American Journal of Botany and Bioengineering
https://biojournals.us/index.php/AJBP
ISSN: 2997-9331

The Nanotechnology Applications in Developing Food Products

Mohammed Saad Naif Abdul Jabbar, Adyan Wael Mohamed Abdel Hamza,
Tuqa Abdul Karim Musa Abbas
Al Qasim green university/ College of Food science/ Department of food science and technology

Received: 2024 29, Jul
Accepted: 2024 28, Aug
Published: 2024 26, Sep

Annotation: Magical spell has the ability to turn everything touched into gold, in real time scenario one such spell is "Nanotechnology" which has the mystical power to revolutionize every field touched by it. Nanotechnology is now invading the food industry and establishing great potential. Nanotechnology applications in food industry include: encapsulation and delivery of substances in targeted sites, increasing the flavor, introducing antibacterial nanoparticles into food, enhancement of shelf life, sensing contamination, improved food storage, tracking, tracing and brand protection. Nano food processing and products can change the color, flavor, or sensory characteristics; they also change the nutritional functionality, removes chemicals or pathogens from food. Nano food packaging materials may extend food life due to high barrier packaging, improve food safety, alert consumers that food is contaminated or spoiled, repair tears in packaging, and even release preservatives to extend the life of the food in the package. Nanobarcodes are used for safety labeling and monitor distribution of food products. Nanosupplements can be easily incorporated by encapsulation techniques for nutritional and drug delivery systems effectively. The health plays a major role in food the disadvantages of the technology is to be concerned. In particular, gold nanoparticles of curcumin were highlighted. To overcome the poor aqueous solubility and bioavailability of curcumin, emphasize its functional features, and broaden its applications in the food and pharmaceutical industries, many nanoscale systems have been widely applied for its encapsulation and delivery. Over many decades, chitosan as a natural biopolymer has been extensively studied due to its polycationic nature, biodegradability, biocompatibility, non- toxicity, and non – allergenic.

Keywords: nanoparticles, curcumin, chitosan, turmeric.

Introduction

In both medicinal and biopharmaceutical science, turmeric (Curcuma longa) is a spice that has been received much interest for its potent medicinal properties as the source of Curcumin [1]. Curcumin is a hydrophobic polyphenol that has advantages in a variety of pharmacological activities such as antioxidant, anti- inflammatory, antitumor, antimicrobial, and anticancer [2]. In the function of Antioxidant, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3,5-dione), also called diferuloylmethane, is a scavenger of oxygen species including hydroxyl radicals, superoxide anions and single oxygen and interferes with lipid peroxidation [3]. In spite of these healthful properties, Curcumin has a low bioavailability in which its absorption in the GI tract is a significant factor. to enhance the curcumin’s therapeutic potential, several encapsulation methods were studied [4]. Nanoencapsulation of curcumin could be a strategy to increase its bioavailability and reduce the dose required for a desired effect in therapeutic use [5]. In nanoencapsulation of curcumin, curcumin will be encapsulated into chitosan nanoparticles. According to potential of chitosan to interact with the negatively charge of the mucosal cells in the gastrointestinal tract. Chitosan helps to extend the residence time while deliver curcumin into human body [6]. Chitosan is a second most abundant polysaccharide on earth, produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell walls of fungi [7]. From further study on the usage of chitosan nanoparticles, it is best for controlled drug release and found to have minimal drug side effects [8]. The bioactive compound curcumin can be tested or evaluated using the spectroscopic method; nanoparticles were determined by dynamic light scattering through a dilution [9]. On the other hand, folate Is one of the naturally occurring form of vitamin B9 which found in foods include leafy vegetables, beans, and eggs. Folate can be synthesized, as well as normally found in supplements and fortified foods, and it is known as “Folic acid”. Folic acid has numerous health benefits. Namely, it helps regulate the amount of sulfur- containing amino acid in the body, a great vitamin for brain health and preventing Alzheimer’s disease, Reduced risk of anemia and high blood pressure [10]. Most importantly, it is known to be very significant for pregnant women which helps developing new cells for the baby and reducing the risk of birth defects, specifically neural tube defects (NTDs). For folic acid’s incorporating application, it is a functional drug vehicle of curcumin compound in order to address the issue of poor water solubility and improve its targeted accumulation at tumor site.

One of the techniques used is folic acid-conjugate micelle in which thin film hydration method is adopted [11]. However, using folic acid-conjugate as a ligand incorporated with chitosan could retain its original physiochemical properties of chitosan and target the folate receptor to up-regulate on the surface of many cancer cells [12]. The nanotechnology of encapsulating curcumin into chitosan with the further of folic acid-conjugate would enhanced the bioavailability and retention time of curcumin in human body. It would also be used as a functional food to improve health benefits. Therefore, this research is aim to maximize the efficiency of curcumin by developing a certain morphology, size, and encapsulation capability as well as to evaluate the bioactivities (i.e. antimicrobial and antioxidant) of the encapsulated curcumin.

Problem statement

Due to the rapid development and advances in the preparation methods of Biopolymeric nanocarriers, the present review focuses on the most recent literature to summarize the innovative CS – based nanocarriers for curcumin delivery that are reported in the past 3 years, including nanocomplexes, self- assembled nanoparticles, nanocomposites, nanoemulsions, nanotubes, and nanofibers. The mechanisms of the molecular interactions between curcumin and CS – based nanocarriers, as well as the in vitro release are illustrated explicitly. Furthermore, their applications as delivery systems in different fields are discussed in detail to offer an updated overview, particularly on the therapeutic efficacy. Compared with previously published review articles that only focus on various methods utilized for CS nanocarriers preparation, or pharmacokinetic findings on the delivery of curcumin using CS nanocarriers, the novelty of the current article is to discuss how physicochemical properties of CS impart functionality to the formation of nanocarriers and encapsulation of curcumin, as well as their pharmaceutical applications for treating chronic diseases. Lastly, research prospects and directions are suggested for the future.

Challenges

The challenges were what we faced was the lack of sufficient equipment to work and complete the results. This tasked us with completing the work in another country, and after that we obtained the results. The potential problems that arose when implementing the project were in order, but we did our best to solve all the problems. Finally, we hope that this project will participate and benefit from other means to achieve food security and safety and preserve people’s health.

Chitosan

In medical field, chitosan is an interesting compound which has been used broadly with the production of encapsulation or a drug carrier. It was produced by deacetylated of chitin_ existed in fungal cell walls and crustacean shells. Chitosan can be obtained via two ways; one is obtained from deacetylated chitin using sodium hydroxide and the other one is obtained from deacetylated chitin using enzymes under favorable conditions. Though, deacetylated chitin under alkaline condition is a better choice of converting chitin into chitosan [13]. Chitosan is biodegradable polymer that functions as an adhesive, antibacterial and antifungal agent. One source stated that, “the elemental composition of the chitosan polymer Is carbon (44.11 %), hydrogen (6.84%) and nitrogen (7.97 %) and the viscosity of average molecular weight of chitosan is ~5.3xl Daltons [14]**.** The degree of deacetylation has an impact on chitosan molecular weight which then affect many properties of its compound in carrying other raw materials into biological cells. Those properties included Solubility, viscosity [15]. Reactivity of proteinaceous material coagulation, and heavy metal ion chelation [16], and physical properties of films Formulated using chitosan such as tensile strength, elasticity, elongation, and moisture absorption [17]. Chitosan has a unique functional property after a slight modification. As it is soluble in an aqueous acidic solution; the amino group could possibly protonate and giving itself a positive charge. Within this property, cationic chitosan compound can interact with other anionic molecules through electrostatic interaction. Usually, chitosan nanoparticles would have formed by interacting with a polyanions such tripolyphosphate (TPP) into chitosan solution [18].

Folate and its bioavailability activity:

Folate (Vitamin B9) is an essential vitamin which body needs; it is widely known as an impo1iant vitamin for pregnant women in order to produce red blood cells and synthesizing certain amino acids. Also, it is recognized as health- beneficial in the prevention of neural tube defects, anemia, cardiovascular disease, poor cognitive performance, and some forms of cancers. Due to its beneficial properties, Folate was used to study its bioavailability, and research shown for human and pig that folates synthesized by colon bacteria is bioavailable. Passive diffusion of folate across the cell membrane is limited and happens only at high doses. Minor extent folate is also absorbed in the colon; and it is suggested that colonic absorption may contribute significantly to total folate absorption [26].

Literature review

There are many sources that focus on the functionality of encapsulated curcumin via chitosan nanoparticles, however, different sources have their own application, for example, not only the stable encapsulated curcumin could be delivered and cured colon cells and breast cancer cells in humans, but it can be also used for treatment of arsenic toxicity. In this review, many ideas, infonnation, methods, and techniques have been generated to produce a stable and efficient encapsulated curcumin, by discussing all advantages and disadvantages of all sources that are useful for this research.

There are many articles and research explaining the role of nanotechnology

Vijayan et al, have been found that functional coating materials can be developed using nanomaterials as their polymeric nanocomposites. In this article, various aspects of antiviral nanolayers are reviewed including the mechanism of interaction with coronavirus, different types of nanolayers developed for various substrates, future research areas, and new opportunities and challenges [34].

Lopez et al, investigate whether chitosan nanoparticles enhance the effects of curcumin and other natural compounds in vitro and in vivo.

In addition to its biocompatibility and biodegradability, chitosan is known for its mucoadhesive properties. Chitosan-based nanosystems enhance most aspects of loaded drugs, including cellular transport and other biological effects. The use of chitosan nanoparticles enhances the penetration, stability and bioactivity of natural compounds. In this review, an overview is provided of the main features of chitosan nanoparticles that have improved in vitro and in vivo effects of natural bioactive molecules, with an emphasis on the results obtained with curcumin [35].

In this review, it was concluded that chitosan-based delivery systems for curcumin: A review of pharmacodynamic and pharmacokinetic aspects to mahsa saheb, Narges Fereydouni, Saeideh Nemati, George E Barreto, Thomas P Johnston, Amirhossein.

Effective drug delivery is one of the most important issues associated with the administration of therapeutic agents that have low oral bioavailability. Curcumin is an active ingredient in the turmeric plant, which has low oral bioavailability due to its poor aqueous solubility. One strategy that has been considered for enhancing the aqueous solubility, and, thus, its oral bioavailability, is the use of chitosan as a carrier for curcumin. Chitosan is a biodegradable and biocompatible polymer that is relatively water-soluble. Therefore, various studies have sought to improve the aqueous solubility of chitosan. The use of different pharmaceutical excipients and formulation strategies has the potential to improve aqueous solubility, formulation processing, and the overall delivery of hydrophobic drugs. This review focuses on various methods utilized for chitosan-based delivery of curcumin[36].There is a clear article chitosan-Polyphenol conjugates for human health ,To Ananya Pattnaik, Sanghamitra Pati , Sangram Keshari Samal.

Human health deteriorates due to the generation and accumulation of free radicals that induce oxidative stress, damaging proteins, lipids, and nucleic acids; this has become the leading cause of many deadly diseases such as cardiovascular, cancer, neurodegenerative, diabetes, and inflammation. Naturally occurring polyphenols have tremendous therapeutic potential, but their short biological half- life and rapid metabolism limit their use. Recent advancements in polymer science have provided numerous varieties of natural and synthetic polymers. Chitosan is widely used due to its biomimetic properties which include biodegradability, biocompatibility, inherent antimicrobial activity, and antioxidant properties. However, due to low solubility in water and the non-availability of the H-atom donor, the practical use of chitosan as an antioxidant is limited.

Therefore, chitosan has been conjugated with polyphenols to overcome the limitations of both chitosan and polyphenol, along with increasing the potential synergistic effects of their combination for therapeutic applications. Though many methods have been evolved to conjugate chitosan with polyphenol through activated ester- modification, enzyme-mediated, and free radical induced are the most widely used strategies. The therapeutic efficiency of chitosan-polyphenol conjugates has been investigated for various disease treatments caused by ROS that have shown favorable outcomes and tremendous results. Hence, the present review focuses on the recent advancement of different strategies of chitosan-polyphenol conjugate formation with their advantages and limitations. Furthermore, the therapeutic applicability of the combinatorial efficiency of chitosan-based conjugates formed using gallic acid, Curcumin, Catechin, and Quercetin in human health has been described in detail [37].

Materials and methods:

Curcumin, Chitosan, hydrochloric acid (HCl), dimethyl Sulfoxides alcohol (DMS), Folic acid 50 mg and Distilled water were purchased from Sigma-Aldrich

Formulation of curcumin-loaded chitosan nanoparticles:

Curcumin-loaded chitosan nanoparticles were created using the ionotropic gelation process based on the electrostatic interaction between positively and negatively charged molecules using dimethyl sulfoxide (DMS) as a crosslinking agent. The following steps were used to make curcumin-loaded chitosan nanoparticles:

1. Distilled water is placed in a 200 ml glass beaker.
2. The machine is placed inside the glass beaker.
3. The glass beaker is placed on the centrifuge and starts rotating at a temperature ranging from 55-65 degrees Celsius.
4. Gradually add 200 mg of chitosan.
5. Add hydrochloric acid, and after 10 minutes, add another 100 mg of chitosan.
6. Weigh 200 mg of curcumin under opaque conditions, as it is a light-sensitive compound, and place it in small bottles.
7. Add dimethyl sulfoxide (DMS) to the curcumin, then close the containers and shake them until the curcumin molecules are completely dissolved.
8. Inject it drop by drop using a needle.
9. After that, it is gradually added to the chitosan solution and left for 15 minutes.
10. Add hydrochloric acid, and after 15 minutes, add 50 mg of folic acid.
11. The centrifuge continues to spin at a speed of 500 degrees to achieve a small particle size.
12. High-resolution transmission electron microscope (TEM) and field emission scanning electron microscope (SEM) are used for particle analysis.

These are the detailed steps that were followed to produce curcumin-loaded chitosan nanoparticles.

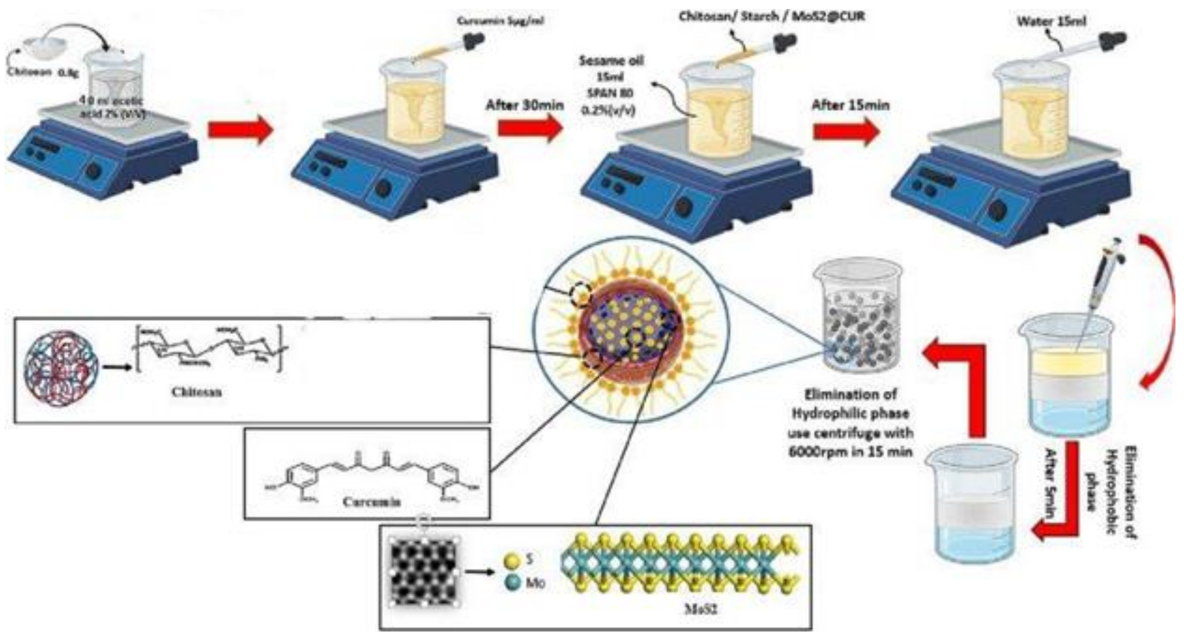


Figure. 7. Procedure of encapsulation of curcumin into chitosan nanoparticles (C-CS-NP s)

Measurements of particle size

The size and polydispersity of Cs-CU nanoparticle were measured with a Zetasizer Nano-ZS (Malvern Instruments, UK). After pre-paring a solution of Cs- CU nanoparticle in double distilled water at 1 mg/ml, the mixture was sonicated for 15 s in an ice cold bath. At RT, every measurement of the Cs-CU nanoparticle nanoformulation was performed using three independent replicates.

Transmission electron microscopy (TEM).

The interior properties of the CU-Cs nanoformulation were determined by high-resolution TEM (JEM-2000 EXII, JEOL, Tokyo, Japan) at 120 kV. A drop of diluted CU-Cs nanoformulation solution was placed on a 200 mesh formvar carbon-coated copper grid (TABB Laboratories Equipment).

Scanning electron microscopy (SEM).

The morphology of the CU loaded Cs was studied by SEM (LEO 1525 field emission scanning electron microscope, Zeiss, Oberkochen, Germany). The dry CU loaded nanocomposite was located on aluminum stubs with double-sided conducting carbon tapes and covered with a 50/50 mixture of Au/Pd. The samples were scanned at an accelerating voltage of 25 kV.

X-Ray Diffraction (XRD) Analysis

XRD analysis was performed to ascertain the crystallo- graphic structure of the nanoparticle formulation. The diffractograms of curcumin, Chi-NPs and Cu- NPs were obtained using an X'pert Pro X-ray diffractometer (Panalytical, Netherlands) equipped with a radiation source Cu K-alpha operated at 45 kV and 40 mA. The analysis was performed from 5 to 75° with a scanning speed of 0.02°/step and the step time was 0.5 s.

Encapsulation Efficiency

Determination of CU encapsulation in the nanoparticles CU-Cs (5 mg) was dispersed in 40 ml of 1mol/L HCL by sonication. After 2 h, the supernatant was collected by centrifugation at 14000 rpm and magnetic separation. The concentration of CU in the supernatant was measured by fluorescence spectroscopy (Hitachi, Japan) in 420 nm absorption and 430-600 nm emission and the gap width of 5 nm. The supernatant from CU-Cs was used as a contrast. The drug encapsulation efficiency of CU-Cs was taken utilizing the following equation:

Encapsulation efficiency (%) = [(drug fed – drug loss) / (drug fed)] × 100% Eq. (1)

Drug Release Profile

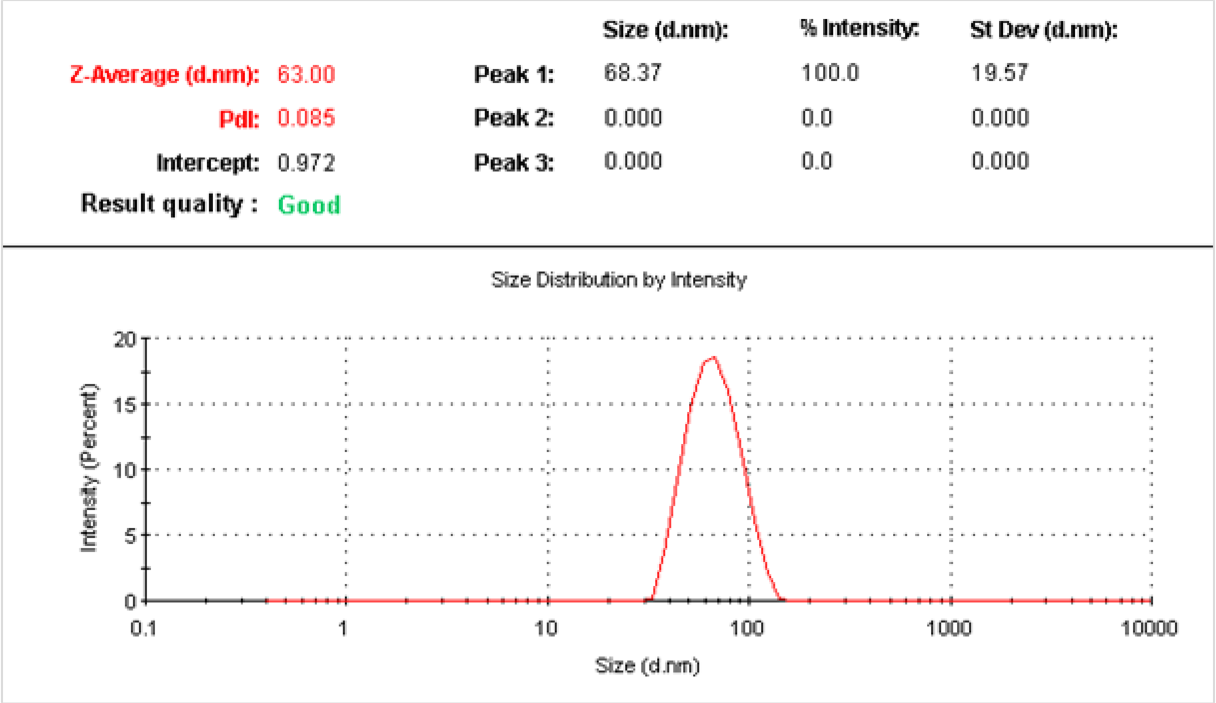
Different buffers such as citrate 0.01 M and pH = 5.4 and phosphate 0.01 M and pH = 7.4 buffers were used in 37 °C to measure drug release value from CU-Cs. Nanoformulation of 1 ml solution was added in the dialyze bag and placed in 100 ml citrate and phosphate buffers separately. The Tween 80 was utilized as an emulsifier to inhibit the sedimentation of released drugs. To perform the release process, shaking water bath was used. The sampling was done at 0, 4, 8, 12, 24, 48, 72, and 96 h. The 500 µl was aliquoted freeze-dried in each sampling process and resolved in 2 ml methanol. The CU release was measured by fluorescence spectroscopy. The CU release was measured using the following equation:

$$R = V \sum n - i\, Ci + V_0\, Cn \,/\, m\ drug$$

where R is the final drug release (%), Ci and Cn are the CU concentrations, V is the volume of each sample, V0 is the initial volume of drug, m drug is the mass of curcumin in nanoparticle, i and n are the sampling times, and precipitated material was rinsed and suspended again with DDW.

Result of size and polydispersity evaluation

The DLS results showed an average diameter of 63.00 ± 19.57 nm with single peak shape and narrow particle size distribution. The polydispersity was 0.085 ± 0.019 that proved the excellent dispersion of nanoparticles.



Z-Average (d.nm): 63.00
PdI: 0.085
Intercept: 0.972
Result quality : Good

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Peak 1:	68.37	100.0	19.57
Peak 2:	0.000	0.0	0.000
Peak 3:	0.000	0.0	0.000

Figure. 8. The particles size and distribution of CU-Cs uses dynamic light scattering (DLS).

Results of SEM and TEM

Furthermore, the high-resolution transmission electron microscope (TEM) and field emission scanning electron microscope (SEM) were applied to recognize the synthesis. nanoparticles' structural order, size, and shape. The result of the synthesized nanoparticles exhibited that the NPs were smooth, ball-shaped, and had no adhesion between them (Fig. 3A, B). It is observed that the particles were obtained in this study uniform in size and spherical. The prepared CU-Cs nanoparticles had a spherical shape and uniform size as observed by TEM, and SEM.

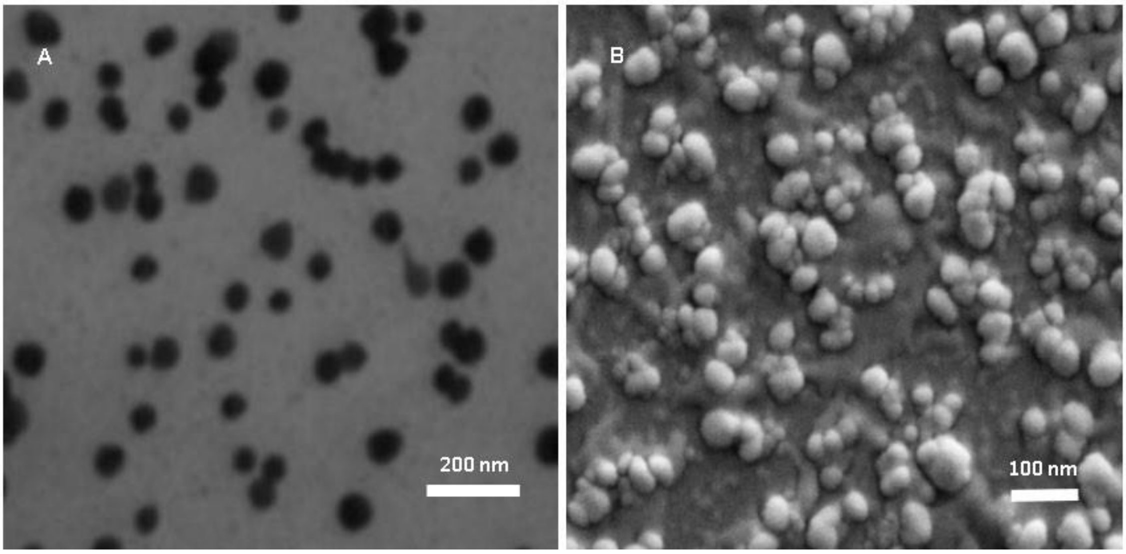


Figure. 9. Microscopic analysis of CU-Cs (A) TEM image of CU-Cs (B) SEM image of CU-Cs

Encapsulation Efficiency

The CU-Cs NPs solution was centrifuged after synthesis, and the supernatant was collected. The supernatant was evaluated by spectrophotometer (UV-Pharma spec, Shimadzu) in 280 nm. The CU encapsulation efficiency in Cs NPs was diagnosed about 80%. The nanoformulation showed high Encapsulation Efficiency

XRD Analysis

The XRD diffractogram of curcumin shows multiple peaks between 5 and 30° which were mainly attributed to its crystalline nature. These characteristic peaks had disappeared in the Cu-NPs (Fig. 4), suggesting the transformation of the crystalline nature of curcumin to an amorphous state. This change in physical characteristics may perhaps result from the molecular interactions between curcumin and chitosan occurring during formulation. It is worthwhile to note that the physical transformation of curcumin in the Cu-NPs did not affect the structural characteristics of curcumin, as confirmed by the FTIR analysis.

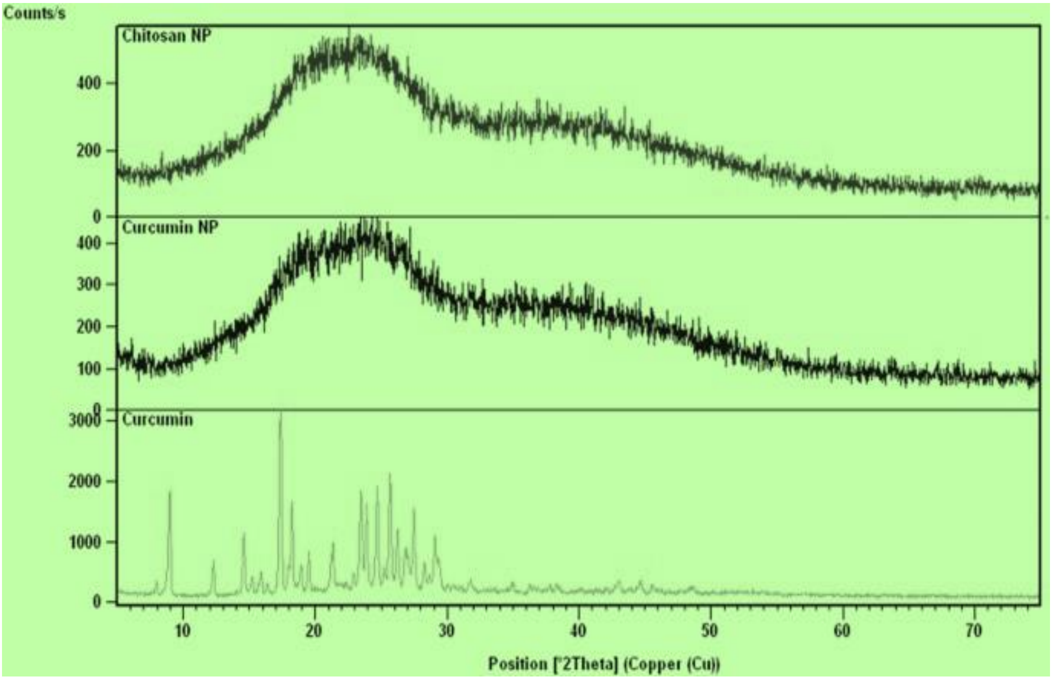


Figure. 10. XRD patterns of curcumin, Cs-NPs and Cu-Cs NPs

Release Profile

CU releases from Cs over a 96 -h period, and the result indicates that release time is slower at pH 7.4, as compared with pH 5.4 as shown in Fig. 4. In comparison with the release profiles of free CU, there are similar release profiles at pH 7.4 and 5.4; we observed a faster CU liberation profile at pH 5.4 under the planned conditions compared with pH 7.4.

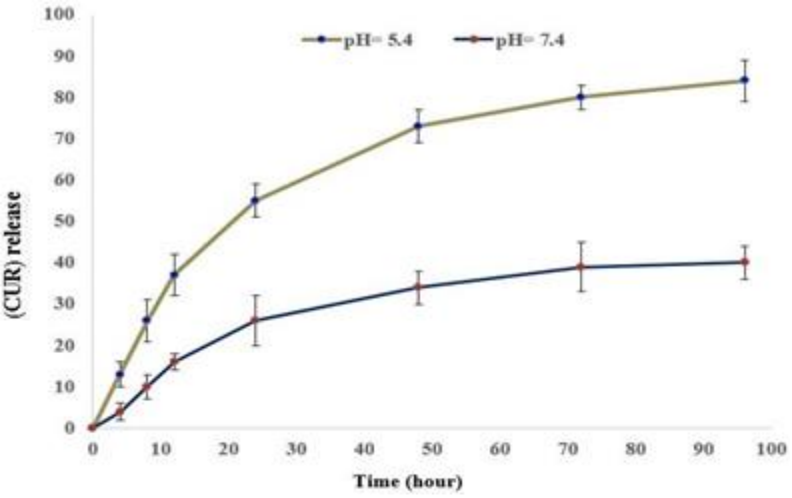


Figure. 11. drug release curve of nano-formulation with CU loaded Cs at different pH (7.4 and 5.4) and 37 °C. The mean values ± SD (n = 3) are defined by the data.

Conclusion and future prospect:

So far, excellent pharmaceutical activities of curcumin including anticancer, antimicrobial, wound healing, neuroprotection, antidiabetic, and nephron protective properties have been well elaborated. Tremendous efforts have been made to develop different types of CS – nanocarriers and try to broaden applications of curcumin in the field of food and pharmaceutical industries.

In conclusion, chitosan nanoparticles are a versatile nanomaterial with numerous applications across various fields. Their unique properties, such as biocompatibility, biodegradability, and antimicrobial activity, make them attractive for a wide range of uses.

The current applications of chitosan nanoparticles in drug delivery, wound healing, cosmetics and skincare, agriculture, food industry, and environmental applications show great promise. However, further research and development are still needed to optimize their properties, explore new applications, and overcome any challenges or limitations.

The future prospects of chitosan nanoparticles are highly encouraging. Ongoing research is focused on enhancing their drug delivery capabilities, improving wound healing properties, developing innovative formulations in cosmetics and skincare, exploring their potential in advanced agriculture techniques, discovering new applications in the food industry, and expanding their use in environmental remediation.

Additionally, as technology advances and our understanding of chitosan nanoparticles improves, there is potential for the development of novel applications that we may not have even envisioned yet. The versatility of chitosan nanoparticles makes them an exciting area of exploration for scientists and researchers in various fields.

In summary, chitosan nanoparticles have already demonstrated their value in several applications, and their future prospects are promising. Continued research and innovation will undoubtedly uncover new possibilities and further enhance their effectiveness in addressing challenges and creating sustainable solutions in various industries.

References:

1. Nugroho, Praptanti S. A, and Umi Nafisah. "Characterization Of Curcumin Encapsulation In Chitosan-Sodium Citrate Nanoparticles". Journal Of Applied Chemical Science, 2017, pp. 370-372. Jacsonline Group Publisher, doi:l0.22341/jacs.on.00402p370.
2. Yadav, Abhishek et al. "Curcumin Encapsulated In Chitosan Nanoparticles: A Novel Strategy For The Treatment Of Arsenic Toxicity". Chemico-Biological Interactions, vol 199, No. 1, 2012, pp. 49-61. Elsevier BV, doi:I0.1016/j.cbi.2012.05.011
3. Souza, Marthyna P. et al. "Quercetin-Loaded Lecithin/Chitosan Nanoparticles For Functional Food Applications". Food And Bioprocess Technology, vol 7, no. 4, 2013, pp. 1149-1159. Springer Science And Business Media LLC, doi: 10.1007/sl1947-013-1160-2.
4. Liang, Jin et al. "Applications Of Chitosan Nanoparticles To Enhance Absorption And Bioavailability Of Tea Polyphenols: A Review". Food Hydrocolloids, vol 69, 2017, pp. 286-292. Elsevier BV, doi:l0.1016/j.foodhyd.2017.01.041.
5. Chen, Hui et al. "Folic Acid Supplementation Mitigates Alzheimer'S Disease By Reducing Inflammation: A Randomized Controlled Trial". Mediators Of Inflammation, vol 2016, 2016, pp. 1-10. Hindawi Limited, doi:l0.1155/2016/5912146.
6. Hao, Jifu et al. "Folic Acid-Functionalized Drug Delivery Platfonn Of Resveratrol Based On Pluronic 127/D-Α-Tocopheryl Polyethylene Glycol 1000 Succinate Mixed Micelles". International Journal OfNanomedicine, Volume 12, 2017, pp. 2279-2292. Informa UK Limited, doi:l0.2147/ijn.s130094.
7. Xu, Yongmei, and Yumin Du. "Effect Of Molecular Structure Of Chitosan On Protein Delivery Properties Of Chitosan Nanoparticles". International Journal Of Pharmaceutics, vol 250, no. 1, 2003, pp. 215-226. Elsevier BV, doi:10.1016/s0378-5173(02)00548-3.
8. Fan, Wen et al. "Formation Mechanism Of Monodisperse, Low Molecular Weight Chitosan Nanoparticles By Ionic Gelation Technique". Colloids And Surfaces B: Biointerfaces, vol 90, 2012, pp. 21-27. Elsevier BV, doi:l0.1016/j.colsurfu.2011.09.042
9. De Campos, Angela M. et al. "Chitosan Nanoparticles: A New Vehicle For The Improvement Of The Delivery Of Drugs To The Ocular Surface. Application To Cyclosporin A". International Journal Of Phannaceutics, vol 224, no. 1-2, 2001, pp. 159-168. Elsevier BV, Doi: 10.1Ol6/s0378-5173(01)00760-8.
10. Shehzad, A. et al. "Curcumin Therapeutic Promises And Bioavailability In Colorectal Cancer". Drugs Of Today, vol 46, no. 7, 2010, p. 523. Clarivate Analytics (US), Doi: 10.1358/dot.2010.46. 7.1509560.
11. Lertsutthiwong, Pranee et al. "Preparation Of Turmeric Oil-Loaded Chitosan-Alginate Biopolymeric Nanocapsules". Materials Science And Engineering: C, vol 29, no. 3, 2009, pp. 856-860. Elsevier BV, doi:l0.1016/j.msec.2008.08.004.
12. Narayanan, Narayanan K. et al. "Liposome Encapsulation Of Curcumin And Resveratrol In Combination Reduces Prostate Cancer Incidence In PTEN Knockout Mice". International Journal Of Cancer, vol 125, no. 1, 2009, pp. 1-8. Wiley, doi:l0.1002/ijc.24336.
13. Ohrvik, Veronica E., and Cornelia M. Witthoft. "Human Folate Bioavailability". Nutrients, Vol 3, no. 4, 2011, pp. 475-490. MDPI AG, doi:10.3390/nu3040475
14. Liu, Buyun et al. "Synthesis, Characterization Of Catechin-Loaded Folate-Conjugated Chitosan Nanoparticles And Their Anti-Proliferative Effect". Cyta – Journal Of Food, vol 16, No. 1, 2018, pp. 868-876. Informa UK Limited, doi:l0.1080/19476337.2018.1491625.

15. Wang, Peter Y. et al. “Carbodiimide Reagents For The Chemical Probing Of RNA Structure In Cells”. RNA, vol 25, no. 1, 2018, pp. 135-146. Cold Spring Harbor Laboratory, Doi:l0.1261/rna.067561.118.

16. Thermofisher.com. (2019). Carbodiimide Cross/inker Chemistry I Thermo Fisher Scientffic – UK. [online] Available at: https://www. Thermo fisher. Com/th/ en/home/lifescience/protein-Biology/protein- biology-learning-center/protein-biology-resource-library /pierce-protein-Methods/ carbodiimide- crosslinker-chemistry .html

17. H, Bangun et al. “Preparation And Evaluation Of Chitosan-Tripolyphosphate Nanoparticles Suspension As An Antibacterial Agent”. Journal Of Applied Pharmaceutical Science, vol 8, No. 12, 2018, pp. 147-156. Journal Of Applied Pharmaceutical Science, Doi:I0.7324/japs.2018.81217.

18. Dube, Denis et al. “Preparation And Tumor Cell Uptake Of Poly(N-Isopropylacrylamide) Folate Conjugates”. Bioconjugate Chemistry, vol 13, no. 3, 2002, pp. 685-692. American Chemical Society (ACS), doi:10.1021/bc010084g.

19. Wang, Han et al. “Folic Acid-Modified Dendrimer-Entrapped Gold Nanoparticles As Nanoprobes For Targeted CT Imaging Of Human Lung Adencarcinoma”. Biornaterials, vol 34, no. 2, 2013, pp. 470- 480. Elsevier BY, doi:l0.1016/j.biomaterials.2012.09.054.

20. Li, Puwang et al. “Synthesis And Characterization Of Folate Conjugated Chitosan And Cellular Uptake Of Its Nanoparticles In HT-29 Cells”. Carbohydrate Research, vol 346, no. 6, 2011, pp. 801-806. Elsevier BY, doi:l0.1016/j.carres.2011.01.027.