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# Discussions on the Properties of Emulsion Prepared by Using an Amphoteric Chitosan as an Emulsifier

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**Featured Application: The amphoteric chitosan successfully substituted sodium acrylates copolymer as an emulsifier in the processes of emulsion production, and the same emulsification effect could be achieved with a smaller dose of the amphoteric chitosan.**

**Abstract:** A typical emulsion contains oil and water phases, and these two phases can be combined by an emulsifier with both lipophilic and hydrophilic groups to form a mixture. If the component of water is more than oil, the mixture is termed as o/w emulsion. The water is called the continuous phase and the oil is called the dispersed phase. Oppositely, if the component of oil is more than water, the mixture is termed as w/o emulsion. The oil is called the continuous phase and the water is called the dispersed phase. Chitosan, which is biocompatible and non-toxic, was modified as an amphoteric emulsifier to replace sodium acrylates copolymer in the preparation of emulsions. Both sodium acrylates copolymer and the modified chitosan were used as emulsifiers, respectively, and the properties of moisturizing, transmittance, the number of bacteria, and emulsion stability were measured. The experimental results showed that the amount of amphoteric chitosan is less than that of sodium acrylate copolymer by 20% under a similar degree of emulsification. The measurement of spatial moisture showed the difference in equilibrium humidity was in the range of 2.05 to 2.20 g$H_2O$/kg dry air, indicating that the moisture retention of the modified chitosan is better. In addition, the calculation of bacterial growth confirmed that the number of bacteria in the amphoteric chitosan emulsion and the sodium acrylate copolymer emulsion were 80 and 560, respectively. The emulsion stability was tested by the separation of oil and water phases in the diluted emulsion and by centrifugal accelerated sedimentation. The results showed that, for both emulsifiers, no separation of the oil and water phases occurred within one hour, and the stability of the modified chitosan emulsion was better. Therefore, the modified chitosan successfully substitutes sodium acrylates copolymer as an emulsifier in the preparation of emulsion.

**Keywords:** chitosan; emulsion; bacterial growth

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## 1. Introduction

Chitosan is a component purified from the shells of shrimp and crabs, and the structure is similar to those of cellulose and chitin [1]. Since chitosan inherently possesses unique biological properties, such as biodegradability, non-toxicity, and high human affinity, it can be applied alone or combined with other natural substances, such as starch, gelatin, and sodium alginate, in food, medicines, textiles, agriculture [2,3], water treatment and cosmetics [4,5]. Chitosan can be used as an adsorbent in wastewater treatment due to the functional groups of hydroxyl and amine [6]. Chitosan can also be used to remove heavy metals [7,8] and dyes [9] from wastewater.

Some studies associated with emulsification processes in recent years are listed in Table 1. Most of these studies focused on the emulsion stability. It is known that emulsifiers

play an important role in the emulsification process, and emulsifiers were modified by some studies to maintain the stability of emulsions. For example, different weight ratios of sodium stearyl 2-lactylate to chitosan were used as emulsifiers [10], and optical microscopy and rheological measurements of emulsions were performed to analyze the stability of the emulsions. Similarly, anionic surfactant–chitosan complexes were used as the emulsifiers to improve the stability of oil-in-water emulsions [11]. To stabilize the emulsions at lower temperature, the chitosan/lithium dodecyl sulfate complex was used as an emulsifier [12], and the turbidity, size, and Zeta potentials of the emulsions were measured to discuss the effects of operating variables on the stability of the emulsions. Unlike the chitosan/surfactant complex, a series of chitosan-graft-oligoN-isopropylacrylamide-graft-oligolysine copolymers have been synthesized and used as the emulsifiers for preparing emulsions with high internal phases, and this kind of emulsion has been applied in pharmaceuticals, food, cosmetics, and biologics [13].

Both chitin and chitosan are non-toxic and antimicrobial and hydrating agents [14]. Since chitosan is a biopolymer made from natural organisms, it has good biocompatibility with biological cells [15,16]. The functional groups of -OH and -$NH_2$ in chitosan can be substituted easily. Therefore, chitosan was used as the backbone to modify the -OH group as a hydrophilic end, such as -COOH, -$SO_3$, or -$H_2PO_4$, and to modify the -$NH_2$ group as a hydrophobic end, such as 1–12 carbon alkyl, so as to form amphoteric chitosan. Higher capacities of amphoteric chitosan in water absorption and moisture retention were previously reported [17,18], and the hollow nanocapsule of acylated carboxymethyl amphiphilic chitosan transformed from solid nanoparticle was observed as a result of the hydrophobic effect, which improved the affinity toward drug encapsulation [19]. As mentioned above, amphoteric chitosan was prepared to replace sodium acrylates copolymer as an emulsifier for the preparation of emulsions to increase the properties of moisturizing, antibacterial activity and stability.

Emulsion stability can be determined according to the method of CIPAC MT 36.3. For instance, the water of different hardness was considered to examine emulsion stability [20]. The result showed that the emulsifiability capacity could be affected when emulsifiable concentrates are exposed to high temperatures. Extended from this method and according to WHO's standard, the emulsion is diluted 20 times and stood for 1 h. The hygienic standards are met when no oil floats on the top and no water sinks at the bottom.

The chitosan has been used as a copolymer, a surfactant, an additive, or an emulsifier by some studies to examine the stability of emulsions. For example, chitosan was used as a copolymer (weak polyelectrolyte), and lecithin was used as a surfactant. Chitosan in different molecular weights and degrees of acetylation were adsorbed on lecithin-stabilized nanoemulsion droplets to examine how the stability was affected by the electrophoretic mobility [21]. Since the cationic surfactants could be generated from chitosan with amine and amino groups in combination with H+ ions, it was used as a cationic emulsifier to examine the suitability in preparing cationic bitumen emulsions [22]. Generally speaking, acetic acids were used to disperse chitosan in aqueous media. To modify their flavor, the oil-in-water emulsions were prepared by dispersing chitosan in lactic acid aqueous solutions first to evaluate their stability [23]. Some studies have found that long-term stable emulsions and Pickering emulsions of food-grade oil could be obtained by using chitosan as an emulsifier at different pH values [24,25]. Besides examining the stability of emulsions, some studies have endeavored toward improving the emulsification efficiency. To obtain a more hydrophobic substrate for bacterial cell surfaces, association of the chitosan solution with *Escherichia coli* was studied, and the results indicated that the emulsification efficiency was increased with increases in chitosan concentration [26]. In addition, a tapioca-starch suspension with addition of hydrophobic substance, such as a combination of chitosan-palm olein emulsion, was used to stabilize the PO (palm olein) droplets, and their stabilization was verified by elongation at break and water vapor permeability [27]. Other emulsion properties including antimicrobial activity, water-binding sites, nanostructural evolution, and emulsion breakdown were also discussed in the literature.

For example, chitosan in different molecular weights and degrees of deacetylation was used in preparing oil-in-water emulsions, and their antimicrobial efficiencies were tested [28]. Carboxymethyl-hexanoyl chitosan amphiphatic hydrogel was synthesized as a carrier for delivering amphiphatic agents, and results showed that the water-absorption ability was affected by the number of water-binding sites and the state of water under low humidity and the fully swollen state, respectively [22]. In addition, their water-absorption ability and drug encapsulation efficiency were defined to assess their performance. Similarly, the self-assembly capability of carboxymethyl-hexanoyl chitosan was employed to increase encapsulation efficiency [29]. Therefore, how the nanostructural stability was affected by the self-assembly mechanism was investigated by the critical aggregation concentration and zeta potential of the amphiphilic chitosan. To explore the mechanism of chitosan binding with fat, images of chitosan–oil aggregation were captured to examine the interaction between chitosan and oil-in-water droplets during the emulsification process [30]. Experimental results showed that the emulsion breakdown was enhanced with increases in viscosity and the degree of deacetylation of chitosan. As mentioned above, the application of amphoteric substances inspired the preparation of amphoteric chitosan to replace conventional emulsifiers in the emulsification process. Therefore, the goal of this study was to modify chitosan as an amphoteric substance to replace sodium acrylates copolymer as an emulsifier in the preparation of emulsion, and to discuss the advantages of amphoteric chitosan as an emulsifier by examining the amount of emulsifier required, the antibacterial effect, the moisture retention, and the emulsion stability. Furthermore, carbon dioxide ($CO_2$)-enhanced oil recovery (EOR), and $CO_2$-enhanced gas recovery (EGR) in shale reservoirs have recently attracted attention; however, both nuclear magnetic resonance (NMR) and compositional simulations of $CO_2$ injection in shales confirmed that adsorption outweighed molecular diffusion in determining $CO_2$ injection rate [31]. Whether the emulsifier, such as amphoteric chitosan, will play a role in solving the problem, is the question behind one of our future studies.

**Table 1.** Some studies associated with chitosan molecule used in emulsification and characterization studies.

| Improved Properties | Modified Method | Test | Reference |
|---|---|---|---|
| stability | SSL (sodium stearoyl 2-lactylate) /chitosan | steady shear rheometry | [10] |
| stability | anionic surfactant–chitosan complexes | adsorption kinetics | [11] |
| stability | chitosan/lithium dodecyl sulfate complexes | turbidity measurements | [12] |
| stability | chitosan-graft-oligoN-isopropylacrylamide-graft-oligolysine (CSNLYS) copolymers | solubility, cloud point and interfacial tensions | [13] |
| stability | different molecular weight and degree of acetylation | electrophoretic mobility | [20] |
| Stability | cationic type surfactant | storage stability, settlement, sieve test | [21] |
| stability | chitosan previously dispersed in lactic acid solutions | rheological assays | [22] |
| stability | pH value control | dynamic interfacial pressure | [23] |
| stability | pH value control | electrophoretic mobility, antibacterial activity | [24] |
| emulsification efficiency | addition of *Escherichia coli* | emulsification index, droplet size | [25] |
| emulsification efficiency | addition of tapioca starch | elongation at break, water vapor permeability | [26] |
| antimicrobial activity | molecular weight of chitosan, degree of deacetylation | bacteria growth | [27] |
| water-binding sites | carboxymethyl-hexanoyl chitosan hydrogel | moisture-retention ability and drug encapsulation | [17] |
| nanostructural evolution | carboxymethyl-hexanoyl chitosan | critical aggregation concentration (cac) and zeta potential | [28] |
| emulsion breakdown | increased degree of deacetylation | chitosan–oil aggregation | [29] |

## 2. Experimental Section

### 2.1. Synthesis of Amphoteric N-octyl-O-sulfate Chitosan

To compare the effects of chitosan with and without deacetylation on the properties of emulsion, chitin purchased from Emperor Chemical Co., Ltd. (Taipei, Taiwan), which had been demineralized, deproteinized and decolorized, was deacetylated with 70% NaOH solution in 1:50 $w/v$ ratio to convert to chitosan. Chitin and chitosan (10 g) were added into 80 mL methanol with stirring at 25–30 °C, respectively, and then octaldehyde (10 mL) was added. An aqueous solution of $KBH_4$, 3 g/10 mL water, was added slowly to the mixture after the mixture had stood for a day. Hydrochloric acid was used to neutralize the mixture, and then the mixture was stirred for 24 h. Finally, the product precipitated and the precipitate was washed with methanol and water. After filtration, the products were dried overnight under vacuum at 60 °C to obtain N-octyl chitosan. The dried N-octyl chitosan was added to 350 mL dimethylformamide (DMF) and the mixture was stirred magnetically for 12 h. Chlorosulfonic acid (60 mL) was added dropwise into another 350 mL of DMF, and the solution was stirred for 60 min at 0 °C. The mixture of N-octyl chitosan and DMF was added to the mixture of chlorosulfonic acid and DMF. The mixed solution was reacted at 10 °C under $N_2$ atmosphere for 36 h. The solution was neutralized by 20% NaOH after reaction, and then the solution was filtered and washed with distilled water. Finally, the product was dried overnight under vacuum at 60 °C to obtain the amphoteric N-octyl-O-sulfate chitosan.

All the chemicals were reagent grade. Potassium borohydride was purchased from Juyi Chemical Co., Ltd., Shanghai, China and sodium hydroxide, octaldehyde, and dimethylformamide were purchased from ECHO Chemical Co., Ltd. (Miaoli, Taiwan). Hydrochloric acid and chlorosulfonic acid were supplied by the SESODA Corporation. Apricot kernel oil and sodium acrylates copolymer were purchased from Emperor Chemical Co., Ltd.

### 2.2. Emulsion Preparation

First, we prepared 10 g apricot kernel oil and 90 g water, and then poured the apricot kernel oil into the water. The oil and water phases were immiscible at first. After that, 0.2 g of the emulsifiers, sodium acrylates copolymer and amphoteric N-octyl-O-sulfate chitosan, were added to the mixture of apricot kernel oil and water, respectively, and the mixtures were stirred magnetically at the same time. The stirrer was controlled at 90 rpm and the transmittance was measured continuously. The emulsification reaction was completed until the unchanged transmittance.

### 2.3. Characterization Method

The structural change resulting from the deacetylation process between chitin and chitosan was examined with a Thermo Nicolet 6700 ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared spectroscopy) spectrometer. The structural difference between chitosan and amphoteric N-octyl-O-sulfate chitosan was also confirmed by ATR-FTIR. The transmittance during the emulsification process was measured with a spectrophotometer, a U-2800A, in the range from 190 nm to 1100 nm. All the skin, emulsion, and air phase contain moisture. On the basis of mass transfer theory, the moisture in skin will be transferred to air phase under the higher moisture content in skin. The higher the moisture retention of the emulsion, the lower the speed transferring moisture to the air phase. Therefore, the system shown in Figure 1 was used to measure the moisture retention of the emulsion in this study. The emulsion was placed in the measuring system, and then the system was evacuated to a vacuum state. The humidity was detected continuously to analyze the moisture retention of the emulsion. The spread plate method was used to examine the number of bacteria. The emulsion was initially diluted 10 times. An inoculate plate containing agar was prepared, and a sample of 1 mL diluted emulsion was spread homogeneously on the surface of the agar medium. The inoculate plate was held in an incubator at 37 °C for 48 h and the number of bacteria was calculated after completion of the cultivation.

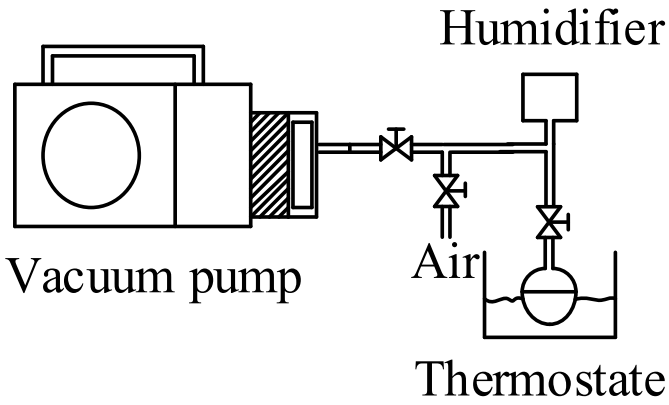



**Figure 1.** Measuring system for the moisture retention of emulsion.

## 3. Results and Discussion

### *3.1. Chitosan Conversion from Chitin to Chitosan*

Chitosan is the product that results from the removal of the acetyl group from chitin. The amine group that is produced upon removal of the acetyl group is an important functional group that gives chitosan a variety of activities [32,33]. When the degree of deacetylation is greater than 65%, the resulting product is soluble in acidic solutions. The FTIR spectra of chitin and chitosan are shown in Figure 2. The peaks at 3450 $cm^{-1}$ for chitin and chitosan were attributed to the stretching vibration and the intermolecular hydrogen bonding of the -$NH_2$ and -OH groups. The peak at 1660 $cm^{-1}$ in the spectra of chitin corresponded to the stretching vibration between the C=O and the N-H groups, and the peak at 1625 $cm^{-1}$ corresponded to the hydrogen bond resulting from the C=O and hydroxyl-methyl groups. In addition, the peaks at 1555 and 1315 $cm^{-1}$ corresponded to N-H and C-N groups, respectively. These results showed that the acetyl group was present in the chitin [34,35].

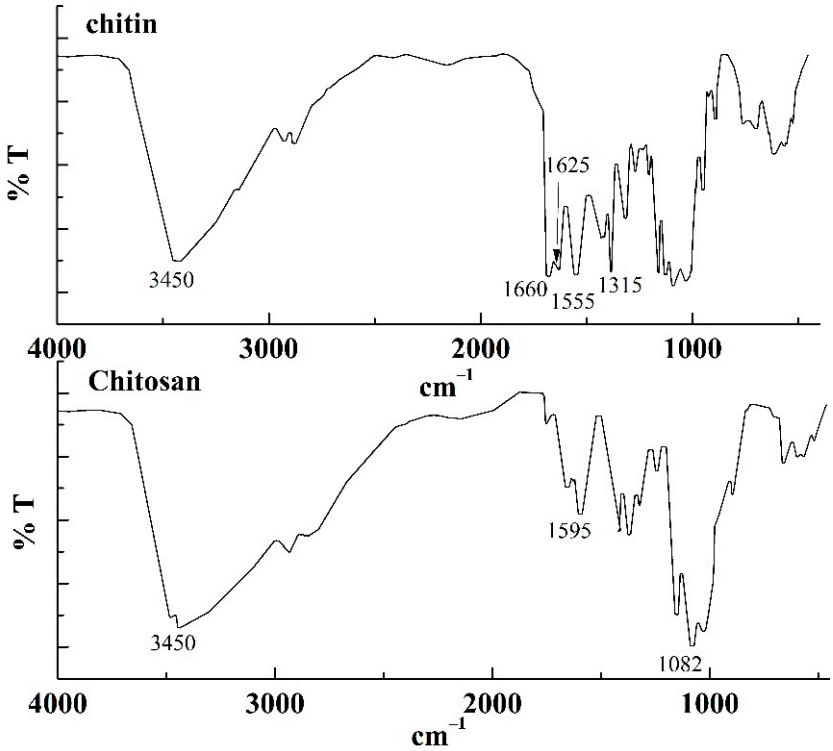



**Figure 2.** FTIR (Fourier Transform Infrared) spectra for chitin and chitosan.

As shown in Figure 2, the peak at 1595 $cm^{-1}$ corresponded to the -$NH_2$ group, meaning that chitin was converted to chitosan, while the peak at 1555 $cm^{-1}$ disappeared. Since the amine group was produced by the removal of the acetyl group, a hydrophobic group, such as the N-octyl contained in aldehydes, can be used to prepare chitosan derivatives. As shown in Figure 3, the absorption bands at 2912 and 2855 $cm^{-1}$, corresponding to the stretching vibration of the –CH group, indicated that the alkylation process was conducted to modify chitosan by octaldehyde [36,37]. The appearance of absorption bands at 1245 and 1212 $cm^{-1}$ was attributed to the presence of a sulfate group [38,39]. The peak at 3450 $cm^{-1}$ in Figure 2, corresponding to the stretching vibration and the intermolecular hydrogen bonding of the -$NH_2$ and -OH groups in the chitosan, was shifted to 3280 $cm^{-1}$ in Figure 3 because of the conjugation in the sulfate group [36,37]. Similarly, the peak at 1082 $cm^{-1}$ in Figure 2, corresponding to the stretching vibration of C-O in the chitosan, was shifted to 985 $cm^{-1}$. These results confirmed that the chitosan was modified as an amphoteric chitosan. Figure 3 shows the FTIR spectra of the modified chitosan and chitin, and the structure of the chitosan and the modified chitosan are shown in Figure 4. Unlike those of the modified chitosan, the absorption bands at 2912 and 2855 $cm^{-1}$, which resulted from the stretching vibration of the –CH group, were insignificant for chitin because no deacetylation process was conducted. However, the absorption bands at 3280 $cm^{-1}$ and 985 $cm^{-1}$, which corresponded to the stretching vibrations of –OH and C-O groups, indicated the presence of a sulfate group in chitin.

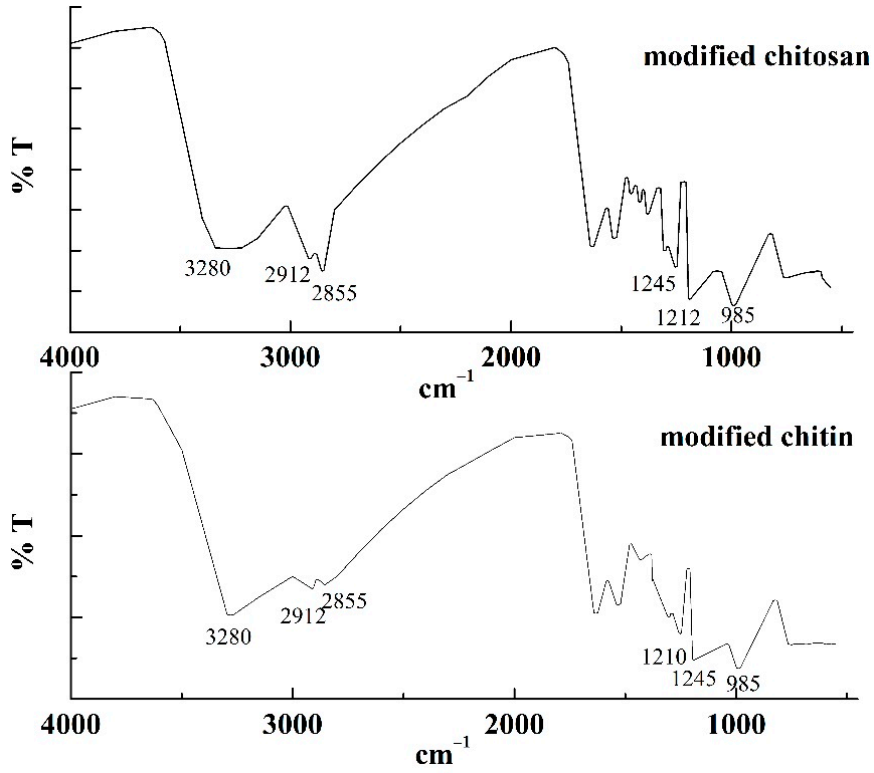



**Figure 3.** FTIR spectra for the modified chitosan and the modified chitin.

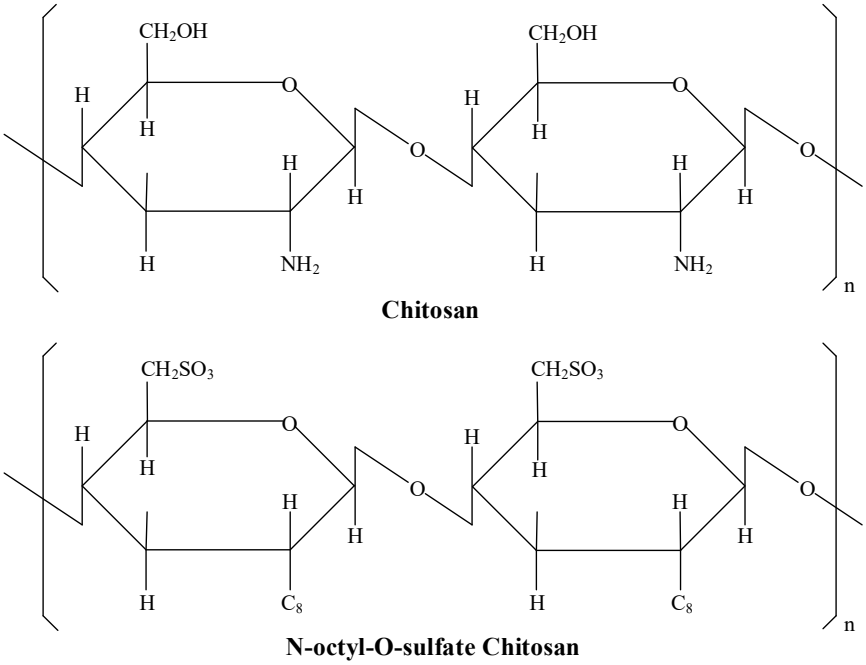



**Figure 4.** Structures of chitosan and the modified chitosan.

### *3.2. Transmittance Test*

The transmittance test was conducted to observe the uniformity through emulsification process in this study. Although the hydrophobic octyl group does not react with chitin, the hydrophilic sulfonate substituting -OH group in the chitin still reacts with water to decrease the transmittance for using the modified chitin as an emulsifier, as shown in Figure 5. Since the remaining water are immiscible with the oil phase, the uniformity is lower than other two emulsifiers, referring to the higher transmittance. Figure 5 also shows that the transmittance of the modified chitosan as an emulsifier was lower than that of sodium acrylates copolymer, and the transmittance decreased with increases in the amount of emulsifier. The results showed that the emulsification effect of the amphoteric chitosan was better, and that a dose of 0.2 g is more suitable. In addition, the same transmittance by a smaller amount of amphoteric chitosan than that of sodium acrylates copolymer can be found in Figure 5.

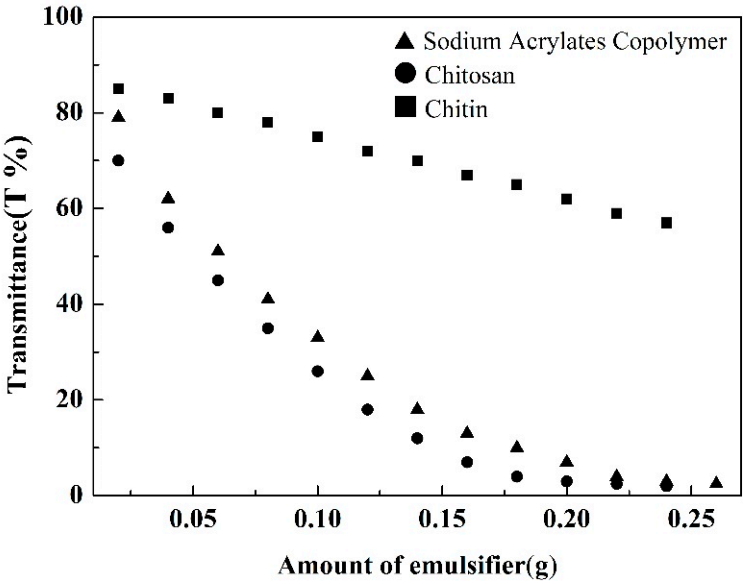



**Figure 5.** Change of transmittance with amount of emulsifier.

Figure 6 shows the change in transmittance over time for a 0.2 g dose of emulsifier. The emulsifiers include sodium acrylates copolymer, the modified chitin, and the modified chitosan. Since the acetyl group in chitin was not removed, only the replacement of -OH group by -$SO_3$ group occurred in the modification; that is, the modified chitin. The deacetylated chitosan was modified as amphoteric N-octyl-O-sulfate chitosan; that is, the modified chitosan. No emulsification behavior occurred when the modified chitin was used as an emulsifier, for it only the sulfonate substituting -OH group in the chitin reacted with water. Therefore, the inhomogeneous phenomenon was observed, and the transmittance was changed by the reaction of sulfonate and water. The transmittance changed from 83 to 59% when the modified chitin was used. Only the hydrophilic sulfonate reacts with water, meaning that the modified chitin cannot be an emulsifier, and leads to the higher transmittance. A lower transmittance indicates a faster emulsification effect. Figure 6 confirms that the emulsification effect was faster when the modified chitosan emulsifier was used, and the suitable time for the emulsification process was about 20 min.

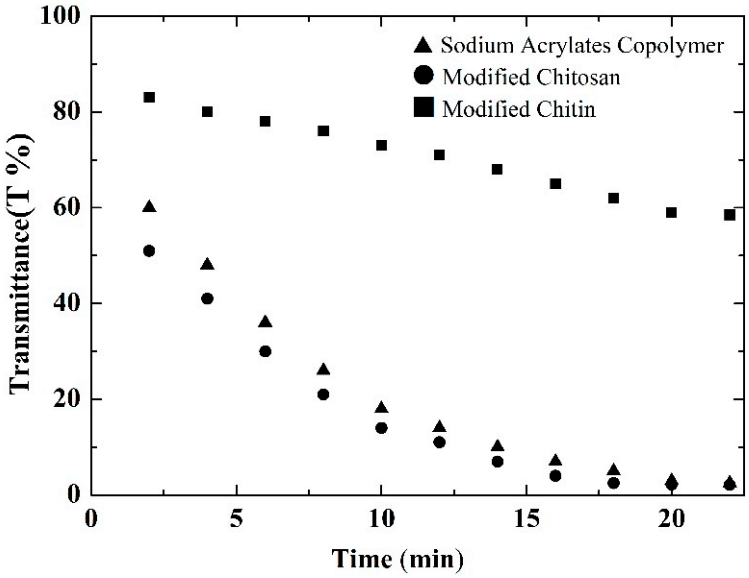



**Figure 6.** Change of transmittance with time.

*3.3. Bacteria and Moisturizing Test*

The spread plate method was used to examine the number of bacteria. Neither antibacterial agents nor antioxidants were added in the production processes of the emulsion products, and the bacteria were cultured under suitable growth conditions. Figure 7 shows that, when the sodium acrylates copolymer emulsifier was used, the number of bacteria within two days was lower than the standard of microbial tolerance in cosmetics, 1000 CFU/mL. Due to the better antibacterial properties of chitosan, when the amphoteric chitosan emulsifier was used, the number of bacteria was much lower than that resulting from the sodium acrylates copolymer emulsifier, as shown in Figure 7.

The moisture retention of the emulsion was measured with the system shown in Figure 1. The spatial humidity was detected continuously after the emulsion was placed in the system. Figure 8 shows the changes in humidity over time for different emulsifiers. A lower spatial humidity indicates greater moisture retention. Since chitosan contains more hydrophilic functional groups, such as -OH and -NH, more moisture was retained in the emulsion made with chitosan. Figure 8 confirms that the moisture equilibrium time between the emulsion and the gas phase was about 40 min, and the moisturizing effect of the modified chitosan emulsion was better than that of the sodium acrylates copolymer emulsion.

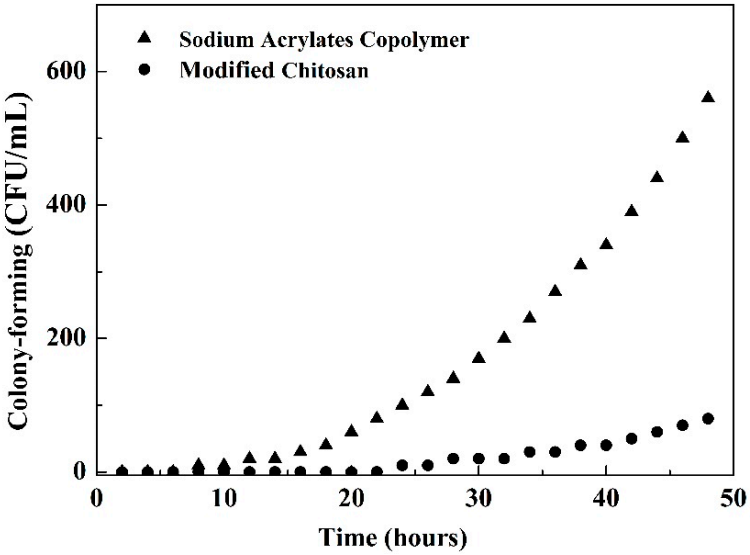



**Figure 7.** Comparison of numbers of bacteria for different emulsifiers.

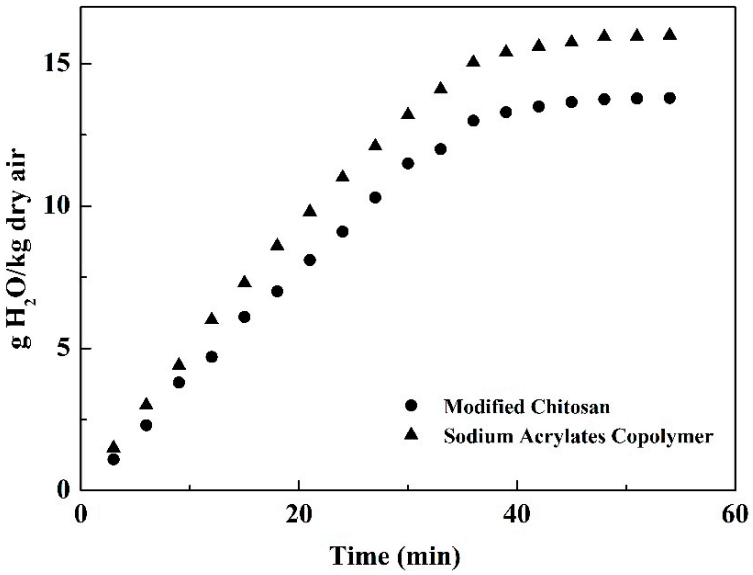



**Figure 8.** Change of spatial humidity with time for different emulsifiers.

### *3.4. Stability Test*

To test the emulsion stability, two methods were used in this study. First, 20 mL of the prepared emulsion was diluted with 400 mL of deionized water, and then the mixture was stirred evenly in a magnetic stirrer for 30 min. The mixture was poured into a tube with a height of 30 cm and a diameter of 2 cm. The height of the oil phase generated at the top of the emulsion was recorded over time, and the ratio of that height to the total height of the tube was calculated. A smaller ratio indicated better emulsion stability. The changes in the ratios of the modified chitosan and sodium acrylates copolymer emulsions are shown in Figure 9. The results confirmed that no separation of the oil and water phases occurred within one hour in either emulsion, and the stability of the modified chitosan emulsion was better. In addition, the storage stability was simulated by the centrifugal accelerated sedimentation test. The centrifugal sedimentation was controlled at 3000 rpm/min for 15 min in a centrifuge. The results showed no sedimentation in either emulsion. Generally speaking, no sedimentation occurring during the tests indicates that the storage stability could persist for at least 6 months.

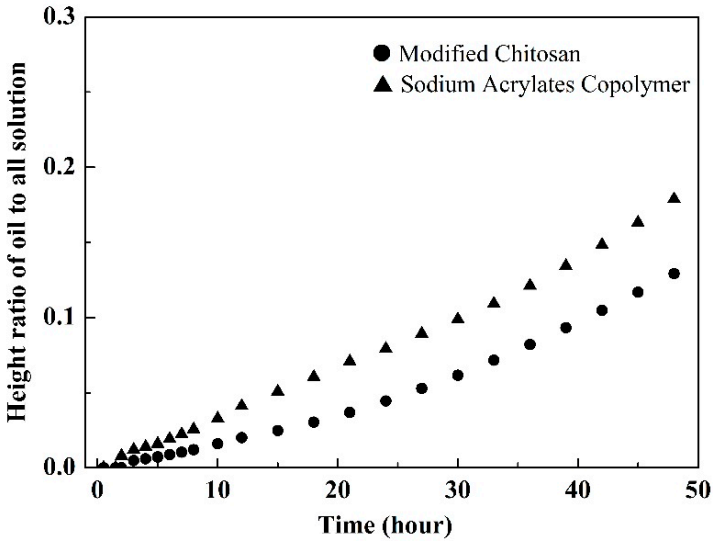



**Figure 9.** Emulsion stability test for different emulsifiers.

## 4. Conclusions

Deacetylated chitosan was prepared by NaOH solution, and the chitosan was modified with chlorosulfonic acid and octaldehyde to produce the amphoteric chitosan. The sodium acrylates copolymer was successfully replaced by the amphoteric chitosan as an emulsifier in the processes of emulsion production, and the moisture retention, emulsification condition, and bacterial growth were examined through spatial moisture measurement, photometric spectrometry, and the spread plate method. A slightly smaller dose of amphoteric chitosan than of sodium acrylates copolymer yielded the same transmittance, indicating that the same emulsification effect could be achieved with a smaller dose of the amphoteric chitosan. Since the moisture retention of the amphoteric chitosan emulsion was better, the spatial humidity of the modified chitosan emulsion was lower than that of the sodium acrylates copolymer emulsion. The number of bacteria was lower when the emulsion using amphoteric chitosan was used than when sodium acrylates copolymer was used because of the inherent antibacterial property of chitosan. The stability tests showed no separation of the oil and water phases within one hour for either emulsifier, as determined from observations of the separation of oil and water phases in the diluted emulsion, and the stability of the modified chitosan was better than that of the sodium acrylates copolymer emulsion. Therefore, the advantages, including the smaller dose, the better moisture retention, the lower number of bacteria, and the better stability for using the amphoteric chitosan, make it a successful replacement for sodium acrylates copolymer as an emulsifier in the preparation of emulsion.

**Author Contributions:** Conceptualization: H.-T.W. and C.-C.C.; Experimental runs and analysis: H.-W.C. and C.-C.C.; Writing: H.-W.C. and H.-T.W. All authors have read and agreed to the published version of the manuscript.

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