



# Competitive adsorption between sugar beet pectin (SBP) and hydroxypropyl methylcellulose (HPMC) at the oil/water interface

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## ABSTRACT

The emulsification performance, stability and competitive adsorption of two natural food emulsifiers, sugar beet pectin (SBP) and hydroxypropyl methylcellulose (HPMC) have been investigated. Both can reduce the surface tension and emulsify oil in water. However, due to their different structure and conformation they operate via different mechanisms. Using 15% middle chain triglycerides (MCTs) oil, the amounts of SBP and HPMC adsorbed in emulsions made with these individually and in mixtures were determined. The interfacial concentration ( $\Gamma$ ) for SBP stabilized emulsion was  $\sim 1.25 \text{ mg/m}^2$  and for HPMC  $3.5 \text{ mg/m}^2$ . The higher adsorption of HPMC was due to multilayer adsorption, whereas SBP adsorbed as a monolayer. Competitive adsorption between SBP and HPMC was also investigated. When the HPMC concentration approached that of adsorbed SBP, the effect of HPMC became dominant and at 1.5 wt.% controlled the behavior of the mixed emulsions, which were then almost independent of SBP. The minor role of SBP was mainly to decrease the proportion of large droplets in the emulsion. A model to describe the competitive adsorption between SBP and HPMC is proposed.

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## 1. Introduction

Competitive adsorption between different emulsifiers such as protein/surfactant (Fang & Dalgleish, 1996; Mackie, Gunning, Wilde, & Morris, 2000; Pugalioni, Dickinson, Ettelaie, Mackie, & Wilde, 2004), protein/polysaccharide (Arbolea & Wilde, 2005; Damodaran & Rammovsky, 2003) and even between different proteins (Dickinson, Rolfe, & Dalgleish, 1988, 1989) has been studied extensively. Two factors influence the competitive adsorption. First, is the difference in surface activity between two competing emulsifiers. For example, the more surface active cellulose derivatives (methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC)) displace adsorbed proteins ( $\beta$ -lactoglobulin and  $\beta$ -casein) and dominate the competitive adsorption at high concentrations (Arbolea & Wilde, 2005). Secondly, is the sequence of adding the polymers in the emulsification process. When added simultaneously the more surface active  $\beta$ -casein displaces gum arabic glycoprotein. But when gum arabic glycoprotein is added first, it forms a thick adsorbed layer which prevents the approach of  $\beta$ -casein and thus reduces its adsorption.

Recently, sugar beet pectin (SBP) has attracted interest as a potential emulsifier (Fishman, Chau, & Cooke, 2008; Funami et al.,

2007, 2008, 2011; Gromer, Kirby, Gunning, & Morris, 2009; Kirby, MacDougall, & Morris, 2006; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Nakauma et al., 2008; Nunez, Fishman, Fortis, Cooke, & Hotchkiss, 2009; Siew & Williams, 2008a; Williams et al., 2005). It can be used at significantly lower concentration ( $\sim 1.5\%$ ) compared with other natural food emulsifiers such as soybean soluble polysaccharide (4%) or gum arabic ( $\sim 10\%$ ) to emulsify 15 wt.% medium-chain triglycerides (MCTs) (Nakauma et al., 2008). Its good emulsification properties have been attributed to the presence of a proteinaceous moiety (Funami et al., 2007; Siew & Williams, 2008a, 2008b) and when the protein is enzymically removed its emulsification ability is lost (Funami et al., 2007). Siew et al. reported that the absorbed SBP fraction has higher contents of protein and ferulic acid than the control sample (Siew & Williams, 2008a, 2008b). Therefore, it was proposed that SBP could form polysaccharide–protein complexes similar to the arabinogalactan protein (AGP) fraction in gum arabic (Funami et al., 2006; Siew & Williams, 2008a). The protein component adsorbs at the oil–water interface, whereas the polysaccharide component extends out from the oil–water interface into the bulk aqueous phase as proposed for gum arabic. Recent atomic force microscopy studies have demonstrated the presence of such protein–polysaccharide complexes in SBP (Kirby et al., 2006; Kirby, MacDougall, & Morris, 2008). However, SBP at the oil interface has been shown to operate via different emulsification mechanism, is capable of faster interfacial tension decrease and forms more elastic films than

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gum arabic (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010). Despite the high emulsification potential, SBP has not been used extensively due to its low emulsion stability (Funami et al., 2008, 2011). Its low emulsion thermal stability has been attributed to: (i) limited adsorption at low SBP concentrations, for example only 0.15–0.20 wt.% SBP is adsorbed on to the oil/water interface in the presence of 15 wt.% middle-chain triglyceride (MCT) or 20 wt.% limonene oil (Funami et al., 2007; Nakauma et al., 2008; Siew & Williams, 2008a, 2008b); (ii) its extended molecular configuration means that it occupies more of the surface of the droplets at lower dosage than spherical soybean soluble polysaccharide and gum arabic (Nakauma et al., 2008).

The competitive adsorption of hydroxypropyl methylcellulose (HPMC) with SBP is studied here. HPMC, is a water-soluble non-ionic cellulose amphiphilic molecule (Perez, Sanchez, Pilosof, & Rodriguez Patino, 2008; Petrovic, Sovilj, Katona, & Milanovic, 2009; Sovilj & Petrovic, 2006) with a high surface activity and which has been extensively used in the food, cosmetic and pharmaceutical industries (Schulz & Daniels, 2000). Its action depends on its lipophilic segments attaching to the oil/water interface and the hydrophilic segments extending into the aqueous phase, forming a train-loop-tail conformation in the adsorbed layer. Wollenweber, Makievski, Miller, and Daniels (2000) determined, the area occupied per adsorbed HPMC molecule and the thickness of the adsorbed HPMC layer at the oil/water interface. Only a few polymer segments are adsorbed at the interface, which further supports the train-loop-tail conformation of the adsorbed layer. From the thickness of this layer it has been concluded that the adsorbed layer is multilayered such that HPMC-stabilized emulsions have strong stabilities (Hayakawa, Kawaguchi, & Kato, 1997; Wollenweber et al., 2000; Yonekura, Hayakawa, Kawaguchi, & Kato, 1998).

The objective of this paper is to investigate the influence of competitive adsorption on the emulsion properties and stability of two emulsifiers, which are structurally different and operate via different mechanisms. To our knowledge, such polysaccharide–polysaccharide systems have not previously been investigated.

## 2. Materials and methods

### 2.1. Materials

Low methoxyl non-amidated SBP (DE of 39%), with a ferulic acid content of 0.29 wt.% and protein content of 10.57 wt.% was supplied from CP Kelco, Denmark. Commercial HPMC was obtained from Dow Chemical Company (USA) with methoxyl content of 27–30% and 4.0–7.5% hydroxypropoxyl contents.

### 2.2. Methods

#### 2.2.1. GPC-MALLS system

GPC-MALLS system was employed to determine the molecular weight and adsorbed polymer concentration. It mainly consists of a DAWN-DSP multi-angle light scattering photometer (Wyatt Technology, Santa Barbara, CA, USA) equipped with He-Ne laser at the wavelength of 633 nm, a differential refractometer (RI 2000, Schambek, Germany) and an ultraviolet detector (Shimadzu SPD-6A, UK) operated at 280 nm. A 100  $\mu$ L of polymer solution was passed through a set of two columns (Shodex OHpak, Japan – exclusion limits  $1 \times 10^5$  and  $2 \times 10^7$  g/mol) namely SB-803HQ and SB-806HQ (8 mm  $\times$  300 mm) protected by a Shodex OHpak SB-LG guard column at a flow rate of 0.45 mL/min with 0.2 M NaNO<sub>3</sub> as the eluent. The eluent was filtered through a 0.2  $\mu$ m cellulose nitrate filter (Fisher Scientific, UK) and was passed through a degasser (ERC-3215 $\alpha$ , ERC Inc., Japan) before being pumped into the GPC

system using a constametric 3200 MS pump (Thermo Separation Products, USA). Calibration of the detectors (light scattering and RI) has been described previously (Al-Assaf, Philips, Aoki, & Sasak, 2007). All samples were filtered through a 0.45  $\mu$ m single use nylon filter (Whatman, UK) prior to injection to the GPC system. dn/dc values of 0.146 mL/g (Fishman, Chau, Hoagland, & Ayyad, 1999) and 0.135 mL/g (Vollmer et al., 2002) were used for SBP and HPMC, respectively.

#### 2.2.2. Preparation of SBP and HPMC stock solutions

Stock solutions of 4.0 wt.% of HPMC and SBP were prepared by dispersing a weighted amount (based on dry matter) of HPMC or SBP into distilled water and leaving the solution on a roller mixer (Barloworld Scientific Ltd., UK) to hydrate fully for 2 days at room temperature. Microorganism growth was prevented by the addition of 0.005% (w/v) of sodium azide to the solvent. Appropriate volume of solvent was used to dilute the stock solution and prepare lower concentration of HPMC or SBP.

#### 2.2.3. Preparation of emulsions stabilized with SBP, HPMC or SBP/HPMC mixtures

In this study various emulsions were prepared either with SBP or HPMC alone or SBP/HPMC mixtures at pH 4.0. Generally SBP/HPMC mixed emulsions containing 1.5 wt.% SBP and 1.0 wt.% HPMC with 15 wt.% MCT oil were prepared as follows. Batches of 50 g of pre-emulsion were prepared by mixing 18.75 g of 4.0 wt.% SBP, 12.5 g of 4.0 wt.% HPMC, 7.5 g of middle-chain triglyceride (MCT), 0.5 g of 10 wt.% benzoic acid and 10.75 g of distilled water using a polytron-type high shear mixer (Kinematica, Switzerland) at 26,000 rpm for 3 min. After adjusting the pH to 4.0 with dropwise addition of HCl, the pre-emulsion was passed twice through a high pressure homogenizer (Nanomiser NM2-L100-D07, Collision type S generator, Yoshida Kikai Co. Ltd., Japan) at 50 MPa to prepare the final emulsion. For other emulsions, different amounts of SBP and HPMC stock solutions were added in the emulsification process.

#### 2.2.4. Laser diffraction

Malvern Mastersizer 2000 (Malvern Ltd., UK) was used to determine the droplet size distributions of emulsions. A glass vial containing the emulsions was gently inverted until homogeneously dispersed. Then a few drops of emulsion were added and dispersed in a small volume sample dispersion unit (Malvern Ltd., UK) at the speed of 2000 rpm until the laser obscuration was between 10% and 15%. The droplet size distribution was determined by fitting the data using the general purpose model. Values of 1.45 and 0.001 were used for MCT refractive index and absorption index, respectively and 1.33 and 0 for the dispersant (water), respectively. The average droplet diameter was characterized as surface–volume mean diameter  $d_{3,2}$  (also known as Sauter mean diameter) and volume–moment mean diameter  $d_{4,3}$ . The surface–volume mean diameter  $d_{3,2}$  was used to estimate specific surface area of freshly made emulsions, while the volume–moment mean diameter  $d_{4,3}$  was used to monitor the changes in droplet size distribution on storage since it is more sensitive to large droplets (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). The specific surface area  $S_v$  of the emulsion was calculated as follows:

$$S_v = \frac{6\varphi}{d_{3,2}} (\text{m}^2/\text{mL}) \quad (1)$$

where  $\varphi$  represents the volume fraction of the dispersed phase. The volume fraction was determined by density measurements as given below:

$$\varphi = \frac{\rho_{aq} - \rho_{em}}{\rho_{aq} - \rho_{oil}} \quad (2)$$

where  $\rho_{aq}$  (mg/mL),  $\rho_{em}$  (mg/mL), and  $\rho_{oil}$  (mg/mL) represent the density of the aqueous phase, emulsion and oil, respectively. Interfacial concentration  $\Gamma$  (mg/m<sup>2</sup>) was calculated as follows:

$$\Gamma = \frac{C}{S_v} \quad (3)$$

where  $C$  is the adsorbed polymer concentration.

### 2.2.5. Extraction and determination of absorbed and non-absorbed fractions

The non-adsorbed polymer fractions were extracted following the method reported by Funami et al. (2007). 5 gm of the final emulsion (made with SBP, HPMC or mixture) was introduced into a centrifugation tube (40 mL capacity) and mixed with 20 g of a mixed solution containing 36 wt.% sugar and 6 wt.% NaCl using a polytron-type high shear mixer (Kinematica, Switzerland) at a speed of 26,000 rpm for 3 min. The emulsion was centrifuged at 4000 ×  $g$  for 30 min which then separated into a thin upper cream phase (containing the adsorbed fraction) and a lower aqueous phase (containing the non-adsorbed fraction). A sample (0.5 mL) of the lower aqueous phase was extracted with a needle connected to a syringe and diluted to 2 mg/mL using 0.2 M NaCl solution and injected into the GPC system. The concentration of the non-adsorbed fraction was determined from the mass recovery (determined by integration of the refractometer signal) and compared with the control. The control (i.e. before emulsification) without the addition of oil was prepared in the same way and 0.5 mL was diluted to 2 mg/mL in 0.2 M NaCl. Similarly 5 gm of mixed emulsion (containing 1.5% SBP and 1% HPMC) was treated the same way. The control was prepared by mixing 1.875 g of 4.0 wt.% SBP and 1.25 g of 4.0 wt.% HPMC and the addition of 1.875 distilled water containing NaN<sub>3</sub> followed by subjecting the mixed solutions to the same method used for the emulsion.

The adsorbed polymer concentration  $C$  was determined by the following equation:

$$C = C_0 \times \frac{C' - C''}{C'} \quad (4)$$

where  $C_0$  is polymer concentration (g/mL) used for emulsification, and  $C'$  and  $C''$  are polymer concentration before and after emulsification respectively used for GPC measurements after dilution.

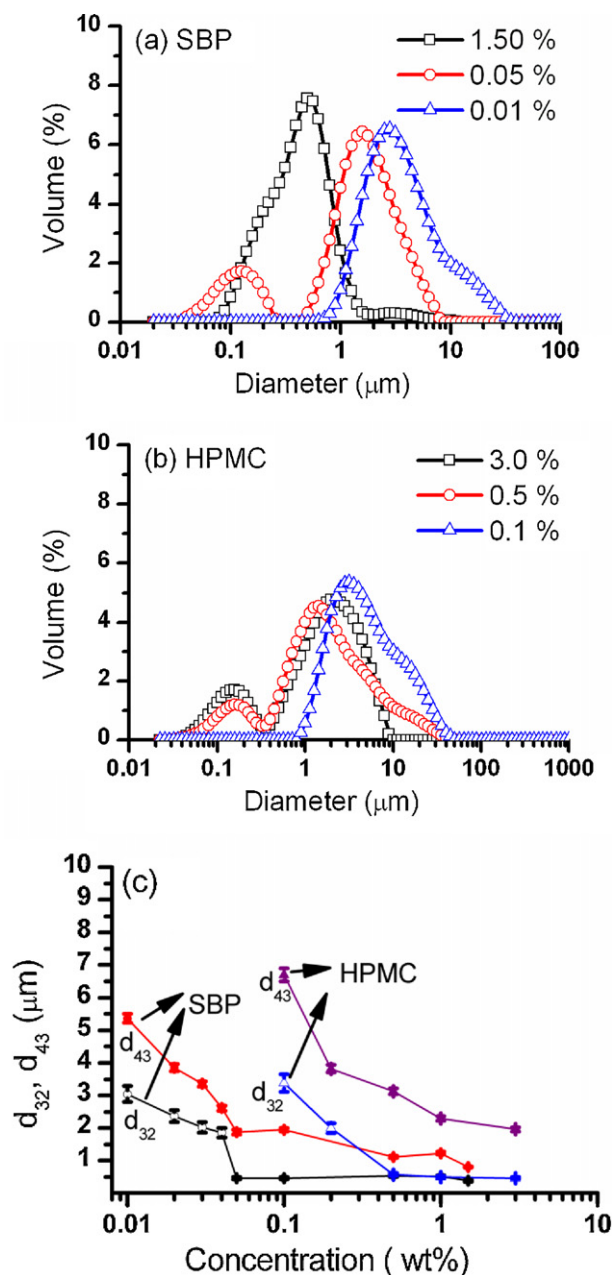
### 2.2.6. Light microscope

Images of emulsion droplets were taken using a BT-1600 image particle size analyser (Dandong Bettersize Instrument Ltd., China) consisting of an optical microscope (Nikon YS100) and a CCD camera (HV2001UC). A small drop of emulsion was placed on to a microscopic slide and carefully covered with a cover slip, left to equilibrate for 2 min and subsequently the photomicrographs (20× magnification) taken.

## 3. Results

### 3.1. Molecular characterization

The weight average molecular weight ( $M_w$ ) of SBP was  $5.3 \times 10^5$  g/mol, with a radius of gyration ( $R_g$ ) of 41 nm and polydispersity index ( $M_w/M_n$ ) of 1.90 when measured by GPC-MALLS. The corresponding values for HPMC were  $0.8 \times 10^5$  g/mol, 33 nm and 1.66. Although the molecular weight of SBP is about 7 times that of HPMC, this difference is not reflected in the  $R_g$  value due to their different molecular conformation. HPMC has a rigid backbone structure and adopts a rod-like conformation in aqueous solution (Wollenweber et al., 2000), whereas SBP adopts random coil conformation due its high content of neutral-sugar side chains (Funami



**Fig. 1.** Droplet size distributions for (a) emulsions stabilized by 0.01 wt.%, 0.05 wt.% or 1.5 wt.% SBP and (b) emulsions stabilized by 0.1 wt.%, 0.5 wt.% or 3.0 wt.% HPMC. (c) Surface–volume mean diameter ( $d_{3,2}$ ) and volume–moment mean diameter ( $d_{4,3}$ ) plotted as a function of SBP or HPMC concentration.

et al., 2007). These results are in agreement with those reported previously (Funami et al., 2007; Perez et al., 2008).

### 3.2. Comparison in emulsification behavior between SBP and HPMC

Fig. 1a shows the droplet size distributions of selected emulsions stabilized by different concentrations of SBP. When SBP concentration is below 0.04 wt.%, there is only a single peak above 1  $\mu$ m. At 0.05 wt.%, a small peak around 0.126  $\mu$ m appears as a consequence of increasing the surface coverage. At 1.5 wt.%, a single peak around 0.5  $\mu$ m appears. A fine and homogenous emulsion was obtained at 1.5 wt.% SBP as previously found (Funami et al., 2007; Nakauma et al., 2008; Siew & Williams, 2008b).



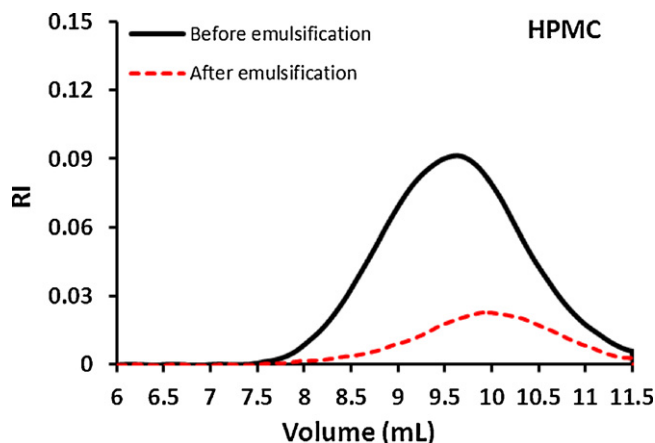


Fig. 2. (a) Elution profiles of HPMC monitored by RI for the control and non adsorbed fraction for 1.5% HPMC stabilized emulsion.

Correspondingly, the effect of increasing HPMC concentration on the emulsion droplet size is shown in Fig. 1b. Below 0.2 wt.% HPMC the emulsions have only droplets larger than 0.5  $\mu\text{m}$ . However, an additional peak at around 0.14  $\mu\text{m}$  appears when the concentration is increased to 0.5 wt.%. All droplets larger than 10  $\mu\text{m}$  disappear at 3.0 wt.% HPMC, indicating that 3.0 wt.% HPMC is necessary to produce a fine emulsion (Fig. 1b). Fig. 1c shows the volume–moment mean diameter  $d_{4,3}$  and surface–volume mean diameter ( $d_{3,2}$ ) as a function of various concentrations of SBP or HPMC. The surface–volume mean diameter levels off from 0.05 wt.% and 0.5 wt.% for SBP and HPMC, respectively. It is significant that these two emulsions have similar  $d_{3,2}$  values at 0.5 wt.% whereas HPMC emulsions have larger  $d_{4,3}$  values than SBP emulsions at the same concentrations. Thus large emulsion droplets are present in the HPMC emulsion, indicating that SBP has stronger emulsifying ability than HPMC.

The amounts of adsorbed polymer in emulsions stabilized by SBP and HPMC were also determined. Fig. 2 shows the RI elution profiles of the non-adsorbed fraction in HPMC emulsion at 1.5 wt.% at pH 4.0 compared with the control (i.e. before emulsification). Although the adsorbed fraction spans over the entire range of molecular weights but there seems to be a distinct shift to higher volumes, suggesting that the higher molecular weight fractions are preferentially adsorbed. Similar trend was obtained for SBP emulsion.

The amount of adsorbed SBP in emulsion was 1.91 mg/mL, which is in agreement with the values reported previously (Funami et al., 2007; Nakauma et al., 2008; Siew & Williams, 2008a, 2008b). The interfacial concentrations of 1.25 mg/m<sup>2</sup> correspond to monolayer adsorption of protein and are consistent with a previous study (Castellani et al., 2010). Our value for SBP interfacial concentration is in agreement with Funami et al. (2007) (1.42 mg/m<sup>2</sup>). On the other hand, the value reported by Siew and Williams (2008a) (ca. 10 mg/m<sup>2</sup>) can be largely explained by the difference in the methods used to prepare the emulsion. The large droplets reported by Siew et al. were the result of pre-emulsion without homogenization thus giving a low value of specific surface area which in turn resulted in the high value of the interfacial concentration (as shown in Eqs. (1) and (3)).

The interfacial behavior of SBP and HPMC stabilized emulsions are shown in Table 1. The adsorbed HPMC concentration is 11.5 mg/mL and corresponds to an interfacial concentration of 3.5 mg/m<sup>2</sup>. This high value, compared to SBP, is a consequence of HPMC high adsorption ability and probably due to multiple adsorption layers as found by Wollenweber et al. (2000).

Table 1

Emulsion and adsorbed layer properties of O/W emulsions stabilized by SBP tin and HPMC at 1.5 wt.%.

Sample	SBP	HPMC
$d_{3,2}$ ( $\mu\text{m}$ )	$0.47 \pm 0.04$	$0.48 \pm 0.03$
Specific surface area ( $\text{m}^2/\text{mL}$ )	$1.53 \pm 0.18$	$3.24 \pm 0.28$
Absorbed concentration ( $\text{mg/mL}$ )	$1.92 \pm 0.12$	$11.50 \pm 0.92$
Interfacial concentration ( $\text{mg/m}^2$ )	$1.25 \pm 0.23$	$3.55 \pm 0.59$

### 3.3. Effect of competitive adsorption

To identify the role of each emulsifier in competitive adsorption, the concentration of one emulsifier was varied while keeping the concentration of the other emulsifier constant.

#### 3.3.1. Varying HPMC concentration with SBP unchanged

Fig. 3 shows the value of  $d_{3,2}$  plotted as a function of HPMC concentration (0–1.0 wt.%) for emulsions containing 1.5 wt.% SBP. The  $d_{3,2}$  value initially increases to a maximum at 0.05 wt.% HPMC. Subsequently, the droplet size decreases with HPMC concentration and reaches a plateau at 0.4 wt.%. Fig. 3 shows the micrographs of four emulsions with 1.5 wt.% SBP and increasing concentrations of HPMC (0, 0.01 wt.%, 0.1 wt.% and 1.0 wt.%). More large droplets are observed when the concentration of HPMC increases above 0.01 wt.% and they become dominant at 0.1 wt.% HPMC. Thereafter, the number of large particles decreases with increasing HPMC concentration up to 1.0 wt.%.

We have also compared the droplet size distribution of mixed SBP/HPMC emulsions with those prepared with only SBP or HPMC alone (Fig. 4). Fig. 4a shows that 0.01 wt.% HPMC is not sufficient to alter the properties of SBP-stabilized emulsion, but the size distribution changed significantly when the HPMC concentration reached 0.1 wt.%. Fig. 4b shows that at 0.1 wt.% HPMC begins to dominate the emulsion properties. When the HPMC concentration is further increased to 1.0 wt.%, the size distribution of the mixed emulsion containing 1.5 wt.% and 1.0 wt.% HPMC almost overlaps with that of the emulsion only containing 1.0% (w/w) HPMC. Thus, the results suggest that the properties of the mixed emulsion are controlled by HPMC even in the presence of high concentration of SBP.

Stress acceleration testing was carried out by incubating the emulsion at 60 °C in order to examine the stability of mixed emulsions containing 1.5 wt.% SBP and various concentrations of HPMC

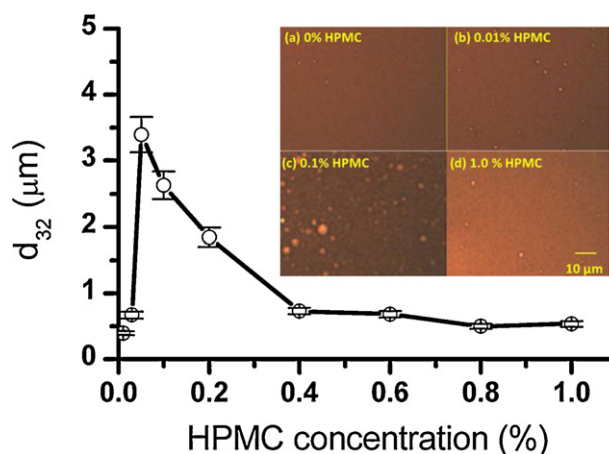
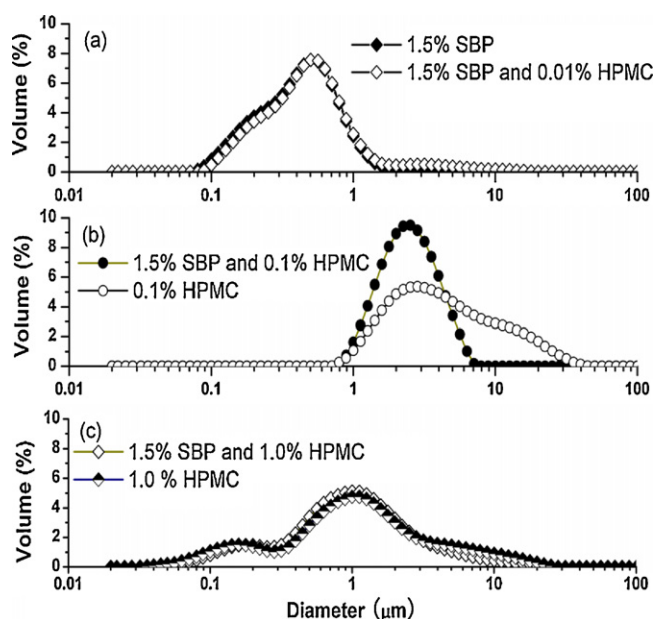


Fig. 3. Surface–volume mean diameter ( $d_{3,2}$ ) plotted as a function of HPMC concentration for SBP/HPMC mixed emulsions prepared with 1.5 wt.% SBP and different concentrations of HPMC. Micrographs of emulsions containing 1.5 wt.% SBP and various concentrations of HPMC: (a) 0 wt.% HPMC; (b) 0.01 wt.% HPMC; (c) 0.1 wt.% HPMC; and (d) 1.0 wt.% HPMC were also inserted in the plot.



**Fig. 4.** Comparisons in droplet size distributions (a) between the mixed SBP/HPMC emulsions containing 1.5 wt.% SBP and 0.01 wt.% HPMC and emulsion stabilized by only 1.5 wt.% SBP, (b) between the mixed SBP/HPMC emulsions containing 1.5 wt.% SBP and 0.1 wt.% HPMC and the emulsion stabilized by only 0.1 wt.% HPMC, and (c) between the mixed SBP/HPMC emulsions containing 1.5 wt.% SBP and 1.0 wt.% HPMC and the emulsion stabilized by only 1.0 wt.% HPMC.

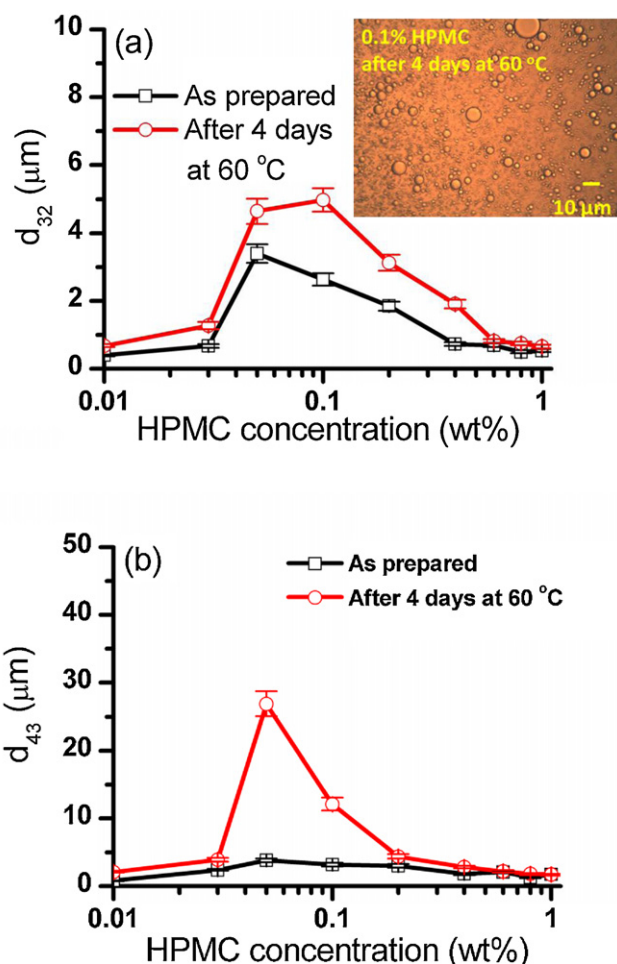
reported above. Fig. 5a and b shows  $d_{3,2}$  and  $d_{4,3}$  before and after stress acceleration testing. The largest increase in the droplet size was found in emulsions containing 0.05 wt.% or 0.1 wt.% HPMC while very small changes were observed at HPMC > 0.2 wt.%. A micrograph for the emulsion containing 0.1 wt.% HPMC is given in Fig. 5a and clearly shows the presence of large droplets. The addition of HPMC not only caused an increase in average droplet size, but also induced creaming of the SBP-stabilized emulsions. For the emulsion prepared with 1.5 wt.% SBP alone, no phase separation was found following acceleration at 60 °C. However, SBP-stabilized emulsions phase separated easily into a cream layer and a serum layer when HPMC was added. The larger the droplet size is the higher the rate of creaming. The creaming rate of the emulsion containing 0.1 wt.% HPMC was the fastest amongst all the emulsions. The creaming rate can be estimated by the following equation (Tadros, 2007):

$$\nu_0 = \frac{2\Delta\rho g R^2}{9\eta_0} \quad (5)$$

where  $\nu_0$  is the creaming rate,  $\Delta\rho$  is the density difference between the water and the oil,  $g$  is acceleration of gravity,  $\eta_0$  is the viscosity of the medium, and  $R$  is the radius of the droplet. The proportion of serum layer in the emulsions as a function of HPMC concentration after 4 days at 60 °C was determined and shown in Table 2. The observed fastest creaming rate of the emulsions containing 0.1 wt.% HPMC is consistent with the droplet size results as shown in Fig. 5a.

### 3.3.2. Varying SBP concentration while keeping HPMC concentration unchanged

The SBP concentration was varied from 0 wt.% to 1 wt.% while HPMC concentration was fixed at 1.5 wt.% in the emulsion. Fig. 6 gives size distributions of three emulsions prepared respectively with 1.0 wt.% SBP, 1.5 wt.% HPMC and mixed SBP/HPMC solution containing 1.5 wt.% HPMC and 1.0 wt.% SBP. The size distribution of the emulsion containing 1.5 wt.% HPMC and 1.0 wt.% SBP resembles that of the emulsion containing 1.5 wt.% HPMC only. This further confirms that the properties of the mixed SBP/HPMC emulsion



**Fig. 5.** Comparison of (a)  $d_{3,2}$  and (b)  $d_{4,3}$  for emulsions containing 1.5 wt.% SBP and various concentrations of HPMC as prepared and following accelerated stress testing for 4 days at 60 °C. (Insert) shows a micrograph for emulsion stabilized by 1.5 wt.% and 0.1 wt.% HPMC.

were controlled by the high concentration of HPMC. Comparison of the size distributions between emulsions stabilized with 1.5 wt.% HPMC alone and mixed SBP/HPMC emulsion clearly demonstrate the role of SBP. The addition of 1.0 wt.% SBP has a minor role in competitive adsorption and its main contribution is the reduction of large droplets with diameters above 3.0 μm (see the micrographs of the two emulsions inserted in Fig. 6).

The values of  $d_{3,2}$ ,  $d_{4,3}$  and volume fraction of large droplets above 3 μm for emulsions containing 1.5 wt.% HPMC and different concentrations of SBP are given in Table 3.  $d_{3,2}$  remains unchanged while  $d_{4,3}$  decreases with increasing SBP concentration. As noted

**Table 2**

The proportion of serum layer in SBP emulsions at 1.5 wt.% as a function of HPMC concentration after 4 days at 60 °C.

HPMC concentration (wt.%)	$H_{\text{serum layer}}/H_{\text{total layer}}$ (%)
0.00	0.00 ± 0.03
0.01	0.02 ± 0.02
0.03	0.02 ± 0.02
0.05	9.80 ± 0.02
0.10	52.94 ± 0.03
0.20	21.43 ± 0.02
0.40	21.28 ± 0.02
0.60	22.86 ± 0.03
0.80	19.35 ± 0.03
1.00	14.29 ± 0.03

**Table 3**

Emulsion properties for mixed emulsions containing 1.5% HPMC and different concentrations of SBP.

SBP concentration in HPMC emulsions (wt%)	$d_{3,2}$ ( $\mu\text{m}$ )	$d_{4,3}$ ( $\mu\text{m}$ )	%Volume fraction above 3 $\mu\text{m}$	$d_{4,3}$ after 4 days at 60 °C ( $\mu\text{m}$ )
0	0.46 $\pm$ 0.03	1.41 $\pm$ 0.04	8.12 $\pm$ 0.43	1.25 $\pm$ 0.03
0.02	0.42 $\pm$ 0.02	1.21 $\pm$ 0.02	5.29 $\pm$ 0.27	1.24 $\pm$ 0.03
0.05	0.42 $\pm$ 0.02	1.10 $\pm$ 0.03	3.41 $\pm$ 0.15	1.07 $\pm$ 0.02
0.1	0.43 $\pm$ 0.03	1.17 $\pm$ 0.02	4.51 $\pm$ 0.22	1.18 $\pm$ 0.03
0.2	0.43 $\pm$ 0.03	1.08 $\pm$ 0.04	3.07 $\pm$ 0.17	1.10 $\pm$ 0.02
0.5	0.41 $\pm$ 0.03	1.03 $\pm$ 0.04	2.41 $\pm$ 0.11	1.21 $\pm$ 0.03
1	0.39 $\pm$ 0.02	0.93 $\pm$ 0.03	1.10 $\pm$ 0.06	0.96 $\pm$ 0.02

above, with increasing SBP concentration the %volume fraction of droplets  $>3 \mu\text{m}$  decreases and again confirms the role of SBP. Stability measurements for the above emulsions were also carried out and the results for  $d_{4,3}$  after 4 days at 60 °C are also given in Table 3. There was no significant difference between fresh and the accelerated emulsions after incubating for 4 days at 60 °C and further confirms the role of HPMC being the dominant emulsifier in the mixed emulsion. The results are in agreement with previous reports about the emulsion stability of HPMC (Hayakawa et al., 1997; Wollenweber et al., 2000; Yonekura et al., 1998).

#### 3.4. Determination of the amounts of adsorbed SBP and HPMC in mixed emulsion

The adsorbed amounts of SBP and HPMC in an emulsion containing 1.5 wt.% SBP and 1.0 wt.% HPMC were also determined. Fig. 7 gives the elution profiles, monitored by UV absorbance at 280 nm, of SBP and HPMC solutions before and after emulsification as well as for HPMC alone. It can be seen that SBP has a strong UV absorbance peak while HPMC has a very weak absorbance peak. The UV absorbance of HPMC can therefore be neglected, and the amount of adsorbed SBP can be estimated from the UV absorbance when HPMC and SBP are both present. The UV absorbance peak area after emulsification is about 95.72% of the original UV absorbance peak area, which indicates that only 4.28% of the total SBP (namely 0.06 wt.% SBP) was adsorbed at the emulsion interface. However, the RI area (data not shown) after emulsification is about 57.5% of the original RI area, which implies about 42.5% of total polymer (namely 1.06 wt.% polymer) was adsorbed at the interface. Apart from the 0.06 wt.% SBP, the remaining 1.0 wt.% adsorbed polymer should be HPMC. This means that the total HPMC was adsorbed

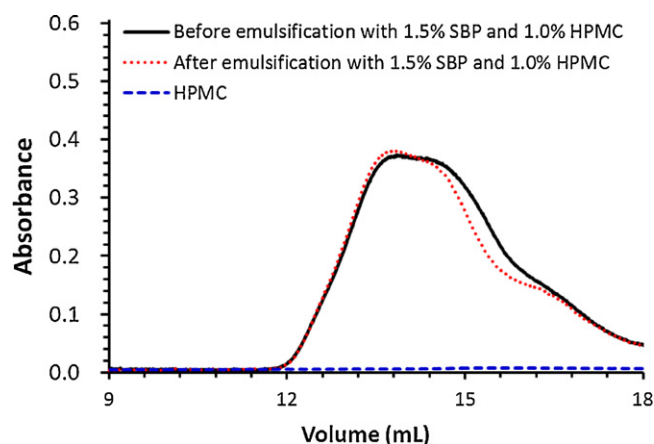


Fig. 7. Elution profile of HPMC and HPMC/SBP monitored by UV absorbance at 280 nm for the aqueous phase before and after emulsification with 1.5 wt.% SBP and 1.0 wt.% HPMC.

onto the interface. Though the amounts of adsorbed SBP and HPMC are only estimated values, the results show clearly that the adsorption of HPMC at the oil–water interface predominates over that of SBP.

#### 4. Discussion

An important factor effecting competitive adsorption is the difference in surface activity between competing emulsifiers. At air/water interface, the reported equilibrium surface tension values for SBP and HPMC are 55–60 (Gromer et al., 2009; Zouambia, Moulai-Mostefa, & Krea, 2009) and 46–48 mN/m (Arboleya & Wilde, 2005) respectively. While values of 17.2 mN/m (Funami et al., 2007) and 14.2 mN/m (Wollenweber et al., 2000) were reported for SBP and HPMC, respectively, at oil/water interface. These results both demonstrate that HPMC is more surface active than SBP. The difference in surface activity between HPMC and SBP may be attributed to the differences in their conformation, size and mechanism to reduce the surface tension.

For SBP, it is predominately carbohydrate and its surface activity is due to the presence of surface-active components such as protein and ferulic acid (Siew & Williams, 2008a, 2008b). Kirby et al. (2006, 2008) revealed through atomic force microscopy that the protein molecule was attached to one end of linear SBP chains and formed tadpole-like protein–polysaccharide complex. Leroux et al. (2003) proposed a model for the adsorbed layer of SBP-stabilized emulsion, where the protein was adsorbed at the oil/water interface whilst the polysaccharide chain extended out into the aqueous phase as shown in Fig. 8a. The tadpole-like complex favors the formation of small emulsion droplets through monolayer coverage. The interfacial concentration determined in this study was around 1.25 mg/m<sup>2</sup>, which also supports the hypothesis about the single adsorbed layer. For HPMC, the emulsifying ability is

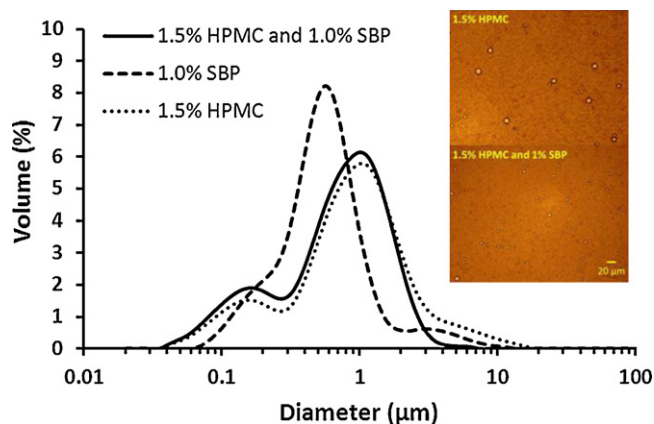
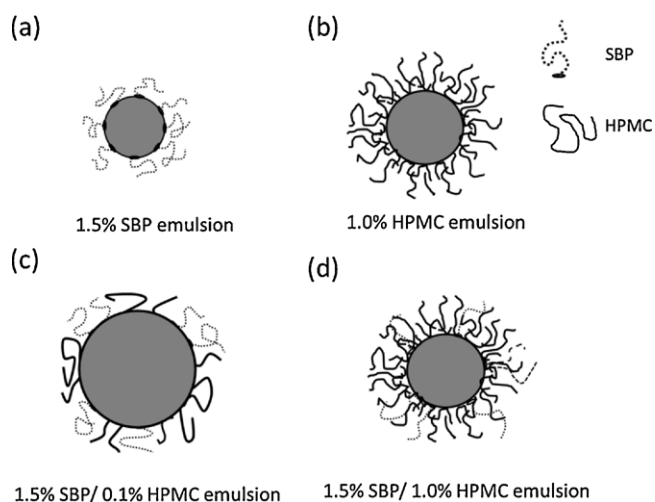


Fig. 6. Droplet size distributions of three emulsions prepared, respectively, with 1.5 wt.% HPMC, 1.0 wt.% SBP, and a mixed solution containing 1.5 wt.% HPMC and 1.0 wt.% SBP. Micrographs of two emulsions prepared, respectively, with 1.5% (w/w) HPMC, and a mixed solution containing 1.5 wt.% HPMC and 1.0 wt.% SBP also given in the plot.





**Fig. 8.** Schematic illustrations of interfacial structures for four emulsions prepared with (a) 1.5 wt.% SBP, (b) 1.0 wt.% HPMC, (c) 1.5 wt.% SBP and 0.1 wt.% HPMC and (d) 1.5 wt.% and 1 wt.% HPMC. Solid lines represent HPMC molecules and dashed lines represent SBP molecules. Solid ellipses attached to one end of the SBP chains represent protein molecules.

due to its high surface activity. It is an amphiphilic biopolymer where the hydrophobic groups (methyl) and hydrophilic groups (hydroxypropyl) coexist in the polymer chains (Perez et al., 2008). Therefore, the proposed emulsification mechanism is that the lipophilic segments along the HPMC chains attach to the interface and form trains, whilst hydrophilic segments extend out into the aqueous phase and form loops or tails as illustrated in Fig. 8b (Wollenweber et al., 2000). Because of its rigid backbone structure, Wollenweber et al. (2000) proposed that it adopts a rod-like conformation in aqueous solution. This conformation is unfavorable for the formation of small emulsion droplets since it requires the lipophilic segment to be bent in order to occupy enough interfacial area. Unlike SBP, high concentrations of HPMC are adsorbed at the oil/water interface and form multilayer (Wollenweber et al., 2000). Its high emulsion stability is due to its capability of forming a highly elastic/viscoelastic layer (Arbolea & Wilde, 2005; Camino, Sanchez, Patino, & Pilosof, 2011; Perez et al., 2008).

During competitive adsorption, HPMC is more rapidly adsorbed onto the oil/water interface than SBP due to its low molecular weight, conformation and the nonionic character. For SBP, only some surface active parts such as proteinaceous moieties can adsorb onto the interface, but they are hampered by the net surface charge from the highly anionic character. Therefore, the interface area occupied by a HPMC molecule (lipophilic segments) presumably is larger than that occupied by a SBP molecule. Consequently, HPMC has an advantage over SBP in the competitive adsorption. When the SBP concentration is fixed at 1.5 wt.%, the emulsion properties and stability are sensitive to HPMC concentration (Figure 5a). The role of HPMC becomes obvious when the amount of HPMC is close to the amount of adsorbed SBP (see Fig. 8c). In the presence of high concentrations of HPMC, the emulsion properties are controlled by HPMC completely because the amount of adsorbed HPMC is much more than that of adsorbed SBP (see Fig. 8d). The emulsion properties are almost independent of SBP concentration when the HPMC concentration is fixed at 1.5 wt.% (Fig. 6). Therefore, the size distribution of the SBP/HPMC emulsion containing 1.5 wt.% SBP and 1.0 wt.% HPMC almost overlap with that of the emulsion prepared with only 1.0 wt.% HPMC (see Fig. 4c). The role of SBP in mixed emulsions is that it can to reduce the number of large droplets (see Fig. 6). Recent AFM study has suggested that the orientation of the SBP chains at the o/w interface changes whereby they seem to lay flat at low concentrations whilst at higher concentration they

stick out from the droplets (Gromer, Penfold, Gunning, Kirby, & Morris, 2010). We propose that these flexible highly proteinaceous molecules can access the oil/water interface in the presence of HPMC rigid chains and therefore contribute to reducing the number of large droplets.

## 5. Conclusion

In this study we have systematically investigated the emulsification performance, stability and competitive adsorption of two polysaccharides emulsifiers. Both are capable of reducing the surface tension and emulsify oil in water emulsion via different mechanisms. HPMC adsorbs onto the oil/water interface more rapidly and occupies more interfacial area than SBP. SBP/HPMC mixed emulsion is therefore sensitive to HPMC. The properties and stability of mixed emulsions is mainly controlled by HPMC when its concentration is close to or higher than the concentration of adsorbed SBP. SBP main role in mixed emulsion is to decrease the number of large emulsion droplets. The results given in study may contribute to the understanding of possible application of mixed emulsion and the mechanism of destabilization.

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