Chitosan Nanoparticles as Drug Carriers

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Abstract- **Chitosan is a natural polymer obtained by deacetylation of chitin. After cellulose chitin is the second most abundant polysaccharide in nature. It is biologically safe, non-toxic, biocompatible and biodegradable polysaccharide. Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method and providing versatile routes of administration. Their sub-micron size is also suitable for mucosal routes of administration i.e. oral, nasal and ocular mucosa which is non-invasive route. Chitosan nanoparticles showed to be a good adjuvant for vaccine. Therefore, the objectives of this review are to summarize the available preparation techniques and to discuss various applications of chitosan.**

Keywords - Nanoparticles, Drug delivery carriers, polymers.

I. INTRODUCTION

Nanotechnology is the engineering of functional systems at the molecular scale. This covers both current work and concepts that are more advanced. In its original sense, nanotechnology refers to the projected ability to construct items from the bottom up, using techniques and tools being developed today to make complete, high performance products.

One nanometer (nm) is one billionth, or 10−9, of a meter. By comparison, typical carbon-carbon bond lengths, or the spacing between these atoms in a molecule, are in the range (0.12–0.15)nm and a DNA double-helix has a diameter around 2nm. On the other hand, the smallest cellular life-forms, the bacteria of the genus mycoplasma, are around 200 nm in length. By convention, nanotechnology is taken as the scale range 1 to 100 nm following the definition used by the National Nanotechnology Initiative in the US.

The lower limit is set by the size of atoms (hydrogen has the smallest atoms, which are approximately a quarter of a nm kinetic diameter) since nanotechnology must build its devices from atoms and molecules. The upper limit is more or less arbitrary but is around the size below which phenomena not observed in larger structures start to become apparent and can be made use of in the nano device. These new phenomena makes nanotechnology distinct from devices which are merely miniaturized versions of an equivalent macroscopic device; such devices are on a larger scale and come under the description of micro – technology.

The advent of scanning tunneling microscope and atomic force microscope in the 1980s has essentially in the nano era. With these powerful tools, scientists were able to see nature at the atomic level. Simultaneously, with increased computing power available, modeling and simulation enabled an understanding of properties at the nanoscale. This powerful combination of atomic scale characterization and detailed modeling has led to the explosion we see today in nanoscale science and technological research. Nanoscale materials have a large surface area for a given volume. The surface properties dominate compared to bulk properties. Quantum phenomena becomes critical at reduced length scales. In most cases, the change in behavior is not a simple extrapolation of bulk behavior as we know. In materials where strong chemical bonding is present, delocation of valence electrons can be extensive. The extent of delocalization can vary with the size of the system. Structure also changes with the size. These two changes can lead to different physical and chemical properties depending on size, for example, magnetic, optical properties, melting point, specific heat, surface reactivity, bandgap, etc. Nanomaterials currently under investigation include nanoparticles, nanotubes, nanowires, powders, quantum dots, nanoporous materials, dendrimers, nanofibers, fullerenes, etc.

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physiochemical and biological properties. Therefore developing a drug delivery system that optimizes the pharmaceutical action of drug while reducing its toxic side effects in vivo is a challenging risk. One of the approaches is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles. Among these carriers liposomes and nanoparticles have been the most extensively investigated. Liposomes present some technological limitations including poor reproducibility and stability, low drug entrapment efficiency.

II. MATERIALS AND REAGENTS

A. Chitosan

Chitosan is a modified natural carbohydrate polymer prepared by the partial deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some microorganisms, yeast and fungi (Illum, 1998). The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose. These units combined by glycosidic linkages,

forming a long chain linear polymer. Although chitin is insoluble in most solvents, chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric it is insoluble in phosphoric and sulphuric acid.

B. *Catharanthus roseus*

Catharanthus roseus, commonly known as the Madagascar periwinkle, rose periwinkle, or rosy periwinkle, is a species of flowering plant in the dogbane family *Apocynaceae*. It is native and endemic to Madagascar, but grown elsewhere as an ornamental and medicinal plant, a source of the drugs vincristine and vinblastine, used to treat cancer. Other English names include '"Cape periwinkle" and "old-maid". It was formerly included in the genus Vinca as *Vinca rosea*.

C. *Copper sulphate*

Copper sulphate, also known as copper sulphate, are the inorganic compounds with the chemical formula $CuSO_4(H_2O)x$, where x can range from 0 to 5.

The pentahydrate (x = 5) is the most common form. Older names for this compound include blue vitriol, bluestone, vitriol of copper, and Roman vitriol.

The pentahydrate ($CuSO_4 \cdot 5H_2O$), the most commonly encountered salt, is bright blue. It exothermically dissolves in water to give the aquo complex $[Cu(H_2O)_6]^{2+}$, which has octahedral molecular geometry. The structure of the solid pentahydrate reveals a polymeric structure wherein copper is again octahedral but bound to four water ligands. The $Cu(H_2O)_4$ centers are interconnected by sulphate anions to form chains. Anhydrous copper sulphate is a white powder.

D. *Chitosan nanoparticle*

Chitosan nanoparticle has been investigated extensively as a potential drug carrier, due to it's biocompatible properties. Some studies have suggested using chitosan to coat nanoparticles made of other materials, in order to reduce their impact on the body and increase their bioavailability.

E. *Acetic acid*

Acetic acid is the simplest carboxylic acid. It consists of a methyl group attached to a carboxyl group. It is an important chemical reagent and industrial chemical, used primarily in the production of cellulose acetate for photographic film, polyvinyl acetate for wood glue, and synthetic fibres and fabrics. In households, diluted acetic acid is often used in descaling agents. In the food industry, acetic acid is controlled by the food additive code E260 as an acidity regulator and as a condiment.

III. TEST REQUIRED

A. *Ultraviolet spectroscopy*

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states ($\Delta E = hf$).

1) Principle of UV spectroscopy

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

The expression of Beer - Lambert law is-

$$A = log (I_0/I) = Ecl$$

Where, A = absorbance
I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute
L = length of sample cell (cm)
E = molar absorptivity

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy.

B. Scanning electron microscopy

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA and considerable overlap in capabilities exists between the two instruments.

C. Fourier transform infrared spectroscopy

Fourier Transform-Infrared Spectroscopy (FTIR) is an analytical technique used to identify organic (and in some cases inorganic) materials. This technique measures the absorption of infrared radiation by the sample material versus wavelength. The infrared absorption bands identify molecular components and structures.

When a material is irradiated with infrared radiation, absorbed IR radiation usually excites molecules into a higher vibrational state. The wavelength of light absorbed by a particular molecule is a function of the energy difference between the at-rest and excited vibrational states.

The wavelengths that are absorbed by the sample are characteristic of its molecular structure. The FTIR spectrometer uses an interferometer to modulate the wavelength from a broadband infrared source. A detector measures the intensity of transmitted or reflected light as a function of its wavelength. The signal obtained from the detector is an interferegram, which must be analysed with a computer using Fourier transforms to obtain a single-beam infrared spectrum. The FTIR spectra are usually presented as plots of intensity versus wavenumber (in cm-1). Wavenumber is the reciprocal of the wavelength. The intensity can be plotted as the percentage of light transmittance or absorbance at each wavenumber.

D. Anticancer activity

a. Preparation of Catharanthus roseus extract

Drying the flowers of Catharanthus roseus in dim light without facing sunlight. And the dried flowers are crushed after two days. Finally the crushed flowers are treated with acidic solution for the preparation of *catharanthus roseus* extract.

b. Comparison of anticancer activity

Comparing the anticancer result of the samples for with and without *catharanthus roseus* extract in nanoparticles. These comparisons gives the effective anticancer activity of the given samples.

c. Treatment groups

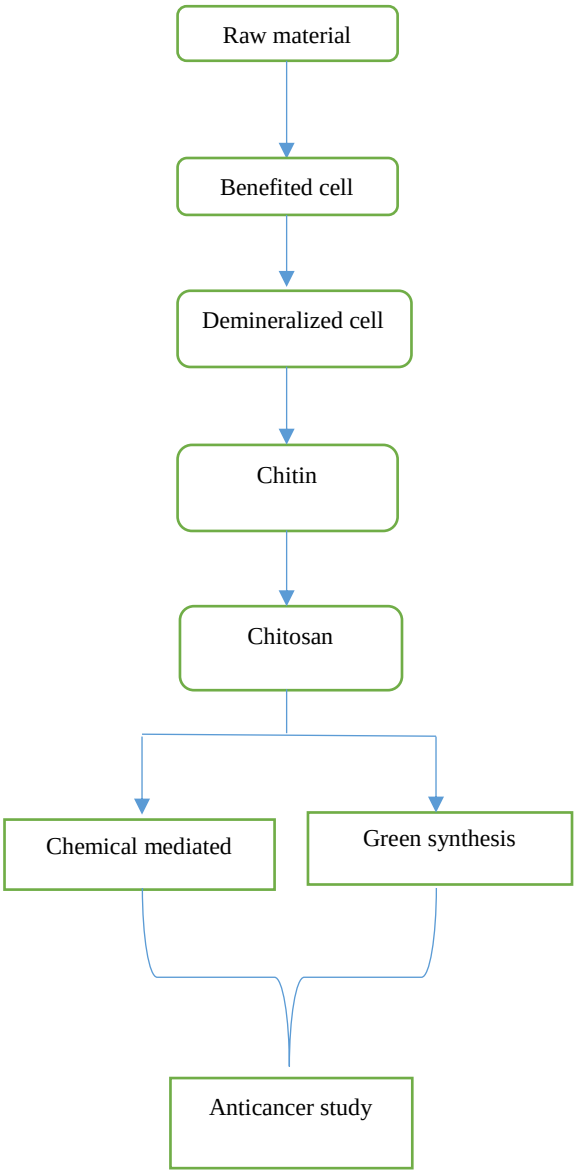
Cell lines were treated with chitosan nanoparticle at different concentration ranges between 10-100µg/mL and incubated for 72 hours, respectively, which was the optimal treatment time of the extracts in each of the cell lines. The following treatment groups are set up of the study. Negative control: cells alone. Positive control: cells + paclitaxel. Test groups: cells + chitosan Np.

d. MTT cell viability assays

After 72 hours, the media of treated cells (100 µL), were removed and the cell culture were incubated with 50 µL of MTT at 37 °C for 4 hours. After incubation, the formazan produced was then solubilized by the addition of 100 µL di-methyl sulfoxide.

The suspension was placed on a microvibrator for 5 minutes and then the absorbance was recorded at 540 nm by an enzyme-linked immunosorbent assay reader and the results were analyzed in triplicate and the percentage was calculated.

IV. FLOW CHART



V. METHODOLOGY

A. *Green synthesis*

Green synthesis is a method for chitosan nanoparticle synthesis which is effective than existing method. The natural method provides the nontoxic chitosan nanoparticle which used as a drug carrier. The anticancer activity of chitosan nanoparticle is tested for further results.

B. *Preparation of chitosan nanoparticle- chemical mediated*

Chitosan solution of different concentration was prepared using 0.1% acetic acid (in distilled water) solution and 25ml of 50mM CuSO4. 5H2O was then added to all samples which given in below calculation and all samples are stirred on magnetic stirrer at 70°C for 12h till the reaction was completed. After 12 hours each samples are colloid was centrifuged at 10,000G for 10 minutes to separate particles from suspension. The precipitate was re suspended in acetone (90%, v/v) and the centrifugation was repeated three times to remove unreacted reagents. Finally, the precipitate was dissolved in water, dried under vacuum overnight and stored and send the samples for UV Spectroscopy, SEM and FTIR testes to identify the effective formation of nanoparticles.

C. *Synthesis of Cu - Chitosan nanoparticle*

In a typical one-step synthesis protocol 100 ml of 0.05. (w/v) chitosan solution was prepared using 0.1% acetic acid (in distilled water) solution and 25ml of 50mM 10% C.roseues extract was added and stirred on magnetic stirrer at 50°C for 12h till the reaction was completed. After 12h the colloid was centrifuged at 10,000G for 10 minutes to separate particles from suspension. The precipitate was re suspended in acetone (90%, v/v) and the centrifugation was repeated three times to remove unreacted reagents. Finally, the precipitate was dissolved in water, dried under vacuum overnight and stored.

D. *determination of anticancer activity*

a. *Culturing of cell lines*

The cells were subcultured in Dulbecco modified eagle medium supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, 1% nonessential amino acids in tissue culture flasks, and incubated in a CO2 incubator in a 5% CO2 and 95% humidity atmosphere. After trypsinization, the cell count was done and the cell viability was tested by trypan blue using a hemocytometer. A known number of cells (2 × 103 cell/well in 100 µl of medium) were seeded into 96-well plates respectively for carrying out a MTT assay.

b. *Treatment groups*

Cell lines were treated with chitosan nanoparticle at different concentration ranges between (10-100)µg/mL and incubated for 72 hours, respectively, which was the optimal treatment time of the extracts in each of the cell lines. The following treatment groups are, Negative control: cells alone. Positive control: cells + paclitaxel. Test groups: cells + chitosan Np.

c. *MTT cell viability assays*

After 72 hours, the media of treated cells (100 µL), were removed and the cell culture were incubated with 50µL of MTT at 37°C for 4 hours. After incubation, the formazan produced was then solubilized by the addition of 100 µL di-methyl sulfoxide. The suspension was placed on a microvibrator for 5 minutes and then the absorbance was recorded at 540nm by an enzyme-linked immunosorbent assay reader and the results were analyzed in triplicate and the percentage was calculated.

E. *Calculation*

Initially four samples were taken with different concentration of chitosan in four conical flasks respectively. To prepare chitosan with different concentration level, samples were taken in 0.01g, 0.5g, 0.1g, 0.375g which produces 0.01%, 0.5%, 0.1%, 0.75% concentration of chitosan respectively.

a. Chitosan

0.01g in 100ml =0.01%

0.5g in 100ml=0.5%

0.1g in 100ml=0.1%

0.75g in 100ml=0.75%

0.75/2g in 50ml=0.75%

Therefore 0.375g chitosan is dissolved in 50 ml to form 0.75% concentration chitosan solution.

In 25ml of 50mm CuSO4. 5H2O is 0.312g and then added to all samples.

b. Copper sulphate

(0.05*249.68*25)/1000 = 0.312

Weight of cuso4 added to solution = 0.312g

c. Tabulation

Chitosan concentration in %	Weight of chitosan in g	Volume of acidic water in ml (with 0.1ml acetic acid)
0.01%	0.01g	100ml
0.05%	0.05g	100ml
0.1%	0.1g	100ml
0.75%	0.312g	50ml

This table represents the different concentrations of chitosan for identifying the analysis of which concentration is more effective for formation of nanoparticles. This effective formation is identified by the test of UV spectroscopy.

VI. RESULT AND DISCUSSION

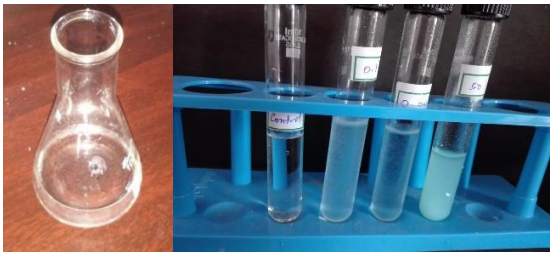


Fig. Standardization of chitosan nanoparticle synthesis

Fig. Synthesis of chitosan nanoparticle

Fig. Green synthesis of chitosan nanoparticle

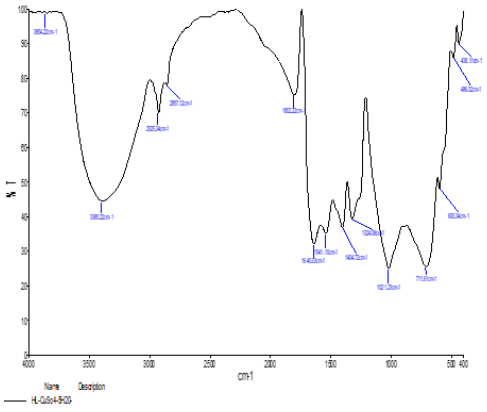


Fig. Output graph of Fourier transfer infrared spectroscopy.

VII. CONCLUSION

Our results indicate that chitosan nanoparticle have the potential of killing cancer cells to a larger extent. Some of the prospects of chitosan nanoparticles in biomedical field have been highlighted and looking potential direction for future research. Furthermore, chitosan nanoparticles possess various natural biological activities and have a considerable potential to be utilized in a number of medical and industrial applications. Formation of nanoparticles can be achieved under extremely mild conditions particle size and surface charge was modulated by changing the proportion and specification of raw materials. The results demonstrated that nanoparticle size was obtained from the lower molecular weight of chitosan and CuSo4 loading. The incorporation of *catharanthus roseus* is a more protective method in opposition to the cancer activities. The biodegradable nature and anti-cancer activity acts as a best carrier for the cancer therapy when compared to other natural polymers available. The further studies should be perform to increase the nanoparticles efficiency. Commercial production of the prepared nanoparticles is in progress.

ACKNOWLEDGEMENT

The conventional method for treating cancer is by drugs which reaches the target cells and then destroy the cancer cell but not in effective manner because it has chances to spread to nearer cells. Our method is greater efficient than the conventional method because it carries the drug and then treat the cancer cell and it has bio degradable in nature. In our method it has a another advantage is we can use the *Cathranthus roseus* as locally called as Nithiyakalyani flowers which are abundant in earth. The decrease in mortality rate is observed by using this method. This method is cost effective which is useful for cancer patient.

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