DEVELOPMENT OF CURCUMIN-LOADED CHITOSAN NANOPARTICLE TO ENHANCE ITS ANTIMICROBIAL PROPERTIES

A PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Under the

Supervision of

Dr. Abhishek Chaudhary

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BY

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DECLARATION

This is to certify that I, Gitika Thakur (235111017), M.Sc Biotechnology, 4th semester, from the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan have personally worked on the project entitled "Development of curcumin-loaded chitosan nanoparticle to enhance its antimicrobial properties". The data mentioned in this project report has been obtained during the genuine work carried out by me. The data obtained from the other agencies has been fully acknowledged. None of the findings pertaining to the research work has been concealed. I also declare that the project report has not been submitted partially or fully to any other agency or institution for the award of any other degree or diploma, title or recognition.

Place: Waknaghat — Gitika Thakur

Date: — 235111017

Supervisor's Certificate

This is to formally certify that the project titled "Development of curcumin-loaded chitosan nanoparticle to enhance its antimicrobial properties," submitted by Gitika Thakur, Roll Number [235111017], at Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan, fulfils the requirements for the degree of Master of Science in Biotechnology in part.

This document constitutes a record of original research conducted under my supervision and guidance. This work is genuine and hasn't been submitted, in whole or in part, to any other university or institution for the purpose of conferring a degree or diploma. Additionally, I affirm that this project adheres to the academic and ethical standards requisite for such scholarly work.

I extend my best wishes to her for success in all future endeavours.

(Supervisor)

Dr. Abhishek Chaudhary

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

Dated: 22-05-2025

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There are emotions of accomplishment and contentment after finishing the current task. I use these happy times to write down my heartfelt appreciation to everyone who has mattered to me.

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Gitika Thakur

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ABSTRACT

Development of curcumin-loaded chitosan nanoparticle to enhance its antimicrobial properties

Curcumin, a bioactive compound derived from Curcuma longa, exhibits broad-spectrum antimicrobial activity but its clinical application is limited due to poor aqueous solubility, low bioavailability, and rapid degradation. Chitosan nanoparticles loaded with curcumin were created by the ionic gelation technique in order to get over these constraints. Chitosan is a natural biopolymer that works well as a carrier system because of its strong antibacterial qualities and high biocompatibility. Using scanning electron microscopy (SEM) and dynamic light scattering (DLS), the nanoparticles were examined for shape, encapsulation effectiveness, zeta potential, particle size, and UV-visible spectroscopy. Using both bacteria strains as Gram-negative and Gram-positive, the Nano-formulation antibacterial effectiveness was assessed. The findings showed that when curcumin was encapsulated in chitosan nanoparticles as opposed to free curcumin, its antibacterial activity was significantly increased. The synergistic effect of chitosan and curcumin within the nanoparticle matrix highlights the potential of this Nano-formulation as a promising antimicrobial agent for biomedical applications.

Keywords

Curcumin, Chitosan nanoparticles, Antimicrobial activity, Nano-encapsulation, Ionic gelation, Bioavailability, Drug delivery, Biopolymer

CHAPTER 1

INTRODUCTION

The increasing incidence of microbial infections and the alarming rise in antibiotic resistance have necessitated the development of novel therapeutic strategies that are both efficient and biocompatible. Natural compounds with intrinsic antimicrobial potential are being increasingly explored as safer alternatives or complements to synthetic antimicrobials (Rai et al., 2020).

Among these, a hydrophobic polyphenolic compound, curcumin extracted from *Curcuma longa* rhizome part which has gained considerable attention due to its diverse biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties. However, the therapeutic capability of it is greatly delayed by poor absorption, aqueous solubility is less, rapid metabolism, and instability under physiological conditions (Khan et al., 2016). These limitations restrict its bioavailability and, consequently, its effectiveness as a therapeutic agent.

Fig 1. Image of Curcumin. Source:- (Food Lab)

In order to overcome these obstacles, drug delivery systems based on nanotechnology have become a game-changing tool that can improve the bioavailability and targeted distribution of medications that are poorly soluble. One of the most promising approaches is the development of polymeric nanoparticles, particularly those composed of natural, biodegradable polymers. Chitosan, which is a cationic polysaccharide produced through the chitin deacetylation (a major component of crustacean shells), has proven to be an ideal candidate for nanoparticle fabrication due to its biocompatibility, biodegradability, low toxicity, and inherent antimicrobial properties. Additionally, its positive surface charge facilitates adhesion to negatively charged bacterial membranes, enhancing its bactericidal effect (Deka et al., 2016).

In recent years, curcumin-loaded chitosan nanoparticles (CUR-CSNPs) have been developed using various techniques, among which the ionic gelation method using sodium tri-polyphosphate (NaTPP) is the most widely employed. This method is simple, mild, and does not require organic solvents or high temperatures, preserving the structural integrity of both curcumin and chitosan. The encapsulation of curcumin within the chitosan matrix not only improves its stability and solubility but also allows for sustained and controlled drug release, increasing its residence time at the site of infection (Jahromi et al., 2014).

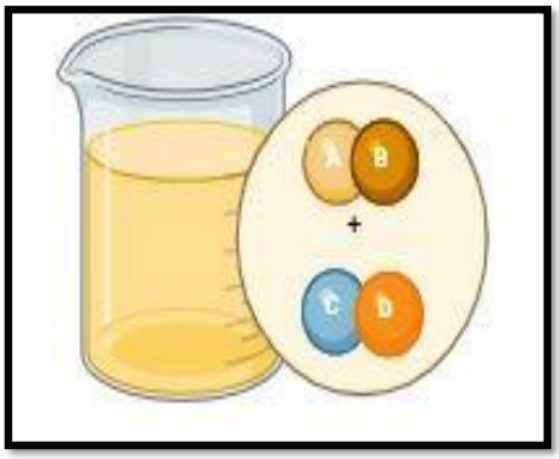

Fig 2: Diagram showing curcumin-loaded chitosan np. Source- (Biorender)

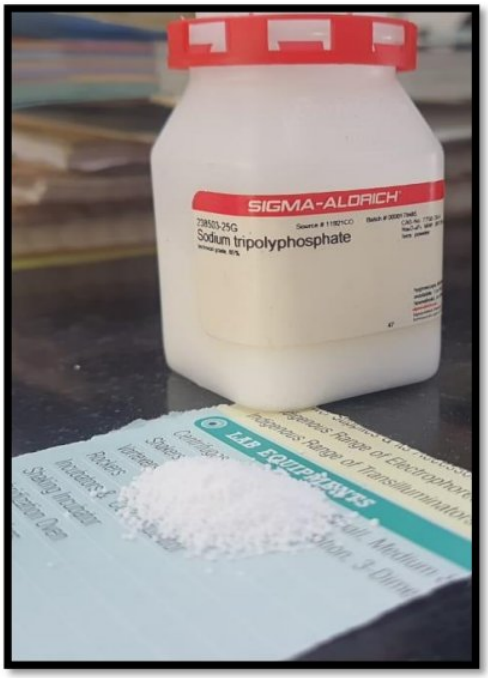

Fig 3: Forms of Sodium tri-polyphosphate. Source- (Food lab)

Moreover, the synergistic antimicrobial effect of chitosan and curcumin in nano-particulate form offers a multifaceted mechanism of action, disrupting microbial membranes, inhibiting biofilm formation, and modulating inflammatory responses (Aziznia et al., 2024). Such nano-formulations show promising applications in treating bacterial infections, especially in cases of

chronic wounds, burns, and antibiotic-resistant microbial strains. These systems also exhibit great potential in topical drug delivery, where localized treatment with minimal systemic exposure is desirable (Thomas et al., 2017).

The formulation and characterization of such nanoparticles involve evaluating parameters like zeta potential, particle size, drug loading, encapsulation efficiency, along with antimicrobial efficacy in vitro against clinically relevant microbial strains. Together, these studies contribute to the optimization and validation of curcumin-loaded chitosan nanoparticles as effective antimicrobial agents.

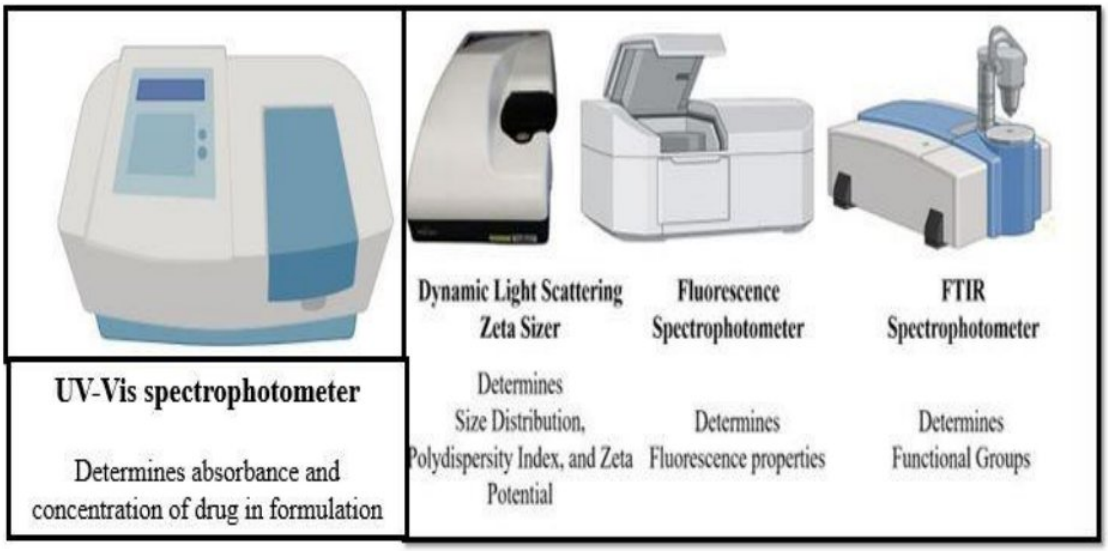

Fig 4. Characterization techniques for drug loaded nanoparticles. (Source – Biorender)

Research Gap –

Despite growing interest in natural polymer-based nanoparticle systems, several critical gaps remain in the development of curcumin-encapsulated chitosan nps for antimicrobial applications:

1. Limited Bioavailability Data: Although many studies have demonstrated improved antimicrobial activity with Cur-CSNPs, comprehensive pharmacokinetic data on bioavailability enhancement remains scarce. There is a lack of in vivo studies correlating enhanced release profiles with therapeutic outcomes.
2. Optimization of Formulation Parameters: Most existing research focuses on basic characterization. However, there is a need for systematic optimization studies involving Design of Experiments (DoE) to fine-tune critical process parameters such as pH, chitosan/TPP ratio, and curcumin loading concentration to achieve stable and reproducible nanoparticle formulations.
3. Synergistic Mechanism Exploration: The synergistic antimicrobial action of chitosan and curcumin has been observed, but detailed mechanistic studies on how these components interact at the molecular and cellular level to inhibit microbial growth are lacking.
4. Targeted and Site-Specific Delivery: There is a need to develop formulations that allow targeted or stimuli-responsive delivery for localized infection treatment, especially for wound healing applications.
5. Clinical Translation Challenges: Although lab-scale studies have shown positive outcomes, few studies have progressed towards clinical translation, including long-term stability studies, scalability of production, toxicity profiling, and regulatory aspects.

The goal of this research is to fill these gaps and help create a strong and efficient system of curcumin-loaded chitosan nanoparticles that has better antibacterial activity, formulation stability, and potential translational value in biomedical applications.

- **Objectives of the study –**

1. To develop and optimize a nanoparticle formulation using chitosan as a biopolymer for the encapsulation of curcumin.

2. To assess the antimicrobial activity of the delivered curcumin nanoparticles against Gram-positive and Gram-negative bacterial strains.

3. To investigate whether the nanoparticle-based delivery system enhances the bioavailability and antimicrobial assay due to action of this compound.

CHAPTER 2

REVIEW OF LITERATURE

[2.1] NANO-TECHNOLOGY:

Nanotechnology, is the science of working with matter at the molecular or atomic level, has the potential to significantly advance environmental protection technologies. The (EPA) U.S. Environmental Protection Agency employs the definition developed by the (NNI) National Nanotechnology Initiative, despite the fact that there are other definitions of nanotechnology. Knowledge from several fields, such as chemistry, physics, and biology, is combined in the multidisciplinary field of nanotechnology. According to the United States National Nanotechnology Initiative, nanotechnology is the study and development of structures, devices, and systems that, because of their small size, have unique properties; the ability to manipulate or control matter on an atomic scale; and research development at the macromolecular, atomic, or molecular levels using a length scale of approximately one to one hundred nm in any dimension. (USEPA, 2007). The pharmaceutical, engineering, medical, manufacturing, biotechnology, manufacturing, information technology, and telecommunications sectors all have great potential for using the technology.

- Development of nanotechnology–

According to R. D. Booker, there are two main reasons why it is challenging to explain the history of nanotechnology: (1) The name "nanotechnology" is ambiguous, and (2) it is unclear how long the early phases of nanotechnology development relate to. The term nanotechnology is explained by a wide spectrum of various technologies that nanotechnology covers, which are based on various types of physical, chemical and biological processes, realized at nano level.

Nanotechnology, though recognized as a modern scientific field, has origins that trace back to ancient times when people unknowingly used nanoscale materials. What differentiates those early, unintentional uses from contemporary nanotechnology is our growing ability to comprehend the fundamental principles that govern nanoscale behaviour, critically evaluate existing knowledge, and plan future advancements based on scientific understanding (USEPA, 2007). The term "nanotechnology" itself was first introduced by Norio Taniguchi during the 1974 International Conference on Industrial Production in Tokyo, where he referred to the fabrication and manipulation of materials with atomic-level precision and nano-meter scale accuracy. Richard Feynman, known as the "Father of Nanotechnology," introduced the ideas of nano-technological strategy in his 1959 address at the American Physical Society session, and Eric Drexler expanded on them in 1986. The early 1980s marked significant progress in the field of nanotechnology, largely due to two major breakthroughs: the emergence of cluster science and the invention of the Scanning Tunnelling Microscope (STM) in 1981. These innovations paved the way for the identification of fullerenes in 1985 and the detailed structural analysis of carbon nanotubes by 1991. Between the late 1980s and early 1990s, several ground breaking discoveries further propelled the growth of nanotechnology. In 1991, the United States initiated its first nanotechnology program through the National Science Foundation. A decade later, in 2001, the U.S. government formally launched the National Nanotechnology Initiative (NNI). Since then, research and development in this field have expanded rapidly across the globe, with significant contributions from countries such as China, South Korea, Japan, Germany, the United Kingdom, France, and more recently, nations within the Commonwealth of Independent States (CIS). Therefore, the entire era up until the 1950s could be regarded as nanotechnology's prehistory.

As a result of the scientific and technological revolution, circumstances for the development of controlled nanotechnology emerged near the end of this time. It is now commonly acknowledged that nanotechnology is considered "the next industrial revolution" and is becoming a significant contributor to 21st-century business success (Banerjee et al., 2020) (Langaroudi and Kazemi, 2019) (Manzoor et al., 2023) (Elshamy et al., 2021) (Cerqueira-Coutinho et al., 2015).

A. Nano formulation: Nano formulation refers to the process of creating nanoscale materials (1–100 nm) for specific applications, such as improving the delivery and efficacy of active agents in medicine, agriculture, cosmetics, and materials science. They are designed to overcome challenges like poor solubility, low bioavailability, and instability of bioactive compounds (Madej et al., 2022).

Key Components of Nano formulations:

1. Active Ingredients:
    - Drugs, bioactive compounds, or other functional molecules are encapsulated, adsorbed, or dispersed in the nano formulation.
2. Carriers**:**
    - Materials like polymers, lipids, or surfactants that form the structural basis of the nano formulation.
3. Stabilizers**:**
    - Substances like surfactants or emulsifiers that ensure stability and prevent aggregation.

Types of Nano formulations:

1. Nanoparticles**:**

    a. Engineered from polymers, metals, or lipids to encapsulate active agents.

b. Types include lipid based nanoparticles, metallic nanoparticles, and polymeric nanoparticles.

2. Nano-emulsions**:**

a. (W/O) Water-in-oil or (O/W) Oil-in-water systems stabilized by surfactants, offering high bioavailability and stability.

3. Liposomes:

a. Spherical vesicles made of lipid bilayers, used for both hydrophilic and hydrophobic drugs.

4. Nano micelles**:**

a. Formed by the self-assembly of amphiphilic molecules, ideal for solubilizing hydrophobic drugs.

5. Nano gels**:**

a. Hydrophilic networks capable of swelling, offering controlled drug release.

[2.2] NANOPARTICLES -

Nanoparticles have garnered significant attention over the past few decades due to their unique physicochemical properties, including quantum effects, enhanced reactivity, and high surface area-to-volume ratio, which distinguish them from their bulk counterparts. These materials, ranges in size typically from 1 to 100 nm, have been extensively studied for applications across diverse fields such as drug delivery, diagnostics, agriculture, and environmental remediation. According to Roco et al. (2003), the interdisciplinary nature of nanotechnology has accelerated innovation in material science and biomedical research. Various types of nanoparticles—including metallic (e.g., gold, silver), metal oxide (e.g., zinc oxide, titanium dioxide), polymeric, and lipid-based nanoparticles—have

been developed to address specific challenges related to bioavailability, controlled release and targeted delivery, of therapeutic agents.

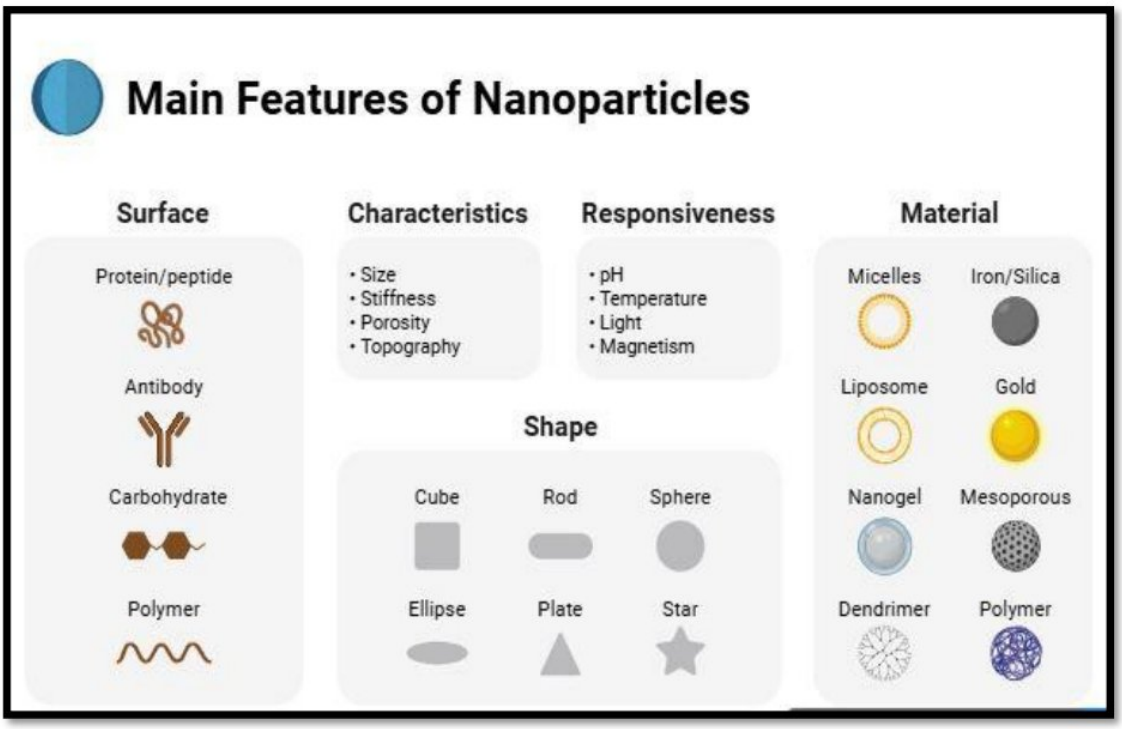

Fig.5. Main features of nanoparticle. (Source – Biorender icons)

Polymeric nanoparticles, in particular, have shown promise due to their biocompatibility and tuneable degradation profiles, which are ideal for biomedical applications. Furthermore, the surface modification of nanoparticles with ligands, antibodies, or polymers enhances their functionality and targeting efficiency, as demonstrated in various cancer therapy and imaging studies. Among the many bioactive compounds explored for nano-formulation, a natural polyphenol produced from *Curcuma longa*- curcumin has emerged as a prime candidate owing to its diverse therapeutic potential and poor aqueous solubility, making it an ideal molecule for nanoparticle-based delivery systems.

Brief Description about Curcumin:

Curcumin has also been shown to be a compound with hypoglycemia, cardio, and neuroprotective properties. It is the most noticeable polyphenol and is frequently ingested daily with food (in countries such as India, Eastern Asia, and some of Africa), improving cooperation. The use of curcumin dates back to ancient times, but it wasn't until 1949 that the journal "Nature" published the very first experimental proof of curcumin's antimicrobial effectiveness at concentrations of very minute levels. Only then did major research on the compound start. There have been over 8000-9000 peer-reviewed studies, reports, inventions, and views of preclinical and clinical trials during the past 20 years that demonstrate curcumin's potential as a medicinal chemical. Furthermore, the Food and Drug Administration of the US (USFDA) considers this chemical as GRAS i.e., generally recognized as safe and curcumin molecule has multi-targeting capacity in diverse pathological situations, allowing the development of nutraceuticals as well as a medicinal drug (Deka et al., 2016).

Fig 6. Curcumin. Source- (Food lab)

Curcumin, like numerous other lipid-soluble therapeutic small pharmacological compounds, has constraints for its effective application in analytical schemes to handle disease states. There are some examples: Minimal hydrophilic nature and inherent dissolution rate(s), low physicochemical instability, absorption with low

bioactive content, minimal pharmacokinetic and related bioavailability, and limited diffusion and targeted potency. These parameters have a substantial impact on the efficacy of curcumin as a medicinal chemical (Jahromi et al., 2014). Thus, various formulations of curcumin, comprising native, modified, and nano-forms, are manufactured as emulsions, creams, solutions, tablets, gel, and so on have been intended for conventional or exploratory administration to acquire the best results in a variety of clinical circumstances. For efficient local, circulation, affinities, or active targeting uses, both non-invasive and invasive methods of administration have been created. To deliver a proper drug concentration of curcumin to the illness site, personalized and customized ways of administration may be required. Despite a few crucial claims which should be taken into consideration, curcumin has many advantages, including its centuries-long use, exceptional pre-clinical and biological activity, and diagnostic, human, and animal trials boost the fast growth of curcumin-based preparations for medicinal purposes (Malik et al., 2022).

All these encouraging results have prompted scientists in creating and refining effective nano curcumin formulations for enhanced soluble, stable, intracellular efficiency, specificity, tolerance, and high therapeutic index. Some of these novel ingredients and formulations, first and foremost, improve the cellular targets' sustained release of drugs and boost the efficiency of therapeutics. Several nano-formulation-based techniques have been developed over the past years to improve all the use using curcumin in vitro and in vivo, and preclinical setup, including adjuvants, polymers, stabilizers, and other chemical moieties, hydrogels, lipids, and nanoparticles. Nanoparticles-based efficient curcumin helps in difficulties with solubility, rapid metabolism of the drug, hydrolysis, and stability of drugs and should also disseminate specific cells at the same time while reducing inadvertent damage at adjacent healthy tissues (Asif et al., 2023).

[2.3] IMPORTANT FINDINGS

Due to an increment in the cases of side effects of synthetic drugs, there is an enormous growth in the utilization of natural products in the past few years, and near about 80% of FDA-approved drugs used for cancer treatment are natural products or their by-products. A few of the substances, including resveratrol, and sulforaphane have demonstrated favourable efficacy against cells of breast cancer. Green tea catechin i.e., EGCG has been demonstrated to increase cell death in breast tumour cells via causing upregulation of genes like caspase 3, 8, 9 inducing pro-apoptotic pathways (Rai et al., 2020). Furthermore, the human telomerase reverse transcriptase i.e., hTERT gets inhibited by sulforaphane in a dosage and time-related manner. The mitochondrial membrane potential is raised by resveratrol and breaks it down to release cytochrome c, which then promotes cell death via multiple cascade enzymes. Curcumin, an active component in the culinary spice used as turmeric, has received special importance because of its numerous pharmacological effects like anticancer, and antioxidant activities. Curcumin's advantages, although, are hampered by its rapid first-pass metabolism from the liver and poor water solubility due to which system circulation is restricted. Due to this several nano based formulations are being developed to overcome such problems (Rezkita et al., 2024).

[2.3.1] Curcumin as a Phytochemical with Hydrophobic Properties in Medicine:

Curcumin Composition:

The chemical moiety of curcumin is (1E,6E)-1,7-bis(4-hydroxy-methoxyphenyl)-1,6-heptadiene-3,5-dione, which consists of 2 aromatic ring structures having methoxy and hydroxy groups linked by a 7-carbon-containing chain of the molecule containing unsaturated diketone. Generally, available commercial curcumin consists of 3 curcuminoids: bisdemethoxycurcumin

desmethoxycurcumin, and di-feruloyl methane. Curcumin exhibits a mechanism of tautomerism with keto and enol form, having keto form prevailing in acidic and neutral settings and the constant enol type prevailing in alkaline environments.

Due to its ionization capabilities, at neutral or acidic ph curcumin is less water soluble. It absorbs alkaline ethanol, methanol, ketone, CH_3COOH, DMSO, etc. Curcuminoids like diferuloylmethane which is responsible for the yellow colour of curcumin show different pharmacological and biological behaviour patterns of the substances (Duse et al., 2018).

Regarding the United States Food and Drug Association (FDA), various studies have been conducted in-depth on the inherent well-tolerance, safety, and biocompatible properties of pure curcumin.

[2.3.2] Curcumin as a therapeutic agent and Its Disadvantages –

However, CUR exhibits limited poor absorption, quick excretion, and bioavailability from the body. It is also not unstable in solutions and soluble in water. With these factors, CUR has been solubilized using solvents that are organically based like methanol, DMSO, ethanol, and acetone). These limitations make it difficult to employ CUR as a medicinal agent in vivo. Native curcumin has been shown to exhibit an unexpected potential in vitro, but due to its limited solubility in lipids of 0.6 g/ml, it exhibits very little to no action in vivo. Its non-specific circulation and insufficient aggregation after intravenous injection restrict its therapeutic effectiveness.

To have the intended therapeutic effects, curcumin must deliver its active ingredient to the most noticeable site of injury at a constant condition and quantity. Depending on each drug's specific therapeutic goal, the amount and dose may range from quick and complete absorption when a rapid commencement of

an action is necessary, as in the case of acute diseases like asthma or heart attacks, to slow and maintained when a longer circulation is required.

Therefore, more study on curcumin is needed to discover potential solutions to these constraints. By far, a number of biochemical developments have been shown as promising candidates for enhancing its bioavailability, including an adjuvant fusion alongside additional dietary variables, metals hybridization, curcumin-based lipid nano-formulations, its phospholipid structures and conjugation with polymeric compositions and artificially made analogs. Yet, all of the above research mentioned uses curcumin in its natural form (Woranuch and Yoksan, 2013).

As a result, many methods have been employed to address the issues with CUR, including the adjuvants use in drug delivery devices. Chitosan, a natural polymer, which is found in crustaceans shell and in chitin found in the cell wall of fungi, have been linked to CUR to increase its bioavailability.

[2.3.3] Antimicrobial Properties

CUR has been shown to have antibacterial activity against Gram-positive and Gram-negative bacteria, including those responsible for human illnesses and antibiotic resistance. CUR also inhibits the formation of microbes like biofilms out of bacteria, which are colonies of cells encased within the created matrix of polymers that are resistant to therapies associated with antimicrobial activity. CUR's antibacterial mechanism of action includes membrane or cell wall damage, disturbance with a cells-related cascade of processes via protein and DNA targeting, and restricts bacterial quorum sensing. Curcumin is effective in helping treat bacterial infections since it possesses antibacterial activity against bacterial pathogens. Curcumin has been shown to possess a variety of antimicrobial actions, including bacterial cell membrane rupture, inhibition of DNA replication, motility impairment, and changes in bacterial gene expression. The fact that curcumin works against both Gram-positive and Gram-negative bacteria makes

it a wide-spectrum antibacterial agent. Curcumin's amphipathic characteristics enable it to pass across bacterial cell membranes and render them permeable to antibiotic absorption.

Both types of bacteria gram positive and gram negative are prevented from growing by curcumin. The MIC (Minimum Inhibitory Concentration) of curcumin required for the prevention in development of Staphylococcus aureus MRSA) is found to be 125-250µg/ml, data collected from the research in vitro studies. In vitro, all of the Helicobacter pylori species were inhibited from growing in individuals with gastroenteritis using curcumin, one of the primary components of turmeric. The pour plate method was used by Negi et al to assess curcumin's antibacterial properties. *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* were among the bacteria against which various curcumin fractions were tested. They discovered that curcumin significantly inhibits all of the bacterial strains they had previously investigated (Keawchaoon and Yoksan, 2011).

Curcumin also works well against 20 different kinds of Candida. According to Pandit et al. assessment of the antibacterial properties of curcumin nano-formulations alongside the synthetic antibiotics gentamycin and chloramphenicol that are available for purchase, curcumin nanoparticles rank amongst the remaining synthetic antibacterial agents.

As a result, lotions containing curcumin or nano curcumin might be created to treat illnesses brought on by different bacteria. The principal infectious bacterium *S. aureus* may be found in the inner elbow, on the bottom of heels and side of the groin area. It is a gram-positive microbe that can cause skin and soft tissue infections, bacteremia, and pleuro-pulmonary infections, among other illnesses. Gram-negative *E. coli* bacteria are found mostly in the small intestine. Inguinal and perineal regions that have been exposed to urine and faeces can contain them. It is generally resistant to antimicrobials due to its robust cell wall containing

biological components like porins. This is because the bacterium's active efflux mechanism could eliminate the antimicrobials before they had a chance to do any damage.

[2.3.4] Antioxidant Properties

Antioxidant defenses, such as enzymes that neutralize free radicals (antioxidants) and antioxidant dietary ingredients, are present in all aerobic organisms and are used to either eliminate or rebuild injured molecules. By delaying the process of peroxidation of lipids, which constitutes one of the main causes of degradation of items related to food and pharmaceuticals while their preparation and preservation, the chemicals have antioxidant properties like scavenging of free radicals that cause damage and extend their shelf life. And the effects of reactive oxygen species and free radicals can be prevented by antioxidants. Both the oxidation of lipids and the progression of many chronic illnesses are slowed down by them. As a result, an alternative, natural, and safe source of dietary antioxidants needs to be identified as having a plant origin, and in recent years, interest in natural antioxidants, particularly those with a plant origin, has grown significantly.

Antioxidants are frequently employed as food additives in order to protect foods from oxidative food deterioration. Currently, propyl gallate, tert-butyl hydroquinone, butylated hydroxy anisole (BHA), and butylated hydroxytoluene (BHT) are the antioxidants that are most often utilized. But inflammation of the liver and tumorigenesis have been linked to BHA and BHT respectively. As a result, demand for natural and nontoxic antioxidants is expanding and one such natural compound is curcumin. Due to its considerable medical potential, it has received a lot of attention recently.

However, curcumin has a diketone moiety that exists in a cis, Trans, or enol form in solutions. The asymmetrical diphenolic dienone series, which contains

molecules that are kept or free of phenol-containing groups, is thought to be responsible for curcumin's antimicrobial characteristics. Curcumin includes hydroxyl, methoxy, and carbonyl, which are related to its antioxidant properties activity due to its ability to scavenge free radicals in vivo, especially peroxyl radicals (ROO) (Yang et al., 2010).

According to studies, the formation of units containing 2 and 4-hydroxyphenyl as well as an alkoxy- in ortho form groups with enhanced antioxidant activity. Few studies have shown that the treatment of curcumin activated endogenous cellular antioxidant mechanisms, which in turn led to the activation of the cytoprotective Nrf2 gene activated the target genes, which helps in protection against free radicals or reactive oxygen species (ROS) by scavenging them and protecting cells from ROS-induced oxidative stress. Therefore, another aim of this study is to investigate the DPPH radical scavenging activity of curcumin. Clarifying the antioxidant, radical-scavenging, and metal-chelating processes of curcumin was another significant major objective of this work.

[2.3.5] Drug Release Study

The properties of nano-curcumin are governed by both their physical and chemical compositions. The primary physicochemical properties of that enable it to be more powerful than conventional curcumin are its particle dimension, charge on the surface, its hydrophobicity and its surface area. Strong pharmacological qualities and effective target specificity, as well as other attributes, have been found to be associated with a better dissolution rate and greater bioavailability when administered orally. Its characteristics alter when curcumin's particle diameter varies on a nanoscale. It was shown that decreasing particle diameter enhanced its efficacy and lifted it above natural curcumin. In comparison to conventional curcumin, nano curcumin is thought to be a better adjuvant for use as a medication because of its larger surface area. It was 16 shown that nano curcumin has a higher capacity for intracellular absorption than

regular curcumin. This skill is necessary for identifying intracellular microorganisms in infectious diseases as well.

Nano-curcumin dissolves more quickly physiologically through tissues and plasma than free curcumin does. Ma et al. demonstrated in an in vivo investigation on rats that nano curcumin enhances biocompatibility and nanoparticle dispersion in tissues by offering a 60 times-fold increase in biologically related half-life in comparison with the administration of native curcumin (Aydogdu et al., 2020).

Numerous types of cancer cell lines have been used to examine the cytotoxicity using curcumin Nano-formulations. Due to the long-term exposure of cells to high static levels of curcumin (whether it's in its free form or in its nano-formulation), which are not always correlated with the concentrations attained in vivo, makes it difficult to interpret relevant results. Yallapu et al. showed that the prolonged release of the active ingredient made the Nano-CUR6 formulation's intracellular retention of the drug superior to that of free curcumin (when dissolved in DMSO).

Furthermore, this formulation dramatically increased cellular absorption in MDAMB-231 metastatic breast cancer cells and A2780CP cisplatin-resistant ovarian cancer cells by two and six times, respectively, in comparison to free curcumin.

[2.3.6] Synthesis of Curcumin Nanoparticles

To generate nano curcumin, a variety of methods have been devised. Wet milling, spray pyrolysis, anti-solvent precipitation, ionic gelation, solvent evaporation, and the Fessi technique are used. Curcumin nanostructures have higher stability and solubility, according to extensive literature evidence. The ionotropic gelation process is based upon the propensity of polymer composites to cross-link mostly when counter ions are present. This strategy has emerged as among the most

effective. This approach has emerged as one of the most efficient ways of creating bioactive, nontoxic, biocompatible, and environment-friendly polymers like chitosan.

As a result, numerous research has been carried out to investigate the ability or usage of organic polymer nanoparticles (alginate/chitosan) for curcumin delivery via oral methods. Das et al. created a nano-formulation that is curcumin-based having chitosan or alginate composites and used an ionic gelation technique to deliver them to malignant cells. Anti-solvent precipitation is another widespread and practical approach for producing curcumin nanoparticles, where success is regulated via agitation temperature, rotation speed, and duration. Biopolymers also increase the stable nature and solubility of curcumin nanostructures. Because it is a simple procedure, it can be employed in the various industrial processes of medicinal nanomaterials.

[2.4] PROPOSED SOLUTIONS

Curcumin-loaded nanoparticle administration has been found that alter its pharmacology profile, hence increasing its chemically induced therapeutics agent's potential. PLGA, chitosan, and alginate biopolymers, conjugated micelles, are some of the nano-drug delivery vehicles used for curcumin distribution. Aside from the composition of the medicine, the selection of material is heavily influenced by its physicochemical qualities and mode of administration.

Current findings from some papers have demonstrated that encapsulating curcumin in Peg-PLGA NPs effectively enhances its liquid suspension and its bioavailable property, resulting in a significant increase in its medicinal and chemo-sensitizing efficiency. However, with PLGA being highly expensive it is not economically beneficial to use it as a drug delivery system for a long duration in chemoprevention. So, to tackle the above problem in this current work chitosan

is being used as it is cost-effective and acts as a biocompatible nano-carrier, and can be given orally in a wide variety of populations Furthermore, chitosan is a mucoadhesive that behaves as a greater nano-based carrier of quercetin and curcumin for cancer prevention than another form of nano based carriers.

Chitosan is a polysaccharide which is linear that is formed via the deacetylation of chitin and its structural components from crabs and shrimp shells or other crustaceans and is composed of β (1,4)-linked N-acetyl glucosamine units. Because of their biodegradability and biocompatibility, as well as their ability to enclose hydrophobic medicines, chitosan, as well as its chemically induced versions, like chitosan carboxymethyl, is a very much studied nano-carrier.

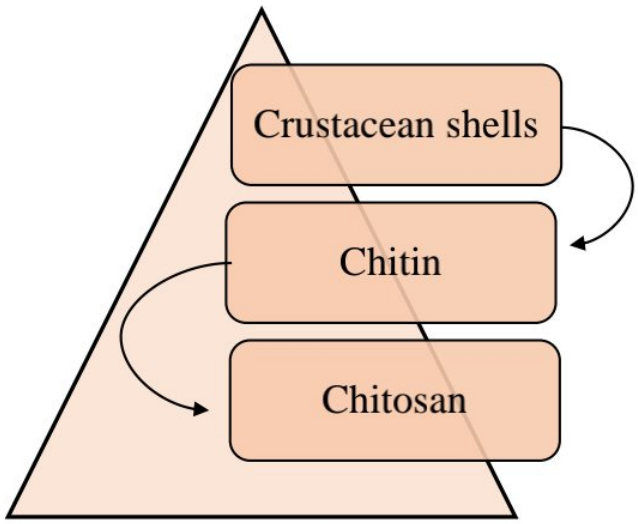

Fig 7. Schematic presentation of chitosan from chitin through crustacean shells.

Chitosan, which is available commercially, has a percentage of deacetylation ranging from 60-100% and molecular weight varying from 3500- 20,000 Da. In drug delivery systems, chitosan formulations with a higher degree of deacetylation are desirable since a higher level of deacetylation is associated with

a faster disintegration rate. The FDA has approved chitosan as nontoxic for utilization in medical applications. The process of ionic gelation has distinct advantages over other forms of nanoparticle creation, such as emulsified extraction, spray drying, or micelle formation. TPP, the cross-linking agent, is non-toxic and has a high gelling capacity. The manufacturing process is straightforward; no harmful chemicals are required, and the zeta potential and particle size may be easily controlled by varying all the concentrations of chitosan and TPP.

Numerous characteristics make CS an appropriate option to be used in the current investigation. For starters, it is regarded as biocompatible with living tissues, which means will not induce hypersensitive reactions within the body. Chitosan is biodegradable, producing non-hazardous and effortlessly removed breakdown products when fermented by bacterial colonies. Chitosan's distinctiveness stems from its multiple beneficial qualities, which allow it to be used in a wide range of industries.

This moderate-cost material is biodegradable, biocompatible, and has low toxicity, making it an FDA-approved substance that may be utilized effectively in the pharmaceuticals, bio-medical, food, or cosmetic sectors. The physiological and biological characteristics, such as antifungal, anti-inflammatory, antibacterial, or antioxidants, actions, are also important.

They can be utilized effectively for medicinal purposes as well as in other industries such as agriculture, food production, and other domains (for example, the textile industry). Furthermore, chitosan's strong adsorption ability is employed in ecological uses to remove hazardous substances from water-based solutions.

Toxic organic solvents don’t require addressing during formulation given that it is quite soluble in an acidic aqueous medium. It is easily cross-linked with diverse

anions because it is a polyamine linear form having lots of free amine groups. It can also be designed as a sustained release matrix. Chitosan works as a concentration enhancer by releasing the epithelial tight junctions. As seen in Figure 8, chitosan enhances either paracellular or drug delivery via transcellular mode. Chitosan is involved in the formation of a compound with negatively charged mucus via noncovalent interactions like ionic bonding, hydrophobic interactions as well as hydrogen bonding. Also, the main amine in chitosan has a pKa of 6-6.5, because of the level of N deacetylation. Amine molecule helps in chitosan's solubility strength in acidic ph environments.

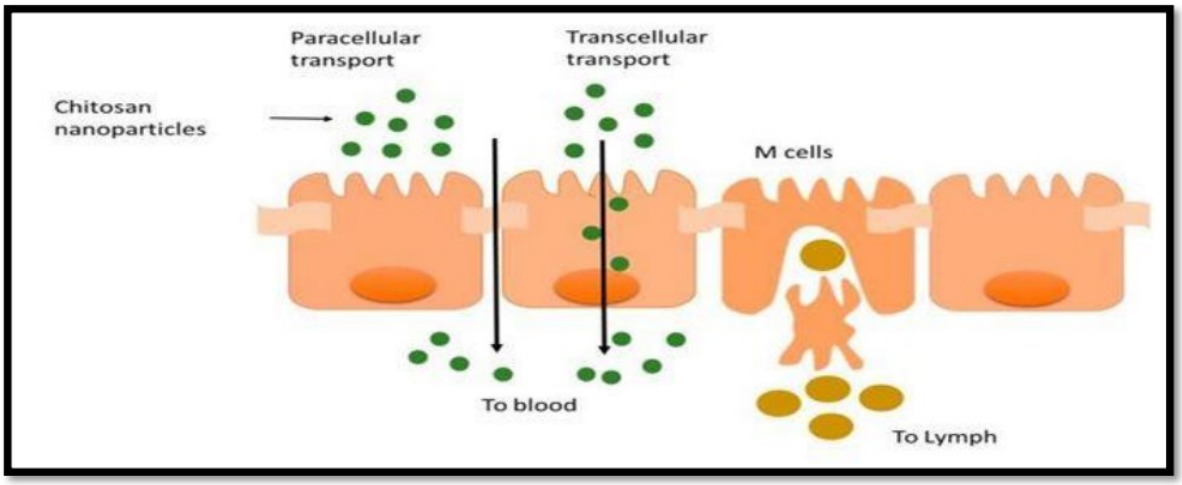

Fig 8. Schematic illustrations of chitosan nps transport. Source: (Pharmaceutics. 2017 Dec; 9(4): 53.)

When considering chitosan for use in pharmaceutical applications, it's also crucial to take its stability into account. Due to its high hygroscopic property, chitosan is extremely sensitive to environmental factors, particularly humidity. Chitosan retains water by hydrogen bonding, which has been shown to alter its mechanical characteristics and partially reduce its mucoadhesive qualities. At both room temperature and 60℃, thermal deterioration of solutions containing chitosan was also seen.

These factors make low-temperature storage (2-8°C) in a dry environment preferable. Due to its muco-adhesive qualities and permeability-improving effects, nanoparticles of chitosan are also especially well-suited for local distribution at the skin's surface mainly dermis and mucosal region. Chitosan nanoparticles are a highly promising and adaptable approach to overcoming bioavailability and stability problems of the majority of active substances. They combine the inherent features of polymers with tuneable dimensions and the potential for surface alteration and other modifications according to custom demands. The production of innovative therapeutic drug release mechanisms with higher bio-availability, greater sensitivity and specific target mechanism, and decreased toxicity from pharmaceutical perspective has made chitosan nanoparticles a hot topic in the field of nano medicine, biomedical engineering, and these days.

The size and shape of CSNP may have an impact on certain activities. The unique chitosan nanoparticles, which are made up of aggregates of NP's diameters ranging from 10 to 100 nm, have prospective applications in the sectors of pharmaceuticals, medical engineering, industrial, and nano medicine. The amazing physicochemical and biological properties of the chitosan-based nano systems, as well as their ability to change protein loading and adjust parameter values while manufacture process, make them suitable for application as high-tech drug delivery systems. Additionally, they may be manufactured as a frozen or lyophilized powder, have excellent protein packing efficiency, and are simple to store and transport.

Chitosan and its analog superficially perforate and exert non-proliferative effects in different ways like antioxidant-based defences, apoptosis, and enzyme modulation via modulating the signalling molecule pathways like nuclear factor k light chain enhancer of activated B cells [NF kb] and chitosan controlling cell cycle markers, TGF and induce apoptosis.

The primary goal of gene therapy is to input any type of genetic substance into the targeted cell paving the path for the control of genomic expression. The oppositely charged gene, on the other hand, is incapable of reaching the plasma and cell membrane via a passive diffusion mode. Furthermore, introducing DNA straight inside the cells results in fast nuclease breakdown.

The success of gene therapy is dependent on several factors, including the ability to target specific cells, protect nucleotides from degradation in the external medium of cells, and transport sufficient quantities of nucleic acid to produce a curative impact.

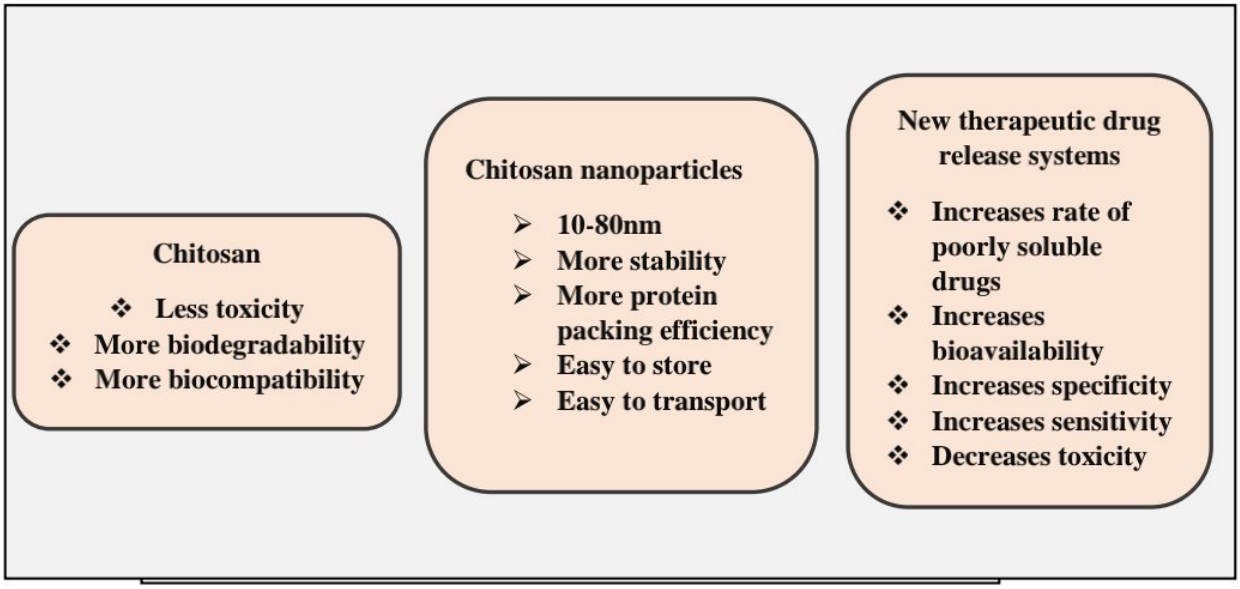

Fig.9. Advantages of chitosan nanoparticles. Sources – (Self made)

It is having low level of toxicity. It has been suggested that taking oligo chitosan/chitosan's may shield the body from oxidative stress brought on by cancer. While their increased penetration qualities are primarily responsible for their anti-metastatic action. A higher chitosan content inhibited the movement of MDA-MB-231 female breast cancer cell line. Another method by which chitosan exerts its anticancer properties is through improving the bio-distribution of

medicines. Due to chitosan's improvement of cell permeation and drug retention duration to low toxicity, the medication accumulates in tumour cells.

[2.4.1] Preparation of Chitosan Nanoparticles

Ionic gelation, which was initially described by Calvo et al., (1997), is one of the most popular production techniques for chitosan nanoparticles, which have been studied for more than 20 years. Its foundation is ionic crosslinking, which takes place in the presence of negatively charged polyanion groups like sodium tripolyphosphate (TPP) and positively charged amino chains of chitosan.

Chitosan is dissolved in an acidic aqueous solution (usually an acetic acid solution), and then aqueous TPP solution is added while being forcefully agitated. Positively charged chitosan molecules spread out and crosslink with one another, creating nanoparticles shows how TPP and chitosan form an electrical connection that causes spherical nanoparticles to develop. After a few centrifugation and water-washing steps, chitosan nanoparticles are created by either freeze-drying or oven-drying. It employs a straightforward process devoid of dangerous cross-linkers and solvents. Furthermore, the process can be carried out at a controlled room temperature. By altering the ratio of chitosan to TPP, the final size of the nanoparticle may be changed, which is a crucial factor that directly affects how well drugs are packaged and delivered.

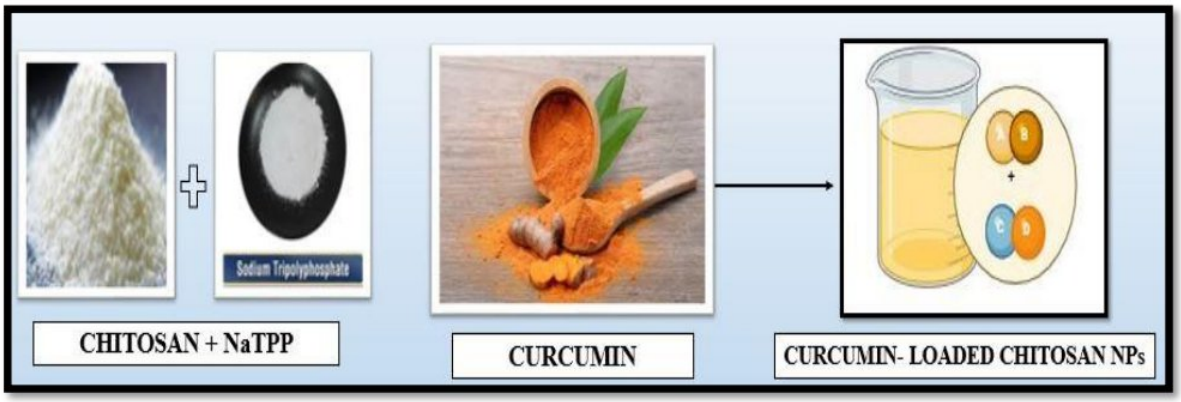

Fig 10. Schematic presentation of curcumin-loaded chitosan nanoparticle. Source- (Self made from Biorender)

To ensure the successful synthesis and application of such nanoparticles, it is essential to thoroughly characterize their physical and chemical properties. Various analytical and imaging techniques are employed to evaluate parameters such as morphology, surface charge, particle size, and structural composition. Some commonly used techniques are :-

2.4.2 UV-Vis spectroscopy

UV-Vis spectroscopy, by stimulating electrons from their ground to excited states (absorption) and relaxing them from their exciting to ground states (emission), one can ascertain the electronic structures of atoms, ions, molecules, or crystals. In gaseous, liquid, and solid states, it studies electronic transitions between the orbitals or bands of atoms, ions, or molecules. It has been shown that tiny objects may both absorb and disperse electromagnetic energy. Since metallic nanoparticles absorb electromagnetic radiation from the coherent oscillation of valence band electrons brought on by an interaction with the electromagnetic field, UV-Vis spectroscopy can be used to study the unique optical characteristics of nanoparticles. These resonances are unique to nanoparticles and are referred to as surface plasmons.

Fig 11. UV-Vis Spectrophotometer

2.4.3. Fourier Transform Infrared spectroscopy (FTIR)

In (FTIR) Fourier transform infrared spectroscopy, photon-species interactions that result in energy transfer to or from the sample via vibrational excitation or de-excitation are used as a vibrational tool for characterization. Chemical bonds in the detected materials are revealed by these vibrational frequencies. It deals with the different frequencies at which chemical bonds in molecules vibrate based on the elements and bond types. When a bond absorbs electromagnetic radiation, its vibration frequency rises, causing it to move between its ground state and a number of excited states. The measurement can be used to look into whether protein molecules are present in the solution because the FT-IR spectra fall between 1400 cm-1 and 1700 cm-1. The infrared portion of the electromagnetic spectrum is represented by the energy connected to these frequencies.

2.4.4 Dynamic Light Scattering (DLS) –

It is among the most popular methods for figuring out the polydispersity index (PDI) and particle size distribution of suspended nanoparticles. The principle of DLS is based on the Brownian motion of particles in a liquid medium and the scattering of a laser light beam as it passes through the colloidal dispersion. The fluctuations in the intensity of the scattered light are measured over time, which allows for the calculation of the hydrodynamic diameter of the particles. DLS provides rapid and non-invasive measurements and is especially useful for assessing nanoparticle stability, size uniformity, and aggregation behaviour. The PDI value obtained from DLS also gives insight into the homogeneity of the sample; a lower PDI indicates a more uniform population of nanoparticles. Due to its sensitivity and ease of use, DLS is an essential tool in the characterization of nano-formulations such as curcumin-loaded polymeric nanoparticles.

2.4.5 Scanning electron microscopy –

A sophisticated imaging method that is frequently used to investigate the surface composition and shape of different materials at high magnification and resolution is scanning electron microscopy, or SEM. It works by moving a concentrated beam of high-energy electrons over a specimen's surface. In order to create detailed images, the electrons interactions with the material produce signals like secondary electrons, backscattered electrons, and distinctive X-rays. The secondary electrons provide fine topographical details, while backscattered electrons offer compositional contrast based on atomic number differences. Since SEM requires samples to be electrically conductive, non-conductive specimens are usually coated with a thin metal layer like gold or platinum. The technique is invaluable in fields such as materials science, nanotechnology, biology, and forensics, enabling researchers to investigate surface structures. And SEM offers significant advantages such as high resolution and depth of field.

Table 1:- Research in the current topics

Study	Findings	References
1. Eugenol-loaded chitosan nanoparticles: I. Thermal stability improvement of eugenol through encapsulation.	The study successfully encapsulated eugenol in chitosan nanoparticles, achieving a loading capacity of 12% and encapsulation efficiency of 20%, with particles smaller than 100 nm, enhancing its thermal stability by 8-fold during extrusion with thermoplastic flour.	(Woranuch and Yoksan 2013)
2. Synthesis of curcumin-loaded chitosan phosphate nanoparticle and study of its cytotoxicity and antimicrobial activity	The curcumin-loaded (CPNs) showed 84% loading efficiency. DLS analysis revealed a size increase from 53 nm (blank CPNs) to 91 nm (curcumin-loaded CPNs), confirmed by TEM (94 nm). It shows significant antimicrobial activity against Gram-positive, Gram-negative bacteria, and fungi, while cyto-compatibility tests with PBMCs and murine cells confirmed their safety for therapeutic applications.	(Deka et al. 2016)
3. Curcumin-loaded chitosan tri-polyphosphate nanoparticles as a safe, natural and effective antibiotic inhibits the infection of staphylococcus aureus and pseudomonas aeruginosa in vivo	The nanoparticles had a size of 160 ± 10 nm with a +7 ± 2 mV surface charge, ensuring stability. They showed 75 ± 2% encapsulation efficiency for curcumin and demonstrated a synergistic antibacterial effect stronger than curcumin alone.	(Jahromi et al. 2014)
4. Preparation, characterization and in vitro release study of Carvacrol-loaded chitosan nanoparticles	The nps had a spherical shape with an average size of 40–80 nm and a positive zeta potential (+25 to +29 mV), and also effectively inhibited S. aureus, B. cereus, and E. coli, with a minimum inhibitory concentration (MIC) of 0.257 mg / mL. EE was from 14–31%, with 3–21% loading capacity (LC). The optimal chitosan-to-carvacrol ratio was 1:1.	(Keawchaoon and Yoksan, 2011)
5. Effect of tween 80 on nanoparticle preparation of modified chitosan for targeted delivery of combination doxorubicin and curcumin analogue	The addition of 0.5% Tween 80 produced nanoparticles with an average size of 111.8 ± 4.11 nm, smaller than those without active substances. Tween 80 as a stabilizer, reduces surface energy and preventing crystal growth, resulting in smaller, more stable particles. The chitosan-FA conjugation was confirmed through UV spectroscopy, ensuring successful binding.	(Sukmawati et al., 2018)

CHAPTER 3

MATERIALS AND METHODS

[3.1] Materials: Chitosan (medium molecular weight), Curcumin, NaTPP (Sodium Tri-polyphosphate) from Sigma Aldrich, Acetic Acid (Loba Chemie), Ethanol, DPPH (Himedia Lab.), Muller Hinton Agar, Muller Hinton Broth, E.coli (ATCC 25922), B.subtilis and Xanthomonas (obtained from CSIR-IHBT, palampur), Deionized water (Millipore Q.) All solvents used in this study are of analytical grade.

[3.2] Methodology

[3.2.1] Preparation of Chitosan Based Nano-formulations

A generalized method used for synthesizing nanoparticles using polymer is the Ion Gelation method, which was first described by (Woranuch and Yoksan, 2013).

Chitosan medium molecular weight (1.2% (w/v) was dissolved in 100 mL of 1% v/v solution of acetic acid with continuous vigorous stirring in a magnetic stirrer at room temperature (RT) at 600rpm for overnight to form a clear CS acidic solution. To create a homogenous mixture, 40 ml of CS solution were mixed with Tween 80 and swirled for 30 minutes at 50°C. Drop by drop, while continual magnetic stirring, 20 ml of TPP solutions [0.5%, w/v] was added into the 40 ml CS acidic solution. The solutions were then agitated for another 30min. The pH of the solutions varied between 3-5.

CS-loaded Curcumin nanoparticles (CSCURNP) were obtained when (30mg/ml) of Cur dissolved in 10ml of ethanol into 40ml CS solution and 20ml of (0.5%) TPP solution. In this study, the concentration of CS and solvent were optimized

by preparing different ratios of mixing concentrations. After centrifuging some of the generated particles for 10 minutes at 25°C at 10,000 rpm, they were occasionally cleaned with water.

[3.3] Physical and Chemical Characterization:

[3.3.1] UV-Visible Spectroscopy

Using a Thermo Fisher Scientific Spectrophotometer, the UV-visible absorption spectrum of the samples was produced in order to investigate the optical absorption characteristics of the photocatalyst. Understanding the spectral characteristics of chitosan nanoparticles was made possible by spectroscopy, that were recorded at constant room temperature within the wavelength of 200-800 nm (Khan et al., 2016).

[3.3.2] Fourier Transformed Infrared Spectroscopy (FTIR)

Then, using a Nicolet IR100 FTIR Spectrometer (Thermo, USA), the Fourier transformed infrared (FTIR) spectra of curcumin (Cur), and curcumin-loaded chitosan-TPP nanoparticles (Cur-CSTPP) were evaluated (Khan et al., 2016).

[3.3.3] Particle Size and Zeta Potential

Dynamic light scattering (DLS) was used to measure NP's hydrodynamic dimension, poly-dispersity index at a particular length, and detection angles. To minimize multiple dispersions, all the samples were liquified/diluted from deionized water. NP comprising curcumin, and curcumin-loaded chitosan nps that have the proper size, and better zeta potential properties were named CSNP, and CSCURNP, and subjected to further testing.

[3.3.4] Scanning Electron Microscopy (SEM)

SEM (Hitachi) was used to investigate the morphology of the particles. Centrifugation was used to separate the CNP for 30 minutes at 40,000 g. The pellet was freeze-dried using a lyophilizer then the supernatant was decanted before being analyzed by SEM at a 15.0 kV accelerating voltage. On a graphite surface, a single drop of nanoparticle was applied, and after the sample had dried, it was covered with gold utilizing ion sputter.

[3.3.6] Drug Release Study

A measured quantity of CSNP, and CSCURNP, filled in dialysis membrane was kept in deionized water of a particular concentration pH 5, pH 7.4, and temperature 40°C and 50°C incubated at room temperature. In a time-lapse of 1,2,3,5,10,24 hrs., the solvent absorbance was measured using UV-VIS spectrophotometer at around 420nm which determined the quantity of drug produced by nanoparticles. To maintain total volume, a constant concentration of D.W. was withdrawn at specific given time intervals and substituted with an equal number of fresh buffers. The quantity of curcumin produced by the nanoparticles will be determined using an ultraviolet spectrophotometer at around 420nm.

Bioactive properties determined by:

[3.4] Antioxidant Assay

The free radical scavenging test to check the capacity of chitosan-based nano-formulations was evaluated using the standard 2,2-diphenyl-1-Picrylhydrazyl DPPH assay. It is a free radical method based on electron transfer). The antioxidant's IC50 was determined. To determine the radical scavenging activity (RSA) formula RSA (%) = [(Acontrol - Asample)/Acontrol] /100 is used.

[3.5] Antibacterial Activity

The antibacterial action of NP will be tested using different gram-positive or negative bacteria and the micro-dilution technique in a 96-well microtiter plate at concentrations ranging from zero to 400 g/mL via serial dilutions, by Clinical and Laboratory Standards Institute (CLSI, 2018). The Minimum concentration at which colour change happens is the minimum inhibitory concentration (MIC).

Aliquots of materials from 96-well plates were utilized to determine the MIC of specimens that were transmitted to Petri plates for further microbial growth culture parameters and the Antibiotic Sensitivity Test (AST) was performed using Kirby Bauer Method at different concentrations and the zone of inhibition (ZOI) was calculated using diameter.

CHAPTER 4

RESULTS AND DISCUSSION

[4.1] OPTIMIZATION OF THE CHITOSAN-BASED NANOPARTICLE

The shift in the colour change of the chitosan solution from faint transparent colour to turbid solution shows the formation of chitosan nanoparticles (CSNP).

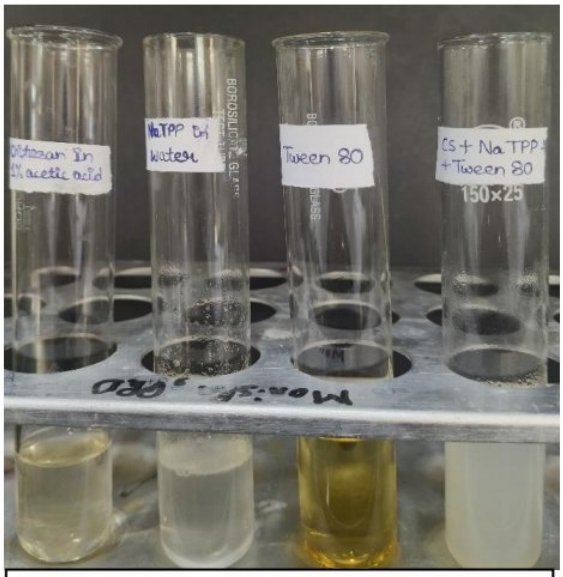

Fig 12. Formation of CSNP

Similarly, colour change in the solutions confirmed one of the parameters for the formation of CSNP, and CSCURNP nano-formulations.

Fig 13. Formation of CSCURNP

[4.2] CHARACTERIZATION OF CS-BASED NANOPARTICLES

[4.2.1] UV-Visible Spectroscopy

The UV spectra of CS nanoparticles, and Cur-CSTPP nanoparticles are shown in the following figures. The scanning of the solution takes place with CSNP provided an Absorption peak at around 350nm which preliminary confirmed the formation of CSNP.

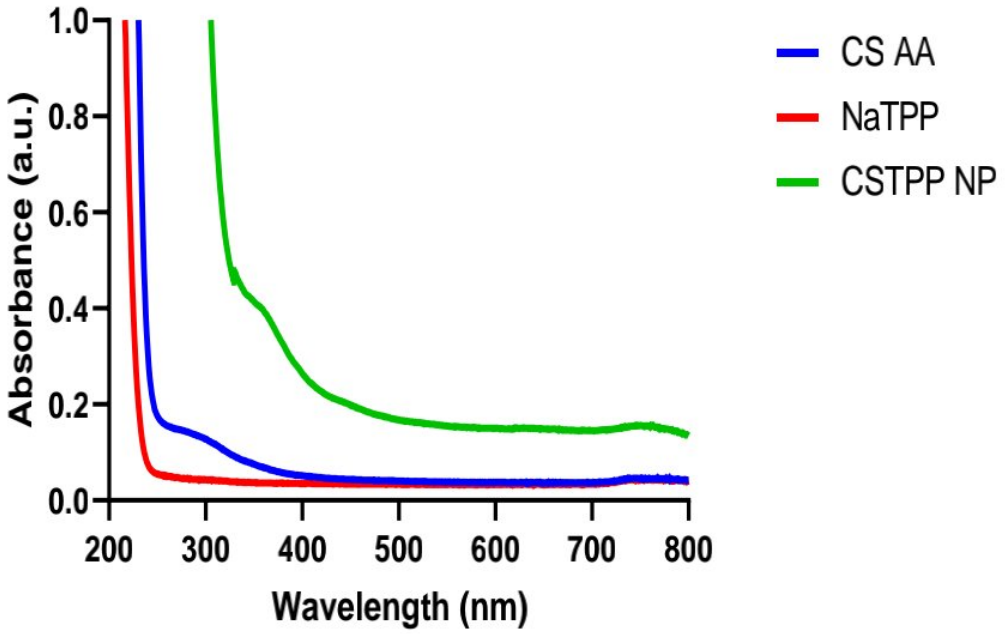

Fig 14. UV-VIS spectra of CSNP

Similarly, the absorption peak of CS-CURNP between 400-500 nm confirmed the formation of chitosan-based curcumin-quercetin nanoparticles. The peak intensity of curcumin-loaded chitosan nanoparticles (CSTPP) was higher in comparison to pure curcumin.

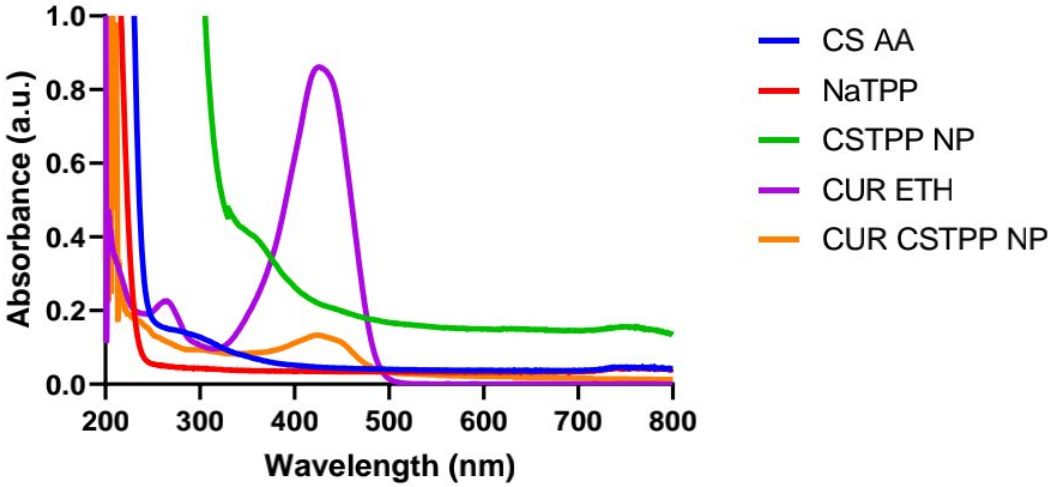

Fig 15. UV-VIS spectra of CUR-CSNP

[4.3] ANTIMICROBIAL ASSAYS

The Antimicrobial activity of chitosan nanoparticles with the incorporation of curcumin and quercetin nanoparticles was investigated against *E. coli DH5α* (ATCC68322), *Xanthomonas*, and *B. subtilis* using well diffusion assay. The antimicrobial activity increased the most in CSNP with curcumin nps in a (1:1) ratio showing the synergistic killing of bacteria and significant zone of inhibition (ZOI). Improved antibacterial activity of CSCURNP was related to their large surface area that arranged to have more interactions on the surface with microorganisms.

S. No.	Nanoformulation	Concentration (µg/ml)	E.coli DH5α ZOI (mm)	B. subtilis ZOI (mm)	Xanthomonas ZOI (mm)
1.	CSNP	50	0	0	0
2.	Curcumin+ Ethanol	150	1	0	0
3.	CSCURNP	150	4± 2	7± 2	11± 3

Table 2. ZOI of different Nano-formulations

Fig 16. AST of CUR-ETH, CUR-CSNP against *E.coli*

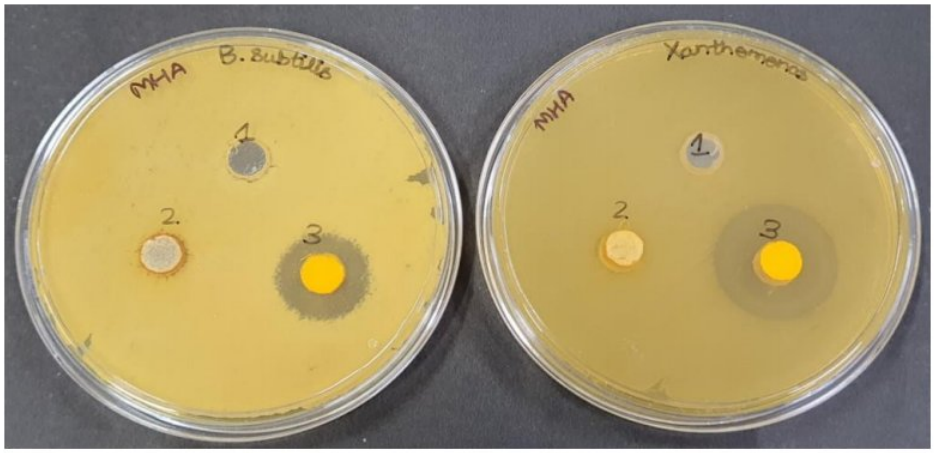

Fig 17. AST of CSNP, CUR-CSNP against *B.subtilis* and *Xanthomonas*.

Another antimicrobial test used to determine the activity is the (MIC) minimal inhibitory concentration using 96 well plates taking E. coli DH5α as culture and (MHB) Muellor Hinton Broth as culture medium. The MIC concentrations of the nano-formulations are depicted in the following table:

S.No.	Nano-formulation	Minimum Inhibitory Concentration (MIC) mg/ml (E.coli DH5α)
1.	CS-TPPNP	0.30
2.	CUR-ETH	0.0321
3.	CUR-CSTPP NP	0.0280

Table 3. MIC of different nano-formulations

4.4 ANTIOXIDANT ASSAYS

DPPH Assay –

The capacity of the suggested antioxidant to donate hydrogen to DPPH free radicals can be determined in part by the DPPH radical scavenging test. At 517.0 nm, the stable radical DPPH glows purple. In several in vitro investigations, curcumin has previously demonstrated its potential to scavenge free radicals against the DPPH radical. However, when curcumin is dissolved in an organic solvent, the majority of documented literature has demonstrated its efficiency in DPPH scavenging (Borra et al. 2013). The IC50 value has a negative correlation with the sample's capacity to scavenge free radicals or function as an antioxidant.

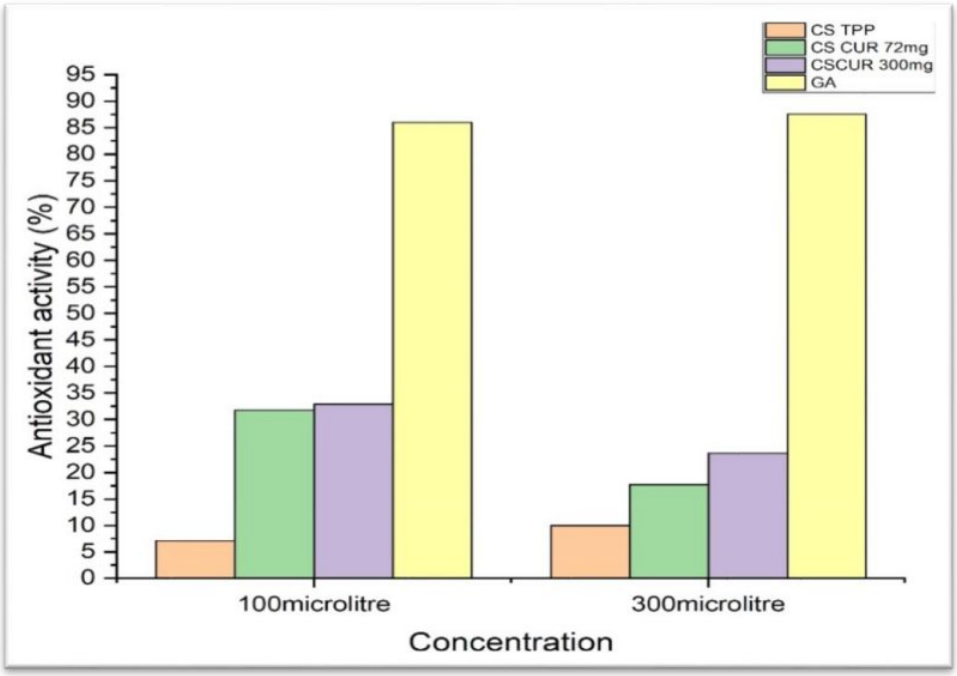

Fig 18. DPPH Scavenging activity of CSNP and CSCURNP

DISCUSSION

Curcumin-loaded chitosan nanoparticles were created primarily to overcome the difficulties in administering curcumin, such as its instability, low bioavailability, and poor solubility. Chitosan nanoparticles loaded with curcumin were successfully synthesized, formulated, and physico-chemically characterized. The drug's controlled release, cellular absorption, bio-distribution, and physical stability all showed notable improvements. The small size and spherical morphology of the nanoparticles enabled efficient passive targeting of tumour tissues, capitalizing on the enhanced permeability and retention (EPR) effect. The encapsulation of curcumin within the nanoparticle matrix was validated through SEM imaging, FTIR spectroscopy, and DLS analysis.

This study proposes a novel strategy for treating and targeting multidrug-resistant cancers and microbial infections by leveraging a drug-loaded nano-particulate system, as supported by various literature (Li et al., 2016). The Noyes-Whitney equation states that decreasing particle size significantly increases the interfacial surface area, which improves the rate of dissolution, water solubility, and eventually the bioavailability of medicinal substances. Moreover, the expanded surface area improves the pharmacological activity of the drugs and their interaction with specific molecular targets (Keawchaoon and Yoksan, 2011).

Since chemotherapeutic agents like curcumin, quercetin, and piperine are inherently hydrophobic, their therapeutic efficacy is often limited by reduced bioavailability. To control this limitation, a nanoparticle-based drug delivery system was engineered to enhance both bioavailability and biological reactivity. Additionally, these natural bio-enhancers possess a wide range of pharmacological activities, including potent anti-cancer properties.

Based on this rationale, curcumin was encapsulated within chitosan nanoparticles to combat multidrug resistance, enhance bioavailability by minimizing intestinal and hepatic metabolism, and achieve a synergistic therapeutic effect. The ionic gelation process was used to create the chitosan nanoparticles (CSNPs). The ratio of chitosan to TPP (tripolyphosphate) was optimized to achieve a uniform nanoparticle size distribution. The biological activity of TPP/CS nanoparticles is largely determined by their physicochemical characteristics. Chitosan, containing amino groups, becomes positively charged in acidic conditions and dissolves more readily, whereas TPP, a negatively charged anion, acts as a crosslinking agent. CSNPs are created by the electrostatic interaction of TPP with chitosan. Because chitosan molecules are arranged closely together at greater concentrations, it was found that increasing the concentrations of both chitosan and TPP initially produced larger nanoparticle sizes. Positively charged nanoparticles in the size range of 100–150 nm were produced by dissolving 40g/100 mL of chitosan in 3% acetic acid and 0.5% TPP solution, which produced the best results (Woranuch and Yoksan, 2013).

Following the making of CSNPs and CSCURNPs (chitosan-curcumin nanoparticles) via ionic gelation, several characterization techniques were employed. UV-Visible spectroscopy confirmed nanoparticle formation with absorption peaks around 300 nm and 400 nm. SEM analysis revealed particle sizes ranges from 80 to 100 nm. While SEM provided projected area diameter, DLS offered measurements of the hydrodynamic diameter, considering the solvent layer around the nanoparticles. This information is crucial for understanding nanoparticle behaviour in liquid media and their interaction with biological systems. Encapsulation efficiency and drug loading capacity were also calculated, confirming successful incorporation of curcumin within the chitosan matrix (Elnawasany et al., 2023).

Drug release profiles were studied using a dialysis membrane method over different time points (1 hr, 2 hrs, 3 hrs, 10 hrs, and 24 hrs), demonstrating sustained release behaviour. Antimicrobial assays showed a synergistic enhancement of antimicrobial activity in CSCURNPs compared to individual formulations, as evidenced by larger zones of inhibition and lower minimum inhibitory concentrations (MICs) (Jin et al., 2019). Strong antioxidant activity was observed using DPPH assays, with CSCURNPs exhibiting high radical scavenging potential, indicating potent antioxidant properties with minimal toxicity at lower concentrations, confirming their potential for therapeutic applications.

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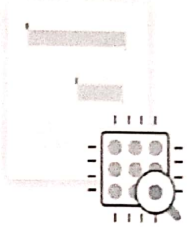

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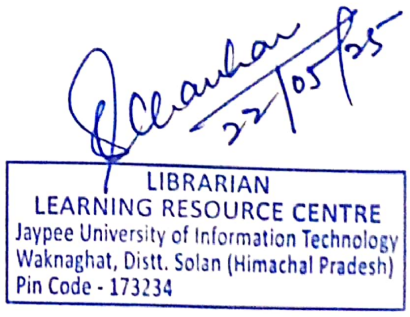

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