Preparation, Assay and Stability Analysis of Curcumin-Polysaccharide Complexes

Yun Qiao*

Hohhot No. 2 Middle School, China

Abstract: Curcumin is a hydrophobic plant polyphenol with various functional properties such as antioxidant, antibacterial, anti-inflammatory, blood lipid regulator, hypotensive and anti-tumour, etc. However, it is sensitive to environmental factors such as light, oxygen and heat, and is prone to oxidative degradation and loss of biological activity during processing, storage and transportation, thus limiting its application. In this paper, chitosan and curcumin were used to form a complex to encapsulate and protect curcumin, and some modern assay techniques were used to examine the structural and functional properties of the complex and analyze its stability. RESULTS: Chitosan and curcumin could form a nanocomplex and the resistance of curcumin to light and heat was greatly enhanced after the formation of the complex. Significance: This study is of great importance to solve the problems of poor water dispersion, unstable physicochemical properties and difficult application of curcumin, and is of academic and practical significance for the future development and utilization of curcumin.

1 INTRODUCTION

Curcumin is a component of the root turmeric, an herb native to Asia, whose roots are used medicinally. Curry is a common and popular Asian delicacy, and its main colour is derived from all the curcumin contained in curry powder. In addition to being used as a spice, curcumin has been recorded by the ancients as being used to repel insects, as an antibacterial agent and to promote wound healing (Kotha, 2019). In addition to the most important active ingredient, curcumin (Yuan, 2012), turmeric also contains water, protein, carbohydrates, fat, mineral volatile oils and curcumin-like constituents. Among the curcuminoids, curcumin makes up the majority, while dideoxymethyl curcumin makes up about a small percentage and deoxylated curcumin accounts for about 3-6% (Prasad, 2014).

Curcumin powder is orange-yellow to the naked eye and is often used as a natural orange or yellow colouring agent in the production of food products. The molecular formula is C_{21} H_{20} O_6 (see Figure 1 for structure) and its main chain contains carbonyl and aromatic groups. In recent years, the interest in health supplements, nutritional products and dietary supplements made from natural plant extracts has led to an increase in research on curcumin (Yuan, 2012). Its anti-inflammatory, anti-diabetic, anti-cancer and anti-ageing potential has made curcumin a hot topic in pharmaceutical research today (Tsai, 2018). However, its sensitivity to light, heat and iron ions as well as its low or even insoluble solubility in water, low bioavailability (i.e. short intestinal half-life) and low pharmacokinetics make it susceptible to deterioration during processing, storage and transport, thus limiting its application, so scientific means are needed to address this issue.

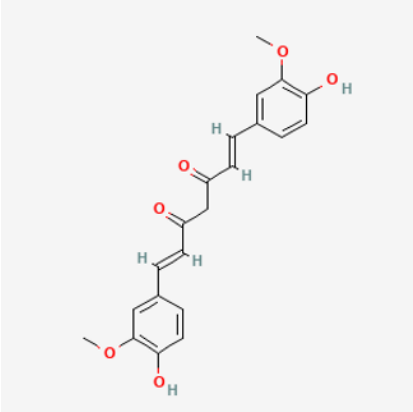

Figure 1. Chemical structure of curcumin

1.1 The Value of Curcumin Applications

1.1.1 Anti-inflammatory

The main anti-inflammatory mechanism of curcumin is the inhibition of inflammatory factors (Bai, 2020). The main inflammatory factor in bronchial asthma is nuclear factor κB, and in Peng et al.'s study, curcumin was found to inhibit the production of NF-κB, thereby regulating the inflammatory signalling pathway and exerting its anti-inflammatory effect (Peng, 2021). Dai et al. showed that curcumin could improve the autoimmune disease Rheumatoid arthritis by inhibiting the mTOR pathway and suppressing the production of TNF-α in serum and

* Corresponding author: rt234456678@163.com

synovial membranes (Dai, 2018). A study by Fan Aiyue et al. showed that the above-mentioned indicators hs-CRP, TNF-α and IL-6 were reduced in the curcumin-treated group by establishing a rabbit model of AS, performing surgery and testing its inflammatory indicators after surgery (Fan, 20200.

1.1.2 Antioxidants

Oxidation reactions lead to the production of large amounts of free radicals in the body. These free radicals are harmless to the body in certain amounts, but if they exceed a certain amount they rob the body of electrons from other substances that are needed by the body, and therefore it may be harmful to the body. In recent years, the antioxidant properties of curcumin have been studied both at home and abroad and it has been found that curcumin exerts antioxidant effects by activating relevant signal transduction molecules in the antioxidant process (Li, 2016).

1.1.3 Anti-tumour

Curcumin is not only a novel antioxidant, but it can also achieve its anti-tumour mechanism through anti-nitrosation, anti-NO and induction of cell cycle arrest and apoptosis. Curcumin can be used alone to combat tumour proliferation and induce apoptosis in tumour cells through multiple molecular targets. It also works in combination with other drugs as a false positive approach to the chemotherapy part of cancer treatment, e.g. the combination of curcumin and 5-fluorouracil plus cisplatin resulted in a reduction in the activity of gastric cancer cells and a significant decrease in their migration rate, along with an increase in the proportion of apoptotic effects of gastric cancer cells and the expression of factors such as caspase family activity associated with apoptosis (He, 2017).

1.1.4 Protection of the liver

Curcumin and turmeric protect against liver damage caused by various toxicants in vitro and in vivo, while curcumin has been shown to be protective against alcoholic liver disease in experiments (Dong, 2017). In experiments by Wu Xiongjian et al. on liver fibrosis in rats, curcumin inhibited ROS, MDA, NF-kB and other factors causing liver fibrosis to varying degrees (Wu, 2015).

1.2 Extraction and Detection

1.2.1 Extraction

Nowadays, with the development of technology, the main extraction methods of curcumin are extraction, column chromatography, etc. Among the commonly used solvents, ethanol is the best organic solvent for the extraction of curcumin. Organic extraction, ultrasonic extraction, supercritical CO2 extraction and microwave extraction are commonly used for industrial extraction (Priyadarsini, 2014). Because supercritical CO2 extraction is green, environmentally friendly, non-toxic and has high extraction rate, it is a new and promising extraction method. In recent years, studies have shown that surfactant-free microemulsion (SFME) extraction using water/hydrotrope/TriA as a solvent is a promising extraction method for aqueous solvent systems commonly used in industry (Degot). Researchers mostly use liquid chromatography (HPLC). A reversed-phase C_{18} column is generally used as stationary phase and the mobile phases are acetonitrile/water or chloroform/methanol solvents with different gradients. For the detection of curcumin, an absorption detector in the wavelength range of 350 ~ 450 nm or a common detection wavelength of 250 ~ 270 nm was selected for the UV region. Three different fractions of the curcumin mixture can be obtained by adsorption of the mixture onto silica gel by column chromatography. The curcumin fractions can be further purified on silica gel using chloroform/dichloromethane and ethanol/methanol mixtures as eluents (Tsedendorj, 2014).

1.2.2 Detection Methods

There are three main methods for determining the presence of curcumin in medicinal plants (1) UV spectrophotometry, (2) thin layer chromatography (TLC), and (3) high performance liquid chromatography (HPLC). Although spectrophotometric methods are widely used for the quantitative analysis of curcumin, this method is susceptible to interference by other impurities and requires a number of preparative steps for impurity removal. TLC is simple and inexpensive to perform. HPLC not only gives the corresponding analytical results, but also separates the various components simultaneously with high accuracy, but requires more time and cost. The fluorometric estimates were found to be closer to those of HPLC only for the bottom optical measurement of GA1000. This suggests that the fluorometric method could potentially be used as an alternative standard for the determination of curcumin in samples and curcumin content in turmeric extracts (Nugraha, 2017).

1.3 Disadvantages and Solutions

The application of curcumin has been plagued by its poor water dispersibility and unstable physicochemical properties. Nowadays, the chemical properties of curcumin are changed to improve its stability, but the molecular structure is altered to produce by-products that can cause food safety problems. In this paper, we choose to make curcumin and polysaccharide to improve its stability, this method does not change the molecular structure and its physiological activity, at the same time, the use of polysaccharide does not cause pollution to the environment, and there is no food safety problem. In this experiment, chitosan was chosen as the polysaccharide to be bound to curcumin, a polysaccharide obtained by the deacetylation of a translucent, robust chitin that is found

in nature. Because the material is naturally sourced, safe and non-toxic, and the complex can be applied to liquid products, as well as to solid products, it can also be used as a material for food or other applications.

1.4 Research Objectives

a. Preparation of complexes of curcumin and chitosan.

b. Analysis of the physicochemical and structural properties of the complexes and revealing the mechanism of interaction between curcumin and chitosan by means of high tech instrumental assays.

c. Study of the effect of combined curcumin-chitosan on the amelioration of the physicochemical instability of curcumin

2 MATERIALS AND METHODS

2.1 Materials

Curcumin (99% purity), chitosan (90% purity, 70%, 80% and 90% deacetylation of low, medium and high deacetylated chitosan respectively); analytically pure anhydrous ethanol; deionised water; magnetic stirrer (XD-D04RCT) Malvern laser nanoparticle sizer (Nano ZS90); turbidimeter (2100N); UV-Visible spectrophotometer (UV- 1800); light box; digital display thermostatic water bath.

2.2 Methodology

2.2.1 Preparation of Curcumin-Chitosan Complexes

Weigh 0.50 g of curcumin powder and dissolve in 1000 mL of 70% ethanol aqueous solution to prepare 0.5 mg/mL of curcumin solution. 2.50 g of chitosan powder with different degrees of deacetylation were weighed and dissolved in 1 L of 0.1% aqueous acetic acid to prepare 2.5 mg/mL of aqueous chitosan solution. The aqueous chitosan solution was then stirred on a magnetic stirrer (see Figure 2 for apparatus) at 400 rpm for 10 hours to fully dissolve. 10 mL of the curcumin solution was slowly injected into 40 mL of aqueous chitosan solution, with high speed stirring at 1200 rpm to allow the curcumin to rapidly adsorb onto the chitosan molecules and facilitate the formation of a complex. The chitosan samples with low, medium and high deacetylation levels were named as: chitosan (low), chitosan (medium) and chitosan (high) respectively; the complexes formed by curcumin and chitosan with low, medium and high deacetylation levels were named as: curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high) respectively.

Figure 2. Magnetic stirrer

2.2.2 Detection of Particle Size and Potential of Curcumin-Chitosan Complexes

The sample dispersions of 1 mL of chitosan (low), chitosan (medium), chitosan (high), curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high) were pipetted into a quartz dish on a Malvern laser nanoparticle sizer (Figure 3) and held at a constant temperature for 2 min before the particle size data were collected. Using a pipette, 1 mL of chitosan (low), chitosan (medium), chitosan (high), curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high) sample dispersions were pipetted into the potentiometric cell of the Malvern Zeta Potentiostat and held at a constant temperature for 2 min before the collection of potential data was initiated. (Note: The Malvern laser nanoparticle sizer and the zeta potential meter are the same instrument and contain both a dynamic light scattering particle size detector and a potential detector, so that both particle size and potential parameters can be measured separately, but not simultaneously).

Figure 3. Potentiostat

2.2.3 Detection of Turbidity in Dispersions of Curcumin-Chitosan Complexes

A measuring cylinder was used to measure 30 mL of chitosan (low), chitosan (medium), chitosan (high), curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high) sample dispersions and placed in the sample bottle of the turbidimeter (Figure 4) and held at a constant temperature for 2 min before collecting and recording turbidity data.

Figure 4. Turbidimeter

2.2.4 Detection of Encapsulation and Loading of Curcumin by Chitosan

Using a pipette, 1mL of the sample dispersions of curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high) were added to 9mL of aqueous ethanol solution with a volume fraction of 70% for dilution, and 1mL of the dilutions were placed in a cuvette using a pipette, and the absorbance was collected by UV-1800 UV-Visible spectrophotometer (Figure 5) The data were obtained from the curcumin standard curve and Lambert-Bier law to obtain the concentration of curcumin, and the mass of curcumin (mg) was converted according to the dilution times and volume. Encapsulation and loading rates were calculated according to Equations 1 and 2.

Formula (1)

$$\text{Embedding rate} = \frac{\text{Mass of curcumin in the sample}}{\text{Total mass of curcumin used in the manufacturing process}} \times 100\%$$

Formula (2)

$$\text{Load rate} = \frac{\text{Mass of curcumin in the sample}}{\text{Quality of curcumin and chitosan used in the manufacturing process}} \times 100\%$$

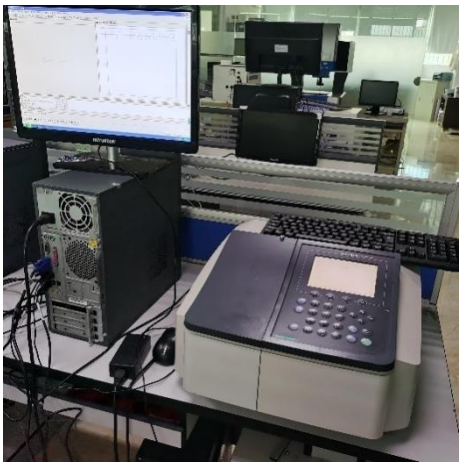

Figure 5. UV-1800 UV-Visible Spectrophotometer

2.2.5 Detection of The Effect of Chitosan on The Photostability of Curcumin

The samples were measured using a measuring cylinder and 30 mL of free curcumin in 70% ethanol aqueous solution and 30 mL of curcumin-chitosan (high) complex dispersion, and the concentration of curcumin in both samples was ensured to be equal, and the samples were placed in a transparent glass sample bottle and sealed with a screw cap. Samples were taken at 30 min intervals and the concentration of curcumin was measured according to the method in 2.2.4. The retention of curcumin by photodegradation was calculated using Equation 3 below.

Formula (3)

$$\text{Photodegradation retention} = \frac{\text{Determination of curcumin in the sample}}{\text{Initial content of curcumin in the sample}} \times 100\%$$

Figure 6. Light box

Formula (4)

$$\text{Thermal degradation retention rate} = \frac{\text{Determination of curcumin in the sample}}{\text{Initial content of curcumin in the sample}} X100\%$$

Figure 7. Digital display thermostatic water bath

2.2.7 Data Statistics and Analysis

All experimental data in this study were averaged over three parallel trials and the graphs were plotted using Origin 8.5 software.

2.2.6 Detection of The Effect of Chitosan on The Thermal Stability of Curcumin

A measuring cylinder was used to measure 30 mL of free curcumin in 70% ethanol aqueous solution and 30 mL of curcumin-chitosan (high) complex dispersion, and to ensure that the two samples contained equal concentrations of curcumin. Samples were taken every 2 hours and the curcumin concentrations were measured according to the method in 2.2.4. The retention of curcumin by thermal degradation was calculated using Equation 4 below.

3 RESULTS

3.1 Particle Size Analysis of Curcumin-Chitosan Complexes

As can be seen from Figure 8, the degree of deacetylation had a significant effect on the particle size of the flavin-chitosan complex. At the same time, its spatial size increased significantly when it was combined with curcumin. In this paper, we suggest that the change in particle size of curcumin-chitosan is due to hydrophobic binding, where both curcumin and chitosan have non-polar groups hydrophobic binding produced by expelling the water atoms between them and binding.

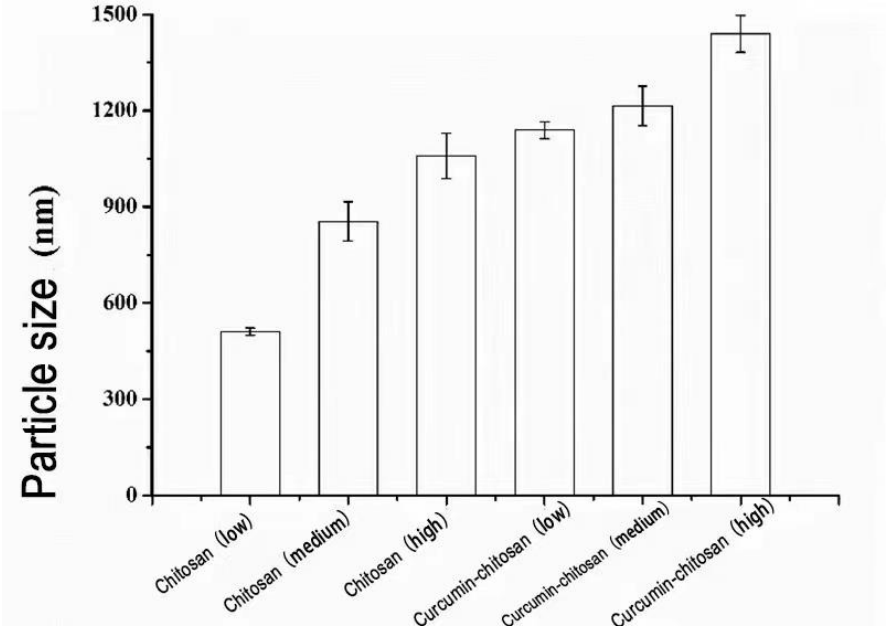

Figure 8. Particle size of chitosan and its complexes with curcumin at different degrees of deacetylation

From left to right, the particle sizes (nm) of the six particles are chitosan (low), chitosan (medium), chitosan (high), curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high). The lowest

particle size is the low purity chitosan with a particle size of approximately 500 nm, while the highest is the high purity chitosan-curcumin complex with a particle size of approximately 1400 nm

3.2 Potentiometric Analysis of Curcumin-Chitosan Complexes

As can be seen from Figure 9, as its deacetylation increases, its potential also increases. Because chitosan is a large molecule and curcumin is a small molecule the forces generated when they combine will change their charge distribution and thus increase their potential.

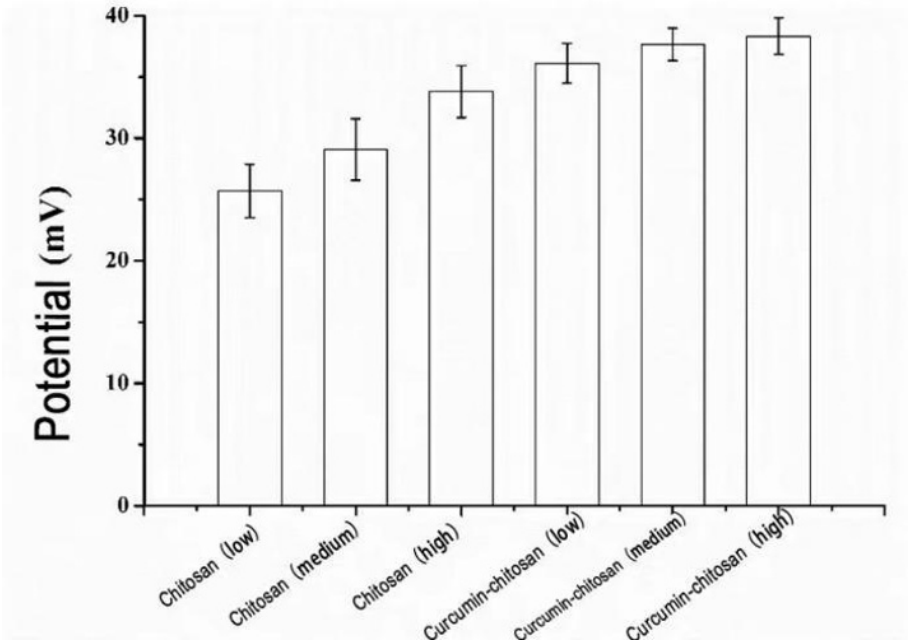

Figure 9. Potentials of chitosan and its complexes formed with curcumin at different degrees of deacetylation.

From left to right, the potentials of the six samples are chitosan (low), chitosan (medium), chitosan (high), curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high), with chitosan (low) having the lowest potential and curcumin-chitosan (high) having the highest potential.

3.3 Turbidity Analysis of Dispersions of Curcumin-Chitosan Complexes

As can be seen from Figure 10, the turbidity of the curcumin-chitosan complex also increases with the degree of deacetylation of chitosan. Because curcumin is poorly soluble and suspended in water, its turbidity increases substantially when combined with chitosan. It has also been shown that the degree of deacetylation is related to the change in chitosan refractive index (He, 2013).

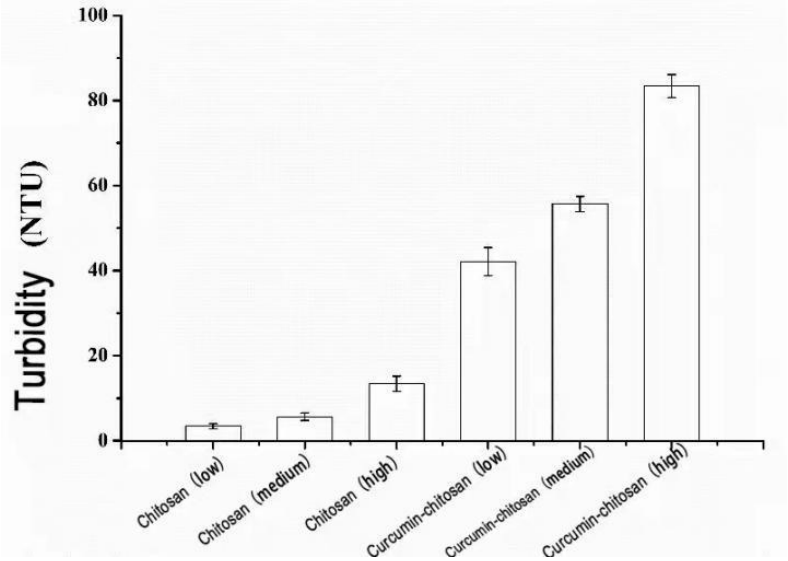

Figure 10. Turbidity of dispersions of chitosan and its complexes formed with curcumin at different degrees of deacetylation.

From left to right, the turbidity of the six samples are chitosan (low), chitosan (medium), chitosan (high), curcumin-chitosan (low), curcumin-chitosan (medium) and curcumin-chitosan (high). The turbidity of chitosan (low) is the lowest and curcumin-chitosan (high) is the highest.

3.4 Encapsulation Rate of Curcumin by Different Degrees of Deacetylation of Chitosan

As can be seen from Figure 11, the encapsulation rate (the ratio of curcumin bound to chitosan to its total number) was measured for different degrees of deacetylation. shows that the degree of deacetylation had a significant effect on the encapsulation rate of the curcumin-chitosan complex.

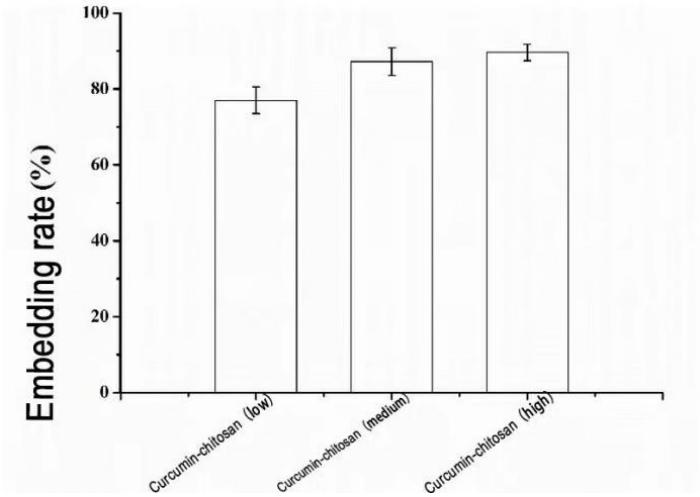

Figure 11. The encapsulation rates of curcumin by different degrees of deacetylated chitosan, from left to right, the encapsulation rates of curcumin-chitosan with low, medium and high degrees of deacetylation, with curcumin-chitosan (low) having the lowest encapsulation rate and curcumin-chitosan (high) having the highest encapsulation rate

3.5 Loading of curcumin by different degrees of deacetylated chitosan

As can be seen from Figure 12, the loading of chitosan (the ratio of the mass of curcumin in curcumin-chitosan to its total mass) increases with its degree of deacetylation.

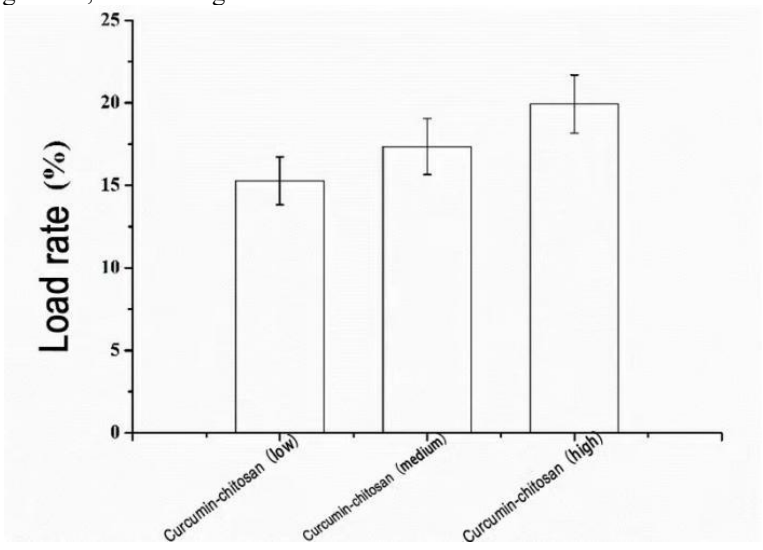

Figure 12. Loading of curcumin by different degrees of deacetylation of chitosan.

From left to right, the loading of curcumin-chitosan with low, medium and high deacetylation, with curcumin-chitosan (low) having the lowest loading and curcumin-chitosan (high) having the highest loading

3.6 Improvement of Curcumin Light Stability by Curcumin-Chitosan Complexes

After a certain amount of time in the light, this results in Figure 13. As can be seen from the figure below, the stability of curcumin is somewhat improved when it is combined with chitosan. There have been experiments

using the free amino group of chitosan to cross-link ions with the anion of sodium polyphosphate (TPP) to form bonds and solidify to form polymers, which can be nanoparticles (NPs) in the nano to micron range, and by using such nanoparticles, it has been shown to improve the stability of curcumin and improve the limitations of its low yield and a number of other problems (Gupta, 2013).

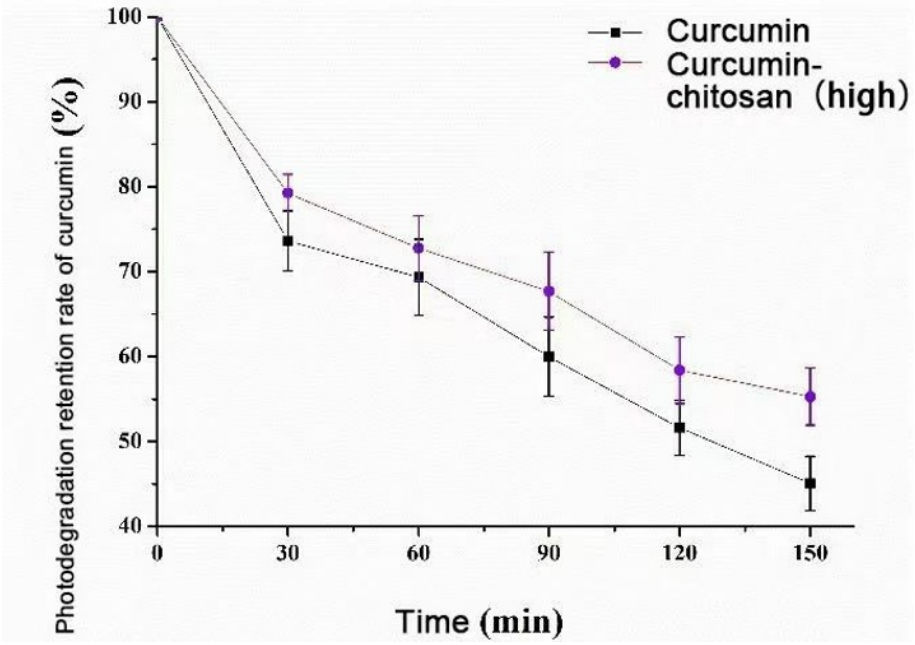

Figure 13. The effect of curcumin-chitosan complex on the improvement of the photostability of curcumin. The black line in the figure shows the retention of photodegradation of curcumin and the red line shows the retention of photodegradation of curcumin-chitosan (high), both of them show a decreasing trend at the same time, but the retention of photodegradation of curcumin-chitosan (high) is slightly higher.

3.7 Improvement of The Thermal Stability of Curcumin by Curcumin-Chitosan Complexes

The data were collected by heating in a constant temperature water bath and Figure 14 shows that when curcumin and chitosan were combined, their thermal degradation retention increased and no great change in the thermal degradation retention of curcumin-chitosan (high) occurred during prolonged heating, with the highly deacetylated chitosan playing a role in the increased thermal degradation retention of curcumin.

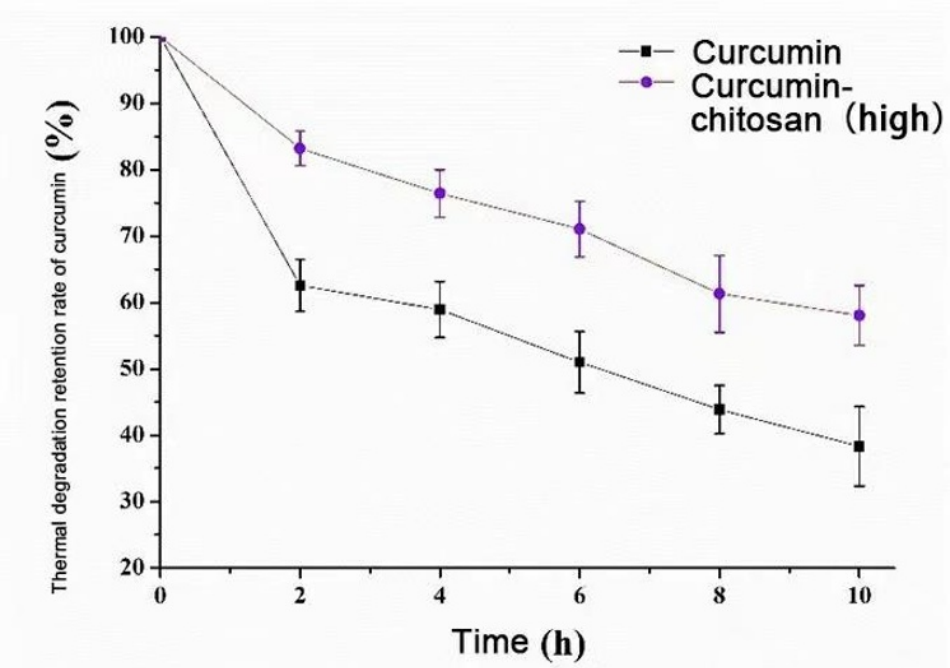

Figure 14. Improvement effect of curcumin-chitosan complex on the thermal stability of curcumin, the black line in the figure is the retention of thermal degradation of curcumin and the red line is the retention of thermal degradation of curcumin-chitosan (high), both of them show a decreasing trend at the same time, and the retention of thermal degradation of curcumin-chitosan (high) is always higher than that of curcumin

4 CONCLUSION, DISCUSSION AND OUTLOOK

4.1 Conclusion

In this paper, by combining chitosan and curcumin, the changes in particle size, potential, turbidity, encapsulation rate and loading rate of curcumin combined with different degrees of deacetylation of chitosan were collected, and the light and thermal stability of curcumin-chitosan complexes and curcumin were tested, and it was obtained that the combination of curcumin and chitosan could improve its light and thermal stability, and it was also found that the degree of deacetylation of chitosan had a significant effect on curcumin-chitosan complexes. The degree of deacetylation of chitosan was also found to have a significant effect on the turbidity, encapsulation rate and loading rate of the curcumin-chitosan complex.

4.2 Discussion

The results of this experiment were not perfect, as it was the first time I had entered the laboratory and I was unfamiliar with the rules and regulations of the laboratory, and I did not know most of the laboratory equipment, so I made several mistakes during the experiment. As a result, the experiment progressed slowly, the results were only replicated three times, and the results were crude compared to other experiments, all due to the operator's lack of familiarity with the laboratory.

4.3 Outlook

Through this test, it was found that the combination of chitosan with curcumin could improve the stability of curcumin. The increased thermal stability allows curcumin to be taken in hot water with other drugs in the future as a medicine, and the increased photostability makes it possible to preserve curcumin in a common transparent medicine bottle. In the future, the stability of curcumin could be encapsulated by making chitosan films to produce curcumin-based drugs that are preserved, easy to transport and easy to take.

Due to time and experimental techniques, only a preliminary study of the curcumin-chitosan complex was carried out in this experiment, but the molecular mechanism of how chitosan protects curcumin was not investigated or explained, and future experiments could be conducted to investigate this. This experiment did not directly address the problems of improving the short half-life and low pharmacokinetics of curcumin in vivo, but it can be speculated through the above experiments that the use of chitosan can improve these problems of curcumin when used as a medicinal material, but specific and more scientific and intuitive results need to be further related experiments and studies.

At the same time, it is possible that other compounds or biomolecules may have been identified in the experiment that may improve the physicochemical properties of curcumin, and this part could not be studied further in the laboratory due to time issues. Apart from this, the safety of the chitosan-curcumin complex as a pharmaceutical or dietary supplement has not been investigated and described in this experiment, and some safety studies such as toxicology and pharmacotoxicology will be required if the relevant methods are required to produce curcumin-based drugs or health products in the future.

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