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pH-Induced Structural Changes of Crystalline Curcumin Enhance Its Encapsulation in Emulsions

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ABSTRACT: The simple and green pH-based method shows promise for encapsulating hydrophobic molecules in delivery systems to enhance their bioavailability. However, there is still a limited understanding of the pH-induced structural changes that are involved. In this study, we combine experimental techniques with molecular dynamics simulations to investigate pH-induced structural changes in curcumin crystals. An alkali-acid pretreatment was introduced to encapsulate curcumin, where curcumin is first dissolved in an alkaline solution and then rapidly acidified to form aggregates. Remarkably, these curcumin aggregates can be spontaneously encapsulated into emulsions, even at high concentrations (1 mg/mL). Microscopy images suggested that this pretreatment disrupts the crystalline structure of curcumin. Molecular dynamics simulations further demonstrated that the hydroxyl groups of curcumin form hydrogen bonds with water molecules, while the hydrophobic interactions dominate within pH-treated curcumin aggregates. The structural changes increase the solvent-accessible surface area and promote the rapid solubilization of curcumin into emulsions or milks.

KEYWORDS: *delivery systems, bottom-up encapsulation, fluorescence imaging, molecular dynamics, hydrogen bonding*

1. INTRODUCTION

Encapsulating and protecting hydrophobic molecules, including nutraceuticals and drugs, presents a formidable challenge in nutraceutical and pharmaceutical fields.[1] These molecules inherently exhibit poor solubility in aqueous environments, which greatly hinders their absorption and distribution within the body upon administration. This issue, known as the solubility-dependent bioavailability problem, significantly complicates drug development processes and compromises the therapeutic efficacy of pharmaceutical compounds.[2] Overcoming this challenge, therefore, requires the development of novel strategies and technologies to enhance the solubility and bioavailability of hydrophobic molecules. A promising solution is to integrate them into nanoparticle-based delivery systems.[3] Nanoparticles offer unique advantages in enhancing the solubility, stability, and bioavailability of hydrophobic compounds. Through precise engineering, nanoparticles can encapsulate hydrophobic molecules within their core or surface, shielding them from degradation and improving their dispersibility in aqueous environments.[4] Additionally, nanoparticles can facilitate controlled release kinetics, allowing for sustained and targeted delivery of encapsulated molecules to specific tissues or cells.[5] Various types of nanoparticles, such as liposomes, polymeric nanoparticles, and lipid-based nanoparticles, have been extensively explored for their potential in food and drug delivery applications.[6,7] These nanoparticle-based delivery systems hold great promise for enabling the effective administration of hydrophobic nutraceuticals and drugs for improved therapeutic outcomes.[8,9]

At present, both top-down and bottom-up technologies are the two primary technical approaches for the production of hydrophobic molecule-loaded nanoparticles.[10] The commonly used top-down techniques include media milling, microfluidization, and high-pressure homogenization.[11] While these methods have demonstrated success on the commercial front, they come with inherent drawbacks. One significant challenge is the considerable amount of heat generated during these operations, which makes the processing of thermolabile materials difficult.[12] The process often necessitates extended processing times to achieve particle sizes below 100 nm. Thus, particle size reduction beyond a certain limit can be challenging when using standard top-down processes.[13] Additionally, using heat to drive hydrophobic molecules into nanoparticles often results in a limited encapsulation efficiency and poses challenges for heat-sensitive nanoparticles.[14] In contrast to top-down approaches, bottom-up methods offer alternative strategies.[15] These methods include solvent-based precipitation, supercritical fluid processing, and emulsion−solvent evaporation.[16] Bottom-up processes provide several advantages over their top-down counterparts. They are characterized by low energy requirements, simplicity in instrumentation, and lower costs, making them particularly suitable for processing thermolabile molecules. However,

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bottom-up methods also have some disadvantages. They often require the use of organic solvents, which can be environmentally unfriendly and toxic, as well as require complete solvent removal from the final product. Furthermore, scaling up may pose challenges due to issues such as solvent removal and processing cost. Despite these drawbacks, bottom-up methods remain promising for applications requiring precise control over particle size and distribution.[15,17]

Recently, a pH-based bottom-up method has been developed for the encapsulation of hydrophobic nutraceuticals into emulsions or milks, due to its simple, fast, and organic-free process.[18−20] It involves a controlled alteration of the pH environment to facilitate an encapsulation process into delivery systems.[21,22] As a consequence, they have successfully applied the encapsulation of hydrophobic molecules into different delivery systems, such as micelles, liposomes, emulsions, protein-based nanoparticles, or nanogels.[23] Recently, this approach has been successfully used to incorporate hydrophobic polyphenols into plant-based milk (e.g., soy, almond, cashew, coconut, or oat milk).[24] First, hydrophobic polyphenols are dissolved or dispersed in an alkaline solution (e.g., pH 12). Then, a rapid addition to the plant-based milk enables the solubilized polyphenols to be solubilized into the hydrophobic phase (e.g., oil droplets).[14] It was noted that the pH-based manipulation induced changes in the charge and conformation for both polyphenol and plant-based milk, so an acidifier (e.g., HCl) is often added to stabilize the pH environment.[25] One most recent study has reported the incorporation of curcumin into different fluid milks (e.g., whole, low-fat, and skim milks), and results showed that this pH-driven encapsulation increased both the *in vitro* bioaccessibility of curcumin and the *in vivo* antioxidant activity of curcumin, compared to a direct addition.[26]

The pH-driven method, therefore, offers a practical strategy for the development of health-promoting milk systems and the enhancement of the bioavailability of phenolic compounds. In milk systems, particularly plant-based or functional dairy products, the encapsulation of hydrophobic phenolic compounds like curcumin via pH-driven techniques improves their solubility, stability, and bioavailability.[18,24] This method allows these compounds to be effectively integrated into the milk matrix without significantly impacting the stability of milk systems, making it ideal for creating nutritionally enriched beverages that offer antioxidant and anti-inflammatory benefits.[19,26] The pH-driven encapsulation can enhance the solubilization of hydrophobic phenolic compounds in nanoparticles, allowing for more efficient absorption in the body.[27] By promoting better solubilization and protecting active ingredients during delivery, this method is valuable for formulating phenolic compounds that require improved stability and controlled release in the gastrointestinal environment.[28]

Despite significant progress in applying the pH-based method for encapsulating hydrophobic nutraceuticals for different applications, the fundamental understanding of how this process alters their structure remains limited. Gaining a deeper understanding of these structural changes is crucial for comprehending the fundamentals of this method and will enhance its practical applications. Additionally, the use of strong alkaline solutions can potentially disrupt the structure of the components designed for delivery systems, so it is desirable to minimize their contacts. In this context, this study proposes an innovative strategy for integrating hydrophobic molecules (e.g., curcumin) into delivery systems (e.g., emulsions) by exclusively introducing an extra pH-based pretreatment. Insoluble curcumin crystals are initially dissolved in an alkaline solution. Subsequently, a rapid acidification process is applied to yield curcumin aggregates for direct addition in emulsions. It is noted that this new pH-based strategy is different from conventional pH-based strategies. This new strategy applies a simple alkali-acid pretreatment to the curcumin crystals and then mixes it with the emulsion-like systems, which completely avoids the contact of the alkaline solution with delivery systems while minimizing processing time to reduce chemical degradation. To understand the potential structural changes, we hypothesize that this pH-based treatment can effectively transform the insoluble crystal structures into new structures that are soluble in emulsion systems. Both experimental and computational approaches will be integrated to validate and understand the underlying mechanisms to promote the applications of this pH-based strategy in nutraceutical and pharmaceutical areas.

2. MATERIALS AND METHODS

2.1. Materials. The curcumin (C2302, purity >97.0%) was purchased from TCI America. The corn oil was purchased from a local grocery store (Mazola, ACH Food Company, Memphis, TN). The chemicals purchased from Sigma-Aldrich (St. Louis, MO, USA) include Tween 80 (CAS number: 9005−65−6), casein (CAS number: 9000−71−9, technical grade), Nile Red (CAS number: 7385−67−3, purity >97.0%, analytical grade), and fluorescein isothiocyanate (FITC, CAS number: 27072−45−3, purity >97.5%, analytical grade). The chemicals purchased from Fisher Scientific (Hampton, NH, USA) include 1.0 N hydrochloric acid solution (HCl, CAS number: 7647−01−0, certified ACS reagent grade) and sodium hydroxide powder (NaOH, purity >98.0%, certified ACS reagent grade). Distilled water was used throughout the study.

2.2. Preparation of pH-Treated Curcumin Solutions. A curcumin/NaOH stock solution (10 mg/mL) was first prepared by fully dissolving 100 mg of curcumin crystals into 10 mL of a NaOH solution (pH = 13). The choice of NaOH solution (pH 13) is sufficiently used to fully deprotonate the curcumin molecules, and its chemical degradation is minimized.[21] To obtain the curcumin/NaCl solutions with a target concentration of curcumin (1.0, 0.1, and 0.01 mg/mL), we first diluted the stock solution with water and then added 1 mL of HCl solution (1.0 N) to acidify the diluted curcumin/NaOH solution. Taking the preparation of a 1.0 mg/mL curcumin/NaCl solution as an example, 1 mL of curcumin/NaOH stock solution was mixed well with 8 mL of water to obtain a diluted solution (9 mL). Sequentially, 1 mL of HCl solution (1.0 N) was rapidly injected into the remaining 9 mL of diluted solution without any agitation. The resulting curcumin/NaCl solution is termed the pH-treated curcumin solution in the following description.

2.3. Preparation of Both Coarse and Nanosized Emulsion Systems. Initially, 2.00 g of emulsifier (Tween 80 or casein) was put in a glass beaker (250 mL), and then, distilled water was added until the weight of the total sample was 90.00 g. After the emulsifier was fully dissolved, 10.00 g of corn oil was added to obtain an initial sample with 10 wt % oil and 2 wt % emulsifier. To fabricate coarse emulsions, a general laboratory homogenizer (Omni International, Kennesaw, GA) was used to mix the sample for 5 min to obtain coarse

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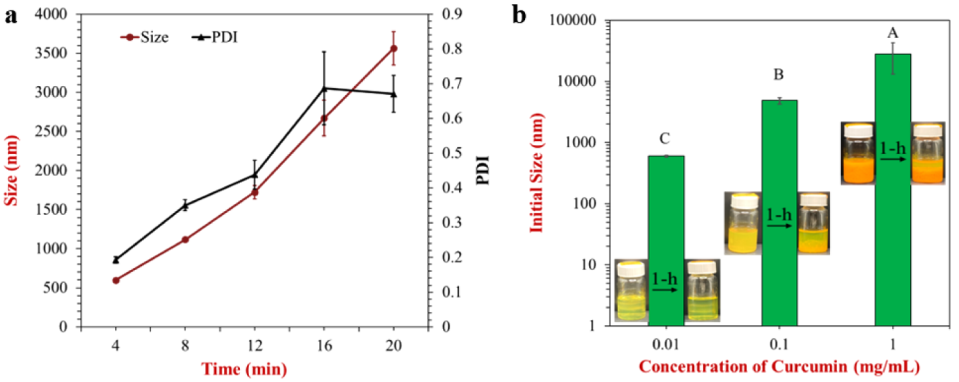

Figure 1. Formulation of curcumin aggregates using a pH-based treatment. a. Z-average size of curcumin particles and their respective polydispersity index (PDI) values over different processing times after acidification. b. The initial (at 4 min) Z-average size of curcumin particles under different concentrations. The inserted images were taken initially and after 1 h. Their initial PDI values, with the curcumin concentration (0.01, 0.1, 1 mg/mL) increased, are 0.19, 0.69, and 0.65, respectively. The uppercase letters (A, B, C) show statistical differences between samples ($p < 0.05$).

emulsions. The coarse emulsions were passed through a high-pressure homogenizer (NanoGenizer, Genizer LLC, Los Angeles) three times to obtain stable nanoemulsions, where the pressure was set at 12 000 psi.

2.4. Formulation of Curcumin-Encapsulated Emulsion Systems. To encapsulate the curcumin into emulsion systems, 1.0 mL of pH-treated curcumin solution was added to 9.0 mL of Tween 80- or casein-stabilized emulsion solutions, and then, fast agitation was applied to mix them well. These two types of emulsions represent surfactant-stabilized and protein-stabilized emulsions, respectively. For comparison, the curcumin crystal was also directly added to 10 mL of emulsion solutions to obtain a reference solution with the same concentration of curcumin.

2.5. Concentration of Curcumin. The UV−visible spectrophotometer (Genesys 150, Thermo Scientific, USA) was used to measure the absorbance values of samples. Curcumin (>97% purity) was used to construct a standard curve, $y = 6.0471x - 0.1043$ ($R^2 = 0.9998$), where x is the absorbance at 425 nm and y is the concentration of curcumin (ppm). The ethanol solvent acidified by adding 1.0 wt % acetic acid was used to dissolve all samples.

2.6. Characterization of Particle Size. Particle sizes of all systems were measured by dynamic light scattering (DLS) using a Zetasizer Pro instrument (Malvern Panalytical, Malvern, UK). To characterize the growth of the curcumin molecules, each sample was directly measured within the given time slots. Additionally, a digital camera was used to take pictures of the samples.

2.7. Microstructures. A Nikon Ti2E widefield fluorescence microscope (Nikon, Tokyo, Japan) was used to observe the microstructures. To obtain bright-field microscopy images of both crystalline and pH-treated curcumin, samples were diluted to 0.1 mg/mL and visualized using the Nikon Ti2 inverted microscope. For fluorescence imaging of curcumin-encapsulated coarse emulsions, we first stained the lipids and proteins of casein sodium-stabilized emulsions using 1 mg/mL Nile Red and FITC, respectively. The proteins were visualized using an excitation wavelength of 550 nm, while the oils were visualized at an excitation wavelength of 621 nm. The curcumin molecules were visualized by using an excitation wavelength of 475 nm. We then mixed 10 μL of each dye with 200 μL of the emulsions and placed 10 μL of this mixture on a glass slide. All observations were conducted using a 40× objective lens. Images were captured and analyzed with the same microscope by using NIS-Elements software from Nikon.

2.8. Molecular Dynamics (MD) Simulations. To create an initial simulation system, a periodic cubic simulation box (5 × 5 × 5 nm) was first created, and then, 16 curcumin molecules were randomly put in the simulation box to simulate the formation of a curcumin aggregate. Next, water molecules were added to solubilize the solute. The water molecules were modeled by a TIP3P model, and the CGenFF was used to generate the force field of the curcumin molecule, because its reliability for the curcumin molecule has been validated by a previous study.[29] We used the particle mesh Ewald method to calculate electrostatic interactions in a periodic box, and the cutoff was set to 12 Å for the calculations of nonbonded interactions. Initially, the entire solute−solvent system was energy-minimized and subsequently slowly heated to 300 K for a 200 ps equilibration period, followed by a 200 ps equilibration under a constant pressure of 1 atm by a Parrinello−Rahman coupling scheme. A sufficient production time (100 ns) was applied to generate the isothermal−isobaric ensembles of the curcumin cluster in water. The hydrogen-involved bonds were constrained in this simulation by using a Linear Constraint Solver (LINCS) algorithm, to allow the 2 fs time step.[30] All energy minimizations and MD simulations were carried out using the GROMACS software.[31] To quantify the interactions between curcumin molecules in a cluster, we calculated the minimum O−O distances between each oxygen atom of curcumin molecules and the oxygen atoms of other curcumin or water molecules. All analysis results were calculated by the use of the MDTraj package[32] and in-house Python scripts, in which the second-half trajectories of all production simulations were used. The visualization of simulation systems was performed using VMD software.[33]

2.9. Statistical Analysis. Three independent trials were employed to characterize the properties of each sample. The standard error and average values were calculated from all measurements (Excel, Microsoft). An ANOVA with a posthoc Tukey HSD test online calculator was employed to ascertain significant differences between samples (https://astatsa.com).

3. RESULTS AND DISCUSSION

3.1. Growth of pH-Treated Curcumin into Aggregates. The use of an alkaline solution, such as NaOH (pH =

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13), proves to be an effective strategy to fully dissolve curcumin crystals into ionized forms. Subsequently, an acidification process can be employed to obtain curcumin molecules. It is anticipated that the negatively charged curcumin will undergo protonation, initiate nucleation and growth, and potentially form aggregates due to its low water solubility. Without agitation, a 1.0 N HCl solution was directly added to the curcumin/NaOH solution, and the mean particle size of the acidified curcumin solution was measured over time (Figure 1a). Initially, at 4 min, the particle size is approximately 600 nm at a curcumin concentration of 0.01 mg/mL. As time progressed, there was a significant increase in the size of the curcumin particles, which indicated rapid growth and aggregation. Although the initial polydispersity index (PDI) value was relatively low (~0.19), which indicated a uniform particle distribution, it increased significantly over time.

We further examined the influence of the curcumin concentration on the initial size of curcumin particles. We found a notable increase in the initial size of the curcumin particles as the curcumin concentration increased. Moreover, as the curcumin concentration increased, there was a clear change in the color of the initial curcumin solution from green to orange. This color transition is a direct result of the increasing concentration of curcumin and reflects the changes in their different optical properties and molecular structures. After allowing the solution to stand for 1 h, we observed clear particle aggregates when the curcumin concentration was either 0.1 or 1 mg/mL (Figure 1b). This observation suggests that a higher concentration promotes curcumin aggregation over time. Overall, it shows that curcumin aggregates can be formed with the increase of initial concentration and time because the hydrophobic curcumin molecules favor to form the aggregates due to the strong hydrophobicity.

3.2. Microstructure of Crystalline and pH-Treated Curcumin. To visualize both curcumin crystals and pH-treated curcumin particles, their bright-field microscopy images are presented in Figure 2. The brick-like characteristic of

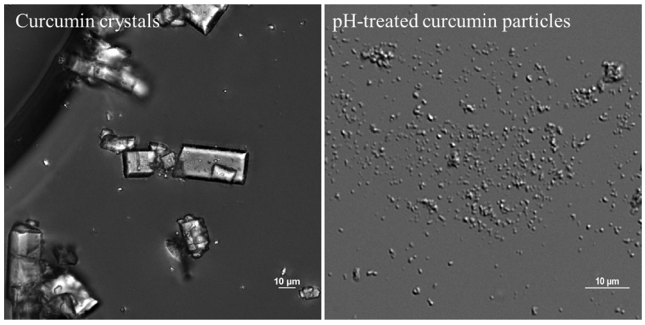

Figure 2. Bright-field microscopy images of curcumin crystals and pH-treated curcumin particles. The scale bars are 10 μm.

curcumin crystals is clearly observed. Previous studies have indicated that curcumin molecules predominantly exist in the β-keto−enol tautomeric form within crystal structures.[34] The hydroxyl groups of curcumin molecules can potentially form both intra- and intermolecular hydrogen bonds within commercially available curcumin crystals. Specifically, phenol O−H···O motifs on both sides can form intra- or intermolecular hydrogen bonds, while also interconnecting with other curcumin molecules via bifurcated C−H···O motifs.[34] Similar binding features were also observed in the keto−enol fragment of the curcumin molecule.[34] The formation of intramolecular hydrogen bonds and aromatic ring structures contributes to the hydrophobic nature of curcumin molecules, which promotes compact molecular packing and thus results in low water solubility.

The inter- and intramolecular hydrogen bonding networks can be readily disrupted in alkaline solutions due to deprotonation. When ionized curcumin molecules are protonated back to neutral molecules, they gain considerable conformational flexibility, which allows the formation of diverse hydrogen bonds in aqueous solutions. Consequently, curcumin molecules are unable to readily pack tightly into the crystal structures. Instead, we observed the formation of small curcumin particles after this pH-based treatment (Figure 2). This observation aligns with the understanding that hydrophobic molecules in aqueous solutions tend to form smaller particles due to enhanced entropy effects, rather than larger crystals, after following bottom-up treatments.[35] However, as curcumin concentration increases, they may grow into larger particles and eventually aggregate because a higher concentration accelerates the collisions of individual small particles. It is worth noting that the addition of stabilizers can be employed to mitigate the growth of larger particles and potential aggregation.[36] Further research is needed to explore the impact of adding stabilizers on the growth of curcumin particles following a pH-based treatment.

3.3. Solubilizing pH-Treated Curcumin into Nanoemulsions. Nanoemulsions serve as versatile vehicles for encapsulating, delivering, and protecting hydrophobic molecules across various applications.[37,38] With their high surface-area-to-volume ratio, nanoemulsions can efficiently encapsulate hydrophobic compounds within their oil phase. This will enhance the stability and solubility in aqueous environments and protect from degradation factors such as light and oxygen. For example, in pharmaceutical applications, nanoemulsions show promise as delivery systems by offering improved solubility, cellular uptake, and targeted delivery for better therapeutic outcomes.[39] In comparison to conventional encapsulation strategies, we propose a simple mixing method to solubilize pH-treated curcumin aggregates into nanoemulsions. Two different nanoemulsions were therefore selected as examples to validate: surfactant-based (Tween 80) and protein-based (casein sodium) (Figure 3). Initially, both nanoemulsions exhibited mean particle sizes of 200−300 nm (216 nm for Tween 80-based and 244 nm for casein-based) and had a milk-like appearance. Upon addition of pH-treated curcumin (1 mg/mL) to both nanoemulsions, the nanoemulsions turned yellow, and surprisingly, the curcumin aggregates were fully dissolved in the nanoemulsions and did not significantly affect their mean particle sizes (Figure 3a). In contrast, when curcumin crystals were added to both nanoemulsions, they made the colors of both nanoemulsions unchanged because they were sedimented at the bottom of the solution (Figure 3b). This suggests that the packing structures of curcumin molecules under both crystalline and pH-treated conditions are different. This suggests that weaker interactions are present in pH-treated curcumin aggregates compared to crystals. This successful solubilization therefore provides an effective strategy for solubilizing curcumin into delivery systems.

Encapsulating curcumin molecules into nanoemulsions or milk systems has been demonstrated to significantly enhance both the bioavailability and stability of curcumin.[19] In a

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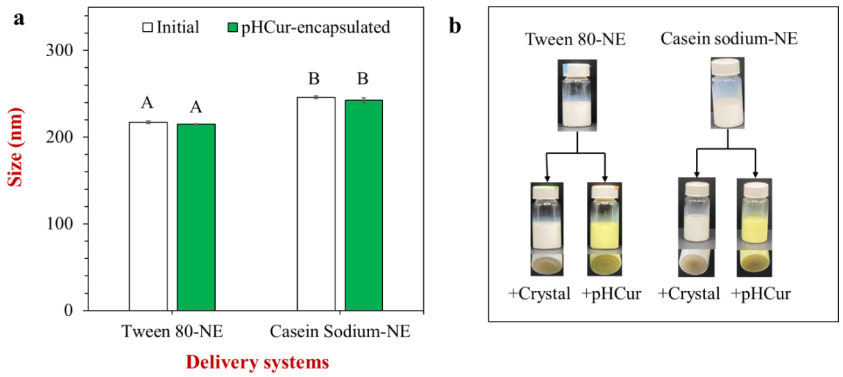

Figure 3. Encapsulation of curcumin into nanoemulsions. a. Mean particle size of nanoemulsion delivery systems: initial versus pH-treated curcumin (pHCur) encapsulated. b. The appearance of curcumin-added nanoemulsions after a direct addition of curcumin crystals versus pHCur samples (after 1 h). Both "Tween 80-NE" and "casein sodium-NE" mean the Tween 80-stabilized and casein sodium-stabilized nanoemulsions, respectively. The PDI values for both nanoemulsions, both with and without pHCur, were as follows: 0.17 and 0.15 for Tween 80-NE and 0.21 and 0.23 for casein sodium-NE, respectively. The uppercase letters (A, B) show statistical differences between samples ($p < 0.05$).

previous study, curcumin was encapsulated into three delivery systems: curcumin nanocrystals, curcumin-loaded nanoemulsions, and curcumin-loaded soy oil bodies.[40] The curcumin nanocrystals formed a cloudy suspension prone to sedimentation and resulted in low bioaccessibility during digestion, but the nanoemulsions and soy oil bodies formed stable dispersions and exhibited high bioaccessibility (>70%).[40] This was attributed to the ability of nanoemulsions and oil bodies to form mixed micelles that enhance curcumin solubilization in the gastrointestinal tract. Furthermore, curcumin-enriched milk systems produced via a conventional pH-driven method showed excellent storage stability at 4 °C, where the curcumin degradation decreased with decreasing storage temperature: 55 °C (43%) > 37 °C (21%) > 20 °C (10%) > 4 °C (5%).[18] These findings highlight the effectiveness of nanoemulsions and milk systems in increasing curcumin stability and bioavailability, making them promising platforms for functional food and beverage development.

3.4. Alkali-Acid Pretreatment and Its Impact on the Encapsulation of Curcumin. Hydrophobic molecules, such as curcumin, often exist in crystallized forms due to the presence of strong intra- and intermolecular hydrogen bonds, coupled with hydrophobic interactions.[34] These crystalline structures pose a challenge for conventional processing methods aimed at increasing water solubility. Traditional methods, such as direct dissolution in water, struggle to break down these crystals into their molecular forms, while organic solvent-based dissolution often introduces undesirable solvent-removal steps.[41] Therefore, alternative strategies are necessary to overcome the inherent insolubility of hydrophobic molecules such as curcumin, particularly in aqueous environments such as emulsion systems. The proposed alkali-acid pretreatment can enhance the solubilization of hydrophobic molecules, such as curcumin, into emulsion systems (Figure 4a). This approach relies on the pH-dependent solubility of these molecules, particularly polyphenols such as curcumin, which contain phenolic hydroxyl groups. The alkaline environment facilitates the conversion of insoluble crystalline forms into soluble ionized forms, thereby enhancing the water solubility. Afterward, acidification can be applied to enable the regeneration of molecular forms for efficient encapsulation within emulsions.[21] This alkali-acid pretreatment is also advantageous for its simplicity, scalability, and compatibility with organic-solvent-free processes, which becomes an

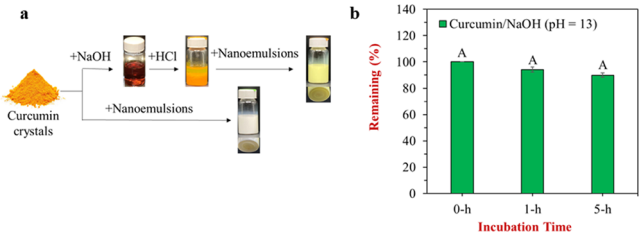

Figure 4. Formulation of curcumin aggregates using a pH-based treatment. a. The pH-based versus direct approaches for incorporating hydrophobic curcumin crystals into emulsion systems (such as nanoemulsions), where both NaOH (0.1 N) and HCl (1.0 N) solutions were used, and Tween 80-stabilized nanoemulsions were employed to demonstrate the difference. b. Effect of incubation time on the stability of curcumin molecules in an alkaline NaOH environment (pH = 13), where a 60 mL solution was added to a sealed beaker (100 mL).

attractive option for encapsulating hydrophobic molecules into emulsions for various applications. It is noted that the processing time can be minimized to simultaneously achieve dissolution and encapsulation, where curcumin crystals are dissolved, acidified, and then rapidly encapsulated into emulsions.

However, numerous factors can influence the encapsulation performance of this pH-based process. One key factor is the selection of the pH solution used for dissolving the hydrophobic molecules. For example, many polyphenols are highly solubilized in an alkaline solution due to the presence of negatively charged phenolic or carboxylate groups, but several hydrophobic molecules are also solubilized in acidic environments, like active ingredient.[42] However, a high pH selection can significantly affect stability, in particular, of polyphenols. For example, quercetin exhibits a very high pH sensitivity.[21] Balancing dissolution and degradation therefore requires further investigation to determine the optimal pH value. Other factors such as oxygen, light, and temperature also influence polyphenol stability under alkaline conditions, where oxygen and light exposure could induce oxidation, while higher temperatures could accelerate chemical degradations.[43] In contrast, the curcumin molecule exhibits improved stability in highly alkaline environments, possibly attributed to the protection provided by the $-O-CH_3$ groups to the phenolic OH group, which is pretty different from other phenolic

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compounds, such as quercetin and resveratrol.[21] This suggests that a higher pH value can likely enhance the encapsulation efficiency of curcumin in emulsions. This was also observed in our study, which showed that ~5% or ~10% of curcumin was degraded in the alkaline solution (pH 13) after 1 or 5 h, respectively (Figure 4b). It suggests that the chemical degradation of curcumin can be neglectable during the alkali-acid process because its operation process can be completed within a few minutes.

3.5. pH-Treated Curcumin Is Hydrophobically Driven into the Lipid Phase of Coarse Emulsions. To investigate the significant difference between crystalline and pH-treated curcumin, we further visualized the samples after solubilizing pH-treated curcumin into casein-stabilized coarse emulsions. As expected, a similar phenomenon appeared in coarse emulsions, with pH-treated curcumin aggregates rapidly dissolved in the coarse emulsions. To visualize the distribution of oil, casein proteins, and curcumin molecules within emulsions, we employed Nile Red and FITC dyes to stain the oil and protein phases, respectively, followed by widefield fluorescence microscopy (Figure 5). Results revealed that pH-

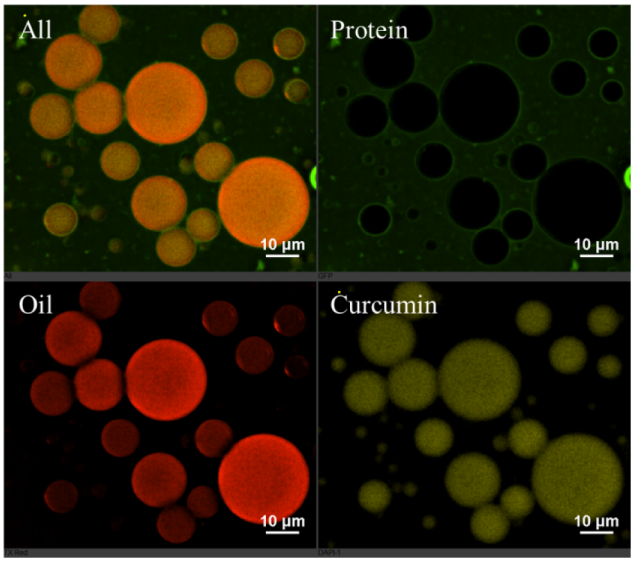

Figure 5. Fluorescence images of curcumin-encapsulated coarse emulsions by a widefield fluorescence microscopy. The corn oil and casein protein phases of coarse emulsions were stained by Nile Red and FITC, respectively. The green, red, and yellow phases show the distributions of protein, oil, and curcumin, respectively. The scale bars are 10 μm.

treated curcumin molecules were predominantly driven into the lipid phase of coarse emulsions due to their hydrophobic nature. This observation suggests that the weaker interactions between curcumin molecules facilitate their hydrophobic incorporation into the lipid phase of the emulsions. In addition, we visualized the locations of the oil phase and curcumin by using different excitation wavelengths, which suggests that the yellow color is contributed by the curcumin, while the red color is contributed by the oil phase. We therefore clearly observed that the curcumin molecules are distributed uniformly inside the lipid phase based on the distribution of yellow color, which indicates that the curcumin molecules can be distributed inside the lipid phase. It was mentioned that the stability of curcumin molecules can be maintained for several months when encapsulated within the lipid phase of nanoemulsions and stored in a refrigerator.[44] This finding thus underscores the feasibility of our pH-based strategy for developing curcumin-encapsulated emulsion systems.

3.6. Mechanisms on the Encapsulation of pH-Treated Curcumin. A driving mechanism is proposed to explain why this alkali-acid pretreatment causes such a significant difference in the solubilization of curcumin in emulsions (Figure 6). First,

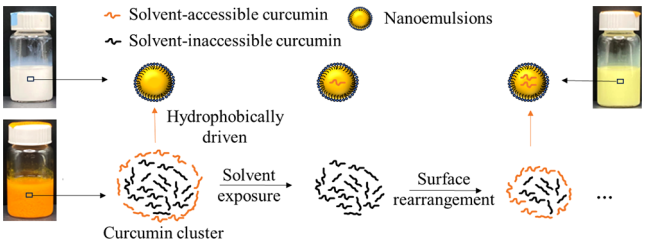

Figure 6. Proposed mechanism for solubilizing pH-treated curcumin aggregates in emulsions. Tween 80-based nanoemulsion and pH-treated curcumin aggregates (1 mg/mL) were selected as examples. Once the curcumin aggregates are added to the emulsions, the solvent-accessible curcumin molecules can be hydrophobically driven into the lipid phase of the emulsions. Afterward, more curcumin molecules are exposed to solvents and thus undergo structural rearrangement, which accelerates the encapsulation of curcumin in emulsions.

this pretreatment leads curcumin molecules to form smaller aggregates compared to their crystalline structures, which have more accessible surface area to accelerate the incorporation of curcumin molecules into emulsions. Additionally, it alters the interfacial chemistry of curcumin aggregates, which allows more hydroxyl groups of curcumin molecules on the surface to be exposed to the solvent and thus reduces hydrogen bonding interactions with the inner curcumin molecules. The synergistic effect significantly facilitates the separation of curcumin molecules from the surface of the aggregates, and then, they are hydrophobically driven into the lipid phase of emulsions. Sequentially, the inner curcumin molecules are exposed to water, followed by a structural rearrangement on the surface that weakens the interactions between curcumin molecules. This will accelerate the size reduction of the curcumin aggregates.

MD simulations were carried out to provide a deep understanding of the interactions of curcumin molecules with other curcumin or water molecules. Figure 7 shows the snapshots of the curcumin cluster before and after a 100 ns MD simulation. We observed that the initially dispersed curcumin molecules rapidly formed a cluster (Figure 7a). Many curcumin molecules had packed against each other and restricted the water penetration between them due to their strong hydrophobicity. Nonetheless, water molecules can be seen approaching the hydrophilic parts of the curcumin molecules on the surface. This means more −OH groups of curcumin are exposed to the solvent, which is consistent with the observations of previous studies.[45] The structure of the curcumin cluster appears much less ordered, where a much less amount of hydrogen bonds were observed. This is different from the crystalline structure of curcumin, in which the hydrogen bonding dominates the interactions by forming both O−H···O and C−H···O structural motifs.[34]

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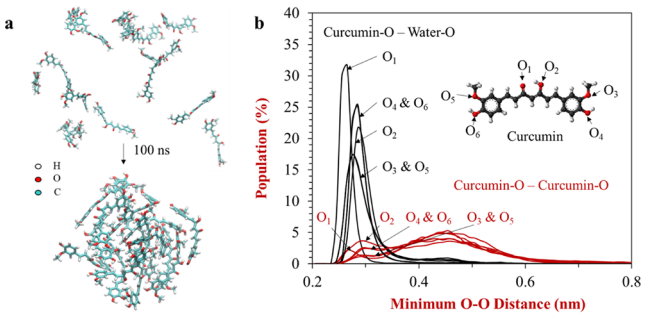

Figure 7. Interfacial behavior of the curcumin cluster in water. a. Snapshots of the curcumin cluster before and after a 100 ns MD simulation. Water molecules are omitted to highlight the structure of the curcumin cluster. b. Population of minimum O−O distances between the oxygen atom of curcumin molecules and another oxygen atom from other curcumin or water molecules.

To further quantify potential interactions, we calculated the population of minimum O−O distances between each oxygen atom of curcumin molecules and the oxygen atoms of other curcumin or water molecules (Figure 7b). We clearly observed that the oxygen atoms of curcumin molecules formed stronger hydrogen bonds with water than with other curcumin molecules due to the presence of dominant peaks near 0.3 nm, in particular for the O_1 atom. It suggests that the −OH groups of curcumin molecules on the surface prefer to form hydrogen bonds with water, rather than with the inner curcumin molecules, which validates our proposed mechanism (Figure 6). We also observed that there are some differences in different types of oxygen atoms. For example, the atom of O_1 has the shortest length of hydrogen bonding. Both O_4 and O_6 (or both O_3 and O_5) also have similar distributions due to their structural similarity. On the other hand, the minimum distances between oxygen atoms of curcumin molecules are distributed in either a hydrogen bonding (0.25−0.35 nm) or hydrophobic (0.35−0.60 nm) range. A larger portion of the hydrophobic range showed that curcumin molecules were mainly packed hydrophobically, and curcumin molecules formed a much less amount of hydrogen bonds. This indicates that it is difficult for the pH-treated curcumin molecules to form a strong hydrogen bonding network in the aqueous solution. Therefore, our simulation results support that this alkali-acid pretreatment significantly alters the structure and interactions of the crystalline curcumin.

This study observed that a simple and green pH-based treatment transformed the structure of crystalline curcumin into smaller particle-like aggregates. These pH-induced structural changes can significantly accelerate the solubilization of curcumin in emulsions. By dissolving curcumin crystals in an alkaline solution, followed by rapid acidification, we successfully formed smaller curcumin particles that eventually grew in size, with initial particle formation closely linked to the curcumin concentration. Notably, even at higher concentrations (1 mg/mL), curcumin aggregates were effectively integrated into the emulsions. Our findings, supported by bright-field and fluorescence microscopy, indicated a transition from crystallized to smaller particle-like structures, with the hydrophobic curcumin molecules migrating into the lipid phase of emulsions. This structural transformation was also confirmed by MD simulations, which showed that more hydroxyl groups of curcumins on the cluster surface were exposed to solvent and had reduced hydrogen bonding interactions with the inner curcumin molecules. These structural changes can enhance the solubilization of curcumin molecules into emulsion-like systems. However, several important studies remain to be conducted, including investigations into how factors such as pH, operating time, and oxygen exposure affect the encapsulation efficiency of curcumin molecules. Additionally, comparisons are needed to determine how this pH-based method outperforms other encapsulation techniques (e.g., heat- or solvent-based methods) in terms of environmental impacts such as energy consumption and carbon emissions. It is important to note that phenolic compounds must be exposed to an alkaline solution in the proposed method, which requires careful attention to minimize their potential degradation. A thorough investigation of the factors that may contribute to the chemical degradation is still necessary.

Overall, this pH-based method not only disrupts the strong hydrogen bonding network of curcumin crystals but also enhances their solvent-accessible surface areas, which drives the curcumin molecules into the lipid phase of emulsions. Therefore, this method allows for an effective and straightforward encapsulation of hydrophobic molecules into emulsions. Representative examples include the development of curcumin-loaded emulsions or milks. We also believe that this study not only provides a simple and efficient bottom-up strategy with extensive potential applications in the pharmaceutical and nutraceutical industries but also advances the goal of developing greener and more energy-efficient production encapsulation technologies.

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■ ABBREVIATIONS

HCl, Hydrochloric Acid; NaOH, Sodium Hydroxide; DLS, Dynamic Light Scattering; FITC, Fluorescein Isothiocyanate; PDI, Polydispersity Index; NE, Nanoemulsion; CGenFF, CHARMM General Force Field; LINCS, Linear Constraint Solver; MDTraj, Molecular Dynamics Trajectories; VMD, Visual Molecular Dynamics; ANOVA, Analysis of Variance

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