

Fabrication and Optimization of Pickering Emulsion Stabilized by Lignin Nanoparticles for Curcumin Encapsulation

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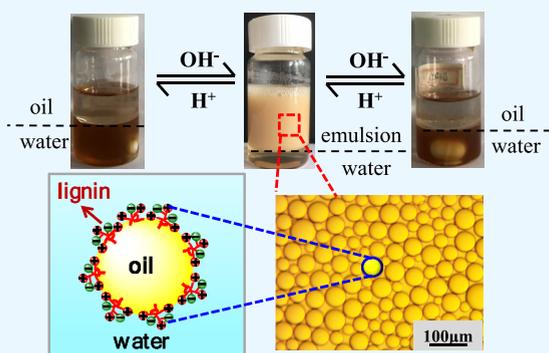


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ABSTRACT: To develop reversible pH-responsive emulsifiers of natural origin, alkali lignin (AL) was used to develop oil-in-water Pickering emulsions. AL was first modified to synthesize quaternized alkali lignin (QAL), which displayed pH-responsive properties and demonstrated solubility in both acidic and alkaline solutions. In contrast, QAL exhibited insolubility and formed particles in neutral solutions, thereby making it a suitable candidate for utilization as an emulsifier in doubly pH-responsive Pickering emulsions. At pH 5–9, the emulsions were stable. Above or below this pH range, the system demulsifies, resulting in a reversible Pickering emulsifier with two pH-controlled transitions. On the basis of this pH-dependent behavior, lignin-based Pickering emulsions (LPE) could be subjected to several cycles of emulsification–demulsification by alternating the pH of the aqueous phase between basic and acidic, while the droplet size and storage stability were maintained. Curcumin was used as a drug model to study the loading/release behavior of LPE, finding that 50.08% of curcumin could be encapsulated in LPE. The *in vitro* release of curcumin was pH-dependent. In addition, LPE exhibited an outstanding protective effect against the ultraviolet-induced degradation of curcumin.



1. INTRODUCTION

Emulsions are composed of two immiscible liquids, one of which is dispersed in the other. Conventionally, molecular surfactants are used to stabilize emulsions.¹ However, surfactants are expensive and recovery is difficult.² Moreover, cells and tissues may be damaged by surfactants, which limits their use in biomedical applications.³ Therefore, the utilization of solid particles as stabilizers in emulsions, commonly referred to as Pickering emulsions, has garnered growing attention due to their enhanced stability, biocompatibility, and environmental friendliness.^{4–6} Pickering emulsions exhibit greater stability compared to classical emulsions that are stabilized by surfactants, which is essential for applications in which long-term emulsion stability is critical, such as bitumen emulsification and food storage. Nevertheless, in other cases, such as catalysis, oil recovery, and controlled release of functional ingredients, only temporary stability is required.^{7–9} Since obtaining ultrastable Pickering emulsions can be challenging, the development of stimulus-responsive particulate emulsifiers for Pickering emulsions, in which the transformation between stable and unstable states can be triggered by external stimuli such as pH, temperature, magnetic field, light irradiation, and CO₂/N₂ addition, is attracting increasing attention. In particular, pH-responsive systems have gained special interest due to their inherent simplicity and practicality. Hence, pH-responsive inorganic, organic, and hybrid nanoparticles have been explored as reversible emulsifiers.^{10–14} However, a vast majority of pH-responsive

emulsifiers are of synthetic origin, with complex synthetic procedures and unexplored physiological safety. Therefore, the development of pH-responsive emulsifiers of natural origin is highly desirable.

In this context, lignin, which is the second most abundant biopolymer found in nature and accounts for 15–35 wt % of lignocellulosic biomass, has recently emerged as a promising biomolecule for application in the fields of adsorption and dispersion.^{15–19} In particular, the nontoxicity, biodegradability, and good biocompatibility of lignin render it particularly suitable for applications in biomedical and cosmetic fields.^{20,21} Although the rapid growth of the lignin market has stimulated the use of lignin to produce high value-added products, the exploitation of lignin still remains undeveloped, being mostly limited to the generation of energy by direct burning of the lignin produced in the paper and bioethanol production industry.^{22,23}

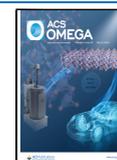
Lignin exhibits an amphiphilic nature due to its hydrophobic aromatic skeleton and hydrophilic groups. As a result, lignin can be adsorbed at air–liquid and liquid–liquid interfaces.^{24,25}

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At present, the utilization of lignin nanoparticles as emulsifiers for Pickering emulsions is observed. For instance, multilayer composite microcapsules were prepared by using lignin nanoparticle-stabilized oil-in-water (O/W) Pickering emulsions as templates, and these microcapsules were used to load isophorone diisocyanate for the preparation of a self-healing coating.²⁶ Polyacrylamide was grafted onto kraft lignin nanoparticles, resulting in the formation of polymer-grafted lignin nanoparticles that exhibit the capability to form Pickering emulsions.²⁷ Zhang et al. prepared spherical lignin nanoparticles and used them as a biobased stabilizer for Pickering emulsions.²⁸ Hong synthesized an alkyl chain-bridged lignin polymer, which was subsequently utilized in the preparation of novel lignin-based Pickering emulsions.²⁹ Ibuprofen could be delivered by a Pickering emulsion, and the embedding rate was 10%. Alkali lignin (AL) is insoluble under acidic and neutral conditions, whereas it dissolves under alkaline conditions and is a pH-responsive polymer. By exploiting this property, Wei et al. used AL extracted from furfural residues as a particulate emulsifier for the preparation of pH-responsive O/W Pickering emulsions.³⁰ It should be noted that Pickering emulsions prepared with unmodified lignin were stable only under acidic conditions. Chen et al. used enzymatic lignin and sodium dodecyl sulfate as emulsifiers to prepare O/W emulsions.³¹ Unfortunately, sodium dodecyl sulfate has severe toxicity against red blood cells, which has greatly hindered its applications.³²

In this paper, a simple pH-responsive Pickering O/W emulsion stabilized using quaternized AL (QAL) was investigated. The effects of pH, oil phase properties, and QAL concentration on the stability of the emulsions were investigated, and the reversible cycling and rheological properties of the emulsions were determined. In addition, the loading rate and release behavior of lignin-based Pickering emulsions (LPE) were investigated using curcumin as a model drug. The ability of LPE in protecting curcumin against UV irradiation and thermal degradation was evaluated. A plausible mechanism for the emulsification–demulsification process of LPE is proposed based on the obtained results.

2. EXPERIMENTAL SECTION

2.1. Materials and Chemicals. AL was separated from the black liquor derived from pine pulping, which was provided by Shuntai Technology Development Co., Ltd. (Jilin province, China). AL samples were acidified, filtered, and washed with distilled water. After purification, the lignin was freeze-dried and was used for further processing. The reagents used in this study, including 3-chloro-2-hydroxypropyltrimethylammonium chloride (Shanghai Aladdin Industrial Corp., China), sodium hydroxide (Guangdong Guanghua Sci-Tech Co., Ltd., China), and hydrochloric acid (Guangzhou Chemical Reagent Factory, China), were of analytical grade. *n*-Hexane, liquid paraffin, toluene, and dichloromethane were procured from Guangdong Guanghua Sci-Tech Co., Ltd. All chemicals utilized in this study were of analytical grade.

2.2. Preparation of QAL. Seventy-five grams of purified AL was introduced into 200 mL of distilled water followed by the adjustment of the resulting mixture's pH to 12 through the addition of 20 mL of NaOH (20 wt %). After the mixture was heated to 85 °C, 55 g of 3-chloro-2-hydroxypropyltrimethylammonium chloride was introduced dropwise. The mixture was stirred for 4 h at 85 °C to facilitate the process of quaternization. NaOH (20 wt %) was added simultaneously to

the above solution to maintain a constant pH (≈ 12). After the reaction, the solution was purified in a dialysis bag with a molecular weight cutoff of 1000 Da. QAL obtained then was concentrated by rotary vacuum evaporation and freeze-dried.

2.3. Preparation of QAL Particles. QAL particles were prepared by adjusting the pH of the aqueous QAL solutions. Briefly, a quantity of QAL was dispersed in deionized water, and the pH was adjusted to 12 using a 20 wt % NaOH until the QAL was completely dissolved. Then, the pH of the QAL solution was adjusted to around 7 by adding 20 wt % HCl to induce the formation of QAL particles.

2.4. Preparation of Lignin-Based Pickering Emulsions (LPE). LPE was typically prepared as follows: In a 25 mL glass bottle, 7 mL of an aqueous phase containing 0.1 wt % QAL particles and 7 mL of an oil phase was homogenized at 11,000 rpm for 2 min using an Ultra-Turrax homogenizer (IKA T18 basic, S18N-10G head). The type of emulsion was judged through observation of the dispersion behavior exhibited by dropping the emulsion into water. To investigate the long-term stability of LPE, the emulsions were kept at room temperature for 1 month.

2.5. Reversible Cycles. The pH-responsive characteristics of LPE were examined by adding small quantities of 20 wt % NaOH or HCl to adjust the pH value of the aqueous phase followed by observing six cycles of the emulsification–demulsification process.

2.6. Rheological Characterization of LPE. The rheological characteristics of LPE were investigated by means of a rheometer (RV-I, Haake, Germany) equipped with a cone and plate configuration (60 mm diameter, 1° cone angle, and 0.05 mm gap). Steady shear tests were conducted in flow mode with shear rates between 0.01 and 100 s⁻¹ at 25 °C. The linear viscoelastic region was determined using a strain sweep (0.01–100% strain at 1 Hz). The viscoelasticity of the samples within the viscoelastic region was analyzed by angular frequency sweep measurements.

2.7. Encapsulation of Curcumin in LPE and *In Vitro* Release Study. Curcumin was dispersed in soybean oil, and the mixture was stirred for 24 h for complete dissolution. The supernatant was obtained by centrifugation, and the concentration of curcumin in soybean oil was determined by HPLC (Agilent ZORBAX, Agilent, USA) with an isocratic elution of methanol–water (88/12, v/v) as the mobile phase. The flow rate was 1 mL/min, and the column temperature was maintained at 40 °C. The detection wavelength was set at 430 nm. Curcumin-encapsulated LPE (LPE@Curcumin) was prepared by processing the oil phase (curcumin in soybean oil) and water phase (0.1 wt % QAL, pH 7) in the Ultra-Turrax homogenizer for 2 min.

The *in vitro* release analysis was conducted according to a previously reported method.³¹ Then, 0.5 mL of LPE@Curcumin was dispersed in 40 mL of phosphate-buffered saline solution (pH 7.4) and acetate-buffered solution (pH 5.0) with 0.5 wt % SDS. The sample was placed in a shaking incubator at 37 °C with shaking at 100 rpm. At specific time intervals, 1 mL of the mixture was collected and an equivalent volume of buffer solution was added to ensure the maintenance of the total volume. The curcumin concentration was determined by HPLC. Three replicates were conducted for all of the samples.

2.8. Photodegradation Studies. To evaluate the photodegradation of curcumin, 10 mL of LPE@Curcumin and 10 mL of curcumin dispersed in soybean oil were poured into a

7.0 cm diameter Petri dish. Subsequently, the samples were exposed to ultraviolet (UV) light emitted by a 30 W, 310 nm lamp positioned at a distance of 30 cm. Samples were removed at different intervals, and the remaining curcumin in LPE@Curcumin was extracted by methanol and analyzed by HPLC. Each experiment was performed three times.

2.9. Other Characterizations. The zeta potential and size of QAL were determined at different pH values using a zeta potentiometer and a laser particle size analyzer (Brookhaven Corp., USA) at 298 K. The three-phase contact angle of the QAL particles was measured across the water phase using the classic captive drop method. Briefly, QAL was pressed into a disk at 10 MPa for 1 min. The QAL disk was then placed at the bottom of a transparent glass vessel. Oil was poured into the vessel followed by a drop of water on the particle layer. Subsequently, the water droplet on the particle layer was promptly captured in a photograph, and the contact angle was directly determined using a protractor.

The Pickering emulsion droplets were observed with an optical microscope (Panasonic, WV-CP470 Japan), and the sizes of the emulsion's droplets were measured by laser diffraction using a Mastersizer (Mastersizer 2000, Malvern Instruments, UK).

3. RESULTS AND DISCUSSION

3.1. QAL Properties at Different pH Values. AL molecules are surface active, and their solubility in aqueous media is dependent on the pH. AL dissolves at high pH and aggregates to form particles in solution at low pH. As mentioned in the introduction, Pickering emulsions prepared using AL are only stable under acidic conditions. Amphoteric QAL exhibits double pH-responsive characteristics and dissolves well at 3–4 and above 10, whereas it becomes insoluble at 5–9, as shown in Figure 1a. The morphology of QAL at different pH values is presented in Figure S1. According to the analysis of the particle size distribution (Figure 1b), the average size of QAL was 120 nm at pH 3. At a pH range of 5–9, the sediment of particles was caused by the

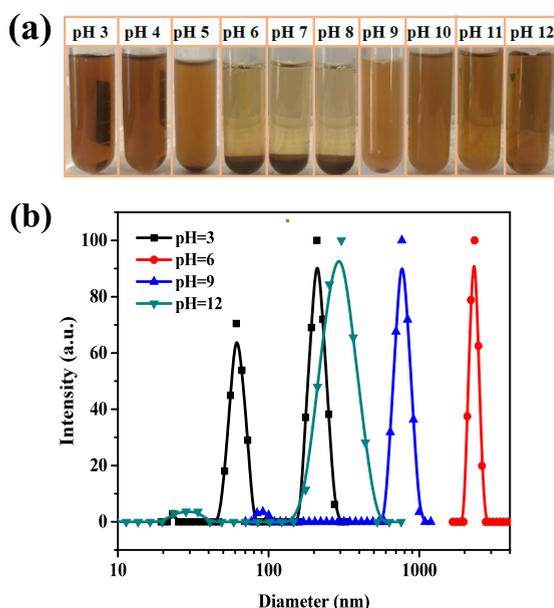


Figure 1. (a) Photographs and (b) diameter of QAL at different pH values.

agglomeration of QAL. At high pH, QAL was dissolved and the average size of QAL was 260 nm at pH 12. The zeta potential of QAL is presented in Figure S2. The zeta potential of QAL shows a gradual decrease (from +24.93 to -18.3 mV) as the pH increases from 3 to 12. At pH below 5, the QAL was positively charged due to the presence of quarternary ammonium groups. At a pH range of 5–9, QAL was insoluble in aqueous solutions owing to the electrostatic interactions between the ionized groups. At high pH, QAL was dissolved due to ionization of the carboxyl groups and phenolic groups. These results show that QAL has a doubly pH-tunable dissolution–precipitation transition.

3.2. Wettability of QAL. The three-phase contact angle θ of a solid particle at the oil–water interface is an important factor to control the type and stability of Pickering emulsions.³³ The optimum stability is obtained when the contact angle approaches 90° . However, it is possible for one phase to have a degree of wettability that is higher than that of another. According to Kaptay,³⁴ the contact angle range for O/W emulsions stabilized by solid particles should fall between 15 and 90° , while for W/O emulsions with hydrophobic particles, the contact angle range should be between 90 and 165° . These conclusions are drawn from considerations of the energy and maximum capillary pressure. The interfacial wetting properties of a solid particle may be changed by varying the pH. The wetting properties of QAL particles were investigated by directly measuring the water-in-oil θ values on a QAL disk. Figure 2 shows images of water droplets attached to a QAL

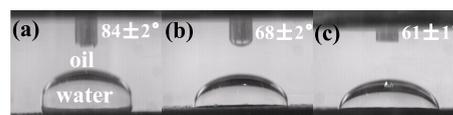


Figure 2. Water droplets attached to a QAL powder disk immersed in *n*-decane at (a) pH 7.0, (b) pH 3.0, and (c) pH 11.0.

disk immersed in *n*-decane. At pH 7.0, the water-in-oil θ is $84^\circ \pm 2^\circ$, as shown in Figure 2a. At low pH and high pH, QAL was preferentially wetted by the aqueous phase, as indicated by the water-in-oil θ of $68^\circ \pm 2^\circ$ (pH 3.0, Figure 2b) and $61^\circ \pm 1^\circ$ (pH 11.0, Figure 2c), respectively. Close to the isoelectric point, at pH 7.0, QAL became more hydrophobic due to the interaction between the majority of the chargeable groups. These results clearly demonstrate that QAL exhibits exceptional suitability as a material for interfacial adsorption and can be used as an emulsifier for pH-responsive Pickering emulsions.

3.3. Effect of pH on the LPE. The alteration in the pH value of the aqueous phase will have an impact on the wettability or electrical characteristics of the particles, consequently influencing the adsorption behavior of said particles at the oil–water interface.³⁵ On the basis of the pH-tunable dissolution–precipitation–dissolution transition of QAL, which was expected to be reversible, the QAL aggregates as a particulate emulsifier for Pickering emulsions were envisaged. *n*-Decane was selected as the oil for the Pickering emulsions. The investigation of the stability of the LPE was conducted by different pH values of the water phase, as shown in Figure 3a,b.

Upon the addition of a drop of the emulsion to water, it was observed that the emulsion dispersed effectively, thereby suggesting its classification as an oil-in-water emulsion. Upon

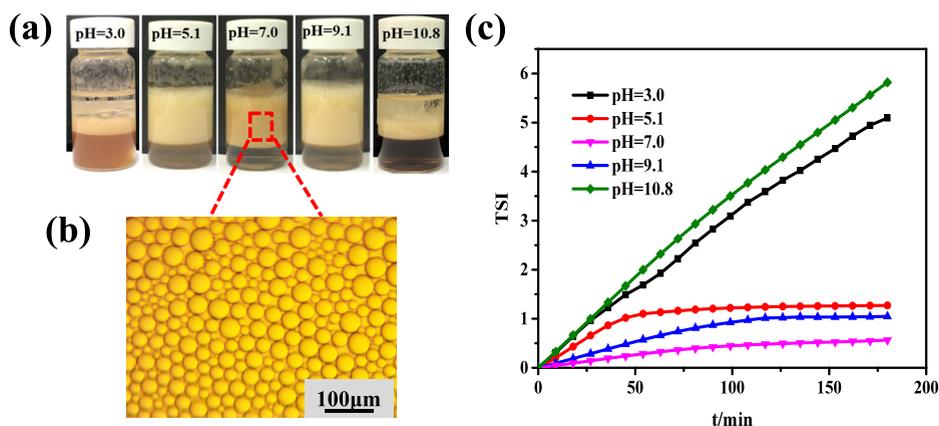


Figure 3. (a) Photographs of *n*-decane-in-water emulsions prepared at different pH values using a 0.1 wt % QAL. (b) Optical micrographs of the emulsion stabilized by QAL at pH 7.0. (c) Effect of pH on the TSI of the lignin-based Pickering emulsion.

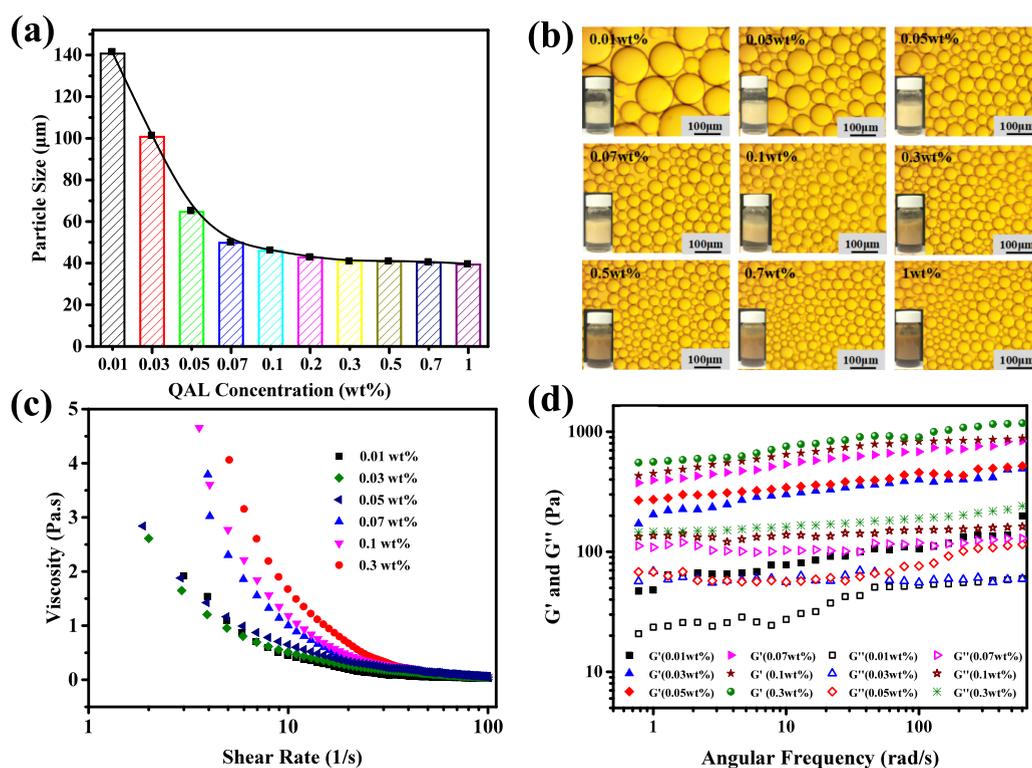


Figure 4. (a) Hydrodynamic diameters of *n*-decane-in-water (pH 7.0) emulsions stabilized by QAL at different concentrations. (b) Optical micrographs and photographs of *n*-decane-in-water (pH 7.0) emulsions stabilized by QAL at different concentrations. (c) Viscosity of the LPE prepared at different QAL concentrations. (d) Storage and loss modulus of LPE prepared at different QAL concentrations.

decreasing the pH of the water phase to 5.0, QAL became soluble in water, and the Pickering emulsions were demulsified after 24 h. As the pH was increased, QAL became insoluble in water and adsorbed at the oil–water interface and Pickering emulsions were formed (Figure 3b). However, at pH above 10, QAL dissolved in water again, and demulsification occurred.

To compare the stability of the emulsion at different pH values, the Turbiscan stability index (TSI) of LPE at different pH values was determined using a Turbiscan Lab Expert stability analyzer, and the result is shown in Figure 3c. At pH 7, the LPE showed the smallest TSI value, suggesting that the emulsion is the most stable. In contrast, under conditions of pH 10.8 and 3.0, the TSI value of the emulsion gradually

increased with time, indicating that the stability of the emulsion was poor.

3.4. Effect of QAL Concentration on the LPE. The stability and droplet size of the Pickering emulsion are affected by the solid particle concentration. Therefore, the effect of the QAL concentration on the particle size of LPE was investigated, and the result is shown in Figure 4a. At QAL concentrations below 0.1 wt %, the particle size gradually decreased with increasing the QAL concentration. Meanwhile, above 0.1 wt % QAL, the particle size of LPE did not change with the QAL concentration. The obtained results are congenial with previous reports that used silica particles as stabilizers to prepare Pickering emulsions.^{36,37} The droplet size of the emulsion gradually decreased with an increase in silica

concentration until it reached the critical micelle concentration, at which point the droplet size exhibited stability.

Figure 4b shows optical micrographs and photographs of *n*-decane-in-water of (pH 7.0) emulsions stabilized by QAL at different concentrations. When the concentration of QAL was lower than 0.1 wt %, QAL particles were all adsorbed at the oil–water interface to participate in the formation of the emulsion, and the color of the water phase was clear. At QAL concentrations higher than 0.1 wt %, excess QAL particles existed in the water phase, and the droplet size did not change significantly with increasing the QAL concentration. However, when the QAL concentration was 0.01 wt %, the emulsion was unstable because the number of QAL particles adsorbed at the oil–water interface was insufficient to stabilize the emulsion. As the number of emulsifying particles decreases, the particle size of droplets increases.³⁸ An insufficient amount of QAL would lead to instability of the emulsion, and an excessive amount of emulsifier would cause waste and pollution. Therefore, QAL with a concentration of 0.1 wt % was selected as the emulsifier for the Pickering emulsion.

In order to investigate the impact of the QAL concentration on the textural properties of LPE, the rheological response of LPE was assessed through oscillatory and flow rheology measurements. Figure 4c illustrates the impact of the QAL concentration on the rheological characteristics of the Pickering emulsion. It was found that the viscosity of LPE decreased upon increasing the shear rate, exhibiting a typical shear-thinning behavior. The viscosity of LPE increased when the concentration of QAL varied from 0.01 to 0.3 wt %. The elevation of viscosity can be attributed to the contribution of the three-dimensional network structure of QAL, which promotes the formation of interchain networks and exhibits strong interactions in the aqueous phase. Figure 4d depicts the dynamic viscoelastic measurements of the emulsion in the presence of different concentrations of QAL. Storage modulus (G') of the LPE was higher than the loss modulus (G''), indicating the formation of emulsion gels. This phenomenon can be attributed to the heightened flexibility and entanglement propensity of lignin at higher concentrations, leading to the formation of compact networks.

3.5. Effect of the Type of Oil on the LPE. To prepare QAL-stabilized emulsions, four oils were selected based on their distinct properties, namely, liquid paraffin, *n*-hexane, toluene, and dichloromethane. A summary of the characteristics of the oil-phase solvents can be found in Table 1. Liquid

Table 1. Dielectric Constant and Viscosity of Different Oils

oil	<i>n</i> -decane	liquid paraffin	<i>n</i> -hexane	toluene	dichloromethane
ϵ	1.9	1.9	1.9	2.4	9.1
viscosity (cP ^a)	0.92	~40	0.33	0.59	0.44

^aIndicates the viscosity value at 20 °C.

paraffin, *n*-hexane, and toluene are characterized as weakly polar oil-phase solvents, while dichloromethane is classified as a highly polar oil-phase solvent. Liquid paraffin exhibits a notably high viscosity, reaching up to 40 cP, whereas the viscosity of the other oil-phase solvents is below 1 cP. Despite the different properties of the oil phases, stable Pickering emulsions were easily obtained in all cases. The optical microscopy images of the corresponding emulsions are shown

in Figure 5. The emulsion prepared by using liquid paraffin as the oil phase had the largest particle size. The high viscosity of

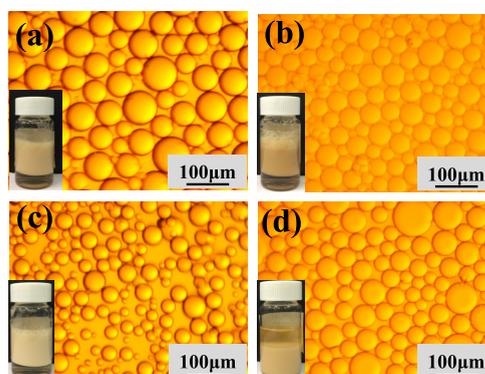


Figure 5. Optical micrographs of 0.1 wt % QAL emulsions prepared using different oils: (a) liquid paraffin, (b) *n*-hexane, (c) toluene, and (d) dichloromethane. The insets are digital photographs of the corresponding emulsions.

the oil phase hinders the flowability of the droplet interface, thereby preventing the formation of a liquid film and the adsorption of particles onto it. Consequently, this decelerates the adsorption rate, leading to inadequate emulsion stability and the presence of larger droplets.³⁹ Due to its higher density compared to water, the emulsion droplets of dichloromethane sunk to the bottom of the vial. It is noteworthy that this type of Pickering emulsion could stabilize diverse oils in water, which may have broad applications in the future.

3.6. Reversibility and Recycling of LPE. In order to develop a pH-responsive emulsion system, the emulsification–demulsification process must be reversible. To investigate this behavior, an *n*-decane–water mixture stabilized by 0.1 wt % QAL particles was subjected to alternate addition of 20 wt % HCl followed by homogenization and 20 wt % NaOH followed by agitation with a magnetic rotator, as shown in Figure 6. In cycle 1, the pH of the aqueous phase was decreased to 7

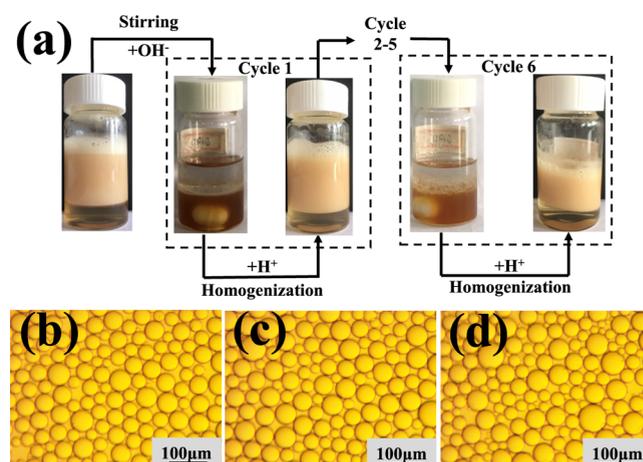


Figure 6. (a) Photographs of *n*-decane-in-water (pH 7.0) emulsions stabilized by 0.1 wt % QAL and then switched between demulsification and emulsification via alternately increasing the pH to ~10.8 by adding NaOH and reducing the pH to ~7.0 by adding HCl. (b–d) Optical micrographs of the emulsion droplets that have undergone (b) zero (freshly prepared), (c) one, and (d) six demulsification–emulsification cycles.

through the addition of HCl, resulting in the formation of a stable oil-in-water emulsion. The complete separation of the emulsion was achieved by increasing the pH of the aqueous phase to 10.8 with the addition of NaOH. Above pH 10.8, QAL was dissolved in the aqueous phase due to the presence of deprotonated phenolic hydroxyl groups. Insufficient adsorption of QAL at the water–oil interface resulted in unstable emulsions. Thus, the emulsion was demulsified by magnetic stirring for 20 min. The aforementioned cycles were repeated six times, during which no discernible alteration in the mean droplet size was observed. The Pickering emulsion, maintained at a pH of 7.0, exhibited exceptional stability for a minimum of six cycles, as substantiated by the consistent average droplet size sustained over a duration of one month.

Similarly, demulsification also occurred at a low pH, as shown in Figure 7. The introduction of 20 wt % HCl to a

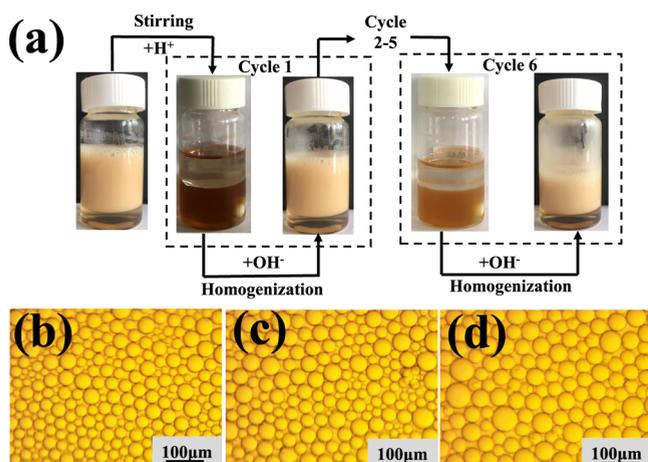


Figure 7. (a) Photographs of *n*-decane-in-water (pH 7.0) emulsions stabilized by 0.1 wt % QAL and then switched between demulsification and emulsification via alternately reducing the pH to ~ 2.9 by adding HCl and increasing the pH to ~ 7.0 by adding NaOH. (b–d) Optical micrographs of the emulsion droplets that have undergone (b) zero (freshly prepared), (c) one, and (d) six demulsification–emulsification cycles.

previously stable emulsion prepared at a pH of 7.0 resulted in a reduction of the pH to 2.9, leading to the demulsification process. Conversely, the addition of an equivalent amount of 20 wt % NaOH caused the pH to nearly revert back to its

initial value, resulting in the formation of a stable emulsion under agitation. Again, no change was observed in the droplet size upon repeating this process six times.

The property of salt tolerance holds significant importance in pH-responsive Pickering emulsions.⁴⁰ During the emulsification–demulsification process, the NaCl concentration would increase as the number of cycles increased. Therefore, the effect of the NaCl concentration in the aqueous QAL solutions on the average droplet diameter of the Pickering emulsion was investigated, with the corresponding findings presented in Figure S3. The particle size of the Pickering emulsion did not change significantly upon the addition of NaCl up to 1 M. At pH 7.0, QAL contains roughly equal amounts of positive and negative charges, and thus, the surface charge of QAL is virtually zero. In a salt solution, the inorganic salt can promote hydrophobic association between lignin molecules, which would form a spatial network structure. As a result, LPE has excellent stability at a high salt concentration.

3.7. In Vitro Release Performance and Photodegradation Studies. Curcumin has been reported to have multitarget antitumor properties and has application in the clinical treatment of cancer.⁴¹ In this paper, curcumin was selected as a model drug to study the encapsulation efficiency and *in vitro* release behavior of LPE. Curcumin was dissolved in soybean oil, and QAL was used as stable particles to prepare a drug-loaded Pickering emulsion. Figure S4 shows a photograph and an optical micrograph of the LPE loaded with curcumin. The drug loading content and encapsulation efficiency were 21.11 and 50.08%, respectively. Then, the release behavior of curcumin from LPE was evaluated in a buffer solution at 37 °C, and the corresponding kinetics are displayed in Figure 8a.

At pH 7.4, the release of curcumin was slow, with only 10.2% of curcumin released after 12 h and 26.5% after 120 h. This slow release rate may be beneficial to decrease drug leakage during LPE circulation in the blood, thereby reducing possible side effects. LPE had a controlled release at pH 5.0 with an increased release rate under acidic conditions. The cumulative release amount of curcumin from LPE was 40.9% within 12 h and 66.2% within 120 h. Under acidic conditions, the carboxylate groups are protonated, reducing the number of electrostatic interactions. The structure of QAL at the oil–water interface becomes loose, and the stability of the LPE decreases, resulting in the rapid release of curcumin. This suggests that the LPE is suitable for the loading of anticancer

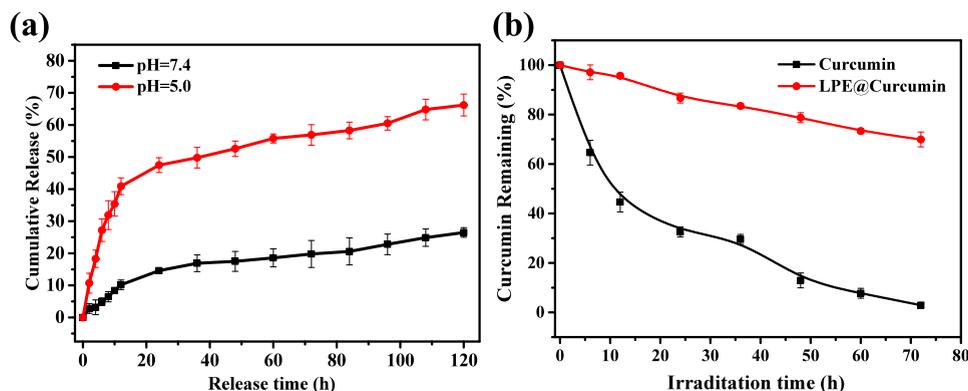


Figure 8. (a) *In vitro* drug release profiles of a curcumin-loaded Pickering emulsion at pH 7.4 and pH 5.0. (b) Remaining percentages of curcumin at different ultraviolet irradiation times.

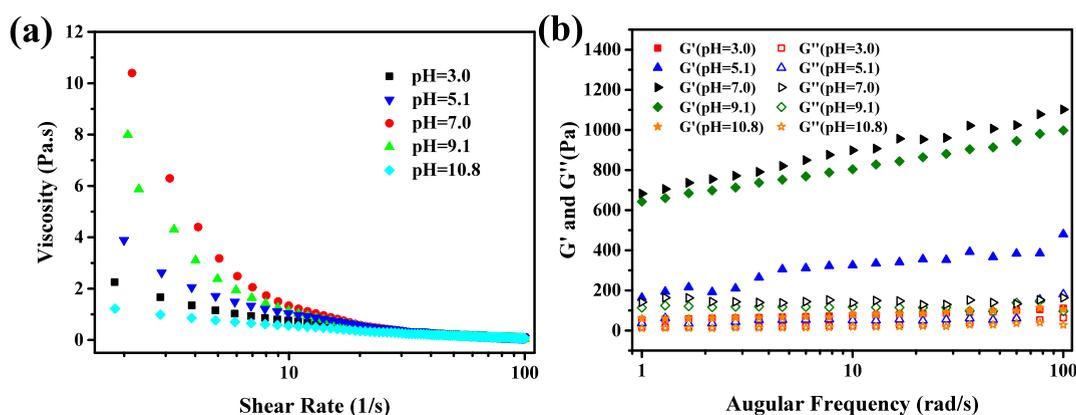


Figure 9. (a) Shear-rate dependence of the viscosity of Pickering emulsions stabilized by 0.1 wt % emulsions with different pH. (b) Storage modulus (G' , filled symbols) and loss modulus (G'' , open symbols) as functions of the frequency for these emulsions.

drugs because it could reduce the burst release of drugs in human normal tissues and release drugs preferentially in the tumor environment and cancer cells.

Curcumin is a phenolic compound that is sensitive to UV radiation. Meanwhile, lignin is a natural anti-UV agent, which can effectively protect drugs from photodegradation.⁴² LPE encapsulation may protect curcumin against UV-induced degradation; the residual curcumin content in LPE upon UV treatment was determined, and the result is shown in Figure 8b. There was only 2.93% residual curcumin in the bulk oil. In contrast, the content of curcumin encapsulated in LPE stabilized by 0.1 wt % QAL maintained 69.94% of its original value after 72 h of UV irradiation. These results demonstrate that LPE has excellent anti-UV properties and could effectively improve the loading efficiency of photosensitive drugs.

3.8. Rheology Properties of LPE. Emulsions possess elasticity because of the existence of an interfacial energy between the droplets. The long-term stability of the emulsion can be evaluated by measuring energy storage. Compressing the emulsion causes deformation of the dispersed phase droplets, leading to an increase in the surface area and storage of elastic energy.⁴³ Therefore, studying the rheological behavior of the emulsion can provide evidence of the stability mechanism of the emulsion.

Figure 9a shows the viscosities of the LPE prepared under different pH. As the shear rate increased, LPE's viscosity decreased, and it exhibited typical shear-thinning behavior. The shear-thinning phenomenon observed in emulsions can be attributed to the existence of weak attractive forces among the droplets, leading to the formation of a weak elastic gel-like network. As a result of shear stress, droplets move away from one another. If the magnitude of the shear stress is less than that of the attractive forces, then the emulsion exhibits an elastic response, whereby the shear energy is elastically stored as an extension of the bonds between the dispersed droplets. At the same shear rate, the apparent viscosity of the LPE first increases and then decreases as the pH increases. At pH 7, the apparent viscosity of the emulsion is the largest, and a three-dimensional network structure of QAL is formed because of the electrostatic interactions between the ionized groups, which formed interchain networks and interacted strongly in the aqueous phase, thereby increasing the viscosity.⁴⁴ When the pH value decreases from 7 to 3, the positive charge of QAL increases and the repulsive force between molecules also increases. The 3D network structure of QAL is loosened, and

the stability of the emulsion decreases. When the pH increases from 7 to 12, the repulsion between the QAL molecules increases, causing a decrease in the viscosity of the LPE.

The quantification of the viscous and elastic responses of the viscoelastic emulsions can be achieved through dynamic oscillatory measurements. Figure 9b shows the dynamic viscoelastic measurements of the emulsion in 0.1 wt % QAL at different pH. G' was greater than G'' throughout the frequency range covered, and the emulsions exhibited typical gel-like behavior. In other words, the emulsion behaved more like a viscoelastic solid, wherein its deformations were predominantly elastic and capable of recovery.

In addition, the G' and G'' of emulsions at pH 7 were greater than those of the emulsions at other pH. The larger storage modulus of the emulsion indicates that it has a rigid volume-filled network structure in the continuous phase. The formation of a viscoelastic film can effectively prevent the coalescence of the emulsion at the O/W interface, thereby stabilizing the emulsion.

A plausible mechanism for the formation of an emulsion is depicted in Figure 10. QAL is an amphiphilic surfactant; it



Figure 10. Proposed emulsification and demulsification mechanisms of the lignin-based Pickering emulsion.

contains not only quaternary ammonium groups, carboxyl groups, phenolic hydroxyl groups and other hydrophilic functional groups but also the hydrophobic skeleton of phenylpropane. Under neutral conditions, the carboxyl group is deprotonated and cross-links with quaternary ammonium groups via electrostatic interactions to form a three-dimensional network structure. Therefore, QAL becomes more hydrophobic and can be stabilized at the oil–water interface to maintain the emulsion stability. Meanwhile, under acidic conditions, the carboxyl group is protonated. The quaternary ammonium cations increase the electrostatic repulsion in the

QAL molecule, which enhances to the enhancement of the hydrophilicity of QAL and the demulsification of the system. Under alkaline conditions, the phenolic hydroxyl group is deprotonated and the electronegativity is enhanced, with the resulting increase in the repulsive force between molecules. The three-dimensional network structure formed by the QAL molecule at the oil–water interface becomes loose, and the hydrophilicity is enhanced, which hinders the stabilization at the oil–water interface, leading to the collapse of the emulsion.

4. CONCLUSIONS

This paper demonstrates a novel and simple approach to prepare a doubly pH-responsive Pickering emulsion based on lignin. Under neutral conditions, QAL was insoluble in water and formed aggregates. These QAL aggregates serve as particulate emulsifiers for stabilizing the O/W emulsions. At high and low pH, QAL was soluble in water, and the emulsions were demulsified. In contrast, the emulsions were stable in the pH range of 5–9. This pH-dependent behavior enabled the realization of an emulsification–demulsification process via alternate addition of an acid or a base while maintaining the droplet size and good storage stability. The prepared LPE exhibited *in vitro* pH-dependent release behavior for curcumin and an excellent protective effect against UV light-induced degradation of curcumin.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c10395>.

Morphology and zeta potential of QAL at different pH values, optical micrographs and particle size of *n*-decane-in-water emulsions stabilized by 0.1 wt % QAL at pH 7.0 in the presence of NaCl, and photograph and optical micrograph of the Pickering emulsion carried with curcumin (PDF)

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Notes

The authors declare no competing financial interest.

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