**Nanoemulsion and Multilayer Nanoemulsion Encapsulated with Chitosan Improving The Stability and Bioaccessibility of Curcumin**

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**Abstract**

Encapsulating lipophilic curcumin improves digestibility and bioavailability. Loading efficiency and droplet size are crucial when encapsulating curcumin into emulsion-based delivery systems. Since the thickness of the emulsifier layer is comparable to that of the radius of the oil droplet, one can deduce that the emulsifier layer is the primary component of the droplet's overall composition. Because the oil droplet radius matches the emulsifier layer thickness. Nanoemulsion (NE) is ideal for encapsulating, protecting, and delivering lipophilic nutraceuticals for food and food-related applications. Conventional emulsion has several-micrometer droplets. Chitosan-coated NE can deliver lipophilic bioactive substances. This is because it will better protect the core material. Phase separation was observed in the NE emulsion sample after it had been stored for three months, in contrast to the CNE emulsion sample. Multilayered emulsion is a multistep procedure that consists of emulsion droplets electrostatically stabilized by layers of alternatively charged emulsifiers and chitosan. The large surface area of nanoemulsions allows the acceleration of the chemical reactions occurring at the oil-water interface such as hydrolysis by lipases. There was a study that investigated the effect of nanosystems' interfacial composition using multilayer chitosan on the stability of curcumin during in vitro digestion, on lipids digestibility, cumin bioaccessibility and antioxidant activity, using a dynamic GI system.

**INTRODUCTION**

The polyphenol known as curcumin has been shown to improve a person's health in a number of important ways. It has already been established that curcumin possesses a wide variety of biological actions, such as anti-inflammatory, anti-cancer, and antioxidant properties (Kocaadam and Şanlier, 2017; Xu *et al.*, 2018). This is one of the many biological actions that have been attributed to curcumin. It was determined that this molecule has the potential to be included in food systems as a functional food due to the significant biological activities that it demonstrates. This conclusion was reached as a result of the fact that this molecule demonstrates these biological activities (Goel, Kunnumakkara and Aggarwal, 2008; Amalraj *et al.*, 2017). The application into functional foods, on the other hand, faces many challenges, including low solubility and a high rate of metabolic breakdown during digestion in the gastro-intestinal tract (Ahmed *et al.*, 2012; Jiang, Liao and Charcosset, 2020).

One of the potential methods for delivering curcumin and improving its stability and release management is encapsulation. Encapsulation is one of the techniques. The delivery of other active ingredients is another potential use for encapsulation (Gómez-Mascaraque *et al.*, 2017; Medina-Torres *et al.*, 2019; Hamad *et al.*, 2020). Emulsions are an excellent choice for encapsulating the lipophilic curcumin, which will both increase the compound's durability as well as its bioavailability. Emulsions can be found in a wide variety of food products. Due to the fact that curcumin can be dispersed into lipid phase, the incorporation of curcumin into a lipid-based capsule will not only improve the dispersibility into aqueous phase, but it will also have the ability to control release into the gastro-intestinal tract (Wu *et al.*, 2017; Kharat and McClements, 2019; Hamad *et al.*, 2020; Hamad, Suriyarak and Boromphichaichartkul, 2020). This will be the case regardless of whether or not the dispersibility of curcumin is improved into aqueous phase. Emulsion delivery systems commonly known as nano-emulsions are typically the encapsulated form of emulsion delivery systems that are used for curcumin (Ahmed *et al.*, 2012). Combining an emulsion with a covering made of biopolymer was yet another approach that was taken into consideration for improving curcumin's level of protection. Chitosan is a type of biopolymer that is cationic and has properties that make it friendly and biodegradable. Chitosan also has properties that make it a biodegradable material. It was discovered that using chitosan as an encapsulating agent could not only increase the stability of curcumin but also limit its release as it traveled through the gastrointestinal tract (Li *et al.*, 2016; Kumar *et al.*, 2017; Cuomo *et al.*, 2018; Hamad *et al.*, 2020). This was one of the findings. In this article, we will talk about how chitosan can be used as a coating material in nanoemulsion (Li *et al.*, 2016; McClements and Jafari, 2018) and multilayer nanoemulsion (Silva *et al.*, 2018). The supplementary data, which were gathered from publishing databases such as PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and Science direct (https://www.sciencedirect.com/), are utilized to further discuss the topic.

**EMULSION - BASED DELIVERY SYSTEM FOR ENCAPSULATION OF CURCUMIN**

The presence of a large number of very small droplets that are dispersed throughout aqueous systems is the defining characteristic of an emulsion. Depending on which phase they are found in, they are typically classified as either water-in-oil (W/O) or oil-in-water (O/W) emulsions. The classifications are based on the order of the phases. Emulsions are examples of thermodynamically unstable systems. This is due to the vast interfacial area that exists between the two immiscible phases that compose the emulsion. Because emulsions are thermodynamically unstable, they have the potential to become destabilized over time, which can result in creaming, sedimentation, flocculation, and coalescence of the systems (Araiza-Calahorra, Akhtar and Sarkar, 2018; Zheng *et al.*, 2018). [Creaming] The process in which an emulsion's constituent parts become separated into distinct layers. Emulsion structures such as standard emulsions that were stabilized with surfactants, monolayer and multilayers of biopolymers, nano-emulsions, and microemulsions have all been utilized in the past decade for the purpose of encapsulating curcumin(Araiza-Calahorra, Akhtar and Sarkar, 2018; McClements and Jafari, 2018). This has been accomplished through the use of a wide variety of emulsion structures. A simplified illustration of the multilayer structure and the type of emulsion is shown in **Figure 1.**



**Figure 1**. The schematics of the nanoemulsion (mean radii 0.2 – 100 µm) (a); multilayer emulsion (b) (Araiza-Calahorra, Akhtar and Sarkar, 2018)

In the process of encapsulating curcumin into emulsion-based delivery systems, two factors that are important to consider are the loading efficiency as well as the droplet size. When we talk about the "loading efficiency" of emulsion systems, we are referring to their capacity to entrap particles. It is the percentage of the total amount of curcumin that has been added that corresponds to the mass of curcumin that has been entrapped inside the emulsion (Zheng *et al.*, 2018). Emulsions, the kind of emulsifier used, and the structural arrangements of the emulsifier at the interface all play a significant role in determining loading efficiency (Ma *et al.*, 2017). The addition of curcumin should not result in a change in the average droplet size of the mixture. This is because the presence of curcumin will have an effect on the emulsion's stability. Both the crystal size of the curcumin and the concentration of the emulsifier can have an effect on the degree to which droplet size increases after the inclusion of curcumin (Ma *et al.*, 2017; Hamad *et al.*, 2020).

**THE USE OF CHITOSAN AS AN ENCAPSULATING AGENT**

Biopolymer is utilized quite frequently in the coating and encapsulating system in order to achieve the goal of improving the system's stability (Tan *et al.*, 2020; Zhong *et al.*, 2020). Chitin is capable of being deacetylated, which results in the production of chitosan, a naturally occurring polymer. Consumption of this polysaccharide does not pose any health risks, and it is also biocompatible and biodegradable. Because of their high level of stability, low level of toxicity, and straightforward and gentle technique of synthesis, chitosan nanoparticles have attracted increased interest as potential delivery vehicles for bioactive substances. This is due to the fact that their level of toxicity is low. Under conditions of neutral pH, the deacetylated chitosan backbone of glucosamine units is able to engage in powerful electrostatic interactions with proteins that have an overall negative charge (Tan *et al.*, 2016; Carvalho *et al.*, 2019). This is possible due to the high density of charged amine groups that the backbone possesses. The rapidly expanding body of research into the valuable physicochemical and biological properties of chitosan led to the discovery that chitosan is a cationic polysaccharide(Kumar *et al.*, 2017; Hamad *et al.*, 2020). The chemical structures of chitin, chitosan, and chitin that has been deacetylated are depicted side-by-side in Figure 2, which shows the various ways in which these three types of chitins can differ from one another.

There is also the possibility that interactions between curcumin and polysaccharides will have an impact on loading efficiency. The influence that the presence of numerous layers of chitosan has on the physicochemical properties of nanoemulsions that are loaded with curcumin has been investigated. It was shown that the loading efficiency had increased to 95.1% when using curcumin at a concentration of 0.548 mg/mL, which was the concentration that was used (Mun, Decker and Mcclements, 2006; Anitha *et al.*, 2011; Bourbon *et al.*, 2018). The electrostatic interaction between the positively charged cationic groups located on the poly-glucosamine chains of the molecule and the negatively charged anionic curcumin may have been facilitated by the interactions between the keto groups of curcumin in either the di-keto or the cis–enol form, and the amine groups of chitosan, which is rich in protonated amino groups. This was one of the hypotheses that could be tested to explain the findings. In addition, it was found that the hydrophobic interactions of curcumin with chitosan were more pronounced at physiological pH (7.4) conditions in the presence of nonionic surfactant (Tween 80) than in the presence of cationic surfactants. This was the case regardless of whether or not cationic surfactants were present. It made no difference whether the conditions were carried out in the presence of cationic or nonionic surfactants; this result was always the same. It was hypothesized that the process of binding was mediated in Tween 80 systems by the formation of hydrophobic, electrostatic, and hydrogen bonds between curcumin and chitosan (**Figure 3**), and presented evidence to support this hypothesis (Chanphai, 2017).



**Figure 2.** Molecular structures of chitin, chitosan, and protonated chitosan polymer (Mathias, Tessier-Doyen and Michaud, 2011).

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**Figure 3**. Hydrogen bonding between curcumin and chitosan molecules (Chanphai, 2017)

**NANOEMULSION ENCAPSULATED WITH CHITOSAN**

Nanoemulsions (NE) have a mean radius that ranges anywhere from 50 to 200 nm, depending on the specific NE (Araiza-Calahorra, Akhtar and Sarkar, 2018; McClements and Jafari, 2018). They have a tendency to be clear or somewhat opaque, and because of the very small droplet size, they have much superior stability against aggregation than traditional emulsions do as a result of the fact that they compare. It is possible to draw the conclusion that the emulsifier layer is the primary component of the oil droplet's overall composition due to the fact that the thickness of the emulsifier layer is comparable to that of the radius of the oil droplet (= r). The process of creating nanoemulsions typically falls into one of two categories: high intensity or low intensity. Additionally, the process can be broken down into two stages: the pre-emulsification stage and the emulsification stage. Examples of high-intensity approaches include the utilization of an ultrasonic bath or sonicator, high-pressure valve homogenizers, microfluidizers, and the use of a high-speed blender (Mcclements, 2012; Hou and Xu, 2016).

In order to keep the integrity of the colloidal system intact, NE necessitates the presence of particular emulsifiers at the oil-water interface. This is because NE possesses a thermodynamic instability. When compared to conventional emulsion, which has droplets that are several micrometers in size, nanoemulsion (NE) is a particularly attractive option for encapsulating, protecting, and delivering lipophilic nutraceuticals for food and applications connected to food. This is because conventional emulsion has droplets that are several micrometers in size. NE that has been coated with chitosan has the potential to be beneficial for the delivery of lipophilic bioactive substances(Sowasod *et al.*, 2013; Araiza-Calahorra, Akhtar and Sarkar, 2018). This is because it will better protect the core material from being damaged.

After curcumin was successfully encapsulated into a nanoemulsion by using MCT oils as the dispersed phase, chitosan was used to cover the nanoemulsion that contained curcumin. Chitosan was used to cover the nanoemulsion. The encapsulated substance was put through heat and ultraviolet (UV) treatments, but the results showed that it did not lose its stability in either of those processes. The degree of stability that the Chitosan Nanoemulsion (CNE) possessed was evaluated based on the changes that occurred during storage in both the size of the particles and the zeta potential of the solution. It was demonstrated that after coating, there was a significant increase in the particle size of CNE, which was dependent on the molecular weight of chitosan. This finding was supported by the findings of the aforementioned study. After being coated, the zeta potential of NE would change from having a negative value to having positive charges. Initially, the zeta potential of NE had a negative value. CNE samples were able to improve the water dispersibility of curcumin by a factor of 1400 when compared to curcumin that was not encapsulated. Both NE and CNE samples were stable for at least one month of storage at room temperature. Phase separation was observed in the NE emulsion sample after it had been stored for three months, in contrast to the CNE emulsion sample (Figure 4). The presence of coated chitosan and surfactant will ensure that the curcumin is protected during the process of heat treatment. Phase separation is inevitable as a result of this, and it will take place even while the substance is being stored (Li *et al.*, 2016; Vecchione *et al.*, 2016; Shin and Kim, 2018).



**Figure 4**: Chitosan nanoemulsion after it has been stored for three months: (A) nanoemulsion, with an oil layer indicated by the red arrow, (B) a chitosan coated nanoemulsion with a low molecular weight, (C) a chitosan coated nanoemulsion with an intermediate molecular weight, and (D) a chitosan coated nanoemulsion with a high molecular weight (Li *et al.*, 2016)

It is likely that the barrier effect that chitosan has on curcumin, which can be described in two different ways, is responsible for the various processes working of chitosan in order to improve the stability of curcumin. One of these ways is that chitosan prevents curcumin from interacting with other molecules. To begin, the water solubility of curcumin increases with increasing temperature, which means that it has a greater potential to dissolve in hot water than in cold water. And if it were to exist outside of the emulsion droplets, it would be susceptible to oxidation and hydrolysis in the aqueous solution; both of these processes would result in a rapid reduction in its concentration. If it were to exist outside of the droplets, however, it would not be subject to either of these processes. It's possible that having a chitosan coating present during the thermal treatment will help prevent the curcumin migration phenomenon from occurring. In addition to this, chitosan is able to inhibit the radicals' ability to metabolize curcumin within the NE, which is a significant benefit. Chitosan coating provides NE with a cationic character, which enables it to reject reactive radicals such as protons and metal ions, thereby preventing oxidation of curcumin [15]. Chitosan coating also gives NE a positive charge, which is known as a negative charge (Mun, Decker and Mcclements, 2006; Woranuch, Yoksan and Akashi, 2015; Li *et al.*, 2016; Hamad *et al.*, 2020).

**MULTILAYER NANOEMULSION ENCAPSULATED WITH CHITOSAN**

It is possible for an emulsion to be multilayered if it contains emulsion droplets that are electrostatically stabilized by layers of emulsifiers that have charges that are in the opposite direction from one another. The layers themselves are made up of the polyelectrolyte that was used to coat each new layer as it was added. A graphical representation of the processes involved in the manufacture of multilayer emulsion is provided in Figure 5. In recent years, there has been a growing interest in the utilization of the layer-by-layer (LbL) electrostatic deposition approach to construct such multilayer emulsion structures. This interest has been spurred on by the advancements that have been made in the field of electrostatic deposition. The following is a breakdown of this process into its individual steps and components: This method makes use of electrostatic attraction, which leads to the adsorption of a charged polyelectrolyte onto the surface of an oppositely charged droplet as a result of the technique's application. By alternating the adsorption of polyelectrolytes or charged emulsifiers with opposite charges, it is possible to create many layers, which ultimately results in the construction of a multilayered structure at the interface (Dickinson and Eric, 2009; Silva *et al.*, 2018). This can be accomplished by alternating the adsorption of charged emulsifiers.

Increasing the physicochemical resistance of nanoemulsions to the effects of their surrounding environment can be accomplished in a few different ways. One of these ways is by employing multilayer nanoemulsions as a method. When deciding which lipid nanosystem to use, it is important to keep in mind the application that will ultimately be carried out with the system. It is common knowledge that the high surface area of nanoemulsions makes it possible to quicken the chemical reactions that take place at the oil-water interface. This is because of the nanoemulsions' ability to increase the rate of chemical reactions. The hydrolysis of lipases is a good illustration of this principle in action. Altering the interfacial properties through the deposition of polyelectrolyte layers, on the other hand, may help control the digestibility of lipids by improving the integrity of the coating and preventing lipase and other enzymes from reaching the lipids that are encapsulated (McClements and Li, 2010). This is accomplished by improving the coating's barrier properties. This is feasible as a result of the improvement in the coating's integrity.



**Figure 5**. The following are the stages involved in the production of multilayer emulsions: (i) in primary emulsions, the oil and aqueous phases are homogenized together in the presence of a charged water-soluble emulsifier; (ii) in secondary emulsions, an oppositely charged polyelectrolyte is added to coat the droplets; and (iii) in multilayer emulsions, sequential polyelectrolyte adsorption steps can be carried out(McClements and Li, 2010).

In one study, a dynamic gastrointestinal (GI) system was used to investigate the effect of nanosystems' interfacial composition using multilayer chitosan and alginate on the stability of curcumin during in vitro digestion, as well as on the digestibility of lipids, curcumin bioaccessibility, and antioxidant activity. Chitosan and alginate were used in the multilayer construction of the nanosystems. Nanoemulsions were successful in producing the most bioaccessible form of curcumin, while multilayer nanoemulsions were able to successfully control the amount of lipid that could be digested. This was accomplished by reducing the amount of lipids that were digested and absorbed as well as significantly slowing down the rate at which lipids were digested. The findings also suggest that the inclusion of polyelectrolyte layers was effective in shielding curcumin from changes that occur in the gastrointestinal tract, thereby better maintaining its antioxidant capacity throughout digestion (Mun, Decker and Mcclements, 2006; Silva *et al.*, 2018; Fang *et al.*, 2019). This is suggested by the fact that the inclusion of polyelectrolyte layers was effective in shielding curcumin from changes that occur in the gastrointestinal tract.

**CONCLUSION**

Chitosan has the ability to improve the stability of encapsulated curcumin as well as the qualities associated with its release. Chitosan also has the ability to improve the quality of the curcumin that is released. When using the nanoemulsion system, covering curcumin with chitosan not only protected it from deterioration during the heat treatment phase, but it also protected it from deterioration while it was being stored. This was possible because of the nanoemulsion's ability to form a protective barrier. When compared to nanoemulsion on its own, multilayer nanoemulsion that included chitosan provided superior protection for curcumin. This was achieved despite the fact that bioaccessibility was reduced.

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