosphate as crosslinking agents based on methods reported by Hamdi *et al.* (1998). For a typical batch, the aqueous phase was prepared by dissolving 2 g of native starch in a 22 g alkaline solution (1N) under stirring which corresponds to 0.5 mol/L of starch, expressed as anhydroglucose units (AGU). The aqueous phase were emulsified in 125 ml of a cyclohexane-chloroform mixture (4:1 v/v) containing varying amounts of sorbitan monooleate (Span 80 or Span 60). The ratio of the organic phase volume to the mass of the aqueous phase was 5:1 and this ratio was then varied. The mixture was homogenized for 3 minutes with a laboratory blender at various stirring rates up to 19,000 rpm. It was blended for 1 minute and rested for 1 minute alternatively. Varying amounts of cross-linker (molar ratios of cross-linker to starch expressed as AGU) were then added under stirring at fixed stirring rate using a magnetic stirrer. The stirring was maintained for 18 h at 40 °C using thermostated water bath.

The resulting starch particles were isolated by centrifugation and washed three times with cyclohexane, and then thoroughly with distilled water until the smell of cyclohexane was gone. Finally, the starch particles were lyophilized in a freeze dryer and kept in closed containers before use.

The effect of various processing parameters on the size of starch particles were investigated including (a) the surfactant concentration, (b) type of cross-linker, (c) the cross-linker/starch molar ratio, (d) emulsifying stirring rate and (e) organic to aqueous phase ratio. Table 1 shows the types of samples prepared in this study using various processing or synthesis parameters.

Table 1: Various synthesis parameters used in the preparation of cross-linked starch particles.

Synthesis Parameters	Types of samples prepared					
	1	2	3	4	5	6
Surfactant Concentration (%)	0.5 ^a	1 ^a	2	3 ^a	4	5 ^a
Type of Cross-Linker*	ECH ^a	TSTP ^a	-	-	-	-
ECH/AGU Molar Ratio*	0.5 ^a	1.45 ^a	2.33 ^a	-	-	-
Emulsifying Stirring Rate (x1000 rpm)*	3.5 ^a	12.0 ^a	14.5	19 ^a	-	-
Organic: Aqueous Ratio (v/w)**	1 ^a	2 ^a	5 ^a	8	-	-

* The Surfactant Concentration is 5% Span 80.

**The Surfactant Concentration is 0.5% Span 60.

^a Characterized Samples.

2.2.2 Characterization of Starch Particles

Physical Characterization

The morphological characteristics and size distributions of starch particles were investigated using scanning electron microscopy (SEM) (JEOL Model JSM 5300LV). SEM micrographs were obtained from two different types of starch particle samples. When powdered dry starch samples were used, they were mounted on stainless steel sheets (1 cm²) with double sided carbon tape. Alternatively, a given starch sample was first dispersed in distilled water. A few drops of the resulting starch dispersion were then deposited and allowed to dry on a glass slide (1 cm x 2 cm). Both types of samples were pre-coated with a thin layer of gold film before being examined using the SEM at an accelerating potential that ranged between 5 to 20 kV. (Soest *et al.*, 2003; Gunaratne *et al.*, 2002).

Equilibrium swelling degree (ESD) of starch particles were determined by swelling a suitable volume (50 & 100 mg) of dried cross-linked starch samples in 5 ml of phosphate buffer saline (PBS) (pH=7.4) contained in a modified syringe overnight. The PBS was prepared by dissolving 7.650 g of NaCl, 0.724 g of Na₂HPO₄ anhydrous and 0.21 g of

KH_2PO_4 in 1L of distilled water. The pH was then adjusted to pH 7.4 by using 1N NaOH. The volume of swollen starch particles were read directly. The ESD was expressed as the ratio of the swollen volume V_s to the mass of dried starch particles m_d (Hamdi *et al.*, 2001).

The pasting temperatures of both native and cross-linked starch samples were studied the using Rapid Visco Analyser (NEWPORT SCIENTIFIC). 2 g of native starch or 1 g of cross-linked starch samples were first dispersed in 25 g or 26 g of distilled water respectively, by stirring at 960 rpm before analysis and 160 rpm during analysis. The heating regime started from room temperature to 50 °C within 10 seconds and was maintained at this temperature until 1 minute. The temperature was then increased to 95 °C with a rate of 12.2 °C/minute, and was maintained at this temperature for 2½ minutes. The temperature was then cooled down to 50 °C with the same rate and was maintained at this final temperature for 2 minutes. The viscogram was obtained by measuring the viscosity of the starch suspension throughout the 13-minutes pre-programmed heating regime. In this study, only the pasting temperature which corresponded to the temperature at which the viscosity increased sharply was determined.

Chemical Characterization

The total organic carbon contents of all starch samples were determined using the Total Organic Carbon Analyser (TOC) (ELEMENTAR high TOC II). The chemical compositions and structures of starch samples were determined using the Fourier Transformed InfraRed (FTIR) spectroscopy (SHIMADZU Model FTIR-8201PC). FTIR spectra were obtained from compressed pellets of KBr starch mixture over the wavenumber range of 400 - 4000 cm^{-1} ,

3. Result and Discussion

3.1 Preparation of Cross-linked Starch Samples

Different types of cross-linked starch samples were prepared using various synthesis parameters and selected samples were characterized as shown in Table 1.

3.2 Characterization

3.2.1 Physical Characterization

Scanning Electron Microscopy (SEM)

The morphological characteristics of various cross-linked potato and sago starch samples prepared in this study were examined mainly by SEM at different magnifications. SEM micrographs were obtained from both water-dispersed samples and freeze-dried powder samples. The formation of nano-sized starch particles under various synthesis conditions was clearly evidenced in micrographs of the well-dispersed as well as less-dispersed but nanoparticulate samples. Nevertheless, due to the inherent magnification capability of the SEM instrument used in this study, it was not possible to quantitatively determine the absolute size and shape of individual starch particles even in well-dispersed samples. Henceforth, based on the nature and extent of dispersion observed for various cross-linked samples, these SEM micrographs were subsequently classified into four broad categories, namely (1) native starch samples; (2) well-dispersed samples; (3) less-dispersed nanoparticulate; and (4) gel-like samples.

a) Potato Starch

The morphology and granule size of native potato starch can be clearly observed under SEM up to 10000x magnification as shown in Figure 1. The shape of native potato starch granules was observed to vary from spherical to oval as shown in Figure 1(a) and 1(b).

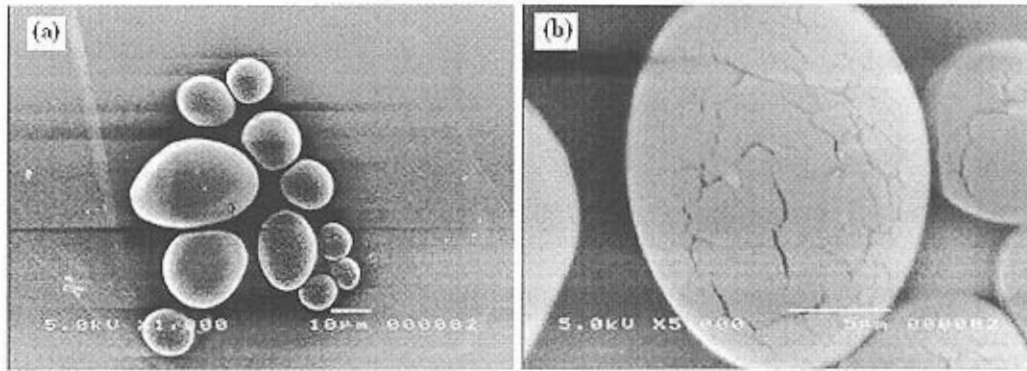


Figure 1: SEM micrographs of native potato starch sample; (a) 1000x, and (b) 5000x magnification.

The size distribution of native potato starch granules was observed to range from 9.6 to 36.0 μm . These results were in agreement with previous works reported by Gunaratne *et al.* (2002) that the granules shape of native potato starch ranged from oval to spherical with characteristics dimension in range of 10-110 μm . The granules surfaces were observed to be smooth with fissures. Baldwin (1995) reported that large protruberences (200-500 nm) were observed on the surfaces of potato starch granules under atomic force microscopy.

Figures 2(a) to 2(f) shows SEM micrographs of cross-linked potato starch samples with well-dispersed particles at various magnifications. These samples were prepared with 1% span 80 (Figure 2a & 2b), 5% span 80 (Figure 2c & 2d), and ECH/AGU ratio of 2.33 (Figure 2e & 2f). Aqueous dispersion of samples was obtained by dispersing the powdered samples in distilled water. These cross-linked potato starch samples showed considerable differences in their morphologies. The shapes of starch particles were observed to range from spherical to polygonal.

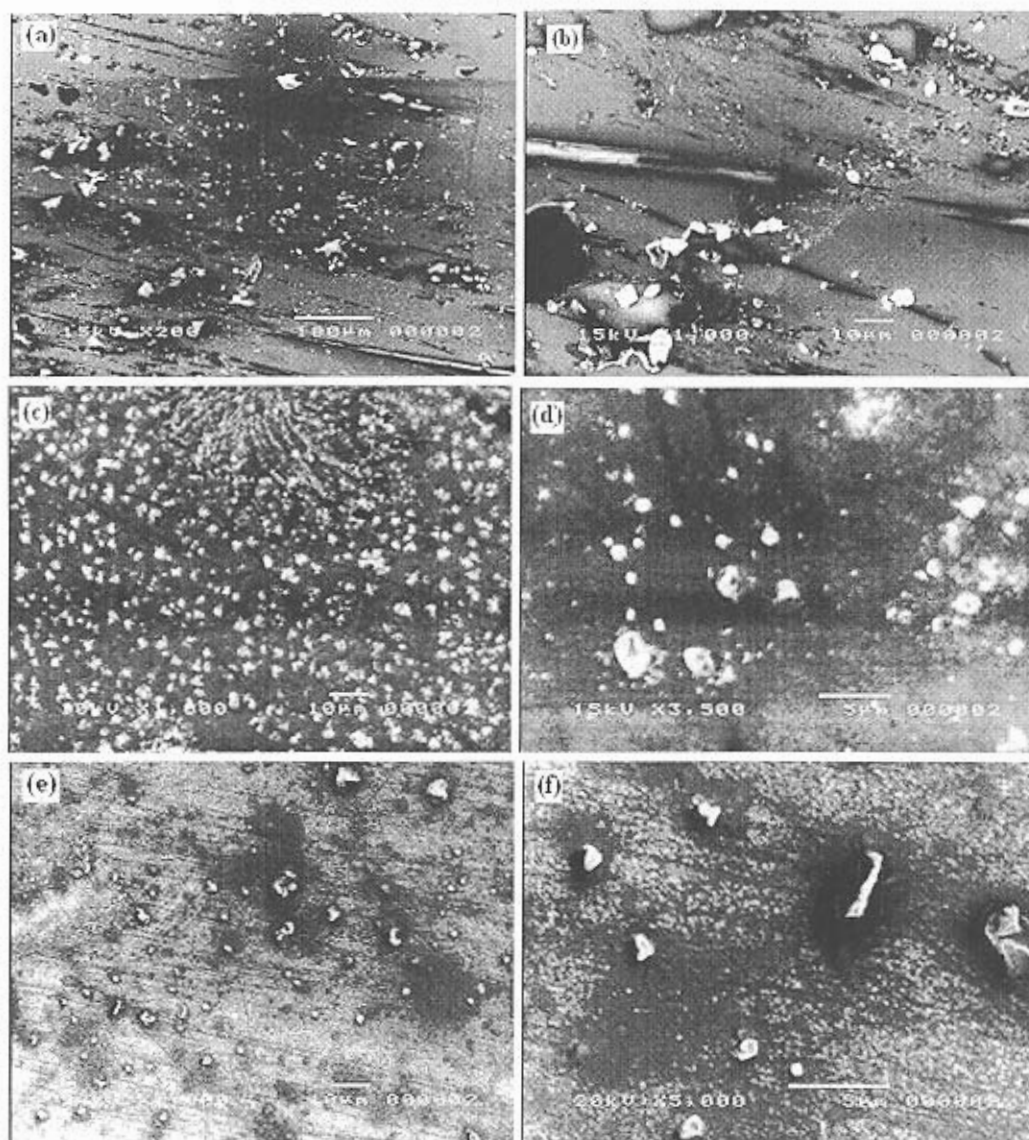


Figure 2: SEM micrographs of well-dispersed cross-linked potato starch samples. (a) 1% span 80; 200x, (b) 1000x magnification, (c) 5% span 80; 1000x, (d) 3500x magnification, (e) ECH/ AGU = 2.33; 1000x, and (f) 5000x magnification.

SEM micrographs show the presence of very fine starch particles of sub-micrometer size range for aqueous dispersed cross-linked potato samples prepared with 1% and 5% span 80 (Figure 2b & 2c). The size distribution of the 1% span 80 sample ranged from 0.9 to 5.5 μm , while the 5% span 80 sample ranged from 0.3 to 3.2 μm . In general, particles are observed to be more irregular in shape in the 1% span 80 sample than that of the 5% span 80 sample. As reported by Hamdi *et al.* (2001), a higher the surfactant concentrations tend to