

Oil–Water Emulsion Flocculation through Chitosan Desolubilization Driven by pH Variation

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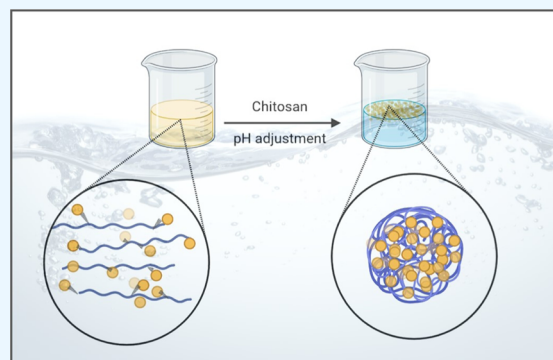


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ABSTRACT: Water pollution is a major concern in our modern age. The contamination of water, as a valuable and often limited resource, affects both the environment and human health. Industrial processes such as food, cosmetics, and pharmaceutical production also contribute to this problem. Vegetable oil production, for example, generates a stable oil/water emulsion containing 0.5–5% oil, which presents a difficult waste disposal issue. Conventional treatment methods based on aluminum salts generate hazardous waste, highlighting the need for green and biodegradable coagulant agents. In this study, the efficacy of commercial chitosan, a natural polysaccharide derived from chitin deacetylation, has been evaluated as a coagulation agent for vegetable oil emulsions. The effect of commercial chitosan was assessed in relation to different surfactants (anionic, cationic, and nonpolar) and pH levels. The results demonstrate that chitosan is effective at concentrations as low as 300 ppm and can be reused, providing a cost-effective and sustainable solution for oil removal. The flocculation mechanism relies on the desolubilization of the polymer, which acts as a net to entrap the emulsion, rather than solely relying on electrostatic interactions with the particles. This study highlights the potential of chitosan as a natural and ecofriendly alternative to conventional coagulants for the remediation of oil-contaminated water.



INTRODUCTION

Environmental pollution is a significant issue that needs to be addressed in this century. Despite the technological advancements that are increasing the life expectancy of people, pollution is reducing the lifespan of billions of individuals across the globe.^{1–4} One of the main contributors to this problem is the unrestricted use of water, which is intensively employed in industrial processes and frequently released into the environment without proper treatment, leading to the contamination of rivers, lakes, and oceans.⁵ Addressing the reduction and remediation of oily water wastes is crucial not just in the petroleum and petrochemical industries but also in the production of food, cosmetics, and pharmaceuticals. These industries rely on large amounts of water that, at the end of the industrial cycle, cannot be reused due to contamination by organic solvents/surfactants, metals, oils, etc. (commonly called industrial emulsion). As a result, these waters become special wastes with high disposal costs, squandering a scarce resource. The production of vegetable oils is a significant contributor to this problem, discharging vast volumes of oily water emulsion.^{6,7} The presence of surfactants slows down or hinders the coalescence process, making oil separation difficult and time-consuming. Usually, oil-processing mills reduce the oil content in the wastewater by sedimentation bath, which generates partially treated oil waste, a colloidal water suspension

containing 0.5–5% of solid fraction (oils, grease, surfactant, solvents, metals).⁶ The removal of this last part of the contaminant is the most challenging and prevents the use of this water for human/animal consumption and traditional or proximal agriculture.⁸

Flocculation/coagulation are gold standards for oily wastewater emulsion treatments. Such a process destabilizes the colloidal structure inducing flocculation, which can be easily settled and removed.⁹ Conventional coagulants are metallic salts such as aluminum chloride, ferrous sulfate, aluminum sulfate, ferric chloride, and hydrated lime. Aluminum-based coagulants are widely exploited for their effectiveness, price, and availability. Such coagulants in water solution generate a positive charge, which neutralizes negatively charged particles, destabilizing oil droplets and thus destroying the emulsion.^{10,11} However, the use of these metallic salts is a topic of debate and is now strictly regulated. In fact, metallic salts are usually used in large excess to ensure coagulation efficiency. Thus, the resulting water is heavily

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	Particle size (nm)	Z potential (mV)	a
SDS	233±97	-47±7	
TWEEN 80	183±78	-15±4	
CTAB	240±97	63±11	

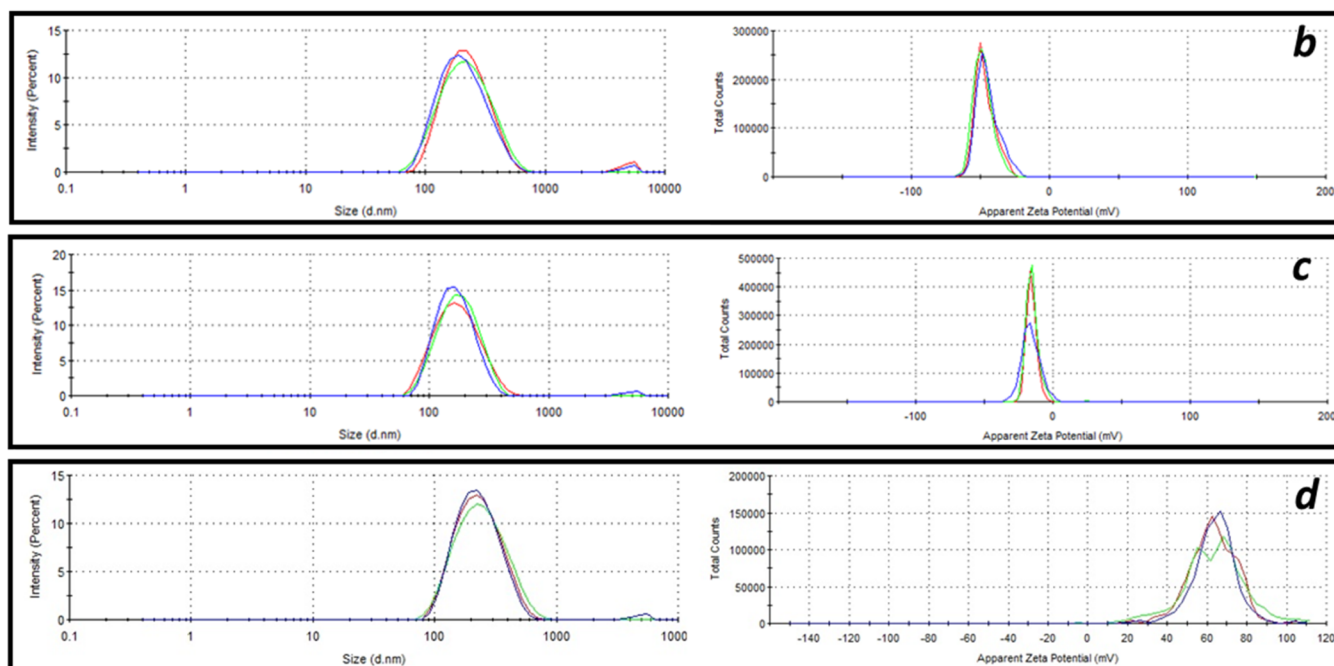


Figure 1. (a) Particle size distribution and ζ potential measured in the emulsion obtained with different surfactants; (b) DLS characterization of SDS emulsion, particle size, and ζ potential distribution are shown from left to right, respectively; (c) DLS characterization of Tween 80 emulsion, particle size, and ζ potential distribution are shown from left to right, respectively; and (d) DLS characterization of the CTAB emulsion, particle size, and ζ potential distribution are shown from left to right, respectively.

contaminated by metallic residuals, which have been associated (in particular aluminum salts) with neurodegenerative diseases such as Alzheimer's.^{12,13} This is driving the research in the development and investigation of new environmentally friendly flocculants. Recently, many natural coagulants have been investigated (e.g., *Moringa oleifera* seed, cactus, rice starch, chitosan).^{14,15} Among all, chitosan (*N*-acetyl-D-glucosamine) is one of the most promising coagulants. Chitosan is a polyelectrolyte polysaccharide derived from chitin, a structural element in the exoskeleton of crustaceans, thus representing a waste of the fish food chain, already used in many bioengineering applications from cosmetics to drug delivery.^{16–18} It is a pH-sensitive polymer, able to solubilize in an acidic environment and desolubilize/flocculate when pH shifts to basic.¹⁹ Chitosan, as other polysaccharides, is reported as a suitable green coagulant biopolymer for its biocompatibility, biodegradability, adsorption property, and flocculating ability.^{20,21} Many studies have reported chitosan as a coagulant for negatively charged emulsion solutions or industrial wastewater rich in metals.²² In this study, chitosan (commercial grade) flocculation properties have been evaluated using emulsions based on anionic, cationic, and nonpolar surfactants, thus owning different ζ potentials, to

understand the mechanism behind the coagulation driven by chitosan. Moreover, the minimum efficacy dose and pH for chitosan coagulation have been evaluated. The obtained data highlighted that more than the electrostatic interaction of chitosan with the nanoparticle, the chitosan coagulation process is driven by its desolubilization, driven by pH changes, which act as a net entrapping the oil particles.

RESULTS AND DISCUSSION

We prepared different stable emulsions using three different surfactants: sodium dodecyl sulfate (SDS), polysorbate 80 (TWEEN 80), and cetyltrimethylammonium bromide (CTAB), which are anionic, nonionic, and cationic, respectively (see Materials and Methods sections for details). The oil–water emulsion contains 2% of oils, which represents the maximum concentration for achieving a stable emulsion with all surfactants. Higher concentrations result in oil-phase separation (see Figure S1). We measured the size distribution and ζ potential of these solutions, and the results are reported in Figure 1. The particle size of all emulsions was ~200 nm, with a single Gaussian distribution. The anionic surfactant SDS generated a colloidal suspension with a negative ζ potential of

~−47 mV, while the cationic surfactant CTAB showed a positive ζ potential of ~63 mV. The nonionic surfactant generated a colloidal suspension with a ζ potential of −15 mV, which may be due to the presence of contaminants, oil, and other impurities (e.g., free fatty acids).^{23–25}

All emulsions were observed under optical microscopy (Figure S2), revealing no significant differences between emulsions with spherical oil particles. To fully evaluate the emulsion characteristics, we assessed the ζ potential in relation to pH variations ranging from 2 to 8, a pH interval where all emulsions remain stable (Figure 2). We observed no particle size

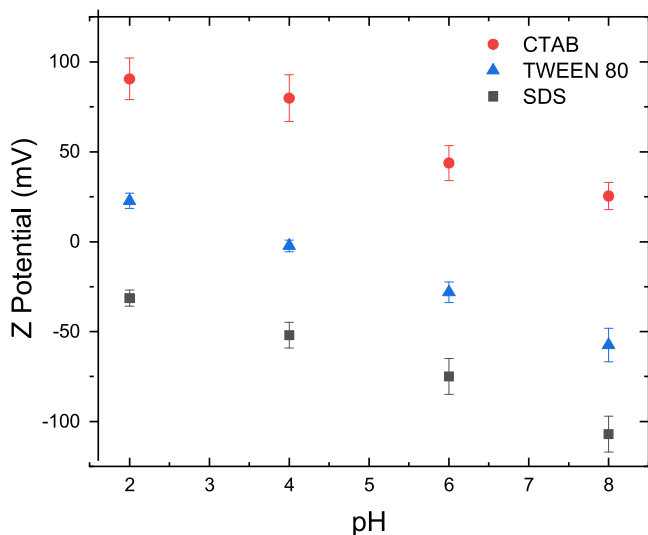


Figure 2. ζ potential characterization of emulsions based on CTAB, Tween 80, and SDS at different pH values ranging from 2 to 8.

distribution variation within the analyzed range. The results demonstrate that droplets at low pH exhibit the highest ζ potential due to an excess of H_3O^+ ions, which stabilize cationic surfactants (CTAB) and increase the positive ζ potential. Conversely, anionic surfactants (SDS) tend to destabilize and are stabilized by increasing the pH (excess of OH^-). Interestingly, Tween 80 demonstrated the ability to cross the instability region typically considered within the range of −20 to 20 mV without particle coalescence.²⁶ This further demonstrates that the stability of emulsions with nonpolar surfactants is independent of the ζ potential. The raw data are reported in Figures S3–S5.

Upon the addition of a chitosan solution and pH modification, the oil fraction in the emulsion undergoes flocculation. In Figure 3a, we present an image of the flocculation process inside the vials along with a schematic representation of the oil coagulation. This flocculation leads to the formation of an oily phase at the top of the vials due to the lower oil density. We have verified that to achieve flocculation with industrial-grade chitosan, two minimum requirements must be met: pH >8 and chitosan concentration >250 ppm.

We evaluated the efficiency of oil removal under different experimental conditions, including variations in pH and chitosan concentration, as shown in Figure 3b,c. To quantify the amount of oil removed, we performed a gravimetric assay of the filtered eluate, as described in detail in Materials and Methods sections. We have demonstrated that chitosan acts as a flocculant in all emulsions, regardless of the starting ζ potential. Notably, the emulsion based on CTAB undergoes flocculation

even without the presence of chitosan. The increase in pH leads to a neutralization of the ζ potential, making the emulsion unstable. However, such flocculation allows for the removal of a lower organic fraction of ~80 and 85% at pH 9 and 11, respectively. Optical microscopy images of the different CTAB emulsions before and after flocculation are shown in Figure 3d–f. Notably, although the CTAB flocculation was driven by pH variation, the eluate still contained oil particles. In the presence of chitosan, such particles were not observable. The increase in pH enhances oil removal in all emulsions due to enhanced chitosan flocculation. However, we also observed that chitosan is less efficient when used with nonpolar surfactants, such as Tween 80, due to its apolar nature. This is likely because chitosan aminic groups are less effective at attracting nanoparticles generated by Tween 80 surfactants, which tend to be more hydrophilic.²⁷ As a result, the chitosan may be less effective with such a surfactant and the emulsion may be less likely to break. We can conclude that the action of chitosan as a flocculant is mainly due to the desolubilization of chitosan, which acts as a net, taking the oil nanoparticles with them, thus breaking the emulsion. We observe that at high pH, the process is more efficient due to the faster kinetic process that prevents the escape of oil particles. In fact, the solubility of chitosan is strictly correlated with pH variation and its solubility drastically decreases within a range between pH 6 and 9,^{28,29} making it more effective for oil removal at higher pH values.

We have investigated the possibility of reusing chitosan for water remediation. We solubilized the flocculate portion of the solutions in 10 mL of acetone by adjusting its pH to 1 with a 0.1 M HCl solution. This resulted in oil and chitosan solubilization. Subsequently, the oil and the chitosan were separated by centrifugation at 4000 rpm for 5 min. The resulting chitosan pellet was solubilized in 0.01 M acetic acid to obtain a 1% chitosan solution, which we reused to drive flocculation in a new SDS emulsion (300 ppm at pH 9). We successfully exploited the same chitosan for three cycles, with a performance reduction of 8% in the second cycle and 30% in the third cycle (refer to Figure 4). These data demonstrate the ability of chitosan to be resolubilized and reused for multiple cycles, despite a performance reduction, probably due to residual oil present in the polymeric network. These results are preliminary, and we intend to investigate other organic solvents for this purpose.

CONCLUSIONS

In this work, commercial-grade chitosan has been investigated as a flocculant agent in oil–water emulsions. The action of chitosan has been verified on anionic, cationic, and nonpolar surfactants. A concentration as low as 300 ppm is sufficient for the removal of up to 97% of the organic fraction. The action of chitosan in the different emulsions, which own different ζ potentials, and pH efficacy dependence suggest that the flocculation action of chitosan is not mainly guided by the electrostatic interaction with the particle, as is demonstrated for already used Al salts but rather from the chitosan desolubilization that traps the oil particle. Furthermore, this study has demonstrated the potential for chitosan to be effectively reused with only a slight reduction in performance, indicating its suitability as a sustainable and cost-effective solution for water remediation applications. The performance of commercial-grade chitosan at such a low concentration and the understanding of the flocculation mechanism could pave the way to the use of chitosan and another polymer as flocculant agents in water waste, reducing the environmental impact of manufacturing plants on water.

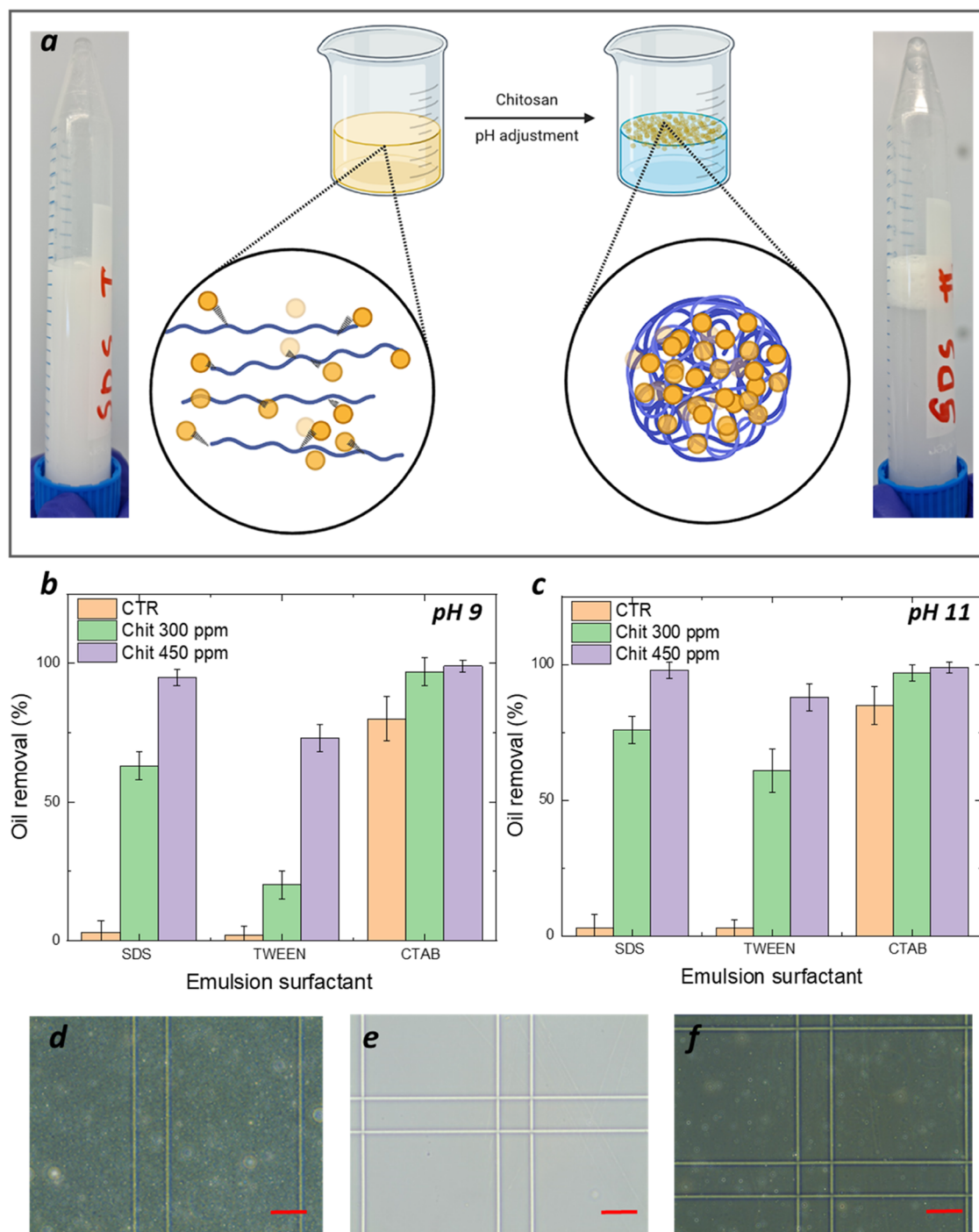


Figure 3. (a) Picture and schematic representation of the chitosan-driven flocculation on an oil–water emulsion. The photos specifically refer to the emulsion and coagulation of an SDS-based emulsion, where flocculation was carried out using 300 ppm chitosan at pH 11. (b, c) Histograms show the oil removal percentages obtained without the presence of chitosan (CTR) and with different concentrations of chitosan (300 and 450 ppm). The left side reports the oil removal obtained at pH 9, while the right side reports the oil removal obtained at pH 11. The pH was adjusted by adding 0.1 M NaOH. (d–f) Light microscopy images of the cell count plate of the CTAB emulsion, CTAB emulsion after treatment with 300 ppm of chitosan at pH 9, and CTAB emulsion after pH adjustment at 9 without chitosan, respectively, with a scale bar of 25 μm in each image.

MATERIALS

The surfactants cetyltrimethylammonium bromide (CTAB, cationic surfactant), polysorbate 80 (Tween 80, nonionic surfactant), sodium dodecyl sulfate (SDS, anionic surfactant), acetic acid, and NaOH were purchased from Sigma-Aldrich. Sunflower oil was purchased from a local supermarket. Chitosan (commercial grade with price <\$20/kg) with a molecular weight

of ≤ 200 kDa and deacetylation of $\geq 80\%$ was purchased from Sinoway Industrial Co.

METHODS

Three different oil/water emulsions were prepared by mixing 0.05% w/w of each surfactant for 1 h in water and then adding 2% w/w of sunflower oil and mixing for 2 h. The stability of the emulsions was visually confirmed, with no phase separation

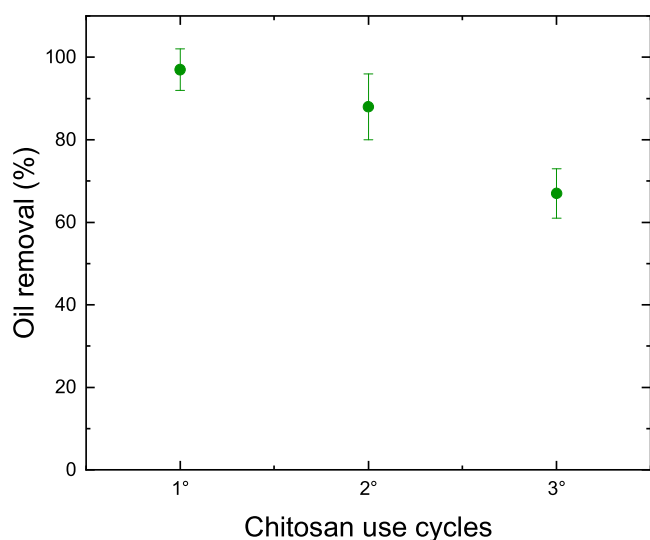


Figure 4. Oil removal percentage of 300 ppm chitosan in a CTAB emulsion over three cycles of reuse.

observed over a time period of 1 week, and further analyzed through ζ potential and particle size distribution measurements, as reported in the main manuscript.

A solution of 1% chitosan was perpetrated by mixing overnight chitosan in a 0.01 M acetic acid water solution.

Each emulsion was poured into a 25 mL falcon, and then different amounts of chitosan were added and vigorously mixed for 1 min. Specifically, we added 800 and 1200 μ L of a 1% chitosan solution to achieve concentrations of \sim 300 and \sim 450 ppm, respectively. The pH was then adjusted to the experimental condition (9 and 11), with 0.1 M NaOH, and mixed for 1 min, allowing chitosan desolubilization. Such solution was filtered using a lab paper filter, and the eluate was dried in an oven at 105 $^{\circ}$ C for 12 h to evaluate the efficiency of the chitosan coagulation.

Particle size and ζ potential were measured using a DLS Malvern Zetasizer 3000 at room temperature (\sim 25 $^{\circ}$ C).

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c01257>.

Images reporting emulsion instability at high concentrations of oil; optical microscopy of different oil–water emulsions using SDS, Tween 80, and CTAB as surfactants; and ζ -potential analysis at different pH values (PDF)

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Author Contributions

[§]L.L. and G.G. equally contributed to this work.

Notes

The authors declare no competing financial interest.

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