OPTIMIZING FUROSEMIDE ENCAPSULATION IN CARBOXYMETHYLCHITOSAN - SODIUM ALGINATE - A POTENTIAL DRUG DELIVERY SYSTEM

Dissertation submitted to

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KOTTAYAM

In partial fulfillment of the

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Bachelor of Science in

CHEMISTRY

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BHARATA MATA COLLEGE, THRIKKAKARA

(Affiliated to Mahatma Gandhi University, Kottayam)

2021-2024

BHARATA MATA COLLEGE, THRIKKAKARA

(Affiliated to Mahatma Gandhi University, Kottayam)

CERTIFICATE

This is to certify that the project report entitled : '**OPTIMIZING FUROSEMIDE ENCAPSULATION IN CARBOXYMETHYL CHITOSAN-SODIUM ALGINATE - A POTENTIAL DRUG DELIVERY SYSTEM'** *is an authentic record of the project work carried out by* ***AMBADY PAVITHRAN (Reg.no:210021024983)*** *in partial fulfillment of the award of the degree of Bachelor of Science in Chemistry at Bharata Mata College, Thrikkakara affiliated to Mahatma Gandhi University, Kottayam under my guidance and supervision.*

FORWARDED BY ***PROJECT GUIDE***

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DECLARATION

We, *do hereby declare that the project report entitled, "**Optimizing Furosemide Encapsulation In Carboxymethylchitosan - Sodium Alginate - A Potential Drug Delivery System"** is a bona-fide record of the work submitted to Mahatma Gandhi University in partial fulfillment of the requirement for the award of the degree of B.Sc in Chemistry carried out by me under the guidance of Dr. LITTY SEBASTIAN, Assistant Professor, Department of Chemistry, Bharata Mata College, Thrikkakara.*

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ABSTRACT

A novel drug delivery system for furosemide was synthesized using a new procedure. The encapsulation of the drug within the delivery system was confirmed through infrared spectroscopy (IR). Optimal encapsulation of the drug was achieved with a ratio of 5:1 of Sodium Alginate to Carboxymethyl cellulose (CMC), resulting in a high encapsulation efficiency of 94%. The stability of the system was demonstrated by a zeta potential value of -21.3 mV. Particle size analysis revealed an average particle size of approximately 800 nm, which is attributed to the encapsulation of the drug within the delivery system. This new drug delivery system holds promise for enhanced drug delivery and may find applications in various pharmaceutical formulations.

INTRODUCTION

Polymeric nanoparticles (NPs) are particles ranging in size from 1 to 1000 nm. They have the capability to carry active compounds either trapped inside or attached to their surfaces. These polymeric NPs hold promise for precisely delivering drugs to treat various diseases. Their advantages as drug carriers encompass controlled release potential, shielding active molecules from environmental factors, and enhancing both bioavailability and therapeutic effectiveness.

Due to their biocompatibility, biodegradability, and low toxicity, natural polymers have garnered considerable interest in the realm of drug delivery. Chitosan, a derivative of chitin, has been extensively studied for drug delivery applications. It is obtained by the deacetylation of chitin, which is abundantly found in nature . Carboxymethyl chitosan, a modified form of chitosan, has been used to develop pH-sensitive hydrogels for drug delivery systems. Additionally, chitosan has been employed in the preparation of hydrogel beads for protein drug delivery systems. Furthermore, carboxymethyl chitosan has been utilized in the development of an injectable composite hydrogel for scarless full-thickness skin regeneration, demonstrating its versatility in drug delivery applications.

Sodium alginate, another natural polymer derived from seaweed, has also been widely investigated for drug delivery systems. It has been reported to be efficiently used in the preparation of pH-sensitive alginate/carboxymethyl chitosan microcapsules for the encapsulation and delivery of drugs such as diclofenac sodium (Omer et al., 2021). Moreover, physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels have been explored for the oral delivery of protein drugs, highlighting the potential of sodium alginate in drug delivery applications (Omer et al., 2021).

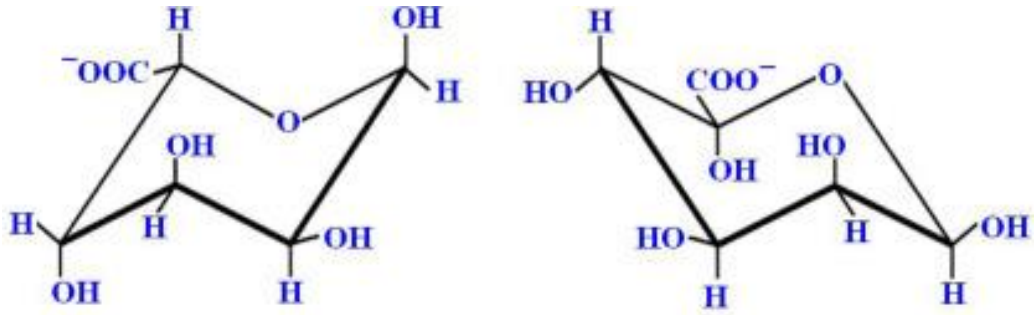
The combination of sodium alginate and carboxymethyl chitosan has shown promise in drug delivery systems. Polyelectrolyte complexes (PECs) based on chitosan and sodium alginate have been applied as a drug delivery system for sustained release of diclofenac sodium, demonstrating the synergistic potential of these natural polymers in drug delivery (Nikolova et al., 2021). In conclusion, natural polymers such as chitosan and sodium alginate have

demonstrated significant potential in drug delivery systems due to their biocompatibility, biodegradability, and versatility in forming various drug delivery carriers.

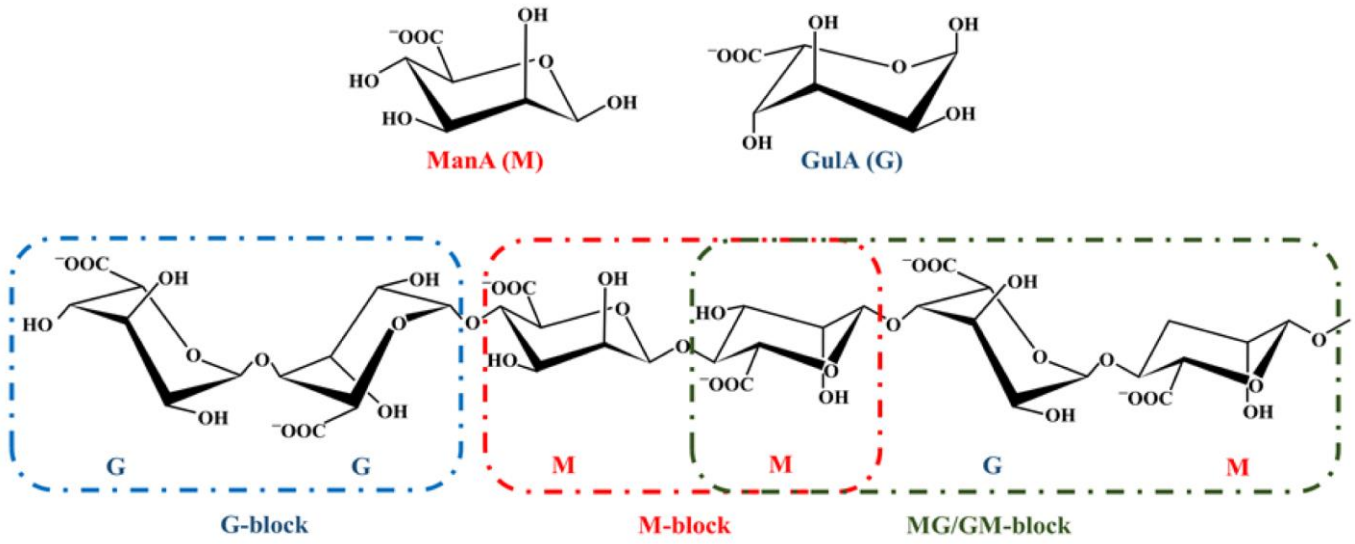
SODIUM ALGINATE

Sodium alginate, sourced from brown seaweeds such as *Laminaria hyperborea, Laminaria digitata, Laminaria japonica, Ascophyllum nodosum, and Macrocystis pyrifera,* is readily available in the market as a powder ranging from white to yellowish-brown. Structurally, sodium alginate is a linear polysaccharide comprising repeating units of two monosaccharides: β-D-mannuronic acid (M) and α-L-guluronic acid (G), forming a chain connected by glycosidic bonds.

The arrangement of these monosaccharide units along the chain varies, influencing properties such as viscosity and gel formation. They may form homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), or alternating M and G-residues (MG-blocks). α-L-guluronate is the C-5 epimer of β-D-mannuronate. The proportion of M and G blocks can differ depending on the seaweed source. For instance, alginate extracted from *Laminaria digitata and Ascophyllum nodosum* exhibits M/G ratios of 1.16 and 1.82, respectively.



Chemical structure of sodium alginate:



In solution, sodium alginate molecules can form gels through a process called ionotropic gelation. This occurs when divalent cations such as calcium crosslink the alginate chains, causing them to form a gel network. This gelation process is widely used in various applications including food, pharmaceuticals, and biotechnology.

CARBOXYMETHYL CHITOSAN

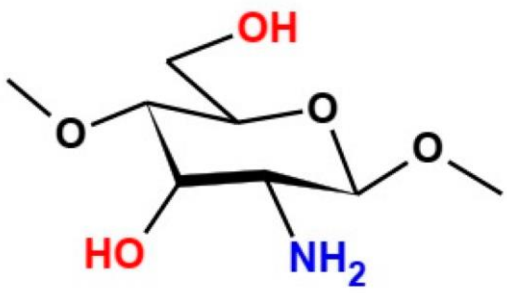
Carboxymethyl chitosan is a derivative of chitosan, a naturally occurring polymer derived from chitin, primarily found in the exoskeletons of crustaceans like shrimp and crab. Chitosan consists of repeating units of glucosamine and N-acetylglucosamine, linked by β-(1→4) glycosidic bonds.

When chitosan undergoes carboxymethylation, carboxymethyl groups (-CH2COOH) are introduced onto the amino and hydroxyl groups of the chitosan molecule. This chemical modification alters the properties of chitosan, making it more soluble in water and providing it with additional functionalities.

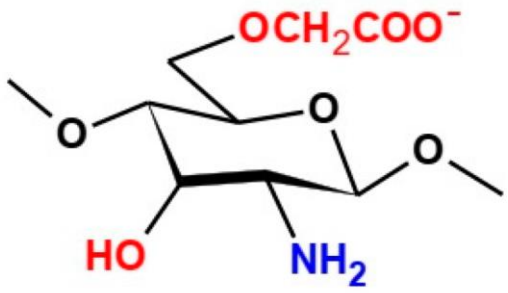
Structurally, carboxymethyl chitosan retains the backbone of chitosan, with the addition of carboxymethyl groups along the polymer chain. These carboxymethyl groups can vary in their distribution along the chitosan backbone, affecting the overall properties of the derivative.

The introduction of carboxymethyl groups increases the hydrophilicity of chitosan, enhancing its solubility in aqueous solutions over a wider pH range compared to native chitosan. This modification also introduces negatively charged carboxyl groups, which can influence the polymer's interactions with other molecules, such as proteins, drugs, and metals.

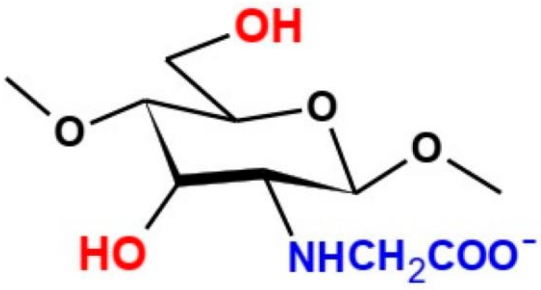
Overall, the structural modification of chitosan to form carboxymethyl chitosan expands its potential applications in various fields, including pharmaceuticals, biotechnology, and materials science, due to its improved solubility and versatility.



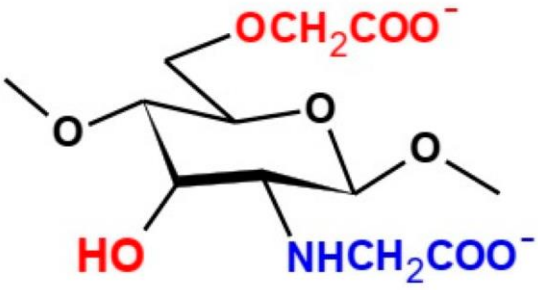
chitosan



O-carboxymethyl chitosan
(OCC)



N-carboxymethyl chitosan
(NCC)



N,O-carboxymethyl chitosan
(NOCC)

SODIUM ALGINATE-CMC SYSTEM FOR DRUG DELIVERY

The combination of sodium alginate and carboxymethyl chitosan has been extensively studied for drug delivery systems. Polyelectrolyte complexes (PECs) based on chitosan and sodium alginate have been applied as a drug delivery system for sustained release of diclofenac sodium (Nikolova et al., 2021). Physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels with calcium have been investigated for oral delivery of protein drugs (Lin et al., 2005). Additionally, physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels have been reported to be efficiently used as a pH-sensitive system for the delivery of proteic drugs to the intestinal tract (Omer et al., 2021).

A pH-sensitive hydrogel based on carboxymethyl chitosan/sodium alginate has been evaluated in vitro as a potential carrier for protein drug delivery (Xie et al., 2018). Furthermore, the amount of sodium alginate and carboxymethyl chitosan has been studied for the preparation of granules (Zhang, 2024). An injectable composite hydrogel of carboxymethyl chitosan and oxidized sodium alginate has been developed for scarless full-thickness skin regeneration (Yang, 2023). Chemical crosslinking between carboxymethyl chitosan and sodium alginate has been successfully achieved, as shown by Fourier transform infrared spectroscopy (Zhong et al., 2019).

A self-healing hydrogel prepared from N-carboxyethyl chitosan and sodium alginate dialdehyde has been reported to enable diffusive transport of C-dots across gel–gel interface for scavenging
reactive oxygen species (Chen et al., 2017). Additionally, paclitaxel-alginate/chitosan microcapsules have been successfully prepared using the high-voltage electrostatic technique as a novel formulation for drug delivery (Chen et al., 2014).

DIURETICS-TYPES OF DIURETIC MEDICINES AND ITS MECHANISM OF ACTION

Diuretics are medications designed to increase the excretion of water and electrolytes from the body by promoting urine production. They are commonly used to treat conditions such as hypertension, heart failure, and edema (fluid retention). Diuretics work by acting on different parts of the kidneys to increase urine production and reduce the volume of fluid in the body. Here are some of the main types of diuretics:

1.Thiazide Diuretics: Thiazides are among the most commonly prescribed diuretics. They work by inhibiting the sodium-chloride symporter in the distal convoluted tubule of the kidneys, which reduces sodium reabsorption and increases water excretion. Examples include hydrochlorothiazide, chlorthalidone, and indapamide. Thiazide diuretics act primarily on the distal convoluted tubule (DCT) of the nephron in the kidneys. They inhibit the sodium-chloride symporter (NCC), which is responsible for reabsorbing sodium and chloride ions from the urine back into the bloodstream. By blocking this transporter, thiazides decrease sodium reabsorption, leading to increased sodium and water excretion in the urine.

2. Loop Diuretics: Loop diuretics act on the ascending loop of Henle in the kidneys to inhibit sodium and chloride reabsorption. This results in a significant increase in urine production. Loop diuretics are often used in the treatment of conditions such as heart failure, renal failure, and severe edema. Examples include furosemide, bumetanide, and torsemide. Loop diuretics act on the thick ascending limb of the loop of Henle in the nephron. They inhibit the sodium-potassium-chloride cotransporter (NKCC2), which is responsible for reabsorbing sodium, potassium, and chloride ions from the urine. By blocking this transporter, loop diuretics prevent the reabsorption of these ions, leading to increased excretion of sodium, chloride, potassium, and water.

3.Potassium-Sparing Diuretics: These diuretics work by either blocking the action of aldosterone (a hormone that promotes sodium retention and potassium excretion) or by directly inhibiting sodium channels in the collecting ducts of the kidneys. They help to increase urine production while retaining potassium, which can be depleted by other diuretics. Examples include spironolactone, eplerenone, and amiloride. Potassium-sparing diuretics act on different parts of the nephron to reduce sodium reabsorption while sparing potassium. There are two main types:

Aldosterone Antagonists (e.g., spironolactone, eplerenone): These drugs competitively block the aldosterone receptor in the late distal convoluted tubule and collecting ducts of the nephron, thereby inhibiting the effects of aldosterone. Aldosterone normally promotes sodium reabsorption and potassium excretion, so blocking its action leads to increased sodium and water excretion while sparing potassium.

Epithelial Sodium Channel (ENaC) Blockers (e.g., amiloride, triamterene): These drugs inhibit the ENaC in the late distal tubule and collecting ducts, reducing sodium reabsorption. By

blocking sodium reabsorption, potassium-sparing diuretics indirectly prevent potassium excretion.

2.

4. Carbonic Anhydrase Inhibitors: These diuretics work by inhibiting the enzyme carbonic anhydrase in the proximal convoluted tubule of the kidneys. By doing so, they reduce the reabsorption of bicarbonate ions, which leads to increased excretion of sodium, potassium, and water. However, they are not commonly used as diuretics due to their limited effectiveness and side effects. Acetazolamide is an example of a carbonic anhydrase inhibitor. Carbonic anhydrase inhibitors (CAIs) work in the proximal convoluted tubule of the nephron. They inhibit the enzyme carbonic anhydrase, which normally catalyzes the conversion of carbon dioxide and water into bicarbonate ions and protons. By inhibiting this enzyme, CAIs reduce bicarbonate reabsorption, leading to increased excretion of bicarbonate, sodium, and water.

3.

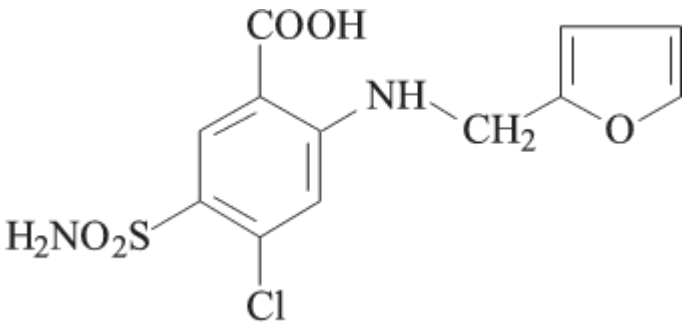
5. Osmotic Diuretics: Osmotic diuretics work by increasing the osmotic pressure of the glomerular filtrate, which inhibits water reabsorption in the nephron and promotes diuresis. These diuretics are often used in the treatment of cerebral edema, acute kidney injury, and glaucoma. Mannitol and glycerin are examples of osmotic diuretics. Osmotic diuretics work by increasing the osmotic pressure of the glomerular filtrate, primarily in the proximal tubule and descending loop of Henle. This increased osmotic pressure inhibits water reabsorption, leading to increased excretion of water, sodium, chloride, and other solutes.

FUROSEMIDE

Furosemide, a potent loop diuretic, is prescribed to address conditions like edema (fluid retention) and hypertension (high blood pressure). It's commonly used in ailments such as heart failure, kidney disease, and liver disease, where there's an accumulation of excess fluid in the body. Furosemide functions by blocking the sodium-potassium-chloride cotransporter (NKCC2) in the thick ascending limb of the loop of Henle in the kidney nephron. This action prevents the reabsorption of sodium, chloride, and potassium ions from the urine, leading to increased excretion of these ions alongside water. Consequently, it induces a substantial diuretic effect, reducing overall fluid volume in the body and alleviating symptoms associated with fluid overload.

Structure of Furosemide

Structurally, furosemide belongs to the class of drugs known as sulfonamide loop diuretics. Its chemical structure consists of a sulfonamide group (SO2NH2) attached to a phenoxy ring. The complete chemical name of furosemide is 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid. The presence of the sulfonamide group is crucial for its diuretic activity as it plays a key role in inhibiting the NKCC2 transporter in the loop of Henle.



Furosemide is typically available in oral tablet and solution forms for systemic administration, as well as in intravenous formulations for more rapid diuretic effect in hospitalized patients. It is usually taken once or twice daily depending on the condition being treated and the response to therapy. Furosemide is known for its rapid onset of action and relatively short duration of action compared to other diuretics. However, due to its potency, it can cause significant electrolyte imbalances, particularly depletion of potassium, so patients taking furosemide may require supplementation with potassium or other electrolytes.

Furosemide, a potent loop diuretic used in the management of conditions such as edema and hypertension, exhibits variable bioavailability depending on the route of administration. When administered orally in tablet or solution form, furosemide demonstrates a bioavailability ranging from approximately 50% to 70%. Absorption from the gastrointestinal tract is relatively rapid, leading to peak plasma concentrations within 1-2 hours post-administration.

However, factors such as gastrointestinal pH, gastric emptying time, and the presence of food can influence the extent of absorption.

SCOPE OF THE WORK

The Biopharmaceutics Classification System (BCS) categorizes drugs based on their aqueous solubility and intestinal permeability properties. According to this system, furosemide falls into class IV, indicating low solubility and low permeability, resulting in poor oral bioavailability and solubility. Despite its rapid absorption from the gastrointestinal (GI) tract, with a half-life ranging from 30 to 120 minutes, its reported bioavailability is only approximately 60% to 70%. To alleviate the poor aqueous solubility of furosemide, many methods have been adopted, including salt formation, prodrug formation, particle size reduction, complexation, and the use of micelles, microemulsions, nanoemulsions, nanosuspensions and solid-lipid nanoparticles as well as 'mixed solvency' using organic solvents. Unfortunately, none of these methods completely resolved the issues, and all of them have their own merits and demerits.

Encapsulating furosemide within a sodium alginate-carboxymethyl cellulose (CMC) system serves several crucial purposes within pharmaceutical formulations. Encapsulation within a polysaccharide-based system like sodium alginate and CMC offers a protective environment for the drug, shielding it from degradation and enhancing its stability. Additionally, the controlled release of furosemide from the encapsulated system can be achieved, allowing for prolonged drug action and reducing the frequency of dosing, which is particularly beneficial for improving patient compliance. Moreover, the biocompatibility and biodegradability of sodium alginate and CMC make them attractive materials for drug delivery applications, ensuring minimal toxicity and compatibility with biological systems. By encapsulating furosemide in a sodium alginate-CMC system, will result in the enhancement of its therapeutic efficacy, and improve patient outcomes in the management of fluid retention and cardiovascular disorders.

CHAPTER -2

EXPERIMENTAL

Preparation of Solutions:

1. Carboxymethyl Chitosan(CMC)

Six different concentrations of Carboxymethylchitosan were weighed out (300, 150, 100, 60, 50, 30 mg) accurately and dissolved in 100 ml of distilled water, a magnetic bead was added and stirred overnight. The pH of the solution was adjusted to 5.4 using dil HCl.

2. Sodium Alginate solution

0.300 grams of sodium alginate was weighed accurately and dissolved in 100 ml distilled water in a beaker. The solution was stirred with a magnetic pellet for 30 to 45 minutes.The pH of the sodium alginate solution was adjusted to 5.1 by using dil HCl using the pH meter.

3. Calcium Chloride Solution

0.332 gms of $CaCl_2$ was weighed out accurately and dissolved in 100 ml water in a standard flask.

4. Preparation of 20 mg/ml Furosemide

1 gram of furosemide was weighed out accurately and dissolved in methanol and made upto 50 ml in a standard flask, so that the concentration of the drug was 20 mg/ml.

Procedure :

To 9 ml sodium alginate solution, one ml of the furosemide drug was added with stirring. 2 mL $CaCl_2$ solution (332 mg/100mL) was added drop wise, at a rate of 1mL/min to this solution, while stirring by a magnetic stirrer at 480 rpm. It was then stirred for 45 min. After that, 4 mL of CMC solution(of various concentrations) was added drop wise and left to stir for one

hour. CMC/ALG NPs formation took place at ambient temperature.The formed NPs were centrifuged using high speed cooling centrifuge at 12,000 rpm at 4 C for 30 min. The supernatant was made upto 100 ml and the drug concentration was determined. The precipitate was washed and dried.

The precipitate was lyophilised and was characterized by IR and the zeta potential and particle size was measured. A description about the characterisation techniques is given below.

LYOPHILIZATION

Lyophilization, commonly known as freeze-drying, is a sophisticated process used to remove water from substances while preserving their structure, integrity, and functionality. This technique finds extensive applications in various industries, including pharmaceuticals, biotechnology, food processing, and preservation of biological samples.

The process of lyophilization involves three main stages: freezing, primary drying, and secondary drying. During the freezing stage, the substance is cooled to a temperature below its freezing point, typically between -50°C and -80°C. Freezing immobilizes water molecules within the substance and prepares it for subsequent drying.

After freezing, the primary drying phase begins. In this stage, the frozen substance is subjected to reduced pressure, typically in a vacuum chamber, while maintaining a temperature slightly above the freezing point. Under these conditions, water molecules transition directly from the solid (ice) phase to the vapor phase through a process called sublimation. This phase change bypasses the liquid phase, thereby avoiding potential damage to the substance's structure and functionality.

Primary drying continues until the majority of the bound water is removed from the substance, resulting in a porous matrix known as a lyophilized cake. While primary drying removes the bulk of the water, some residual moisture remains trapped within the matrix.

The final stage of lyophilization is secondary drying. In this phase, the temperature is gradually increased, and the pressure is further reduced to facilitate the removal of residual moisture through a process known as desorption. Secondary drying is essential for achieving low moisture content and ensuring the long-term stability of the lyophilized product.

Lyophilization offers several advantages over conventional drying methods such as air drying or spray drying. One of the primary benefits is the preservation of the substance's structural integrity and bioactivity. By removing water under gentle conditions, lyophilization minimizes the risk of denaturation, degradation, or loss of functionality in sensitive materials such as proteins, enzymes, and vaccines.

Furthermore, lyophilized products exhibit enhanced stability and shelf life compared to their liquid counterparts. The absence of water reduces the likelihood of chemical reactions, microbial growth, and degradation, allowing for long-term storage at room temperature or refrigerated conditions.

The versatility of lyophilization extends across various industries. In the pharmaceutical sector, lyophilization is widely used for the formulation of injectable drugs, vaccines, and biologics. Lyophilized products offer improved stability, ease of reconstitution, and extended shelf life, making them ideal for applications requiring long-term storage and transportation.

In the biotechnology and life sciences fields, lyophilization plays a crucial role in the preservation of biological samples, including cells, tissues, and diagnostic reagents. By removing water without compromising the integrity of biomolecules, lyophilization enables the long-term storage and transportation of valuable biological materials for research, diagnostics, and therapeutic applications.

UV -VISIBLE SPECTROMETER

A UV-Visible Spectrophotometer, short for Ultraviolet-visible spectrometer, is a sophisticated scientific instrument designed to analyze the interaction of matter with ultraviolet (UV) and visible light. It plays a crucial role in various fields such as chemistry, biochemistry, physics, environmental science, and materials science. This instrument measures the absorbance or transmittance of light by a sample across a range of wavelengths within the UV and visible spectrum.

At its core, a UV spectrometer consists of several key components. The light source emits a broad spectrum of UV and visible light, typically ranging from 200 to 700 nanometers (nm). This light is then directed through a monochromator, which selects a specific wavelength or range of wavelengths for analysis. The sample, contained in a cuvette or other transparent vessel, interacts with the light, leading to absorption or transmission depending on its composition and properties.

One of the fundamental principles underlying UV spectroscopy is Beer-Lambert Law, which describes the relationship between the absorbance of light by a sample, its concentration, and the path length of the light through the sample. By measuring the absorbance at different wavelengths, researchers can obtain valuable information about the molecular structure, concentration, and other properties of the sample.

UV spectrometers offer several advantages over other analytical techniques. They are relatively fast, allowing for rapid analysis of samples. Moreover, they require minimal sample preparation, making them suitable for a wide range of applications. Additionally, UV spectroscopy is highly sensitive, capable of detecting even trace amounts of analytes in a sample

IR SPECTROMETER

Fourier Transform Infrared (FTIR) spectrometry is a powerful analytical technique used to identify and characterize the chemical composition of substances based on their interaction with infrared radiation. This versatile instrument is widely utilized across various fields including chemistry, materials science, pharmaceuticals, environmental science, and forensic analysis.

At the heart of an FTIR spectrometer lies the Michelson interferometer, which splits incoming infrared radiation into two beams and recombines them after they have traveled different optical paths. As one beam passes through the sample while the other serves as a reference, any differences in their infrared absorption spectra reveal information about the sample's chemical composition.

The sample is typically prepared as a thin film or pellet to ensure uniformity and maximize interaction with the infrared radiation. When the sample absorbs infrared radiation, specific vibrational modes of its molecular bonds are excited, causing characteristic absorption peaks in the resulting spectrum. By analyzing these absorption peaks, researchers can deduce valuable information about the functional groups, molecular structure, and chemical bonds present in the sample.

FTIR spectrometers cover a broad spectral range, typically spanning from the near-infrared (NIR) region (approximately 780 nanometers) to the far-infrared (FIR) region (up to several hundred micrometers). This wide spectral coverage allows for the analysis of various chemical bonds, including those associated with organic compounds, inorganic materials, polymers, and biomolecules.

One of the key advantages of FTIR spectrometry is its high sensitivity and specificity. The technique can detect even trace amounts of substances in a sample and distinguish between similar chemical compounds based on their unique infrared absorption spectra. This capability makes FTIR particularly valuable for qualitative and quantitative analysis in research, quality control, and forensic investigations.

FTIR spectrometers are equipped with advanced software for data acquisition, processing, and interpretation. Spectral analysis software allows researchers to manipulate and visualize infrared spectra, perform baseline correction, peak fitting, and spectral comparison with reference libraries. These software tools facilitate the identification and characterization of unknown substances, as well as the quantification of analytes in complex mixtures.

Applications of FTIR spectrometry are diverse and encompass a wide range of industries and scientific disciplines. In pharmaceuticals, FTIR is utilized for drug formulation, quality control, and counterfeit detection. In materials science, FTIR enables the analysis of polymers, ceramics, composites, and coatings for structural elucidation and performance evaluation. Environmental scientists use FTIR to monitor air and water quality, identify pollutants, and assess environmental contamination. Additionally, FTIR is employed in forensic laboratories for the analysis of trace evidence, illicit drugs, and unknown substances encountered in criminal

PARTICLE SIZE ANALYZER

Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS), is a non-invasive optical technique used to measure the size distribution of particles or molecules in suspension or solution. A DLS particle size analyzer utilizes the principles of light scattering to determine the Brownian motion of particles and infer their size.

The operation of a DLS particle size analyzer involves illuminating a sample with a laser beam and measuring the intensity fluctuations of scattered light over time. As particles within the sample undergo random Brownian motion, they cause fluctuations in the scattered light intensity due to changes in their position relative to the detector. These intensity fluctuations, known as the dynamic light scattering or autocorrelation function, contain information about the size of the particles.

The DLS instrument collects and analyzes the autocorrelation function to extract parameters such as the diffusion coefficient, which is inversely proportional to the particle size. By applying mathematical models such as the Stokes-Einstein equation, which relates the diffusion coefficient to particle size, the DLS particle size analyzer calculates the size distribution of particles within the sample.

One of the key advantages of DLS particle size analysis is its ability to measure a wide range of particle sizes, from a few nanometers to several micrometers, depending on the instrument's specifications. This versatility makes DLS suitable for characterizing various types of particles, including nanoparticles, colloids, proteins, polymers, liposomes, and biological vesicles.

Moreover, DLS particle size analyzers offer rapid measurement times, typically ranging from seconds to minutes, depending on the sample concentration and optical properties. This rapid data acquisition makes DLS an efficient tool for high-throughput screening and quality control in research, development, and manufacturing processes.

In addition to size distribution, DLS particle size analyzers can provide information about the polydispersity or heterogeneity of particle populations within a sample. Polydispersity index (PDI) or dispersity index (DI) quantifies the breadth of the size distribution, with lower values indicating a more monodisperse sample and higher values indicating greater heterogeneity.

Applications of DLS particle size analysis span a wide range of industries and scientific disciplines. In pharmaceuticals, DLS is used for the characterization of drug delivery systems, protein aggregates, and nanoparticle formulations. In materials science, DLS enables the analysis of colloidal suspensions, emulsions, and polymer solutions for product development and quality assurance. In biotechnology and life sciences, DLS plays a vital role in the study of biomolecular interactions, vesicle dynamics, and protein folding.

ZETA POTENTIAL

Zeta potential is a key parameter i that describes the magnitude of the electrostatic repulsion or attraction between charged particles dispersed in a fluid medium. It is a measure of the potential difference between the surface of a particle and the surrounding liquid phase.

When particles are dispersed in a solution, they acquire an electrical double layer consisting of a compact layer of ions near the particle surface (the Stern layer) and a diffuse layer extending into the bulk of the solution. The potential at the shear plane within this double layer is known as the zeta potential.

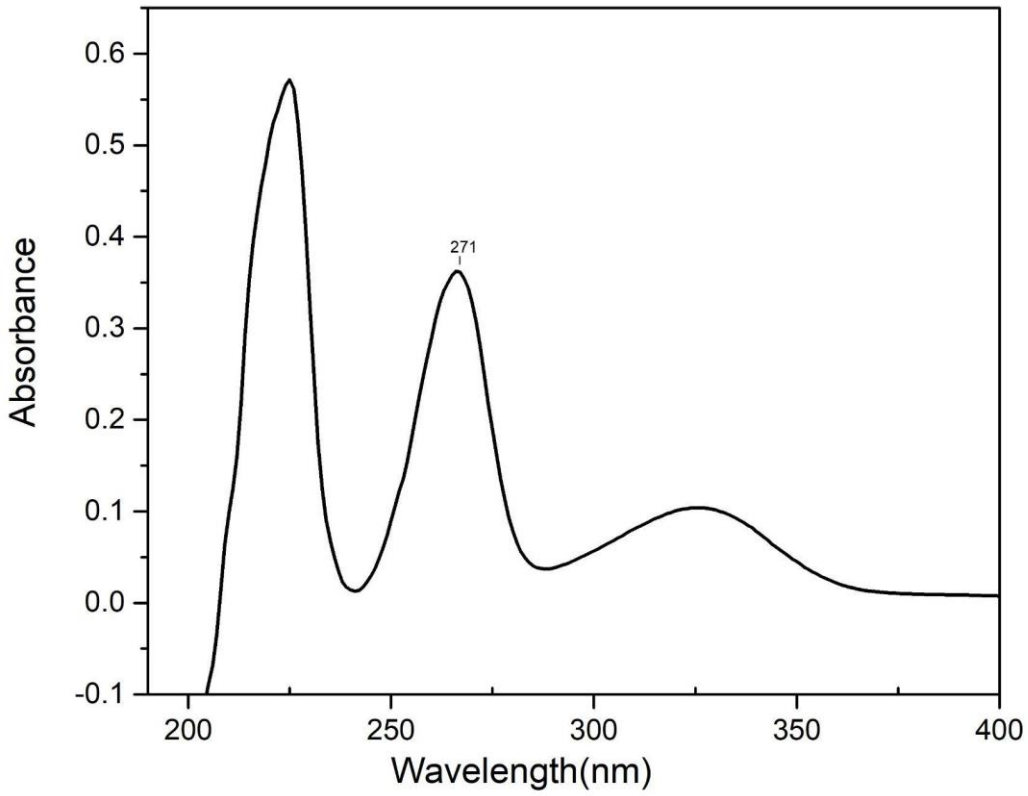
Zeta potential plays a critical role in determining the stability of colloidal dispersions. Particles with high zeta potential experience strong repulsive forces, preventing them from aggregating or flocculating. Conversely, particles with low or opposite zeta potentials may attract each other, leading to particle aggregation and eventual sedimentation or phase separation.

The range of zeta potential values for stability varies depending on the specific system and particles involved. Generally, higher absolute values of zeta potential (either positive or negative) indicate greater stability due to stronger electrostatic repulsion between particles, preventing aggregation or flocculation. Typically, zeta potential values above ±30 mV are considered indicative of stable colloidal dispersions, while values closer to zero may lead to particle aggregation and instability. However, it's important to note that the optimal zeta potential range for stability can vary based on factors such as particle size, surface chemistry, and the composition of the dispersing medium. Therefore, a comprehensive understanding of the system's characteristics is essential for determining the appropriate range of zeta potential to ensure stability in a particular application.

CHAPTER-3

RESULTS AND DISCUSSION

Furosemide was found to be soluble in methanol and an UV of furosemide in methanol was recorded. The graph showed a characteristic absorption at 271 nm.



The drug encapsulation was studied by determining the concentration of the supernatant solution. A calibration curve was made by preparing 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4,1.6 and 1.8 mg% of furosemide in methanol.

Preparation of the standard solution for calibration curve

Stock Solution

Stock solution was prepared by taking 0.005g (5 mg) in 250 ml standard flask and made up in methanol.

The concentration of the stock solution was 0.005g/250 = 0.00002 g/m = 0.02 mg/ml.

Standard Solutions

The standard solution was prepared in 10 ml standard flasks using methanol as a solvent.

0.2 mg% = 0.2 mg in 100 ml = 0.02 mg in 10 ml.

Concentration in 1 ml = 0.02 mg/10 = 0.002 mg in 1 ml.

Calculation of the Stock Solution to be taken:

For 0.2 mg%

$$\frac{0.002\ x\ 10}{0.02} = 1 \text{ ml}$$

1ml of the 0.02 mg/ml stock solution was added to a 10 ml standard flask and made upto 10 ml using methanol.

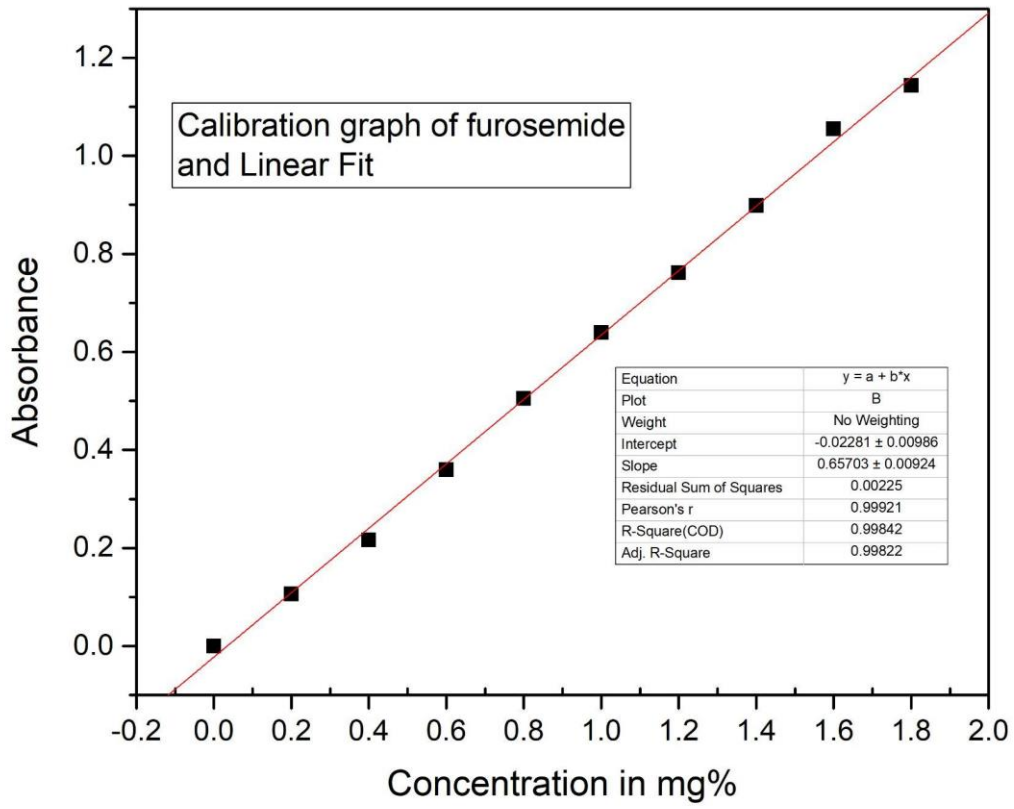
Similar calculations were done for all the other concentrations.

Label No.	**Volume of Stock Solution taken and made upto 10 ml (ml)**	**Concentration of the Standard solution In mg%**	**Concentration of the standard solution per ml**	**Concentration of the standard solution in µg/ml**
L0.2	1	0.2	0.002 mg in 1 ml	2µg/ml
L0.4	2	0.4	0.004 mg in 1 ml	4µg/ml
L0.6	3	0.6	0.006 mg in 1 ml	6µg/ml
L0.8	4	0.8	0.008 mg in 1 ml	8µg/ml
L1.0	5	1.0	0.010 mg in 1 ml	10µg/ml
L1.2	6	1.2	0.012 mg in 1 ml	12µg/ml
L1.4	7	1.4	0.014 mg in 1 ml	14µg/ml
L1.6	8	1.6	0.016 mg in 1 ml	16µg/ml
L1.8	9	1.8	0.018 mg in 1 ml	18µg/ml

UV measurement of the Standard solution of Furosemide in Methanol

Concentration of the solution in mg%	Absorbance at 271 nm
0	0
0.2	0.1063
0.4	0.2164
0.6	0.3595
0.8	0.505
1	0.6394
1.2	0.7618
1.4	0.8984
1.6	1.055
1.8	1.1433

Calibration graph was plotted



The concentration of the supernatant was determined from this calibration graph.

% Entrapment Efficiency was calculated from the formula,

%EE = [(Drug added - Free unentrapped drug)/Drug added] *100

Sodium Alginate	CMC	Ratio Alginate: CMC	Absorbance	Concentration from the graph	In mg:	Entrapment Efficiency
300	300	1:1	1.8028	2.77861	0.0277861	90.18
300	150	2:1	1.432	2.21424	0.0221424	92.17
300	100	3:1	1.380	2.13509	.0213509	92.45
300	60	5:1	0.947	1.47606	.0147606	94.7
300	50	6:1	1.602	2.4729	0.024729	91.26
300	30	10:1	1.868	2.87783	.0287783	89.8
Pure Drug			0.442	0.70745	.0070745	

Calculation

If the drug concentration measured was 0.0070745 mg%, the original drug concentration is = 28. 298 mg per ml.

The calculation was done like this:

Concentration of the drug solution taken is 20 mg/ml, So, 1 ml contains 20 mg, and 0.5 ml contains 10 mg.
0.5 ml of the drug was diluted to 100 ml and then, taken 5 ml was again diluted to 100 ml and measured the absorbance, back calculating the drug concentration.

10 mg(0.5ml) is made to 100 ml, so the concentration of the drug per ml is 10/100 = 0.1 mg/ml, 5 ml of it will contain 5 x 0.1 mg = 0.5 mg which was again diluted to 100 ml, so the concentration comes out to be 0.005 mg, which is equal to 0.5 mg%.
—----------
So, if Z is 10 mg, 2Z is(20mg/ml) is our drug concentration.

Z x 0.5/100 x 100 =0.005 mg.

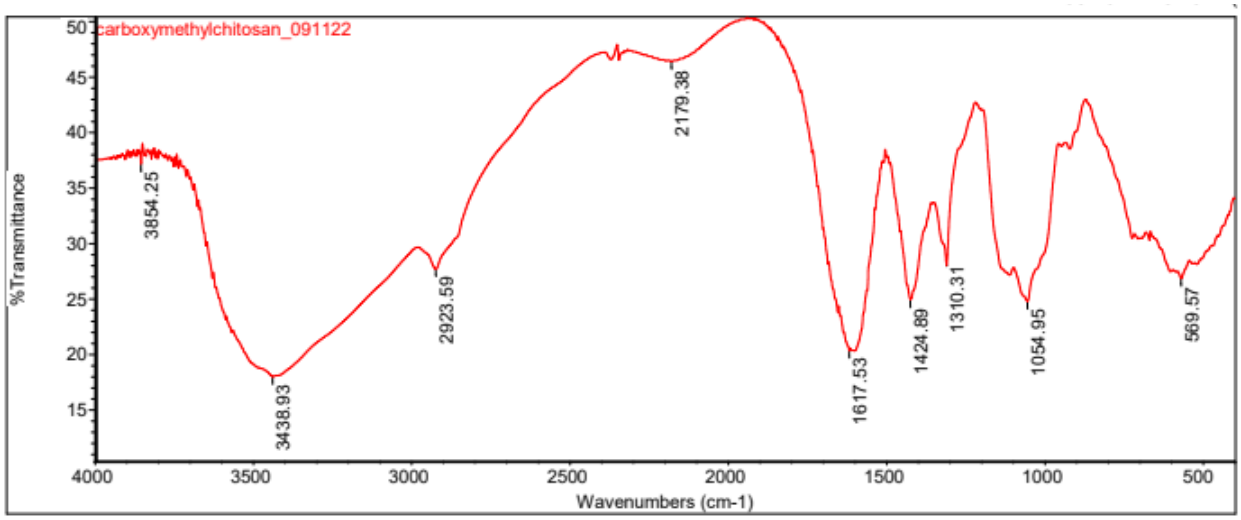
In our case,
Z x 0.5/10000 = 0.0070745 mg
2Z comes out to be 28.298 mg/ml.

We have added 1 ml of the drug containing 28.298 mg/ml, so in 100 ml, the concentration of the drug per ml was 28.298 mg/100 = 0.28298 mg.

Encapsulation Efficiency is
For 60 mg/ml: 0.28298-0.0147606/0.28298 = 94.7%

IR STUDIES

IR of carboxymethyl chitosan

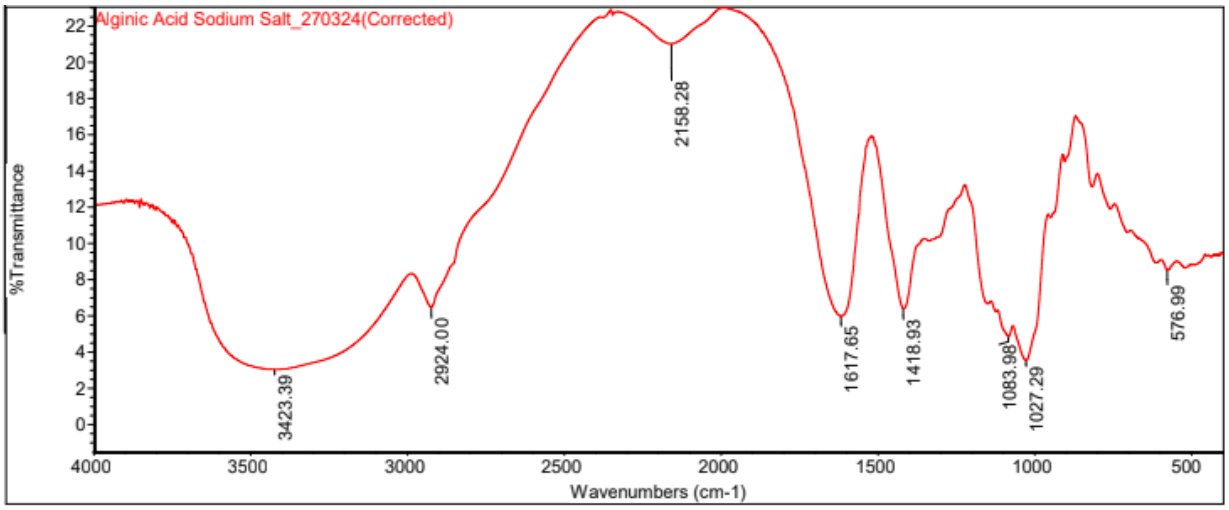


The broad peak in CMC at 3400–3200 cm^{-1} is caused by both O–H and N–H stretching vibrations and the peak at 2900 cm−1 is due to the C–H stretching vibrations. The spectra of CMC shows a strong peak at 1424 cm^{-1} which could be assigned to the symmetrical COO− group stretching vibration. The asymmetrical stretching vibration of the COO− group near 1550 cm^{-1} is overlapped with the deforming NH2 vibration at 1600 cm^{-1} to give a very strong peak.

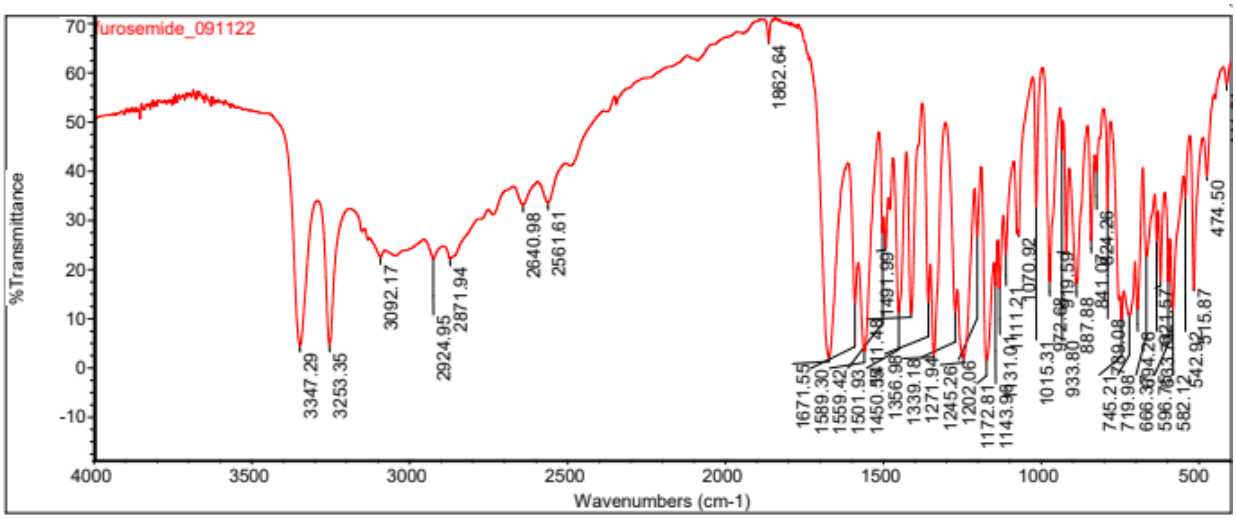
The graph of the sample with the peaks marked is given below:

IR of sodium alginate

Sodium alginate exhibits absorption band characteristics at 3423 cm^{-1} , which can be due to hydroxyl group (–OH), 1617 cm^{-1} (asymmetric stretching vibration of COO groups), 1418 cm^{-1}(symmetric stretching vibration of COO groups), and 1027cm^{-1} (of C-O groups).

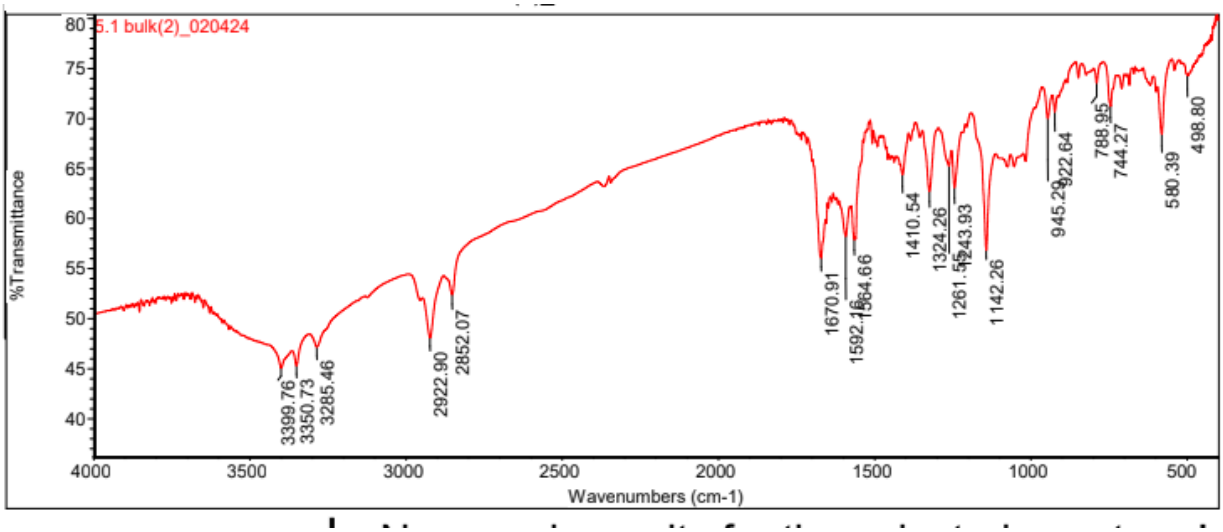


IR of Furosemide

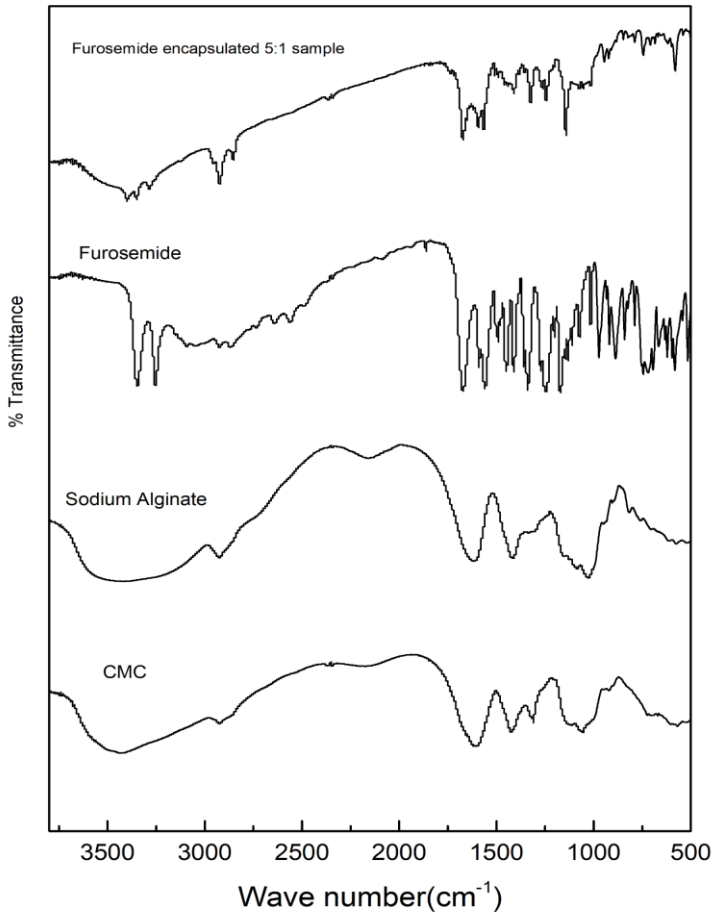


FTIR spectrum of pure furosemide displayed the characteristic peaks at 3347.29 cm^{-1} (O-H stretch), 3253.36cm^{-1} (N-H stretch), 1559 cm^{-1} (C=O stretch), 1671.51 cm^{-1} (N-H bending) etc, consistent with the literature values.

IR spectra of the furosemide loaded 5:1 sample



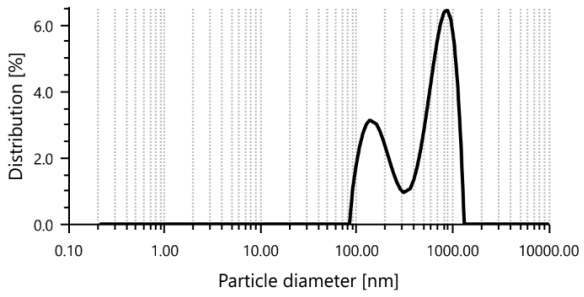
The drug loaded sample showed the characteristic peaks of furosemide appeared in the furosemide loaded 5:1 nanoparticles, indicating that the drug was entrapped in the system.



The particle size and Zeta potential

The particle size measurement gave a hydrodynamic diameter of 791.4 nm, which indicates that the drug interaction leads to an increased particle size.

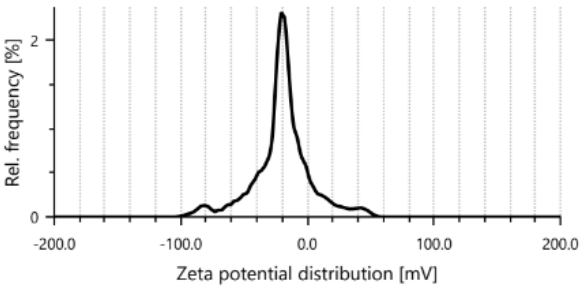
Particle size distribution (intensity)



Result

Hydrodynamic diameter	791.4 nm	**Mean intensity**	232.8 kcounts/s
Polydispersity index	30.7 %	**Absolute intensity**	58183.1 kcounts/s
Diffusion coefficient	0.6 µm²/s	**Intercept g1²**	0.2953
Transmittance	56.8 %	**Baseline**	1.058

Zeta potential distribution



Result

Mean zeta potential	-21.3 mV	**Mean intensity**	785.7 kcounts/s
Standard deviation	0.6 mV	**Filter optical density**	2.4988
Distribution peak	-20.0 mV	**Conductivity**	0.019 mS/cm
Electrophoretic mobility	-1.6632 µm*cm/Vs	**Transmittance**	56.5 %

A zeta potential of -21.3 mV was observed, showing that the system is stable.

CHAPTER-4

CONCLUSIONS

A new drug delivery system for furosemide was synthesized. The procedure is new.
The drug encapsulation was confirmed by IR.
Maximum encapsulation of the drug was shown when we take the ratio of 5:1 Sodium Alginate: CMC ratio.
A high drug encapsulation of 94% was observed.
A zeta potential value of -21.3 mV was observed, showing that the system is stable.
Particle size measurement shows a particle size value of (~800 nm), which might be due to drug encapsulation.

CHAPTER-5

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