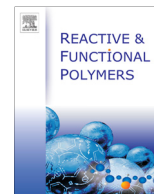


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## pH-triggered phase inversion and separation of hydrophobised bacterial cellulose stabilised Pickering emulsions

Koon-Yang Lee<sup>a,\*</sup>, Jonny J. Blaker<sup>b</sup>, Jerry Y.Y. Heng<sup>c</sup>, Ryo Murakami<sup>d</sup>, Alexander Bismarck<sup>e,f</sup><sup>a</sup> Department of Chemical Engineering, University College London, Torrington Place, WC1E 7JE London, United Kingdom<sup>b</sup> Materials Science Centre, School of Materials, The University of Manchester, Grosvenor Street, M1 7HS Manchester, United Kingdom<sup>c</sup> Surfaces and Particle Engineering Laboratory (SPEL), Department of Chemical Engineering, Imperial College London, South Kensington Campus, SW7 2AZ London, United Kingdom<sup>d</sup> Department of Chemistry of Functional Molecules, Konan University, 8-9-1 Okamoto, Kobe 658-8501, Japan<sup>e</sup> Polymer and Composite Engineering (PaCE) Group, Institute of Materials Chemistry and Research, Faculty of Chemistry, University of Vienna, Währinger Strasse 42, A-1090 Vienna, Austria<sup>f</sup> Polymer and Composite Engineering (PaCE) Group, Department of Chemical Engineering, Imperial College London, South Kensington Campus, SW7 2AZ London, United Kingdom

## ARTICLE INFO

## Article history:

Received 30 June 2014

Received in revised form 19 September 2014

Accepted 19 September 2014

Available online 2 October 2014

## Keywords:

Pickering emulsions  
Bacterial cellulose  
Esterification  
Phase inversion  
Phase separation

## ABSTRACT

The pH-triggered transitional phase behaviour of Pickering emulsions stabilised by hydrophobised bacterial cellulose (BC) is reported in this work. Neat BC was esterified with acetic (C<sub>2</sub>-), hexanoic (C<sub>6</sub>-) and dodecanoic (C<sub>12</sub>-) acids, respectively. We observed that C<sub>6</sub>- and C<sub>12</sub>-BC stabilised emulsions exhibited a pH-triggered reversible *transitional phase separation*. Water-in-toluene emulsions containing of 60 vol.% dispersed phase stabilised by C<sub>6</sub>- and C<sub>12</sub>-BC were produced at pH 5. Lowering the pH of the aqueous phase to 1 did not affect the emulsion type. Increasing the pH to 14, however, caused the emulsions to phase separate. This phase separation was caused by electrostatic repulsion between modified BC due to dissociable acidic surface groups at high pH, which lowered the surface coverage of the water droplets by modified BC. When the pH was re-adjusted to 1 again, w/o emulsions re-formed for C<sub>6</sub>- and C<sub>12</sub>-BC stabilised emulsions. C<sub>2</sub>-BC stabilised emulsions, on the other hand, underwent an irreversible pH-triggered *transitional phase separation and inversion*. This difference in phase behaviour between C<sub>2</sub>-BC and C<sub>6</sub>-/C<sub>12</sub>-BC was attributed to the hydrolysis of the ester bonds of C<sub>2</sub>-BC at high pH. This hypothesis is in good agreement with the measured degree of surface substitution (DSS) of modified BC after the pH-triggered experiments. The DSS of C<sub>2</sub>-BC decreased by 20% whilst the DSS remained constant for C<sub>6</sub>- and C<sub>12</sub>-BC.

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## 1. Introduction

Emulsions are dispersions of two immiscible liquids, which can be kinetically stabilised by suitable emulsifiers, such as surfactants. A suitable surfactant will adsorb at the liquid–liquid interface, usually oil and water, lowering the interfacial tension and therefore the driving force for phase separation [1]. The internal phase droplets could also be charged if ionic surfactants are used. This produces a repulsive force between approaching droplets and reduces the rate of droplet coalescence. In addition to surfactants, colloidal particles are also effective emulsifiers [2]. The use of colloidal particles as emulsifiers dates back to the early 20th century and was described in the pioneering works of Ramsden [3] and

Pickering [4,5]. These colloidal particle-stabilised emulsions are more commonly known as “Pickering emulsions”.

The formation of either a water-in-oil (w/o) or oil-in-water (o/w) emulsion is determined by the curvature of the interface. For the case of surfactants, this curvature is determined by the packing parameter of the surfactants at the oil–water interface [6]. Unlike surfactant-stabilised emulsions, the curvature of particle-stabilised emulsions is determined by the wettability of the particles [2]. If the particles possess intermediate wettability, they tend to adsorb at the oil–water interface and if the interface is sufficiently occupied by the particles, the particles will bend the oil–water interface towards the more poorly wetting liquid [7]. Scarlett et al. [8] put forward a relationship describing the curvature of particle stabilised oil–water interfaces as function of the three phase (particle/oil/water) contact angle ( $\theta$ ). If  $\theta < 90^\circ$ , the particles will curve the oil–water interface towards the oil phase, leading to the formation of o/w emulsions. Conversely, if  $\theta > 90^\circ$ , the particles will curve the

\* Corresponding author. Tel.: +44 (0)207 679 2412; fax: +44 (0)207 383 2348.  
E-mail address: [koonyang.lee@ucl.ac.uk](mailto:koonyang.lee@ucl.ac.uk) (K.-Y. Lee).

oil–water interface towards the water phase, leading to the formation of w/o emulsions. Pickering emulsions offer several advantages over surfactant-stabilised emulsions [9]: (i) particles are virtually irreversibly adsorbed at oil–water interface, i.e. significant energy is required to remove the particles from the oil–water interface, (ii) reduced rate of sedimentation/creaming as a result of particle aggregation in the continuous phase and (iii) improved emulsion stability towards droplet coalescence by forming a mechanical barrier between two approaching droplets.

The relationship relating the energy  $\Delta E$  required to remove a particle from the oil–water interface  $\Delta E$  to  $\theta$  has been derived and re-derived by numerous authors [10–12]. It is expressed as:

$$\Delta E = \pi\gamma r^2(1 \pm \cos \theta)^2 \quad (1)$$

where  $\gamma$  is the interfacial tension and  $r$  the particle radius. The positive and negative signs refer to the removal of a particle into the oil phase and water phase, respectively.  $\Delta E$  is usually several orders of magnitude larger than the thermal energy,  $kT$ , for particles with intermediate wettability. Furthermore, at high particle concentrations, the particles could form a three-dimensional network in the continuous phase and/or act as mechanical barrier between droplets when adsorbed at the oil–water interface [13,14]. These two factors contribute to the improved stability of Pickering emulsions. Even at low particle concentrations, Pickering emulsions are stable against phase separation due limited coalescence and the formation of bridging particle monolayers between the droplets [13,15].

A vast range of colloidal particles were shown to be efficient Pickering emulsifiers (for a comprehensive list of colloidal particles that could serve as Pickering emulsifiers, the readers are referred to [9]). However, very few authors have studied the use of renewable particles as Pickering emulsifiers. For a recent review of this topic please refer to [16]. In this context, cellulose fibrils or particles are a potential candidate because of its wide availability and non-toxic nature. The use of starch nano-crystals as Pickering emulsifier was also recently demonstrated [17]. The first use of cellulose as Pickering emulsifiers was reported by Oza and Frank [18], who used microcrystalline cellulose to stabilise mineral oil-in-water emulsions. Various types of cellulose have since been explored, focusing on different oil types and dispersed phase volume fractions ( $\phi$ ). Table 1 provides an overview of various types of cellulose, which were used to emulsify different oils [18–31]. It can be seen from this table that the hydrophilic nature of cellulose often result in the formation of o/w emulsions. To produce w/o emulsions stabilised by cellulose, the hydrophilic cellulose surface has to be hydrophobised.

We recently reported the phase behaviour of w/o high internal phase emulsions stabilised solely by hydrophobised bacterial cellulose (BC). BC fibrils were hydrophobised by esterification with acetic acid (C<sub>2</sub>-BC), hexanoic acid (C<sub>6</sub>-BC) and dodecanoic acid (C<sub>12</sub>-BC), respectively [31]. We showed that the maximum dispersed water volume fraction ( $\phi_w$ ) stabilised by hydrophobised BC is a function of time after the emulsions were prepared. This was attributed to the disentanglement of larger BC bundles into smaller bundles, thereby stabilising a larger oil–water interface over time. Furthermore, these emulsions do not undergo *catastrophic phase inversion*, which was observed for silica-stabilised emulsions [32]. Instead, these hydrophobised BC stabilised emulsions undergo *reversible catastrophic phase separation*. In this work, we show that in addition to *reversible catastrophic phase separation*, these hydrophobised BC stabilised w/o emulsions will also undergo a pH-triggered *transitional phase separation* and *inversion*, depending on the degree of hydrophobicity of modified BC.

## 2. Materials and methods

### 2.1. Materials

Acetic acid (analaR, purity  $\geq 99\%$ ), pyridine (analaR NORAMPUR, purity  $\geq 99.7\%$ ), methanol (GPR, purity  $\geq 99\%$ ), ethanol (GPR, purity  $\geq 99\%$ ) and toluene (analaR NORMAPUR, purity  $\geq 99.5\%$ ) were procured from VWR International Ltd (Lutterworth, UK). *p*-Toluenesulfonyl chloride (purity  $\geq 99\%$ ), dimethyl carbonate (Aldrich Reagent Plus, purity  $\geq 99\%$ ), hexanoic acid (purity  $\geq 99.5\%$ ) and dodecanoic acid (purity  $\geq 98\%$ ) were purchased from Sigma–Aldrich (Dorset, UK) and sodium hydroxide (purum grade, pellets) from Acros Organics. All materials were used without further purification. BC was extracted from commercial grade *nata de coco* (CHAOKOH coconut gel in syrup, Ampol Food Processing Ltd., Nakorn Pathom, Thailand).

### 2.2. Extraction and surface-only modification of BC

The extraction of BC nanofibrils from *nata de coco* is described in our earlier papers [30,33]. Briefly, *nata de coco* gel from 10 jars (each jar containing 500 g) was repeatedly washed with de-ionised water to remove the sugar syrup. The washed *nata de coco* gel was then blended for 1 min using a Waring blender (LB20EG, Christison Particle Technologies, UK), followed by 2 min of homogenisation using a laboratory homogeniser (Polytron PT 10–35 GT, Kinematica, Switzerland). The BC was concentrated by removing the excess water using centrifugation. The extracted BC was further purified by redispersing the extracted BC in 0.1 M NaOH and heated to 80 °C for 20 min under stirring, which removes soluble polysaccharides [34]. The purified BC was then successively centrifuged and washed to neutral pH.

In order modify only the surface of BC fibrils by esterification with alkanic acids of increasing aliphatic chain length (acetic acid: C<sub>2</sub>–, hexanoic acid: C<sub>6</sub>– and dodecanoic acid: C<sub>12</sub>–), BC had to be solvent exchanged from water into the subsequent reaction medium (pyridine) since severe BC bulk modification occurred when directly dispersing freeze-dried BC into pyridine [35]. Therefore, 2 g (dry weight) of purified BC was solvent exchanged from water through methanol into pyridine. At each stage, the dispersed BC was homogenised at 20,000 rpm for 1 min to ensure that BC was dispersed in each solvent. Centrifugation at 14,000 g was used to retain the BC at each stage before re-dispersing it in the subsequent solvent. A final solvent exchange step was conducted to adjust the BC concentration in pyridine to 0.005 g mL<sup>-1</sup>. This mixture was then placed into a 1 L 3-neck round bottom flask. 92 g of *p*-toluenesulfonyl chloride and an equimolar amount of organic acid (C<sub>2</sub>–, C<sub>6</sub>– or C<sub>12</sub>–) was added to the BC dispersion. The reaction was conducted under continuous stirring for 2 h at 50 °C under nitrogen atmosphere. Ethanol was used to quench the reaction and the hydrophobised (C<sub>2</sub>–, C<sub>6</sub>– or C<sub>12</sub>–) BC washed with ethanol, followed by water using the previously described homogenisation–centrifugation steps. Afterwards, hydrophobised BC was dispersed in dimethyl carbonate at a concentration of 4 g L<sup>-1</sup> and freeze-dried (Heto PowerDry LL1500 Freeze Dryer, Thermo Scientific, UK). The BC modified with acetic, hexanoic and dodecanoic acids are termed C<sub>2</sub>-BC, C<sub>6</sub>-BC and C<sub>12</sub>-BC, respectively.

### 2.3. Preparation of w/o emulsions stabilised by modified BC

The emulsions stabilised by modified BC were prepared as follows. Firstly, freeze-dried and hydrophobised BC was homogenised at 20,000 rpm for 1 min in toluene at a concentration of 0.005 g mL<sup>-1</sup>. De-ionised water was then added into this dispersion to adjust the water volume fraction ( $\phi_w$ ) to 60%. The modified

**Table 1**  
Pickering emulsions stabilised by various types of cellulose [18–31]. Obtained from Lee et al. [31] with kind permission from ACS.

Type of cellulose	Chemical modification	Oil phase	Type of emulsions	$\phi^d$ (vol.%)
Microcrystalline cellulose		Heavy mineral oil	o/w	20
		Sunflower oil	o/w	20
		Vegetable oil	o/w	50
Nanofibrillated cellulose <sup>a</sup>	Silylation with chlorodimethyl isopropylsilane Neat <sup>b</sup> Modified with octadecylamine <sup>b</sup> Modified with poy(styrene-co-maleic anhydride) <sup>b</sup>	Kerosene	o/w	50
		Toluene	w/o	20–50
		Diesel	w/o	10–20
			w/o	10–20
Microfibrillated cellulose <sup>a</sup>		Vegetable oil	o/w	50
		Kerosene	o/w	50
Cellulose nanocrystals	Hydrolysed with H <sub>2</sub> SO <sub>4</sub> Hydrolysed with H <sub>2</sub> SO <sub>4</sub> , followed by desulfonation step Hydrolysed with HCl Hydrolysed with HCl, followed by sulfonation step Hydrolysed with HCl	n-hexadecane	o/w	30
			o/w	30
			o/w	30
			o/w	30
			o/w	30
			o/w	92
Bacterial cellulose	Hydrolysed with H <sub>2</sub> SO <sub>4</sub> , followed by poly(NIPAM) grafting	Heptane	o/w	50
		Vegetable oil	o/w	50
		Kerosene	o/w	50
		Soybean oil	w/o or o/w <sup>c</sup>	30–60
	Silylation with chlorodimethyl isopropylsilane Esterified with acetic acid Esterified with acetic acid Esterified with hexanoic acid Esterified with dodecanoic acid		w/o	60
		Toluene	w/o	71
			w/o	81
			w/o	77

<sup>a</sup> The difference between nanofibrillated cellulose and microfibrillated cellulose is the dimension of the fibres.

<sup>b</sup> In these formulations, a combination of glycerol monooleate and sorbitan monolaurate were also added into the emulsions along with the cellulose particles to stabilise the emulsions.

<sup>c</sup> The type of emulsions depends on the volume fraction of the oil phase used.

<sup>d</sup> The volume fraction of dispersed phase.

BC/water/toluene mixture was emulsified for 10 min by hand shaking at a frequency of about 4 Hz. Previously, we showed that the modified BC stabilised w/o emulsions exhibited time dependent behaviour [31]. Therefore, the emulsions were left at room temperature for one week prior to adjusting the pH of the emulsions, either by the addition of HCl or NaOH, to pH 1 or 14.

#### 2.4. Characterisation of the degree of surface substitution and water-in-air contact angle of hydrophobised BC

In order to quantify the degree of surface substitution (DSS) of hydrophobised BC used as emulsifier, the emulsions were freeze-dried to remove water and toluene after the pH-triggered phase inversion. The freeze-dried, hydrophobised BC was then re-dispersed in water using the previously described homogenisation-centrifugation steps to remove any water-soluble contaminants. This hydrophobised BC-in-water dispersion was then solvent exchanged from water through ethanol into benzene to remove any water-insoluble compounds, mainly the acids used for hydrophobisation of BC prior to freeze-drying from benzene. The DSS was determined using hydrogen–deuterium exchange (H/D) employing dynamic vapour sorption (DVS) [35–37]. Briefly, 15 mg of the purified hydrophobised BC was placed in the sample pan of DVS. The DVS sample chamber was conditioned at 0% relative humidity (RH) for 5 h to remove any adsorbed volatiles. The RH of the chamber was then increased to 90% for 2 h to allow for the H/D exchange between accessible –OH groups to take place. The RH of the chamber was then decreased back to 0% for another 2 h to desorb excess D<sub>2</sub>O. This cycle was repeated 10 times such that all accessible and available –OH groups can be exchanged into –OD groups. A short cycle was used to avoid D<sub>2</sub>O sorption in bulk BC from occurring in the hydrophobised BC. The sample was then post-conditioned to 0% RH for another 5 h to remove any excess D<sub>2</sub>O. The mass change of the sample as a result of D<sub>2</sub>O exposure was recorded in situ during the measurement. The amount of

accessible and available –OH groups was then back calculated from this mass increase using the following equation:

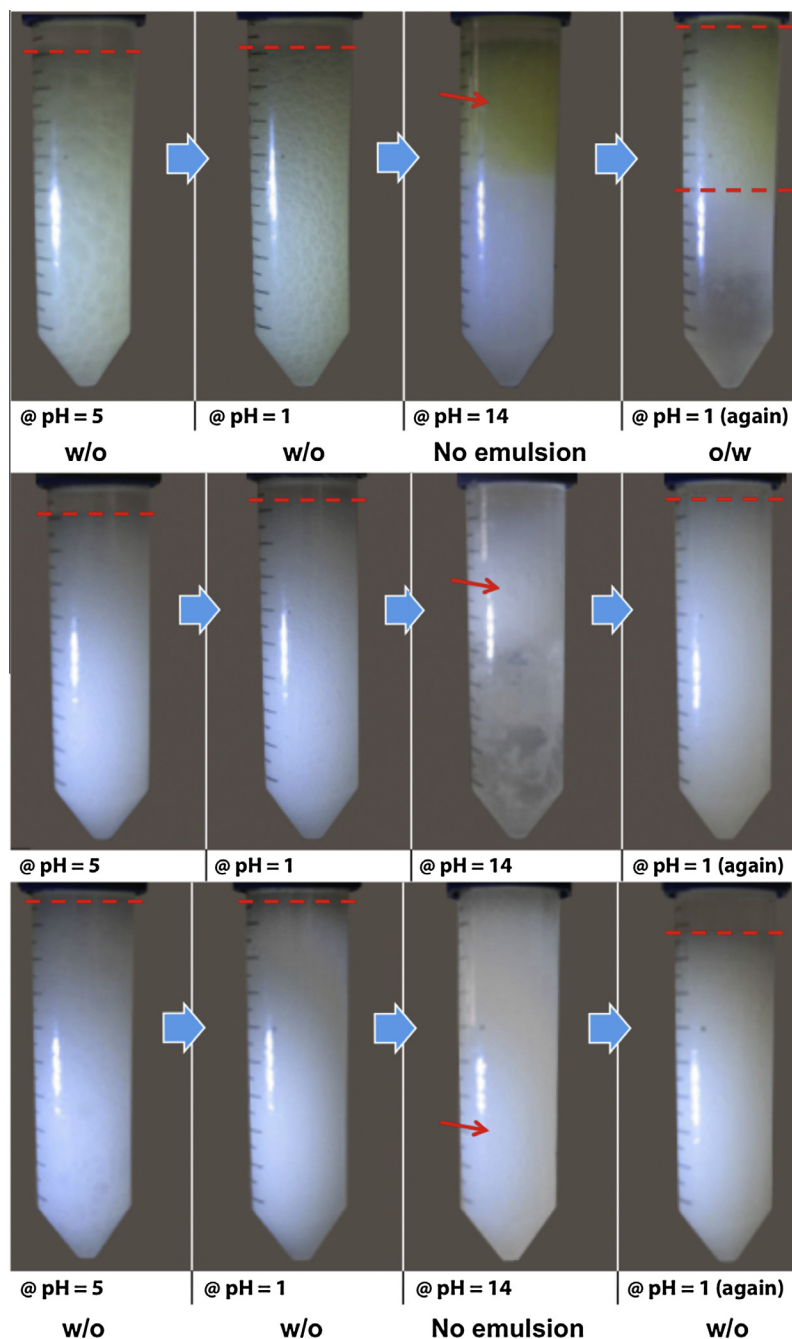
$$\Delta m = \frac{[\text{OH}] \cdot m_i \cdot A \cdot m_n}{162140} \quad (2)$$

where  $\Delta m$  is the mass increase after H/D exchange (mg), [OH] the concentration of accessible and available hydroxyl groups,  $m_i$  the initial mass of the sample (mg),  $A$ , Avogadro's number and  $m_n$  the mass of a neutron (mg). In this equation, we assumed that cellulose is composed of single glucose unit with a molecular mass of 162,140 and neglected the slight difference in the molecular structure of the cellulose chain end group containing a reducing group on one end of the molecule and a non-reducing group on the other end.

The advancing  $\theta_a$  and receding  $\theta_r$  water-in-air contact angle of the hydrophobised BC were measured on nanopapers made of hydrophobised BC. The contact angles were measured using the sessile drop method (DSA 10 Mk 2, Krüss GmbH, Hamburg, Germany).  $\theta_a$  was determined by increasing the drop volume to 20  $\mu\text{L}$  at a rate of 6  $\mu\text{L min}^{-1}$ . The same rate was also used to reduce the drop volume for the measurements of  $\theta_r$ . Images of the sessile drops were processed using DSA software version 1.80.1.12. At least five measurements were taken for each nanopapers. The nanopapers was produced following a previously described protocol [36].

### 3. Results and discussion

Fig. 1 shows the phase behaviour of C<sub>2</sub>-BC-stabilised emulsions of water and toluene seven days after they were prepared. At pH 5, the initial pH of the aqueous phase, a w/o emulsion was produced. When the pH was reduced to 1 by the addition of concentrated HCl followed by hand shaking as described previously, w/o emulsion still persisted. However, when we adjusted the pH from 1 to 14 by the addition of NaOH followed by hand shaking, the Pickering



**Fig. 1.** The transitional phase behaviour of C<sub>2</sub>-BC (top), C<sub>6</sub>-BC (middle) and C<sub>12</sub>-BC (bottom) stabilised emulsion of water and toluene. The arrow shows flocculated modified BC. Note that the slightly yellowish colour of C<sub>2</sub>-BC stabilised emulsion is a result of the colour of C<sub>2</sub>-BC, which is slightly yellowish after the esterification of BC with acetic acid.

emulsion phase separated. Hydrophobic C<sub>2</sub>-BC could be seen aggregated in the top (toluene) phase. This can be explained by the electrokinetic behaviour of C<sub>2</sub>-BC (see Fig. 2) [36]. When the pH of our C<sub>2</sub>-BC stabilised w/o Pickering emulsion was reduced to 1 initially, the negative surface charge of hydrophobised BC reversed. However, the magnitude of  $\zeta$ -potential is only slightly smaller than the  $\zeta$ -potential at pH = 5. Therefore, the intrinsic hydrophobic nature of C<sub>2</sub>-BC led to the formation of a w/o emulsion, as observed.

On the other hand, when the pH was increased to 14, the magnitude of the  $\zeta$ -potential increased significantly and a  $\zeta$ -potential plateau was reached. This increase in  $\zeta$ -potential is a result of the dissociation of functional groups on C<sub>2</sub>-BC. Furthermore, the

intrinsic hydrophobic nature of C<sub>2</sub>-BC surface also led to the preferential adsorption of Cl<sup>-</sup>, increasing the zeta-potential magnitude. The reason for adsorption of anions is because anions are smaller, more polarisable, less hydrated and, therefore, have higher tendency to adsorb at the intrinsically hydrophobic solid-liquid interface [38]. As a result, strong repulsion force arose between C<sub>2</sub>-BC particles adsorbing at the toluene-water interface. Similar observations were also reported by Aveyard et al. [39]. They reported a repulsion force arising from dissociable sulphate functional groups on particles at oil-water interface. This effect is postulated to lead to the high order of C<sub>2</sub>-BC particles at the toluene-water interface in our study, similar to that observed by Aveyard et al. [40] in a different study. The surface coverage of the water droplets

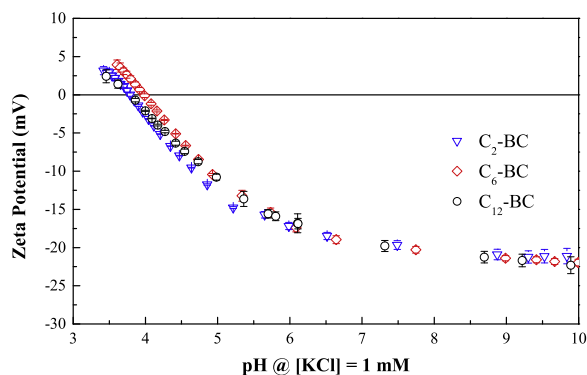


Fig. 2. Streaming  $\zeta$ -potential of modified BC. Obtained from Lee et al. [36] with kind permission from Springer.

decreased and ultimately led to the phase separation of the emulsion. Due to the intrinsic hydrophobic nature of  $C_2$ -BC, the particles aggregated in the top (toluene) phase.

To investigate whether this pH-triggered transitional phase separation was reversible, we then re-adjusted the pH of the emulsion to 1. Contrary to our expectation, the emulsion phase inverted from w/o to o/w, indicating that the transitional phase separation of  $C_2$ -BC stabilised Pickering emulsion was not reversible. This unusual feature was hypothesised to be due to the hydrolysis of the ester bonds of the cellulose acetate by hydrolysis [41], causing the degree of surface substitution (DSS) to decrease (see later sections) and, therefore,  $C_2$ -BC became more hydrophilic. As aforementioned, an o/w emulsion is more favourable to be formed by the more hydrophilic ( $C_2$ -)BC. This is also consistent with the observation of o/w Pickering emulsions formed by hydrophilic neat BC [21–23,42].

The phase behaviour of  $C_6$ - and  $C_{12}$ -BC stabilised emulsions, on the other hand, differed slightly compared to  $C_2$ -BC (see Fig. 1). Starting from w/o emulsions at pH = 5, both  $C_6$ - and  $C_{12}$ -BC still stabilised w/o emulsions when the pH was reduced to 1. This is not surprising as the  $\zeta$ -potentials of  $C_6$ -BC and  $C_{12}$ -BC are small (estimated to be 5 mV) and similar to that of  $\zeta$ -potential of  $C_2$ -BC. The intrinsic hydrophobic nature of  $C_6$ - and  $C_{12}$ -BC still enables the formation of w/o emulsions. When the pH was increased to 14, both emulsions phase separated. The hydrophobised BC can be seen aggregating either in the oil phase (for  $C_6$ -BC) or on the wall of the vessel ( $C_{12}$ -BC). The latter observation is most likely due to the more hydrophobic nature of  $C_{12}$ -BC (see Table 2) compared to  $C_6$ -BC and  $C_2$ -BC [36], which favours neither the toluene or water phase. When the pH was reduced to 1 again, we observe the re-formation of w/o emulsions for both  $C_6$ -BC and  $C_{12}$ -BC. Both  $C_6$ - and  $C_{12}$ -BC underwent pH-triggered reversible transitional phase separation. This behaviour differs from the irreversible transitional phase separation observed for  $C_2$ -BC stabilised

Table 2

The advancing water-in-air contact angle ( $\theta_A$ ), receding water-in-air contact angle ( $\theta_R$ ) and degree of surface substitution (DSS) of modified BC measured using dynamic vapour sorption with heavy water as the solvent.

Sample	$\theta_A$ ( $^\circ$ ) <sup>a</sup>	$\theta_R$ ( $^\circ$ ) <sup>a</sup>	DSS before pH-triggered experiments (%) <sup>a</sup>	DSS after pH-triggered experiments (%)
$C_2$ -BC	75 ± 1	35 ± 2	99 ± 1	82 ± 4
$C_6$ -BC	92 ± 1	45 ± 1	58 ± 1	61 ± 2
$C_{12}$ -BC	133 ± 4	80 ± 4	52 ± 1	54 ± 2

<sup>a</sup> Data obtained from Lee et al. [36].

emulsions caused by the in situ modification of  $C_2$ -BC by ester hydrolysis. Whilst the acetic acid esters of  $C_2$ -BC undergo hydrolysis, this was not observed for  $C_6$ -BC and  $C_{12}$ -BC (see Table 2). This is thought to be due to the longer chain length of the grafted acid resulting in higher hydrophobicity.

To further verify our hypothesis of hydrolysis of the organic acid cellulose esters of our modified BC, the DSS of  $C_2$ -,  $C_6$ - and  $C_{12}$ -modified BC were measured. The results are tabulated in Table 2. As can be seen, the DSS of  $C_2$ -BC decreased from 99% to 82% after the pH-triggered experiments. However, the DSS of  $C_6$ - and  $C_{12}$ -BC was not affected significantly. These results corroborate with our initial hypothesis, that hydrolysis of  $C_2$ -BC caused the irreversible transitional phase separation of  $C_2$ -BC stabilised Pickering emulsion. This observation is also in good agreement with the water-in-air contact angles of  $C_2$ -,  $C_6$ - and  $C_{12}$ -BC (Table 2). The relatively hydrophilic nature of  $C_2$ -BC, in comparison to  $C_6$ - and  $C_{12}$ -BC is thought to enhance the saponification of  $C_2$ -BC.

#### 4. Conclusions

pH-triggered transitional phase behaviour of medium internal phase w/o Pickering emulsions stabilised by  $C_2$ -,  $C_6$ - and  $C_{12}$ -BC was reported in this work. Transitional phase inversion of these emulsions was induced by varying the pH of the emulsions between 1 and 14. We observed that in addition to the previously reported reversible catastrophic phase separation of  $C_6$ - and  $C_{12}$ -BC stabilised emulsions [31], these Pickering emulsions also undergo pH-triggered reversible transitional phase separation. Starting from w/o emulsions containing an aqueous phase with pH = 5, the  $C_6$ - and  $C_{12}$ -BC stabilised emulsions still were w/o emulsion when the pH was adjusted to 1. When the pH was increased to 14, these emulsions phase separated but w/o emulsions re-formed when the pH was reduced to 1. However,  $C_2$ -BC stabilised emulsions did not undergo such reversible transitional phase separation. Instead, a pH-triggered irreversible transitional phase separation and inversion was observed for  $C_2$ -BC stabilised emulsion. A w/o emulsion was observed at pH = 1 for  $C_2$ -BC stabilised emulsion. When the pH of  $C_2$ -BC stabilised emulsion was increased to 14, the emulsion phase separated. However, an o/w emulsion formed when the pH was reduced to 1 again. The phase separation at high pH was associated with the increase in electrostatic repulsion between modified BC fibrils. As a result, the surface coverage of the droplets by the particles decreased, which led to the coalescence of the dispersed droplets. Furthermore, the difference in the exhibited phase behaviour between  $C_2$ -BC and  $C_6$ -/ $C_{12}$ -BC was attributed to the difference in the wettability of the modified BC.  $C_2$ -BC, being the most hydrophilic among all of the modified BC, underwent saponification at high pH. This was further verified by the degree of surface substitution (DSS) of the modified BC, which was quantified by hydrogen-deuterium exchange. The DSS of  $C_2$ -BC after the pH-triggered experiments decreased from 99% to 82%. However, no significant changes in DSS were observed for  $C_6$ - and  $C_{12}$ -BC after the pH-triggered emulsion phase transition. The saponification of the relatively hydrophilic  $C_2$ -BC led to the pH-triggered irreversible transitional phase separation and inversion of the  $C_2$ -BC stabilised emulsion.

#### Acknowledgements

The authors would like to thank the UK Engineering and Physical Science Research Council (EPSRC) for funding KYL (EP/F032005/1) and the EPSRC Challenging Engineering Programme for funding JJB (EP/E007538/1). We also greatly appreciate the University of Vienna for continuing the support for KYL.

## References

- [1] R.J. Hunter, Introduction to Modern Colloid Science, Oxford University Press Inc., New York, 1993.
- [2] B.P. Binks, Curr. Opin. Colloid Interface Sci. 7 (2002) 21–41.
- [3] W. Ramsden, Proc. Roy. Soc. London 72 (1903) 156–164.
- [4] S.U. Pickering, J. Chem. Soc. Trans. 91 (1907) 2001–2021.
- [5] S.U. Pickering, Z. Chem Ind Koll. 7 (1910) 11–16.
- [6] R. Aveyard, B.P. Binks, J. Mead, J. Chem. Soc.-Faraday Trans. I (82) (1986) 1755–1770.
- [7] P. Finkle, H.D. Draper, J.H. Hildebrand, J. Am. Chem. Soc. 45 (1923) 2780–2788.
- [8] A.J. Scarlett, W.L. Morgan, J.H. Hildebrand, J. Phys. Chem. 31 (1926) 1566–1571.
- [9] R. Aveyard, B.P. Binks, J.H. Clint, Adv. Colloid Interface Sci. 100 (2003) 503–546.
- [10] S. Levine, B.D. Bowen, S.J. Partridge, Colloid. Surface. 38 (1989) 325–343.
- [11] A. Scheludko, B.V. Toshev, D.T. Bojadjiev, J. Chem. Soc.-Faraday Trans. I (72) (1976) 2815–2828.
- [12] A.F. Koretzki, P.M. Kruglyakov, Izv. Sib. Otd. An SSSR, Seriiia him. nauk. 2 (1971) 139–141.
- [13] T.S. Horozov, B.P. Binks, Angew. Chem.-Int. Edit. 45 (2006) 773–776.
- [14] S. Abend, N. Bonnke, U. Gutschner, G. Lagaly, Colloid Polymer Sci. 276 (1998) 730–737.
- [15] S. Arditty, C.P. Whitby, B.P. Binks, V. Schmitt, F. Leal-Calderon, Eur. Phys. J. E 11 (2003) 273–281.
- [16] K.-Y. Lee, A. Bismarck, S.D. Stoyanov, V.N. Paunov, Colloidal and nanocellulose-stabilized emulsions, in: K. Oksman, A.P. Mathew, A. Bismarck, O. Rojas, M. Sain, P. Quintus (Eds.), Handbook of Green Materials, Vol 3: Self- and Direct-Assembling of Bionanomaterials, Materials and Energy, vol. 5, 2014, pp. 185–196.
- [17] S.B. Haaj, W. Thielemans, A. Magnin, S. Boufi, ACS Appl. Mater. Interfaces 6 (2014) 8263–8273.
- [18] K.P. Oza, S.G. Frank, J. Dispersion Sci. Technol. 7 (1986) 543–561.
- [19] K.P. Oza, S.G. Frank, J. Dispersion Sci. Technol. 10 (1989) 163–185.
- [20] K.P. Oza, S.G. Frank, J. Dispersion Sci. Technol. 10 (1989) 187–210.
- [21] I. Kalashnikova, H. Bizot, P. Bertoncini, B. Cathala, I. Capron, Soft Matter 9 (2013) 952–959.
- [22] I. Kalashnikova, H. Bizot, B. Cathala, I. Capron, Langmuir 27 (2011) 7471–7479.
- [23] I. Kalashnikova, H. Bizot, B. Cathala, I. Capron, Biomacromolecules 13 (2012) 267–275.
- [24] H. Ougiya, K. Watanabe, Y. Morinaga, F. Yoshinaga, Biosci. Biotechnol. Biochem. 61 (1997) 1541–1545.
- [25] M. Kargar, K. Fayazmanesh, M. Alavi, F. Spyropoulos, I.T. Norton, J. Colloid Interface Sci. 366 (2012) 209–215.
- [26] J.O. Zoppe, R.A. Venditti, O.J. Rojas, J. Colloid Interface Sci. 369 (2012) 202–209.
- [27] A. Lif, P. Stenstad, K. Syverud, M. Nyden, K. Holmberg, J. Colloid Interface Sci. 352 (2010) 585–592.
- [28] M. Andresen, P. Stenius, J. Dispersion Sci. Technol. 28 (2007) 837–844.
- [29] K. Khanari, K. Syverud, P. Stenius, J. Dispersion Sci. Technol. 32 (2011) 447–452.
- [30] J.J. Blaker, K.Y. Lee, X.X. Li, A. Menner, A. Bismarck, Green Chem. 11 (2009) 1321–1326.
- [31] K.-Y. Lee, J.J. Blaker, R. Murakami, J.Y.Y. Heng, A. Bismarck, Langmuir 30 (2014) 452–460.
- [32] B.P. Binks, S.O. Lumsdon, Langmuir 16 (2000) 2539–2547.
- [33] K.-Y. Lee, J.J. Blaker, A. Bismarck, Compos. Sci. Technol. 69 (2009) 2724–2733.
- [34] H. Toyosaki, T. Naritomi, A. Seto, M. Matsuoka, T. Tsuchida, F. Yoshinaga, Biosci. Biotechnol. Biochem. 59 (1995) 1498–1502.
- [35] K.Y. Lee, A. Bismarck, Cellulose 19 (2012) 891–900.
- [36] K.-Y. Lee, F. Quero, J.J. Blaker, C.A.S. Hill, S.J. Eichhorn, A. Bismarck, Cellulose 18 (2011) 595–605.
- [37] L. Rautkari, C.S. Hill, S. Curling, Z. Jalaludin, G. Ormondroyd, J. Mater. Sci. 48 (2013) 6352–6356.
- [38] R.A. Van Wagenen, D.L. Coleman, R.N. King, P. Triolo, L. Brostrom, L.M. Smith, D.E. Gregonis, J.D. Andrade, J. Colloid Interface Sci. 84 (1981) 155–162.
- [39] R. Aveyard, B.P. Binks, J.H. Clint, P.D.I. Fletcher, T.S. Horozov, B. Neumann, V.N. Paunov, J. Annesley, S.W. Botchway, D. Nees, A.W. Parker, A.D. Ward, A.N. Burgess, Phys. Rev. Lett. 88 (2002).
- [40] R. Aveyard, J.H. Clint, D. Nees, V.N. Paunov, Langmuir 16 (1999) 1969–1979.
- [41] G. Zemplén, E. Pascu, Berich. Deut. Chem. Ges. 62 (1929) 1613–1614.
- [42] I. Capron, B. Cathala, Biomacromolecules 14 (2013) 291–296.