Nanosponges based delivery of Andrographolide:Formulation and Characterization .

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Abstract

Andrographolide is a bioactive natural product that has been found to have potent anti-inflammatory, anti-cancer, and anti-viral properties. However, its poor solubility and bioavailability have limited its therapeutic application. To overcome this issue, nanosponges technology has been utilized to enhance the solubility and bioavailability of andrographolide.

Nanosponges are porous polymeric nanoparticles that can encapsulate both hydrophilic and hydrophobic drug molecules. Cyclodextrin-based nanosponges have been developed as an effective drug delivery system for various drugs, including andrographolide. The use of cyclodextrin as a building block in the nanosponge structure can improve the solubility and stability of the drug molecule.

In present research, nanosponges complexed with andrographolide were developed using the hot melt method with polymer beta cyclodextrin and diphenyl carbonate as the crosslinking agent. The andrographolide was incorporated into the nanosponges through lyophilization. The resulting nanosponges were evaluated for various parameters such as production yield, zeta potential, particle size, and entrapment efficiency. The optimized nanosponge formulation was also subjected to SEM analysis to study its surface morphology.

The study demonstrated that the andrographolide-loaded nanosponges had improved drug release and enhanced targeting at specific sites. These findings suggest that cyclodextrin-based nanosponges can serve as an effective drug delivery system for poorly soluble bioactive

compounds such as andrographolide**.**The study indicates that the rate of drug release can be improved by incorporating drug into the nanosponges and thus it can improve the targeting of the drug at the specific site.

Key words: Nanosponge, Formulation, Optimization,crosslinking agent,liophilization,zeta potential

Introduction

Nanosponges are a type of nanoporous structure with a mesh-like pattern that can be used to encapsulate or suspend a wide range of substances for incorporation into a medication form. They have a spherical colloidal nature and are highly effective at solubilizing poorly soluble drugs due to their inclusion and non-inclusion behavior. Nanosponges are a relatively new technology for drug delivery, offering prolonged release and improved bioavailability of poorly water-soluble drugs. They have hydrophobic cavities and hydrophilic branching, allowing them to load both hydrophilic and hydrophobic drug molecules, providing unparalleled flexibility. Nanosponges resemble a three-dimensional network or scaffold, composed of a long length of polyester mixed in solution with crosslinkers that act as tiny grappling hooks to connect different parts of the polymer. The nanosponges encapsulate drug molecules within their core, making them an ideal choice for drug delivery.The unique porous structure of nanosponges, with pores on their surface and no continuous membrane surrounding them, allows for the addition of active ingredients in an encapsulated form. The encapsulation permits the active ingredient to move freely from the particles into the vehicle until equilibrium is achieved. Upon application of the product onto the skin, the vehicle containing the active ingredient becomes unsaturated, disrupting the equilibrium and causing a flow of the active ingredient from the sponge particle into the vehicle and subsequently onto the skin. Even after the nanosponge particles have been retained on the skin surface, the release of the active ingredient continues for an extended period.

The nanosponge technology is the most effective for controlled and prolonged release of drug products on the skin, particularly for antifungal, antibiotics, and anti-inflammatory topical applications. Conventional products may release the drug in high concentrations, which can lead to serious side effects. However, the nanosponge drug delivery system releases the drug in a

sustained and predictable manner. The nanosponges can be formulated into various forms, such as ointments, gels, creams, and lotions.[1]

Andrographolide is a natural bioactive compound found in the leaves of the Andrographis paniculata plant, which has been traditionally used in Ayurvedic and Chinese medicine for various therapeutic purposes.However, despite its potential therapeutic benefits, andrographolide has low solubility in water, which limits its bioavailability and effectiveness as a drug. This is where beta-cyclodextrin comes into play. Beta-cyclodextrin is a cyclic oligosaccharide composed of seven glucose units linked by alpha-1,4 glycosidic bonds, resulting in a truncated cone-shaped structure with a hydrophobic cavity in the center. This unique structure allows beta-cyclodextrin to form inclusion complexes with hydrophobic compounds.[2]

Material & Methods [3][4][5]

Pre-formulation studies

The objective of preformulation studies is to gather information about the physical and chemical properties of a drug and its interaction with excipients, which is essential for developing a stable and effective dosage form. This step is crucial in the development of medicinal substances. Various investigations such as Vyas et al. (2002) and Kerimoglu et al. (2013) are considered as part of preformulation research. The aim is to collect data that can aid in creating a bioavailable dosage form that is consistent and can be manufactured on a large scale.

Organoleptic Properties

Organoleptic properties of Andrographolide were performed by human sensory organs.The organoleptic studies of andrographolide like general appearance like color, odor, state, etc. were performed.

Solubility study

Qualitative solubility of andrographolide in different solvents was determined according to USP NF, 2007. Small amount of Andrographolide transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, ethanol, DMSO, chloroform and water)

Melting Point

The purity of a drug can be determined by various means, one of which is its melting point. The melting point of a pure substance is specific and consistent. However, medicines contain multiple compounds, so their melting points may vary within a particular range. To ascertain the melting point, the open Capillary method using Thiele's tube was employed. A minute amount of andrographolide was positioned in a capillary tube with thin walls, which was then hung into the thiele's tube filled with liquid paraffin oil and gradually heated. A thermometer was attached to monitor the temperature, and the temperature at which the sample began to melt was recorded as its melting point.

Fourier transmission Infra-Red Spectroscopy

FT-IR spe***ctrum of Andrographolide,*** and Andrographolide + polymer was obtained using the KBr pellet method. For this, a KBr disc was prepared by mixing 1 mg of each substance (Andrographolide, Beta cyclodextrin, Diphenyl carbonate, and Andrographolide + polymer) with 100 mg of spectroscopic grade KBr, which was dried using an IR lamp. The mixture was subjected to hydraulic pressure to form a disc, which was then placed in the FT-IR chamber.The infrared spectrum was recorded in the range of 4000 to 400 cm-1 region.

Formulation of Drug Delivery System

Formulation of β-cyclodextrin Nanosponges.[6][7]

To prepare Beta-cyclodextrin nanosponges, the hot melt method was employed, which involved using various ratios of Diphenyl carbonate (DPC cross-linker) and Beta-cyclodextrin (β-CD polymer). First, the anhydrous β-CD polymer and DPC cross-linker were homogenized and heated gradually with magnetic stirring for 6 hours at a temperature range of 90 to 100 ºC. This allowed for the crosslinking reaction to take place between the β-CD and DPC, resulting in the formation of the nanosponges.

After 6 hours, the reaction mixture was cooled to ambient temperature. The solid obtained was washed multiple times with double distilled water to remove any unreacted β-CD. Finally, the placebo nanosponges were dried at 40 ºC and stored in a desiccator for further use.

Loading of andrographolide in β-cyclodextrin nanosponges.[8][9]

Andrographolide was loaded in the prepared nanosponges by lyophilization. Placebo NS (1 gm) was dispersed in double-distilled water (50 mL) with help of magnetic stirrer. Approximately 100 mg of Andrographolide was added to above dispersion. The obtained dispersion was then,

sonicated (for 10 minutes), and subsequently kept for 24 hours (under stirring). The resulting suspensions were centrifuged (2000 rpm; 10 minutes) in order to separate the un-entrapped Andrographolide, as a residue below the colloidal supernatant. Next, the supernatant was lyophilized at -81 ^{O}C and 0.0010 mbar (pressure). The dried Andrographolide loaded NS powder was stored for future use.

Evaluation parameters of andrographolide loaded nanosponges.[10][11][12]

Zeta potential

To determine the particle charge and the velocity of particle movement in an electric field, the zeta potential was measured. The nanosponges were diluted 10 times with distilled water and examined using the Zetasizer Malvern instrument in this study. Prior to conducting the zeta potential measurements, all samples underwent sonication for 5-10 minutes.

Particle size

Dynamic light spectroscopy was employed to determine the particle size distribution of andrographolide in β-cyclodextrin nanosponges using a zeta sizer (Malvern Instruments, UK). The particle size was reported as the z-average value, which represents the particle size diameter. The polydispersity index (PDI) was used to analyze the particle size distribution, with a lower PDI indicating a more uniform particle size distribution (Ağardan et al., 2020). To achieve an appropriate scattering intensity at 25°C, the dispersions were diluted with Millipore filtered water and placed in a disposable sizing cuvette (Sharma and Pathak, 2011).

Entrapment efficiency

To calculate the entrapment efficiency accurately weighed the quantity of nanosponges (10 mg) with 5 ml of methanol in a volumetric flask was shaken for 1 min using vortex mixer. The volume was made up to 10 ml. Then the solution was filtered and diluted and the concentration of Andrographolide was determined spectrophotometrically at 224 nm.

Loading efficiency = Actual drug content in nanosponges / Theoretical drug content × 100

Surface morphology

The surface morphology of Andrographolide loaded nanosponges was examined using a scanning electron microscope (SEM). To achieve this, the nanosponges were coated with a thin layer of metal such as gold, palladium, or platinum using a sputter coater under vacuum. The coated specimen was then bombarded with an electron beam to create secondary electrons called auger electrons, and only the electrons scattered at 90° were selected to acquire images of the surface morphology. The images were obtained using a scanning electron microscope (Model: Supra55 Zeiss). This technique allowed for the visualization and study of the nanosponge's surface structure and features.

FTIR

For the initial characterization of Andrographolide loaded nanosponges, FTIR spectroscopy was utilized. The spectra of the nanosponges were collected using an FTIR spectrophotometer (Parkin Elmer) and the KBr method was employed for recording the IR spectra. The dried potassium bromide pellet was used to make baseline corrections, and the sample pellet was placed in the IR compartment and scanned within the wavelength range of 4000 cm-1 to 400 cm-1.

Result & Discussion:

Pre-formulation study of drug

The first step in the systematic development of a medication's dosage form is preformulation research. This involves analyzing the physical and chemical properties of the drug ingredient, both alone and in combination with excipients. The objective of preformulation testing is to generate information that can be used by the formulator to create stable and bioavailable dosage forms that can be produced in large quantities.

Organoleptic properties

Table1: Organoleptic properties of andrographolide

Drug	**Organoleptic properties**	**Observation**
	Color	White to brown

Andrographolide	Odor	Odorless
	State	Crystalline powder

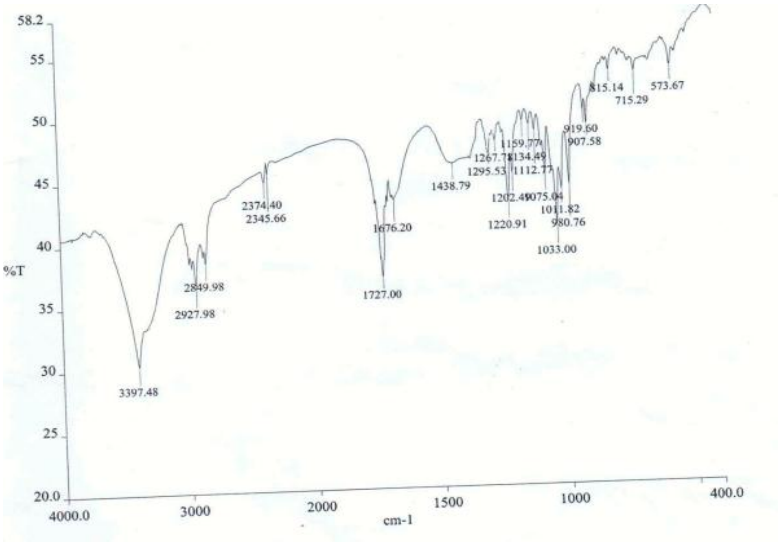
Solubility study

The solubility of andrographolide was investigated in different types of liquid vehicles, both volatile and non-volatile. The vehicles tested included Dimethyl sulfoxide, methanol, ethanol, chloroform, and water. The results showed that andrographolide is highly soluble in Dimethyl sulfoxide, soluble in methanol and ethanol, and only slightly soluble in chloroform.

Melting point

The capillary method is used to determine the melting point of a substance. The melting point of the andrographolide was found to be 235ºC, which is well within the limits of the drug specification.

FTIR of Andrographolide

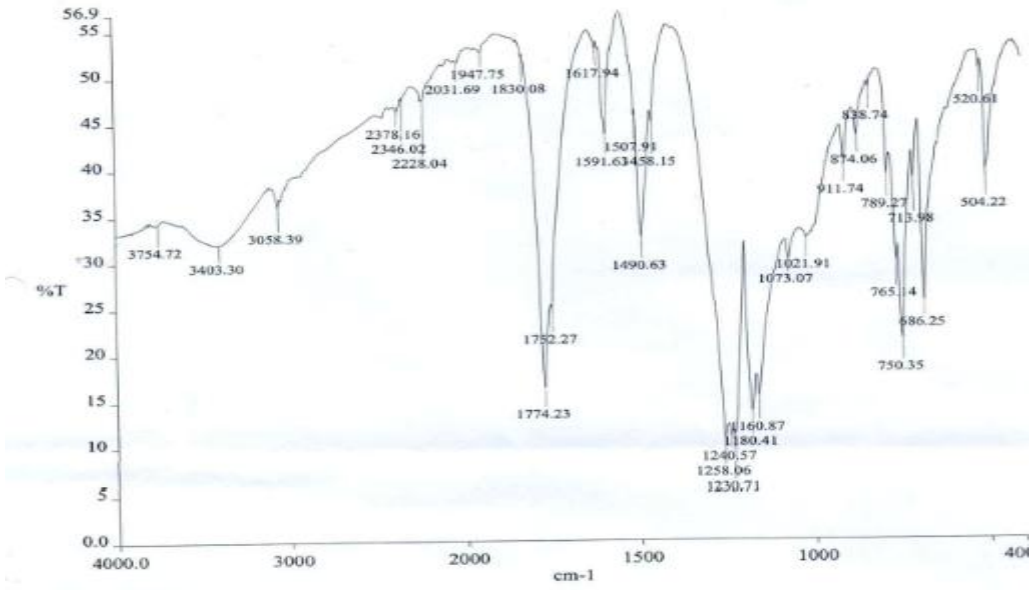


Graph 1: FTIR of Andrographolide

Table1: Interpretation of FTIR of Andrographolide

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3397.48	3500–3200	O–H stretching	alcohols
2	2927.98	3000–2850	C–H stretch	alkanes
3	2849.98	2830–2695	C–H stretch	aldehydes
4	2345.66	2349	O=C=O stretching	carbon dioxide
5	1727.00	1750–1735	C=O stretching	esters
6	1676.20	1680–1640	–C=C– stretching	alkenes
7	1438.79	1470–1450	C–H bending	alkanes
8	1220.91	1320–1000	C–O stretch	esters
9	907.58	1000–650	=C–H bend	Alkenes

Drug +Excipient



Graph 2 : FTIR of Drug +Excipient

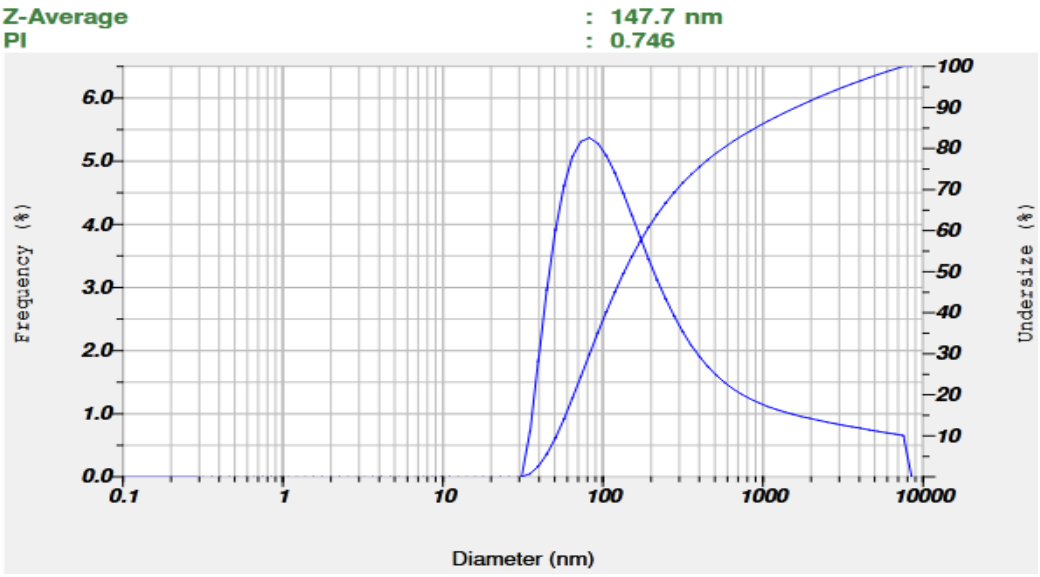
Table 2: Interpretation of FTIR of Drug + Excipients

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3754.50	4000- 3000	O- H stretching	alcohols
2	3403.30	3500–3200	O–H stretch, H–bonded	alcohols, phenols
3	3054.39	3100–3000	C–H stretch	aromatics
4	1774.23	1760–1665	C=O stretch	carbonyls
5	1591.63	1600–1585	C–C stretch	aromatics
6	1490.63	1470–1450	C–H bend	alkanes
7	1258.06	1335–1250	C–N stretch	aromatic amines
8	1160.87	1320–1000	C–O stretch	ethers
9	1073.07	1000–650	=C–H bend	alkenes
10	750.35	725–720	C–H rock	alkanes

The IR spectrum of a mixture of Andrographolide drug and excipient was analyzed to identify the functional groups present. The spectrum showed a prominent phenolic alcohol (-OH) peak at 3398.29 cm-1, which falls within the typical range of 3550-3200 cm-1. Ester (C=O) stretching and (C-O) stretching peaks were found at 1728.06 cm-1 and 1221.36 cm-1, respectively, within the standard range of 1750-1735 cm-1. Alkane (C-H) stretching and alkene (=C-H) bending peaks were observed at 2929.73 cm-1 and 1675.82 cm-1, respectively. The IR spectrum of the extract revealed functional groups such as alcohols, phenols, carbonyls, ethers, and aromatic groups. The peaks of these functional groups were observed at 3754.50, 3403.30, 1774.23, 1160.87, 3054.39, and 1591.63 cm-1, respectively, representing alcohol (O-H stretching), phenol (O-H stretching), carbonyl (C=O stretching), ether (C-O stretching), aromatic (C-H stretching), and aromatic (C-C stretching).Similarly, the IR spectrum of the prepared Andrographolide loaded nanosponges was analyzed, and the following peaks were observed: 3448.11 cm-1 for the alcohol (O-H stretch) peak, 1774.11 cm-1 representing carbonyl (C=O stretching), 1488.83 cm-1 for the alkane (C-H stretching) peak, and 1075.41 cm-1 for the alkene (=C-H bending) peak.

Characterization of optimized nanosponges

Particle Size

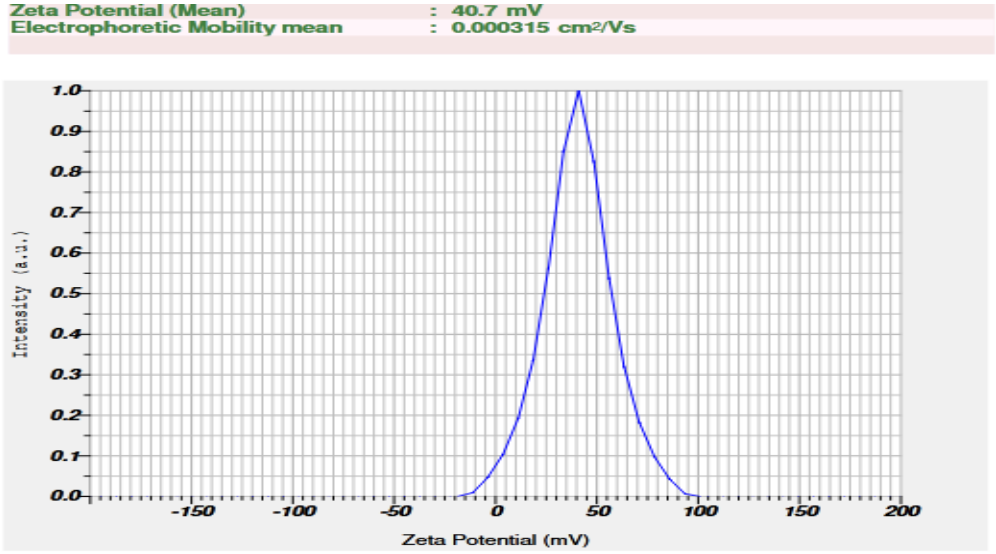


Graph 3: Particle size

Determining particle size is a critical aspect in characterizing nanosponges. To measure the average particle size of Andrographolide loaded nanosponges, Malvern zeta sizer was used. The

particle size analysis revealed that the average particle size of Andrographolide loaded nanosponges was 147.7 nm with a PI (polydispersity index) value of 0.746.

Zeta potential



Graph 4: Zeta potential

Zeta potential analysis was conducted to determine the surface charge of particles and assess their stability during storage. The zeta potential magnitude can provide insight into the colloidal stability of the particles. Nanosponges with zeta potential values greater than +25 mV or less than -25 mV are generally considered highly stable. When particles in nanosponges have a large positive zeta potential, they tend to repel each other, which prevents them from coming together. However, when particles have low zeta potential values, there is no force to keep them apart, causing them to come together and clump. In the case of the nanosponges examined, the zeta potential was measured to be 40.7 mV with a peak area of 100% intensity, which indicates that the Andrographolide nanosponges are stable.

Entrapment efficacy

This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer concentration and difference in the degree of cross linking. The prepared Optimized Andrographolide nanosponges possess high drug entrapment efficiency and were found to be in the range of 79.21%

Scanning electron microscope (SEM)

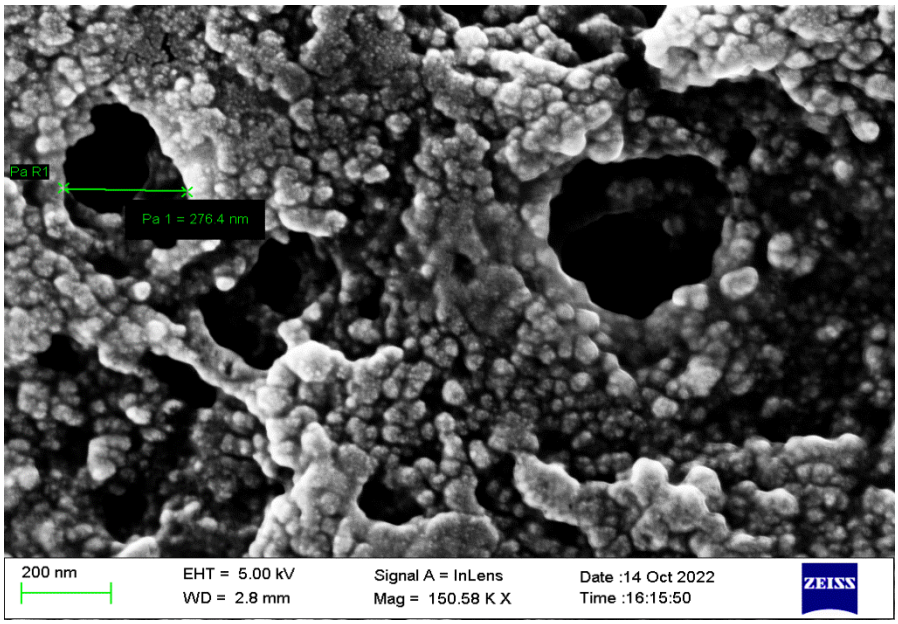
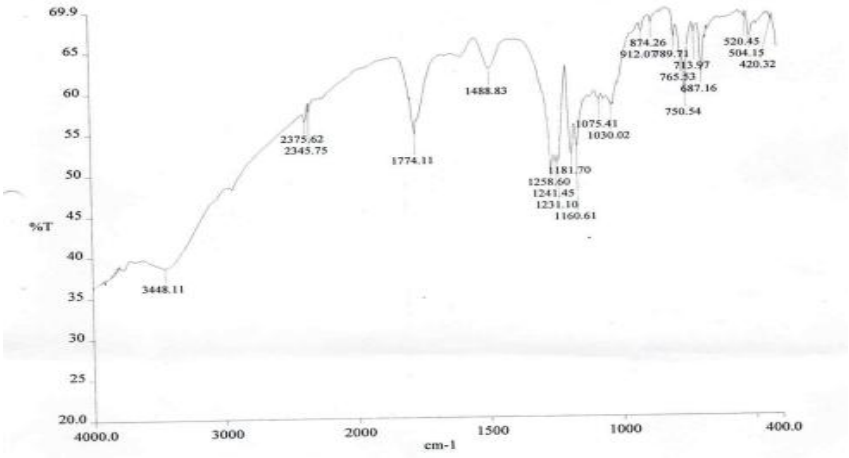


Figure1: Scanning electron microscope

The microscopic characteristics, including the shape and morphology, of the prepared nanosponges were investigated using SEM analysis. The nanosponges were prepared and dried to eliminate any moisture content, and then observed using scanning electron microscopy. The SEM images captured at a magnification of 150.58 kx revealed that the nanosponges had a spherical shape and a smooth surface morphology, and were porous in nature. The spongy and porous nature of the nanosponges was clearly evident from the SEM images. These findings provide important insights into the structure and properties of the prepared nanosponges, which could be relevant to their potential applications in various fields such as drug delivery or other biomedical applications.

FTIR of prepared nanosponges



Graph5: Andrographolide loaded Nanosponges

IR of prepared andrographolide loaded nanosponges of extract was interpretated and the peaks were found to be at 3448.11 of alcohol (O–H stretch) peak, 1774.11 represents carbonyl (C=O stretching), 1488.83 of alkane (C-H stretching) and 1075.41 of alkene (=C–H bending) peak. It confirms that the main functional group alcohol, carbonyl and aklene group are present in the nanosponges.

The information provided by the IR spectrum analysis is important for understanding the chemical composition and properties of the andrographolide loaded nanosponges. It confirms that the nanosponges were successfully synthesized and contain the expected functional groups, which could affect their behavior in various applications.

Conclusion:

The successful preparation of Andrographolide-Beta Cyclodextrin Nanosponges was achieved using the hot melt method. Various ratios of Diphenyl carbonate (DPC cross-linker) and Beta Cyclodextrin (β-CD polymer) were employed to prepare the nanosponges.

Further characterization of the Andrographolide-Beta Cyclodextrin Nanosponges was performed, including FT-IR spectroscopy, which revealed that a good complex was formed between Andrographolide and the β-CD polymer. The FT-IR spectrum obtained confirmed that the interaction between Andrographolide and β-CD polymer was through the formation of hydrogen bonds. The study also revealed that the Andrographolide was effectively entrapped within the

nanosponges, as evidenced by the absence of any Andrographolide peaks in the FT-IR spectrum of the nanosponges. The thorough characterization of the nanosponges through various analytical techniques including SEM, FT-IR confirmed the successful preparation and stability of the nanosponge formulation. The results demonstrated the formation of a stable complex between Andrographolide and beta cyclodextrin, which is crucial for improving the bioavailability and therapeutic efficacy of the drug.

Overall, the successful preparation and characterization of the Andrographolide-Beta Cyclodextrin Nanosponges have shown that this is a promising approach for enhancing the solubility and bioavailability of Andrographolide. The nanosponges have the potential to be used in drug delivery systems to increase the therapeutic efficacy of Andrographolide.It has the potential to improve the solubility and bioavailability of Andrographolide, and provide sustained release of the drug, which could lead to improved therapeutic outcomes. Further studies are needed to evaluate the efficacy and safety of this nanosponge formulation in vivo, but the results so far are promising.

Andrographolide-beta cyclodextrin nanosponges represents a significant step forward in improving the therapeutic potential of Andrographolide. The use of nanosponges as drug delivery systems could also pave the way for the development of similar formulations for other poorly soluble drugs, ultimately leading to improved therapeutic outcomes for patients.

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Conflict of Interest:

The authors declared that there is no conflict of interest.

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