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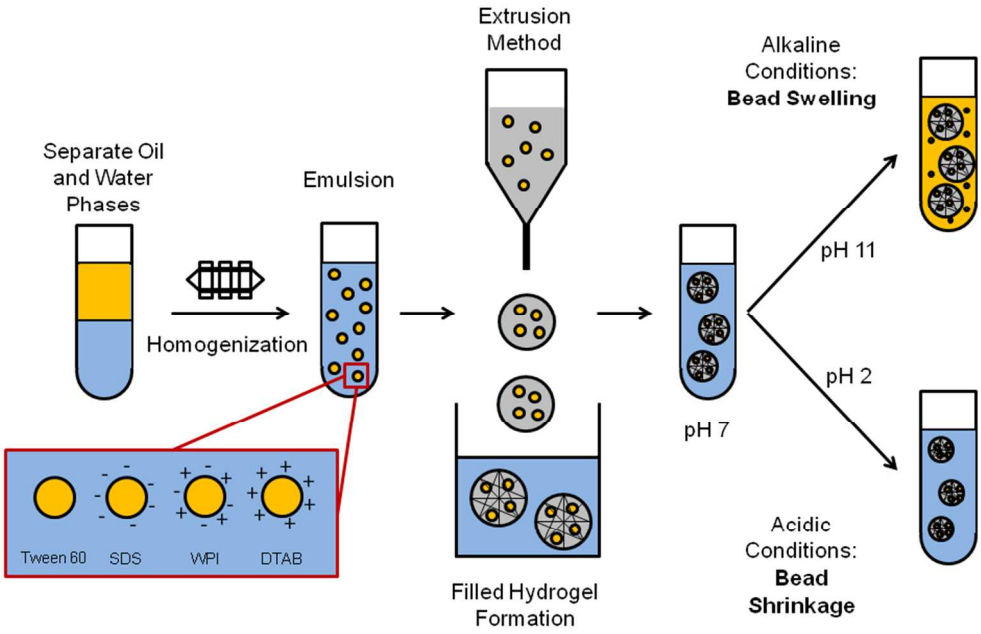

Retention and Release of Oil-in-Water Emulsions from Filled Hydrogel Beads composed of Calcium Alginate: Impact of Emulsifier Type and pH

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Running Title:

Alginate beads as controlled release systems for oil-in-water emulsions stabilized by differently charged emulsifiers

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ABSTRACT

Delivery systems based on filled hydrogel particles (microgels) can be fabricated from natural food-grade lipids and biopolymers. The potential for controlling release characteristics by modulating the electrostatic interactions between emulsifier-coated lipid droplets and the biopolymer matrix within hydrogel particles was investigated. A multistage procedure was used to fabricate calcium alginate beads filled with lipid droplets stabilized by non-ionic, cationic, anionic, or zwitterionic emulsifiers. Oil-in-water emulsions stabilized by Tween 60, DTAB, SDS, or whey protein were prepared by microfluidization, mixed with various alginate solutions, and then microgels were formed by simple extrusion into calcium solutions. The microgels were placed into a series of buffer solutions with different pH values (2 to 11). Lipid droplets remained encapsulated under acidic and neutral conditions, but were released under highly basic conditions (pH 11) due to hydrogel swelling when the alginate concentration was sufficiently high. Lipid droplet release increased with decreasing alginate concentration, which could be attributed to an increase in the pore size of the hydrogel matrix. These results have important implications for the design of delivery systems to entrap and control the release of lipophilic bioactive components within filled hydrogel particles.

Keywords: Hydrogel Beads; Oil-in-water emulsion; Alginate; DTAB; SDS; WPI; Tween 60 Microfluidization; Release

1. INTRODUCTION

There is considerable interest within the food industry in the development of functionalized delivery systems for bioactive components, such as vitamins, minerals, and nutraceuticals.[1, 2] For lipophilic bioactives, emulsion-based delivery systems are particularly suitable because they can be designed to have specific functional properties, such as improved dispersion, compatibility within food matrices, protection against chemical degradation, resistance to environmental stresses, and controlled release profiles.[3-7] The nature of the release profile required for a particular commercial application depends on the precise product, *e.g.,* burst, sustained, or triggered release.[8, 9]

In recent years, there has been considerable interest in the utilization of filled hydrogel beads as food-grade delivery systems for lipophilic bioactives.[6, 10] The nature of the hydrogel matrix can be designed to swell or disintegrate under a specific set of environmental conditions (*e.g.,* pH, ionic strength, temperature, or enzyme activity), thereby allowing release of any encapsulated lipid droplets.[11] In addition, interactions between lipid droplets and hydrogel matrices (such as electrostatic, hydrogen or hydrophobic bonds) can be modulated to retain or release the lipid droplets under different environmental conditions.

Hydrogel beads composed of alginate are capable of encapsulating a wide range of sensitive bioactives because their interior is chemically inert.[12] Alginate beads can be used to control the stability, retention, and release of encapsulated lipids by modifying their porosity and degradability.[13-15] Alginate is an unbranched biopolymer consisting of

(1→4) linked β-D mannuronic acid (M) and α-L-guluronic acid (G) residues that may vary in composition and sequence depending on its origin.[16, 17] It can form ionotropic gels in the presence of multivalent cations such as Ca^{2+}, St^{2+}, Ba^{2+}, or Fe^{3+} due to the formation of cationic bridges between the guluronic-rich regions along the biopolymer backbone. The resulting hydrogels have an egg-box structure consisting of zig-zag alginate molecules (box) held together by cations (eggs).[14, 18] In general, the bioactive to be encapsulated is mixed with an alginate solution, and the mixture is then injected into a solution containing divalent cations, which results in the formation of hydrogel beads.[10] Alginates are acceptable for use in the food, cosmetic, and pharmaceutical industries as functional ingredients to create hydrogels.[19]

Various studies have demonstrated that the encapsulation efficiency of alginate beads depends on bead properties such as size, shape, pore size, and surface morphology.[20] It was reported that the encapsulation efficacy of lipid droplets depends on the degree of alginate crosslinking and emulsion stability.[21] Photographic images revealed that the bead size increased with increasing oil loading.[22] The properties of alginate beads can be further tailored by coating them with oppositely charged biopolymers, such as chitosan.[23] Recent research has focussed on the utilization of hydrogel beads to improve the oral bioavailability of lipophilic compounds, as well as to control the release of water-insoluble molecules within the human gastrointestinal tract. It was shown that the release of free fatty acids was reduced from around 100% to 12% when the lipid droplets were encapsulated within calcium alginate beads.[14, 24] The authors proposed that the bead matrix was able to restrict the access of digestive enzymes and other surface-active components to the surface of the encapsulated lipid droplets, which

resulted in a decreased rate and extent of lipid digestion. Moreover, it has been demonstrated that the porosity of alginate hydrogels is reduced when exposed to acidic conditions, but is increased when they are exposed to alkaline conditions - a fact that has important consequences for the development of delivery systems with triggered release profiles.[13, 14]

The objective of the present study was to establish a better understanding of the factors influencing the release of lipid droplets from filled alginate beads. The pore size of alginate beads might be tuneable by altering pH, whereas the surface charge of lipid droplets might influence their interaction with the hydrogel matrix.[13] To this purpose, oil-in-water emulsions stabilized by differently charged emulsifiers (non-ionic, anionic, cationic, and zwitterionic) were prepared by microfluidization. The emulsions were then mixed with alginate solutions that were dripped into calcium solutions to induce hydrogel bead formation. To study the influence of pH on the release characteristics, filled alginate beads were placed in buffer solutions with different pH values. We hypothesized that lipid droplets having an oppositely charged surface to the alginate matrix would remain trapped within the beads due to electrostatic attraction, whereas similarly charged droplets might freely diffuse out of the beads (provided the pores are big enough). The information obtained from this study will be useful for designing hydrogel beads that can release encapsulated bioactive lipids in response to different environmental conditions.

2. MATERIALS AND METHODS

2.1. Materials

Sodium alginate (alginic acid sodium salt from *Macrocystis pyrifera*, #50K0180, medium viscosity, 20 - 40 cps of 1% aqueous solution) was purchased from Sigma-Aldrich Co. (St. Louis, USA). Medium chain triglyceride (MCT) oil (MIGLYOL®812) was purchased from Warner Graham Company (Sasol GmbH, Germany). Whey protein isolate (WPI) was donated from Davisco Foods International Inc. (Le Sueur, MN56058, USA). Polysorbate 60 (Tween 60, #MKBJ0348V), sodium dodecyl sulfate (SDS, #SLBG6615V), dodecyltrimethylammonium bromide (DTAB, #BCBM9657V, purity ≥ 98%), and calcium chloride anhydrous (purity > 96.0%) were obtained from Sigma-Aldrich Co. (St. Louis, USA). Double-distilled water was used for the preparation of all samples. All concentrations are expressed as mass percentage (% w/w).

2.2. Solution preparation

Aqueous emulsifier solutions were prepared by dispersing 1% Tween 60, SDS, and WPI into 5 mM phosphate buffer (pH 7), respectively, whereas DTAB was dissolved in 5 mM phosphate buffer (pH 3) followed by stirring for at least 2 hours. A stock alginate solution (3%) was made by dispersing powdered alginate into double-distilled water and stirring overnight. Stock hardening solution was prepared by dissolving 1 M $CaCl_2$ into double-distilled water followed by stirring for at least 30 min.

2.3. Emulsion preparation, characterization, and stability

The emulsions were prepared by homogenization of 10% MCT and 90% aqueous emulsifier (1%, 5 mM phosphate buffer, pH 3 or 7) solution using a high shear blender (Barmix, Biospec Products, Bartlesville, OK) for 3 min followed by five passes at 10,000 psi (68.95 MPa) through a microfluidizer (M-110L, Microfluidcs, Newton, MA).

2.3.1. Particle size measurement

Dynamic light scattering was used to determine the particle sizes of emulsions (Nano ZS, Malvern Instruments, Malvern, UK). Samples were diluted to a droplet concentration of approximately 0.005% with an appropriate buffer to prevent multiple scattering effects. The foundation of this technique is based on the scattering of light by moving particles due to Brownian motion in a liquid.[25] The movement of the particles is then related to the size of the particles. The instrument reports the mean particle diameter (z-average) and the polydispersity index (PDI) ranging from 0 (monodisperse) to 0.50 (very broad distribution).

2.3.2. Surface charge measurement

Surface charge (ζ-potential) was measured using an electrophoresis instrument (Nano ZS, Malvern Instruments, Malvern, UK). Samples were diluted approximately 1:1000 with an appropriate buffer to avoid particle interaction effects. Diluted samples were loaded into cuvettes, placed into the measurement chamber, and then the ζ-potential was determined by measuring the direction and velocity that the particles moved in the applied electric field. The ζ-potential measurements were reported as the average and standard deviation of measurements made from two freshly prepared samples, with 3 readings made per sample.

2.3.3. Influence of pH on base emulsion stability

Oil-in-water emulsions stabilized by charged emulsifiers were diluted with 5 mM phosphate buffer (pH 3 and 7) to the same final oil droplet concentration (5%), and then

the pH was adjusted from 2.0 to 11.0 using 0.1 and 1 M HCl and/or NaOH. All samples were kept for 2 min after reaching the final pH value before transferring (10 ml) into glass test tubes and then stored overnight at room temperature before analysis.

2.4. Alginate bead preparation, characterization, and stability

2.4.1. Unloaded beads

An extrusion technique was utilized to generate unloaded alginate beads. A programmable automated pipette (Rainin SE4, Mettler Toledo, Oakland, CA) was used to inject 2 mL of sodium alginate solution (0.5% and 1.5%) into 15 mL of $CaCl_2$ hardening solution (50 mM) with continuous stirring at 200 rpm. An injection rate of 1.4 s per drop was used, whereas a collecting distance of 2 cm between dripping tip and liquid surface was enough to form spherical hydrogel beads. The beads formed were allowed to crosslink with divalent calcium ions for 30 min at room temperature.

2.4.2. Filled beads

Filled alginate beads were accordingly prepared as mentioned in section 2.4.1, whereby stock emulsions stabilized by differently charged emulsifiers were mixed with stock alginate solutions. The final oil droplet concentration obtained was 5% MCT, whereas the alginate concentration varied between 0.5% and 1.5%.

2.4.3. pH stability - Size determination

The alginate beads formed as mentioned above were placed into a series of continuously stirred buffer solutions with different pH values (2 - 11) and stored for 6 h at room

temperature before analysis. Please note, that we used double-distilled water and adjusted the pH values accordingly using 0.1 and 1 M HCl and NaOH. A magnetic stirrer at 300 rpm was used to maintain a homogenous suspension of hydrogel particles. The dimensions (diameter) of the beads were determined using a digital micrometer (0 - 300 mm, EC10, High Precision Digital Caliper, Tresna Instruments, Guilin, China). The bead diameter of at least 10 individual beads was measured and the mean and standard deviation was calculated. A digital camera (PowerShot SX110 IS, Canon, USA) was used to assess the appearance of the alginate beads as a function of pH.

2.4.4. pH stability - Surface charge determination

The alginate beads prepared using the extrusion method described earlier were too large to analyze using the particle electrophoresis instrument. Consequently, we prepared smaller alginate beads for these experiments using a commercial encapsulation unit (B-390, Büchi, Switzerland) under the same conditions mentioned above (0.5% alginate, 50 mM $CaCl_2$). The beads formed were allowed to crosslink with divalent calcium ions for 30 min at room temperature, placed into a series of continuously stirred buffer solutions with different pH values (2 - 11) and stored for 6 h at room temperature before analysis.. The surface charge (ζ-potential) was then measured using the particle electrophoresis instrument (Nano ZS, Malvern Instruments, Malvern, UK).

2.5. Release of oil-in-water emulsions from alginate beads

Turbidity measurements were used to determine the release of lipid droplets stabilized by various emulsifiers (WPI, SDS, DTAB, Tween 60) from alginate beads into the surrounding aqueous phase. Filled beads (0.5% or 1.5% alginate, 50 mM $CaCl_2$, 30 min

hardening) were gently separated from the aqueous phase using a Nutsch-type filter (pore size < 1 mm) and placed into a series of continuously stirred buffer solutions with different pH values (2 and 11). Aliquots were taken from the aqueous phase at regular time intervals and the turbidity was measured at 600 nm using a UV/vis spectrophotometer (Ultrospec 3000 Pro, GE Healthcare Bio-Sciences, Piscataway, NJ).

2.6. Statistical analysis

All experiments were repeated at least 2 times using freshly prepared samples. Means and standard deviations were calculated from a minimum of three measurements using Excel (Microsoft, Redmond, VA, USA).

3. RESULTS AND DISSCUSION

3.1. Properties of lipid-droplets coated by different emulsifiers

In general, the release of colloidal particles trapped within alginate beads may be retarded through two different mechanisms: (i) restricted diffusion due to the small dimensions of the pores in the hydrogel matrix; (ii) restricted diffusion due to attractive interactions between the particles and the hydrogel matrix.[13] The relative importance of these two mechanisms may change when solution pH is changed, since this may change the pore size and/or electrostatic interactions of the hydrogel matrix. Previous studies have reported that the pore size of hydrogel beads may range from around 5 to 200 nm depending on their composition and preparation method, as well as the prevailing environmental conditions.[26, 27] We therefore produced emulsions that contained lipid droplets within this size range so as to determine the influence of hydrogel matrix

properties as well as emulsion surface properties on their release. Four differently charged emulsifier types were utilized to stabilize oil-in-water emulsions: Tween 60 (nonionic), SDS (anionic), DTAB (cationic), and WPI (zwitterionic). A microfluidizer was used to prepare base emulsions under constant homogenization conditions (10,000 psi, 5 passes). The particle size distributions of freshly produced samples were assessed immediately after homogenization and are shown in **Fig. 1**, whereas mean particle diameters and ζ-potentials of all emulsions are reported in **Table 1**. The results indicate that emulsions containing relatively small droplets could be produced using the different emulsifiers. In comparison to WPI, low molecular weight surfactants such as DTAB, SDS, or Tween 60 are known to be more effective in reducing the interfacial tension between the oil and water phase, and adsorb more rapidly to droplet surfaces during homogenization, thus resulting in smaller particle sizes, as shown in **Table 1**.[28] The particle size distributions of all emulsions were fairly similar, which is an important prerequisite for comparing the release of lipid droplets based on charge characteristics. The polydispersity indices ranged between about 0.116 and 0.159 which indicates that the particle size distributions were fairly narrow, which may also have an impact on their release behaviour.

3.2. pH-stability of base emulsions

The purpose of this series of experiments was to identify the influence of pH on the mean particle size and charge of the base emulsions stabilized by WPI, DTAB, SDS, and Tween 60 (**Fig. 2**). For the protein-stabilized emulsions, the typical pH dependence of interfacial charge was observed (**Fig. 2a**): the charge on the WPI-coated lipid droplets went from highly negative (-60 mV) at pH 11 to highly positive (+46 mV) at pH 2, with a

point of zero charge around pH 5, which is close to the isoelectric point (p*I*) of the adsorbed protein.[29] As expected, WPI-coated lipid droplets were highly susceptible to droplet aggregation near the p*I* because the electrostatic repulsive forces were insufficiently strong (**Fig. 2b**).[29, 30] In contrast, emulsions stabilized by low molecular surfactants such as SDS, DTAB, and Tween 60 remained stable over the entire pH range (**Fig. 2b**) which is also in agreement with previously published studies.[31, 32] For example, *Surh et al* (2005) demonstrated that the mean particle diameter of SDS-stabilized emulsions remained relatively small when the pH was shifted from 3 to 8.[33] This effect can be attributed to the strong electrostatic and/or steric repulsion between the surfactant-coated lipid droplets. The relatively high negative charge on the droplets coated with Tween had been reported in numerous other studies, where it has been attributed to adsorption of hydroxyl ions or anionic impurities. Moreover, changes in the charges in the droplets coated with ionic surfactants could be due to electrostatic screening effects associated with the addition of acid or alkaline solutions to adjust the pH.[31]

3.3. pH-induced changes of unloaded hydrogel beads

In this section, we characterized the influence of pH on the properties of unloaded calcium alginate beads so as to better understand their pH dependent release properties. Beads were prepared under neutral conditions using two different alginate concentrations (0.5% and 1.5%) but similar hardening conditions (50 mM $CaCl_2$, 30 min) to potentially alter pore size.[21] The prepared beads were separated from the hardening solution and then placed into a series of continuously stirred buffer solutions with different pH values (2 - 11) for 6 h at room temperature before analysis (**Figs. 3 to 5**). At both alginate concentrations, bead dimensions remained fairly constant when they were incubated in

solutions ranging from pH 3 to 9 (**Fig. 3**). However, bead shrinkage occurred when the solution pH was reduced from 3 to 2, whereas bead swelling was observed when the pH was increased from 9 to 11. Our results therefore agree with previous studies that have reported that alginate beads shrink when stored under acidic conditions and swell when stored under basic solutions.[13, 18, 20] It has been proposed that acid shrinking occurs due to a decrease in the repulsive electrostatic charges due to protonation of any free carboxyl groups on the alginate molecules.[34] In addition, calcium ions are known to dissociate at low pH, allowing the alginate chains to come closer together leading to the formation of hydrogen bonds.[35] However, the alginate beads maintained their overall spherical shape regardless of the storage conditions (**Fig. 4)**.[10, 36] The swelling observed at high pH values may have been due to increased electrostatic repulsion between similarly charged biopolymer chains.

Surface charge measurements were conducted to gain further insights into the origin of the shrinking and swelling behavior of calcium alginate beads as a function of pH. The alginate beads used in the other studies were too large to measure using the particle electrophoresis instrument, and so we prepared some smaller alginate beads (d = 500 μm) under similar hardening conditions (0.5% alginate, 50 mM $CaCl_2$, 30 min) utilizing an instrumental encapsulation device. The pH-dependence of the ζ-potential is shown in **Fig. 5**. The results indicate that the observed changes in bead dimensions could be attributed to the electrical characteristics of the biopolymer molecules in the hydrogel beads. The beads were negatively charged at all pH values, indicating that there was an excess of anionic groups on the alginate molecules. However, the magnitude of the negative charge decreased with decreasing pH, which can be attributed to a loss of

negative charge on the carboxyl groups at pH values around their pK_a value (≈ 3.5).[14] These data support the theory that the beads shrink at low pH due to a reduction in electrostatic repulsion between alginate chains, but swell at high pH due to an increase in electrostatic repulsion.

3.4. Release behaviour of lipid droplets trapped in alginate beads

In this section, we examined a number of factors influencing the release of charged lipid droplets from the hydrogel beads: alginate concentration; pH; and emulsifier charge. At equivalent alginate and calcium concentrations, filled hydrogel beads had similar dimensions and shapes as unloaded hydrogel beads, which indicated that lipid droplet loading did not affect overall bead morphology.[21] After incubation in the calcium bath (for 30 min), the filled hydrogel beads were separated from the surrounding aqueous phase by filtration and suspended in buffer solutions having pH values ranging from 2 to 11. Information about lipid droplets released from the beads was provided by turbidity measurements of the aqueous solution surrounding them: a higher turbidity indicated that more lipid droplets were released. The results of these experiments are summarized in **Figures 6 to 8**.

3.4.1. Effect of alginate concentration

In this section, we examined the influence of alginate concentration (0.5 or 1.5%) on lipid droplet release from the hydrogel beads (**Fig. 6 and 7**). At all pH values, the turbidity of the aqueous phase (which is a measure of the amount of lipid droplets released) was appreciably higher at the lower alginate concentration. Enhanced droplet release may have occurred because the hydrogel beads with the lower alginate concentration had

larger pores.[10] Previous studies have reported that the internal bead structure becomes more porous at lower alginate concentrations, which would facilitate the movement of lipid droplets through the hydrogel matrix and into the surrounding aqueous phase.[20, 27]

3.4.2. Effect of pH

In this section, we examine the influence of pH on lipid droplet release from the hydrogel beads (**Fig. 6**). In general, the amount of lipid droplets released from the hydrogel beads increased at higher pH values for both alginate concentrations and all surfactant types, which can be attributed to an increase in the pore size of the hydrogel matrix.[13, 14] Previous studies have reported that a looser hydrogel network is formed within calcium alginate beads under alkaline conditions, which would account for the faster rate of lipid droplet release at higher pH levels observed in our study. [17, 20]

3.4.3. Effect of surface charge

The purpose of these experiments was to determine the influence of emulsifier type on the release of lipid droplets from alginate beads. The nature of the emulsifier clearly had a pronounced influence on the release characteristics of the lipid droplets from the beads (**Fig. 6**). In the more porous beads (0.5% alginate), the release of the surfactant-coated lipid droplets followed the following order: Tween > SDS > WPI. This effect may be attributed to the electrostatic interactions between the lipid droplets and hydrogel matrix (Fig. 6A). The non-ionic lipid droplets (Tween) would be expected to have a relatively weak interaction with the anionic biopolymer molecules and therefore be released rapidly. The anionic lipid droplets (SDS) would be expected to have an electrostatic repulsion with the biopolymer molecules in the hydrogel matrix, but they may have been

some attraction due to salt bridging with calcium ions that hindered their movement. The protein-coated droplets (WPI) were released more at high pH than at low pH, which may have been due to an increase in pore dimensions at high pH, as well as the fact that there would be an electrostatic attraction between lipid droplets and hydrogel matrix at low pH (where they have opposite charges), but a repulsion at high pH (where they have similar charges). However, mixing alginate solutions (0.5%) with DTAB-stabilized emulsions caused the mixture to from aggregates which hindered a subsequent hydrogel formation. Similar phenomena are observed when multilayered emulsions are formed using electrostatic depositioning: at sufficiently low polymer concentrations, bridging flocculation between oppositely charged biopolymers and lipid droplets occurs leading to the formation of large aggregates that rapidly form a cream layer.[37] This would account for the fact that the turbidity of the surrounding aqueous phase remained relatively high at all pH values for the DTAB system (**Fig. 7**). In general, the differences between the emulsifiers was less at the higher alginate concentration used, which may have been because the release rate was slower due to the smaller pore size (Fig. 6B).

3.4.4. Effect of surface charge on the release kinetics

Information about the kinetics of lipid droplet release were obtained by measuring changes in turbidity with time. After hydrogel formation, the beads were dispersed in either pH 2 or pH 11 buffer solutions, since the most notable changes in bead dimensions were observed under these two conditions (**Figs. 3 and 4**). As expected, the release of the lipid droplets was appreciably higher after 6 hours incubation in the buffer solution at pH 11 than at pH 2 regardless of emulsifier type used to stabilize the lipid droplets (**Fig. 8**). However, the kinetic experiments indicated that the lipid droplets were not continuously

328 released from the hydrogel beads over time. Instead, it appeared to be a delay time of
329 around 2 to 3 hours where no release was observed, followed by a rapid release, and then
330 a relatively constant value. The release behaviour was fairly similar for all four
331 emulsifiers used, which suggested that changes in hydrogel pore size were more
332 important than electrostatic effects.[13, 27] It is possible that the electrostatic interactions
333 were weakened at this high pH because of the relatively high ionic strength associated
334 with adding alkali (NaOH) to increase the pH. We hypothesize that the beads swelled
335 slowly at pH 11, until the pore size was large enough for the lipid droplets to be easily
336 released. However, further investigations are needed to refine the system.

337 **CONCLUSIONS**

338 The present study has shown that filled hydrogel beads composed of calcium alginate can
339 be used as delivery systems for lipid droplets. The release behaviour of the lipid droplets
340 is mainly dominated by the alginate concentration and solution pH, which can be
341 attributed to changes in hydrogel pore size and electrostatic interactions between lipid
342 droplets and biopolymer molecules within the hydrogel matrix. This study suggests that
343 alginate beads will be able to encapsulate lipid droplets over a wide range of conditions
344 that might occur in foods and in the human body. However, one would expect these
345 beads to be broken down by microbes within the lower gastrointestinal tract of humans.
346 Consequently, these filled hydrogel beads may be particularly useful as colonic delivery
347 systems for lipophilic bioactive molecules. In addition, it may be possible to develop
348 triggered release systems by altering the electrostatic interactions between lipid droplets
349 and biopolymer molecules in the hydrogel matrix, but further work is required to refine
350 these systems. In general, filled hydrogel beads produced by extrusion provide a cost-

351 effective and simple to scale-up method that might be easily implemented in food
352 processing lines.

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Table 1.

Mean particle (z-average) diameter, polydispersity index (PDI), and ζ-potential of oil-in-water emulsions (10.000 psi, 5 passes) stabilized by differently charged emulsifiers. Note: all emulsions were initially prepared at the given pH.

Emulsifier	pH	Z-Average Diameter (nm)	PDI (-)	ζ-Potential (mV)
WPI	7	188.8 ± 2.6	0.159 ± 0.014	-53.4 ± 2.0
SDS	7	137.9 ± 1.2	0.131 ± 0.003	-82.2 ± 2.1
Tween 60	7	180.7 ± 2.6	0.116 ± 0.004	-9.7 ± 2.4
DTAB	3	145.2 ± 1.7	0.155 ± 0.010	+56.6 ± 0.9

FIGURE CAPTIONS

Fig. 1. Particle size distribution of oil-in-water emulsions (10% MCT, 1% emulsifier, 10000 psi, 5 passes) stabilized by differently charged emulsifiers: WPI, SDS, Tween 60, and DTAB.

Fig. 2. (**A**) ζ-potential and (**B**) mean particle diameter (z-average) of base emulsions (5% MCT, 0.5% emulsifier (WPI, SDS, Tween 60, DTAB), 10000 psi, 5 passes) as a function of pH (2 - 11). Emulsions were stored 24 h prior to analysis.

Fig. 3. Mean particle diameter of alginate beads (0.5% and 1.5% alginate, 50 mM $CaCl_2$, 30 min hardening) as a function of pH (2 - 11). An average was calculated out of 10 beads.

Fig. 4. Visual appearance of alginate beads depending on pH (2 - 11). Black grid is 5 x 5 mm, whereas white grid is 2 x 2 mm.

Fig. 5. Surface charge (ζ-potential) of alginate beads (0.5% biopolymer, 50 mM $CaCl_2$, 30 min hardening) depending on pH (2 - 11). Note: Alginate beads were prepared using a commercial encapsulation unit.

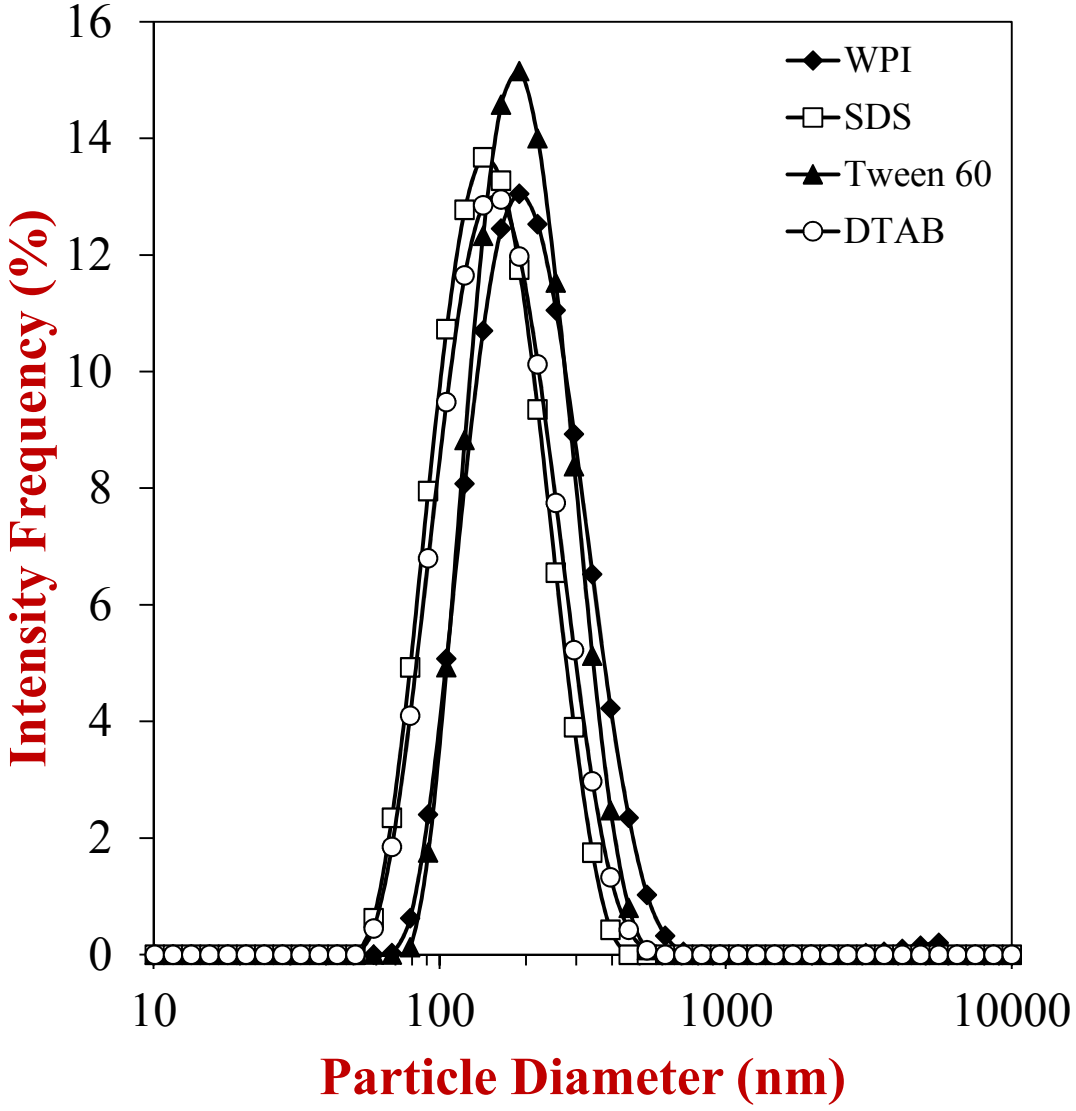
Fig. 6. Turbidity development of hydrogel beads composed of 0.5% (**A**) and 1.5% alginate (**B**) (50 mM $CaCl_2$, 30 min hardening) loaded with oil-in-water emulsions (5% MCT, 0.5% emulsifier (WPI, SDS, Tween 60, DTAB), 10000 psi, 5 passes) as a function of pH (2 - 11). (Please note that bead

formation using 0.5% alginate in combination with DTAB-stabilized emulsions was not possible due to heavy aggregation).

Fig. 7. Visual appearance of filled alginate beads (5% MCT, 0.5% emulsifier, 10000 psi, 5 passes) depending of pH (2 - 11). Each cuvette contains 50 beads. (* Bead formation was not possible due to heavy aggregation).

Fig. 8. Release kinetics of filled hydrogel beads (1.5% alginate, 5% MCT, 0.5% emulsifier, 10000 psi, 5 passes) depending emulsifier type: (**A**) WPI, (**B**) SDS, (**C**) Tween 60, and (**D**) DTAB.

465 **Fig. 1.**



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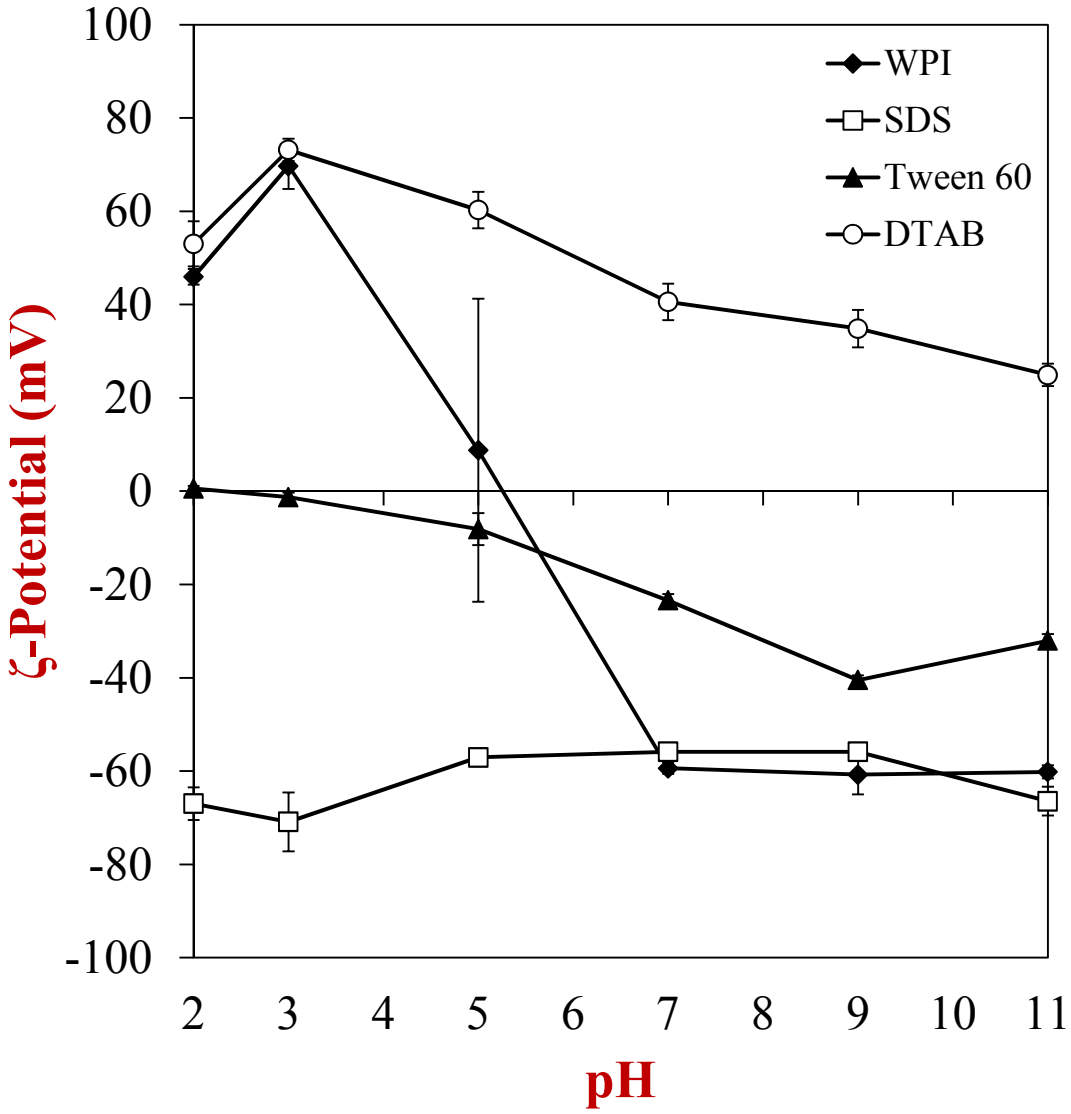
468 **Fig. 2a.**



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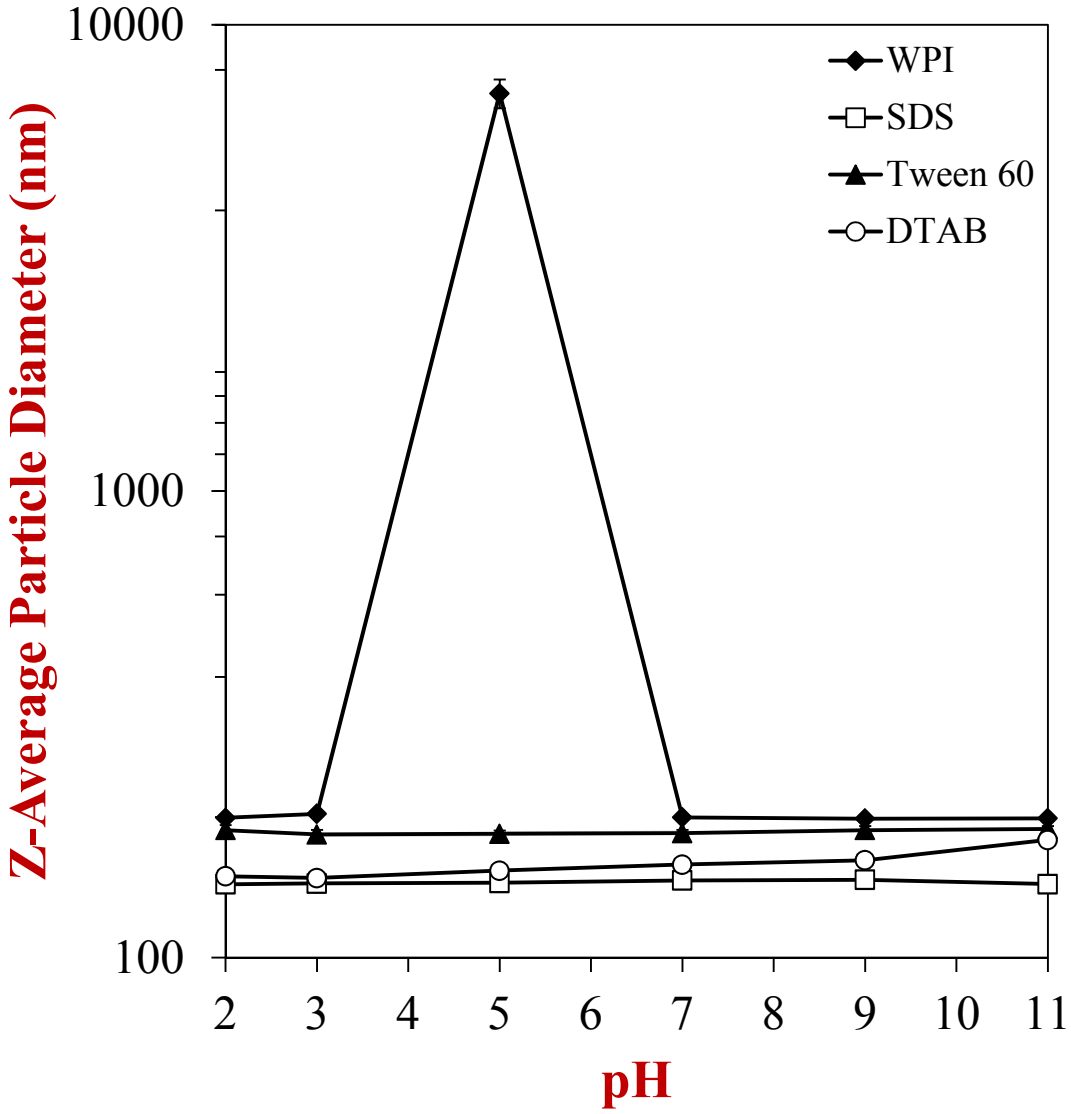
471 **Fig. 2b.**



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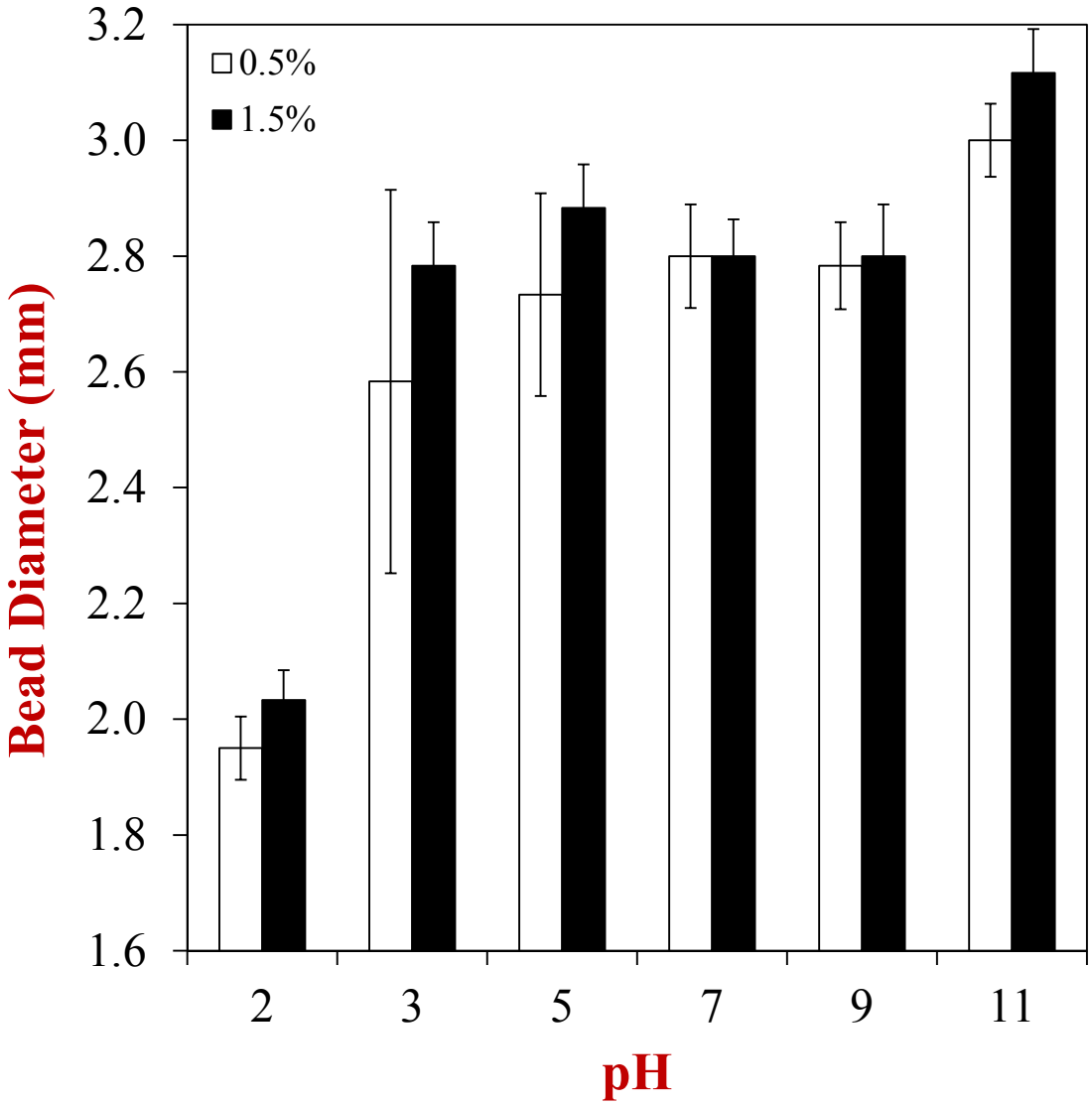
474 **Fig. 3.**



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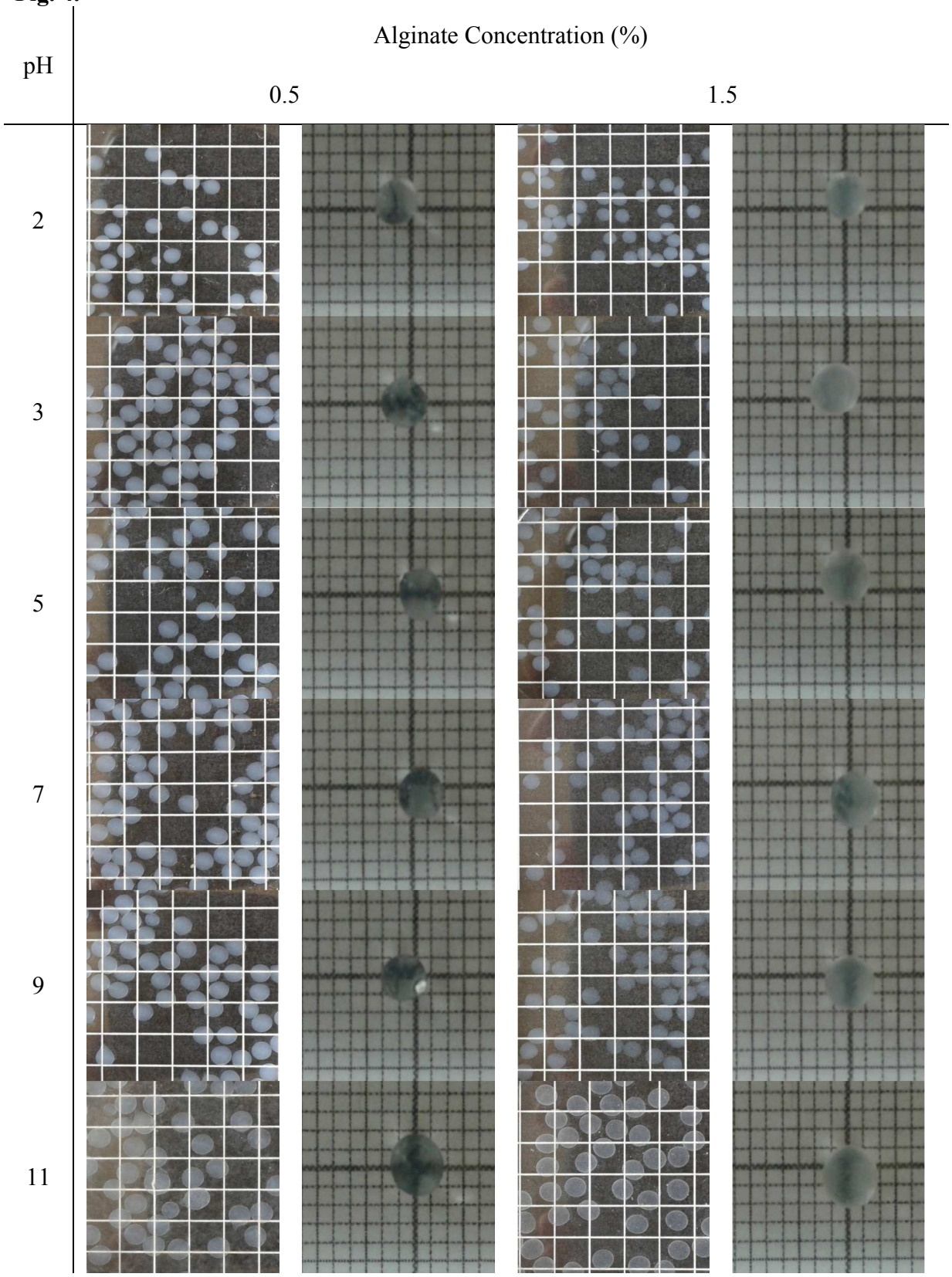
477 **Fig. 4.**



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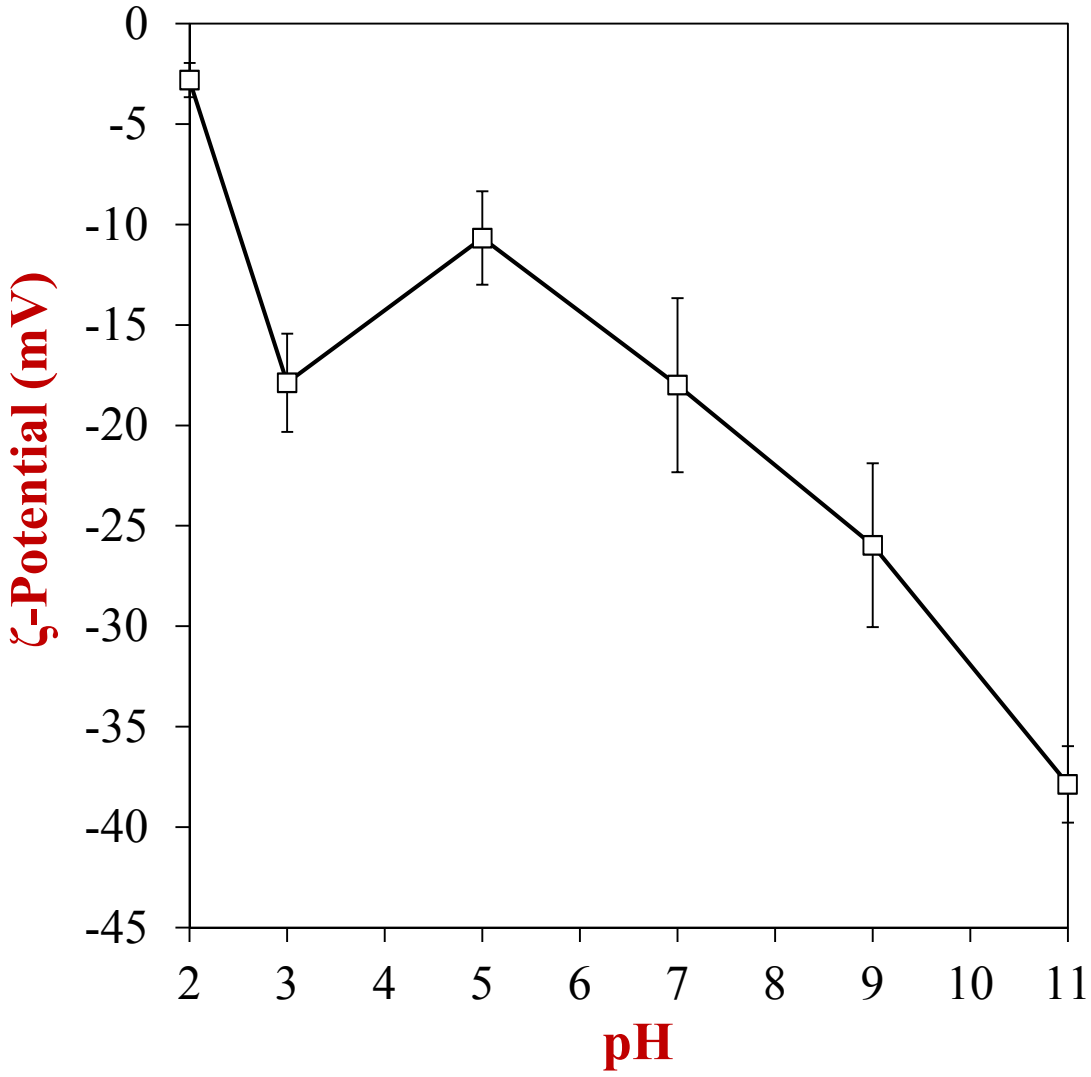
479 **Fig. 5.**



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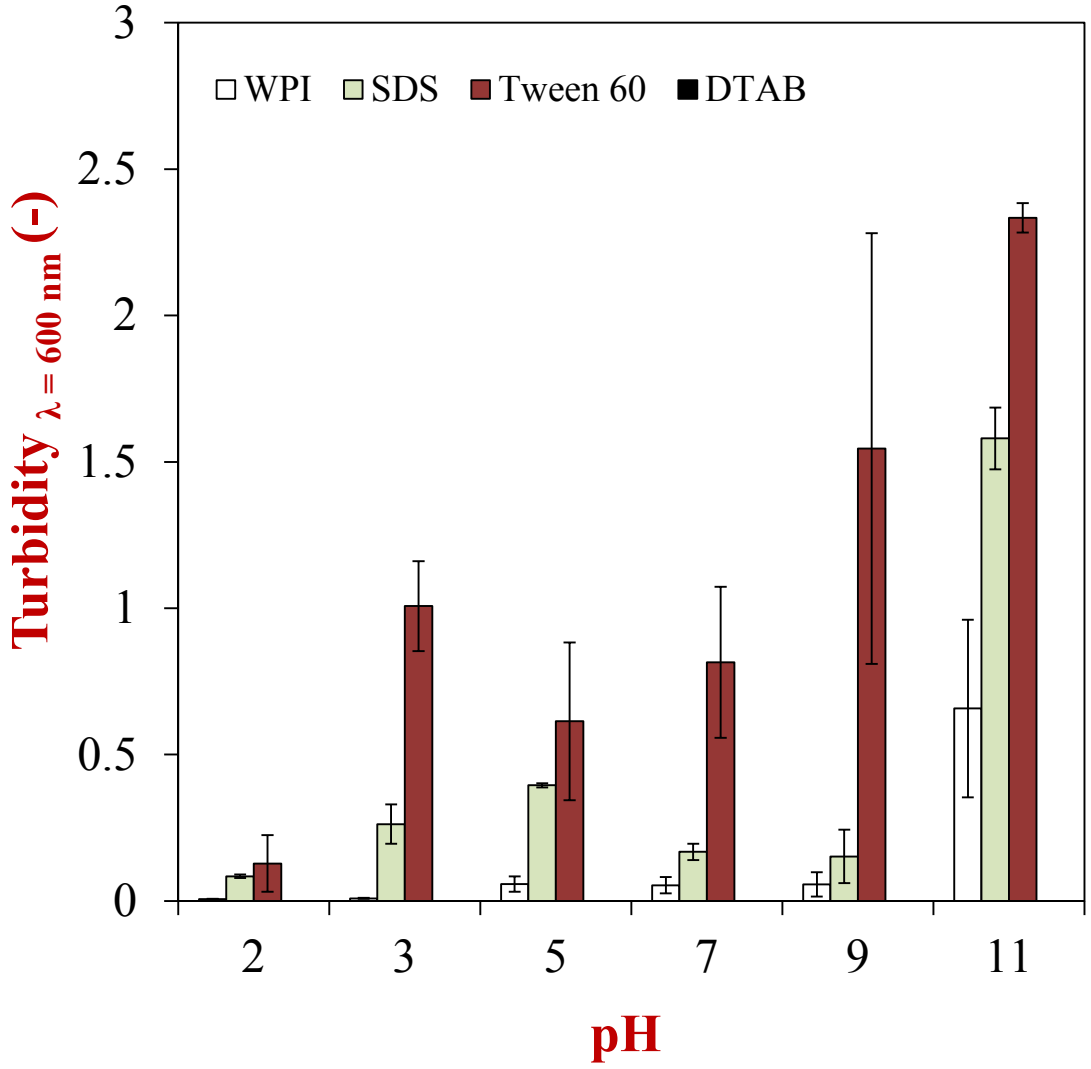
482 **Fig. 6a.**



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485 **Fig. 6b.**

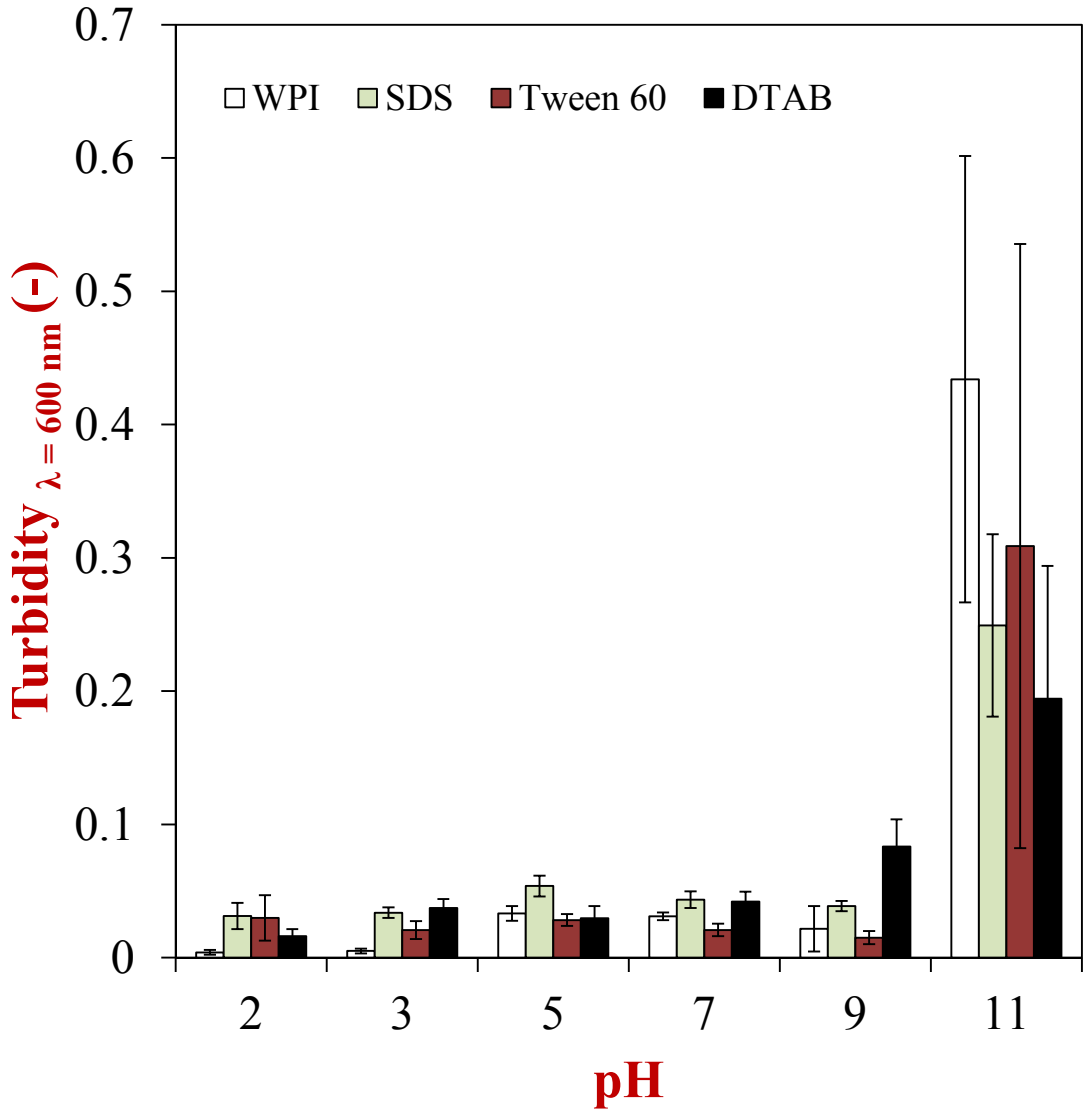

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Fig. 7.

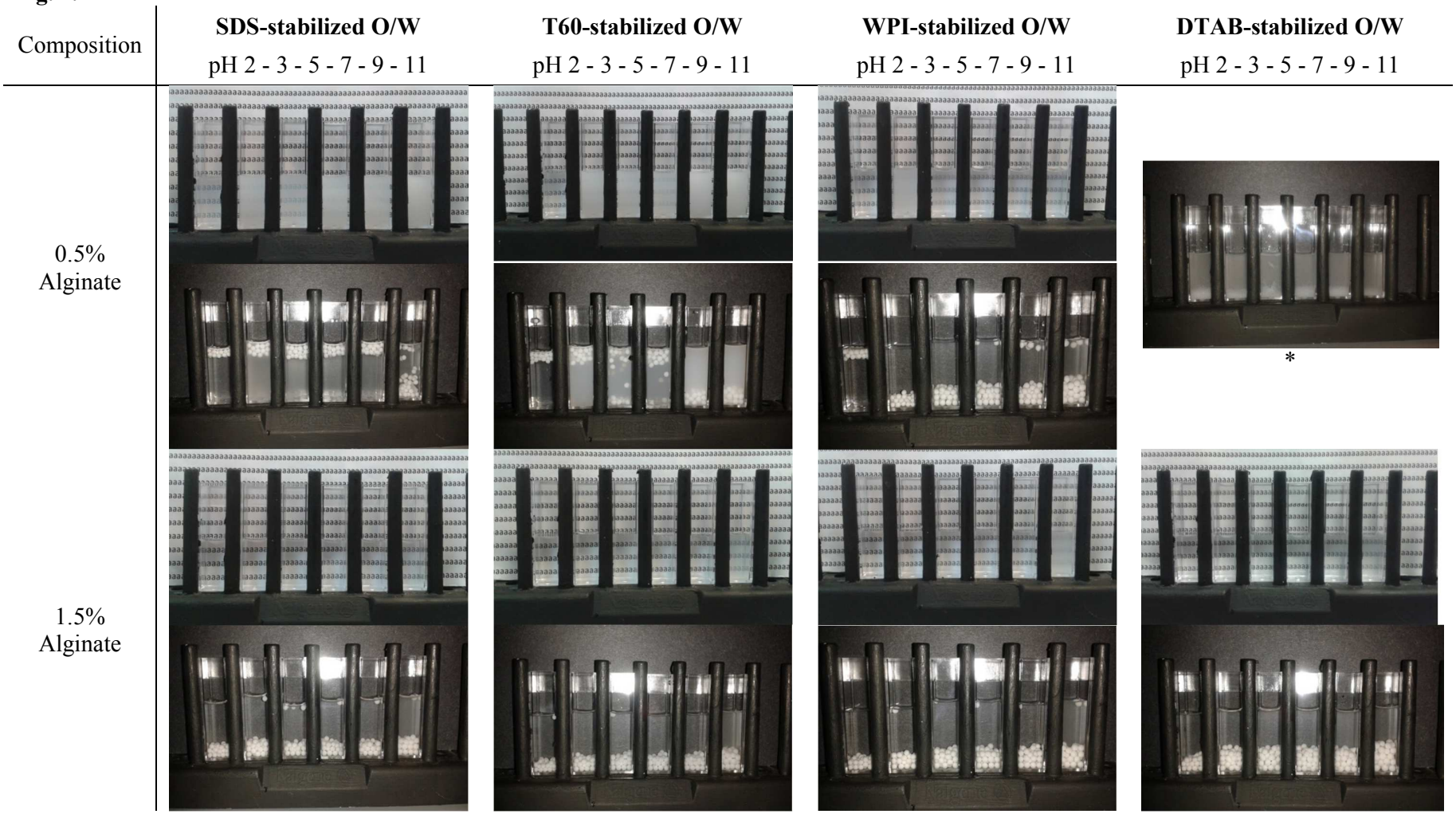

Fig. 8a.

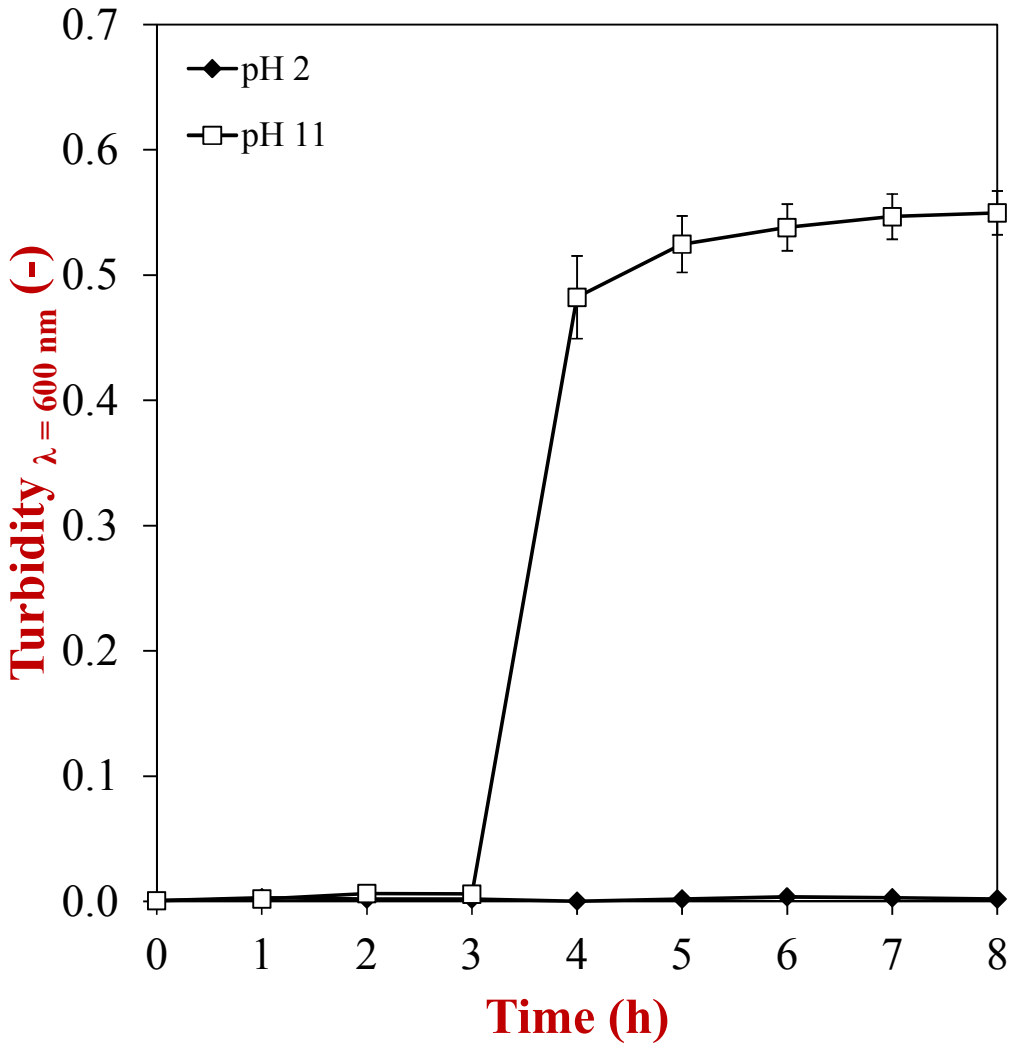

Fig. 8b.

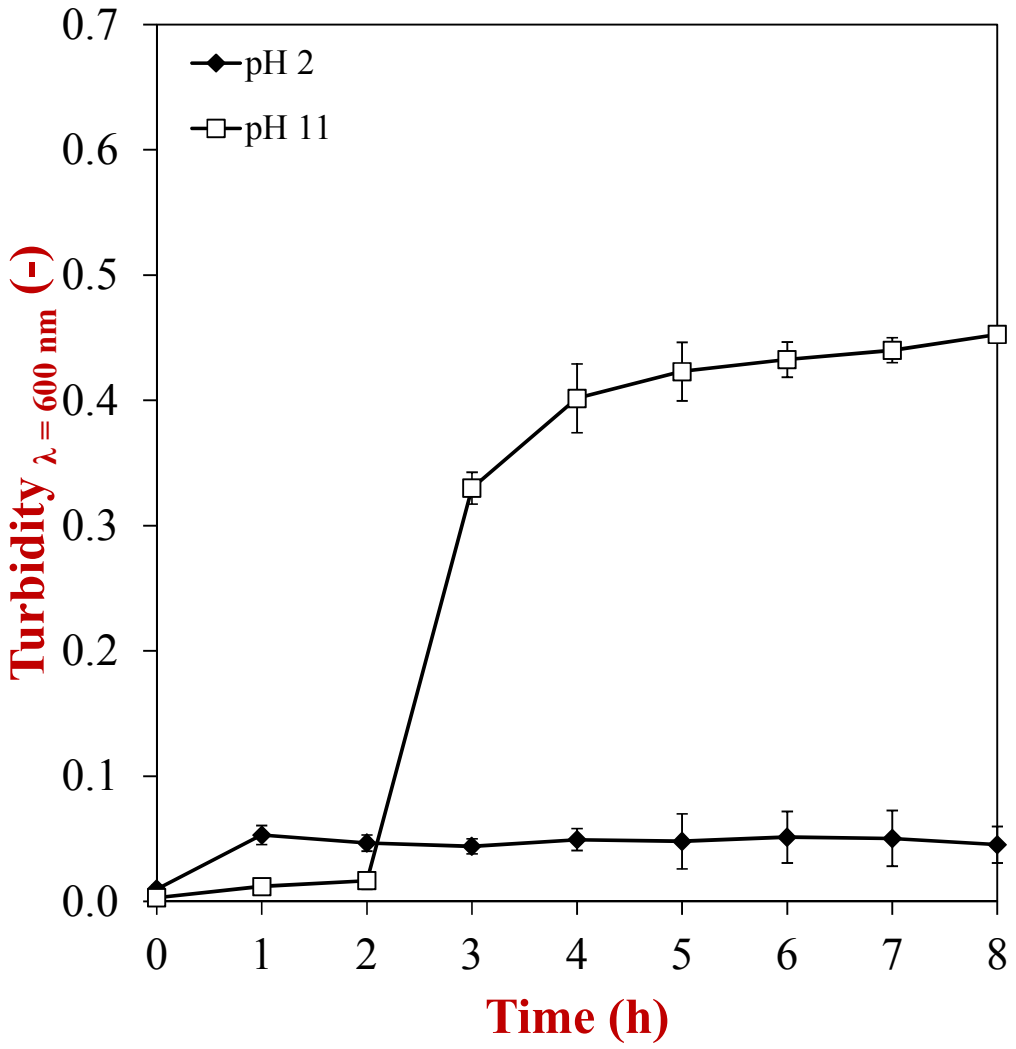

Fig. 8c.

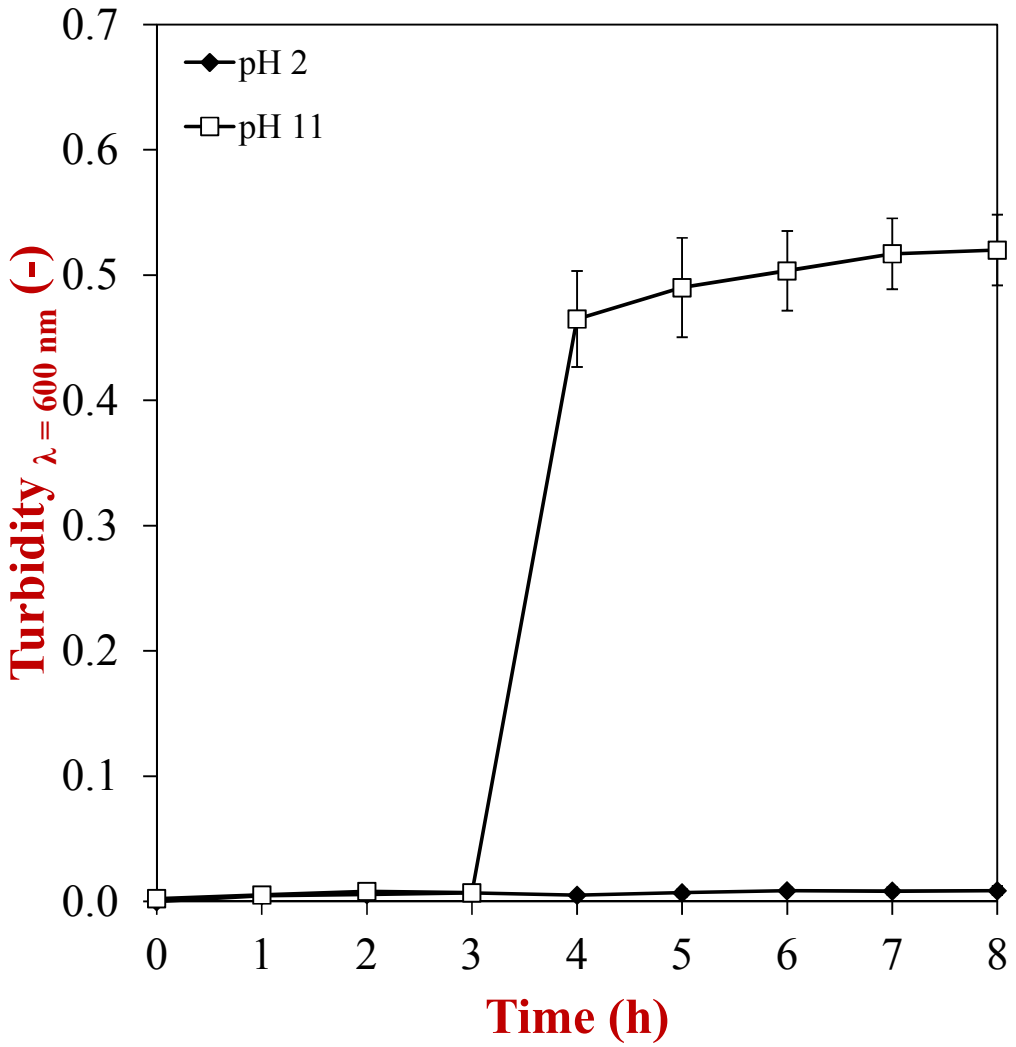

Fig. 8d.