RESEARCH ARTICLE

Incorporating Kappa-carrageenan in Liposomes Facilitates Development of Lipopolysaccharide Hybrid Nanoplatform

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ABSTRACT

Sulfated polysaccharides (kappa-carrageenan) integration in lipids provides diverse structural and physicochemical properties in nanotechnology. Such a lipopolysaccharide hybrid (LPHS) system offers superior applications in the food and pharmaceutical industry. This study investigates the synthesis and characterization of sulfated polysaccharide (kappa-carrageenan: κ-CG) integrated smart liposomes (cubosomes) for establishing cubic tendency in the LPHS system. The κ-CG integrated smart liposomes were prepared using the modified thin film hydration approach. Characterization studies (liposomes and smart liposomes) were performed using dynamic light scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). DLS studies confirmed the nano-range of both delivery systems. The SEM and TEM investigation revealed the formation of classical cubic morphology of the smart liposomes. Sulfated polysaccharide (κ-CG) integrated smart liposomes exhibited an enhanced κ-CG release profile compared to the liposome-free κ-CG alone. This was due to improved entrapment efficiency (68%) and better overall stability established by the κ-CG incorporation.

Keywords: Kappa-carrageenan, Nanoplatform, Drug-delivery system, Smart liposome, Self-assembly.

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INTRODUCTION

Nanocarrier systems have emerged as promising tools to overcome the limitations of traditional chemotherapy and targeted therapies. Among the lipid-based nanocarrier systems, liposomes, cubosomes, and smart liposomes have garnered considerable attention for their ability to encapsulate and deliver bioactive molecular entity (BME) due to their biocompatibility, non-immunogenicity, and no toxicity.[1] Liposomes are vesicular structures consisting of an amphipathic lipid bilayer that allows encapsulation of both hydrophobic and hydrophilic BME. On the contrary, smart liposomes are self-assembled liquid crystalline cubic phase nanocarrier systems that respond to certain stimuli such as pH, temperature, and magnetic fields, releasing the drug in a controlled manner. Smart liposomes offer superior physicochemical advantages over conventional liposomes, such as enhanced precision, minimized off-target effects, and augmented drug release of the BME payload.[2,3]

Lipopolysaccharide hybrid systems (LPHS) are a type of nanocarrier that integrates both polymers and lipids. These systems harness the complementary properties of polymeric and lipid-based nanocarriers while overcoming their inherent limitations. By combining the strengths of each, LPHS offers a versatile and effective solution for the targeted delivery of therapeutic agents. This innovative and promising technology shows excellent potential for application in medicine and biotechnology. The novel approach used in this study involves the usage of sulfated polysaccharides (kappa-carrageenan), such as carrageenan (CG), as a critical polymer ingredient for the synthesis and stabilization of the smart liposome vesicular system as they are biocompatible. CG, a highly sulfated polysaccharide commonly found in red algae, specifically in *Chondrus*, *Gigartina*, and various *Eucheuma* species within the Rhodophyceae family, exhibits a jelly-like consistency when dispersed in water. It is derived from red algae or seaweed and possesses the unique property of forming durable and rigid gels when mixed with water and cooled. The natural polysaccharide is well known for its ability to increase viscosity, prevent phase separation, and form firm and brittle gels that are thermo-reversible.[4] The number and position of the sulfated groups determine two main subtypes of CG: κ- and λ. Kappa-carrageenan (κ-CG: BME) is a common food additive in various products, including ice cream, processed

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meats, and pharmaceutical formulations.[5] This motivated the use of this biopolymer in this study involving the synthesis and characterization of κ-CG integrated LPHS. Sulfated polysaccharides, such as κ-CG, can induce the formation of smart liposomes through "self-assembly" between them and the phospholipids. Hydrophobicity, hydrogen bonding forces, electrostatic interactions and van der Waals are triggered by sulfated polysaccharide self-assembly (SPSA), among other noncovalent interactions.[6,7] The non-polar interactions occur between alkyl chains, whereas the polar interactions occur between the ionized or zwitter ionic heads of the phospholipids and the polar medium. Typically, this energy-driven process results in spherical structures diffusing inside polar liquids, retaining a similar composition in both the surrounding medium and the core of the structures.[8] Nanostructures with controlled release, such as those employed in drug administration, can be produced by an SPSA approach that achieves spontaneous disorder-to-order transition. For example, the electrostatic layer-by-layer (LbL) self-assembly of carrageenan, an anionic sulfated polysaccharide, with chitosan has been used to create hollow nanocapsules.[6,7] Chemical functionalization and intermolecular interactions can be used to design these SPSA nanostructures into nanoplatforms that react to pH, temperature, or light. They help deliver active pharmacological BMEs in a time and target-controlled manner.[7,9]

κ-CG has been reported *in-vitro* to have a cytotoxic effect on cervical cancer (HeLa) and colon cancer (HCT 116) cells.[10,11] Nonetheless, including κ-CG in smart liposomal formulations may provide significant biological activities, including improved stability, dosage concentration, and prolonged drug release kinetics. Such attributes offer a considerable advantage over conventional liposomes, which have limitations regarding stability and control over drug release. This study explores developing κ-CG integrated smart liposomes as LPHS with their potential application in enhancing sustained κ-CG release.

MATERIALS AND METHODS

Chemicals and Reagents

Soya lecithin, κ-CG, cholesterol, and solvents like phosphate-buffered saline (pH 7.4) and chloroform were purchased from Sisco Research Laboratories Pvt. Ltd, India. All the other chemicals used in this study were of analytical grade.

Liposome Synthesis

We used the thin film hydration method to create liposomes. To begin, we dissolved 270 mg of soya lecithin and 30 mg of cholesterol in 10 mL of chloroform. Afterward, we hydrated the solution in PBS with a pH of 7.4 and kept it overnight at 55°C. The solution was then subjected to sonication and extrusion processes, resulting in the formation of liposomes.

κ-CG integrated smart liposome synthesis

To initiate hydration, we dissolved the κ-CG sulfated polysaccharide polymer in pH 7.4 PBS. We mixed 5 mg of κ-CG with 10 mL of PBS and allowed it to self-assemble overnight. The sample was then subjected to sonication at 40 Hz for 45 minutes at 38°C, followed by extrusion using a 0.22 µm syringe filter. We collected the filtrate for further studies.

Dynamic Light Scattering

The Malvern/Nano ZS-90 zeta sizer was utilized to determine the mean size, zeta potential, and poly-dispersion index (PDI) of both control liposomes and κ-CG integrated smart liposomes. This instrument is a well-established and widely used device that employs dynamic light scattering (DLS) to measure the size distribution of particles in a sample. The results obtained from the analysis of the liposomes and smart liposomes are crucial in characterizing their physicochemical properties.

Scanning electron Microscopy

To observe the surface morphology of liposomes, including nanocarrier control and smart liposomes, a high-resolution scanning electron microscopy (SEM) (Thermo Scientific Apreo S) was used. The sample was dried using a hybridizing oven set at 50°C. The resulting powdered sample was coated with carbon and placed on a metal stub before being observed under the scanning electron microscope at an accelerating voltage of 30 KV. Photomicrographs of suitable magnification were obtained for analysis.

Transmission Electron Microscopy

The investigation of the inner surface morphology of κ-CG integrated smart liposomes was carried out using high-resolution transmission electron microscopy (TEM) operated by JEOL Japan. High-energy electrons were transmitted to the nanoparticles to provide crucial information about their composition and crystallography. The application of this technique is highly advantageous in its ability to provide a detailed observation of the nanostructure, leading to a better understanding of the characteristic properties of the system.

Fourier Transmission Infrared Spectroscopy

The presence of soya lecithin, cholesterol, and κ-CG was studied based on their functional groups using a fourier-transmission infrared spectroscopy (FTIR) spectrometer (IRTracer –100 –Shimadzu) for κ-CG (drug) compared to κ-CG integrated smart liposomes and measured at 4000 to 400 cm^{-1}.

Entrapment Efficiency

The κ-CG integrated smart liposomes were centrifugated at 12,000 g at 4°C for 30 minutes. The supernatant obtained was treated with ethanol and sonicated for 15 minutes. The resulting sample was subjected to re-centrifugation, and the absorbance of the supernatant was measured at 205 nm using UV spectroscopy.

In-vitro Drug Release Studies

An *in-vitro* study was conducted to determine if κ-CG (drug) is released in a controlled manner over a 24-hour period into the release medium. For this purpose, 5 mg of κ-CG (drug) was dissolved in 10 mL of PBS and κ-CG integrated smart liposome nanoparticles containing dialysis bags were suspended in

150 mLof PBS (pH 7.4). The mixture was diffused through the dialysis membrane into the release medium. The absorbance of κ-CG (drug) was measured at 205 nm as it diffused through the dialysis membrane and was released into PBS in a time-dependent manner. A standard graph was plotted using this data and the slope equation was calculated. The R2 value was then recorded from the standard graph to determine the efficiency, and the release of κ-CG (drug) was calibrated accordingly.

RESULTS AND DISCUSSION

Chemistry Behind the Synthesis of LPHS – κ-CG Integrated Smart Liposomes.

When κ-CG interacts with phospholipids, several chemical mechanisms are triggered in liposomes. We hypothesize that the sulfate groups present on the κ-CG monomer unit (D-galactose 4-sulfate) can interact with the polar head groups of phospholipids. These electrostatic interactions are responsible for the association between the two molecules. Hydrogen bonds may form between the hydroxyl groups of galactose units and the phosphate groups of phospholipids. κ-CG also acts as a stabilizer for lipid structures due to its gel-forming properties. When incorporated into lipid systems, such as liposomes, κ-CG can enhance stability by thickening the aqueous phase around lipid droplets. This helps in reducing droplet coalescence by forming a protective gel network and also prevents phase separation in emulsions. We strongly believe that all the above interactions between κ-CG and phospholipids contribute to the overall stability and maintenance of membrane integrity in the LPHS.

Dynamic Light Scattering

The average particle size of nanocarrier control (liposome alone) and κ-CG integrated smart liposome was evaluated to be approximately 80 to 120.2 nm and 40 to 50 nm. The decrease in the size of κ-CG containing smart liposomes is because of the electrostatic LbL and anionic ability of κ-CG to polymerize, integrate and stabilize the membrane architecture. Zeta potential is the overall charge of a particle. The higher the negative value of the zeta potential, the better the stability of the nanoparticle. The zeta potential of the nanocarrier control (liposome alone) and κ-CG integrated smart liposome is -36.6 and -36.1 mV. PDI is an important parameter that describes a sample's heterogeneity based on size. The PDI of nanocarrier control (liposome alone) and κ-CG integrated smart liposome was 0.1 and 0.42. PDI closer to 0.1 implies monodisperse particle distribution. A PDI greater than 0.1 indicates polydisperse particle size distribution.

Scanning Electron Microscopy

The surface morphology of the liposome (nanocarrier control) and κ-CG integrated smart liposome were observed in SEM analysis. The morphology of the carrier control (Figure 1A) is primarily spherical and aggregated. In contrast, κ-CG integrated smart liposome morphology had a cubic structure (Figure 1B). It was dispersed and segregated. We strongly believe that blending κ-CG into the liposomes, allowing it to self-assemble, resulted in the smart liposome's cubic phase hollow architecture. When the sulfated polysaccharide (κ-CG) is mixed with the liposomes, it can interact with the anionic lipids, reorganizing the disorganized to organized LbL lipid bilayers. The interaction between the sulfate groups (hydrophilic) of the polysaccharide and the lipid molecules (hydrophobic) can enable the LbL integration into the regular bilayer (amphipathic) structure. However, the internal structure was hollow and not multilamellar. In the case of κ-CG integrated smart liposomes, this was observed to lead to the formation of cubic phases resembling cubosomes. TEM analysis further confirmed the liposome (nanocarrier control: spherical shape) and κ-CG integrated smart liposome (cubic shape) morphology.

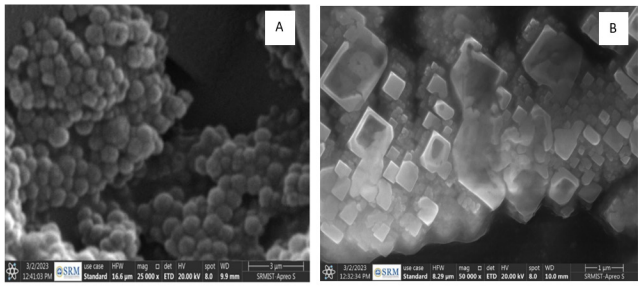

Figure 1: Representative images of (A) SEM for liposome and (B) κ-CG integrated smart liposomes

Transmission Electron Microscopy

The morphology of liposomes (nanocarrier control: Figure 2A) and κ-CG integrated smart liposomes (Figure 2B and 2C) were compared using TEM. TEM analysis further confirmed the morphology of the κ-CG integrated smart liposome to have a cubic tendency. The transformation in the size (40–50 nm) and morphology of the liposomes could be because of the high κ-CG, allowing it to self-assemble, polymerize, and interact with the anionic liposome membrane, enabling it to generate the cubic tendency of the smart liposomes.

Fourier Transmission Infrared Spectroscopy

FTIR data was analyzed using Origin pro software (Figure 3). The peak produced at 2845.0 cm^{-1} in κ-CG integrated smart liposome and 2843.07 cm^{-1}, as shown in Table 1, were due to the presence of aliphatic structure in soya lecithin which is used in the formulation of liposome nanocarrier control. The peak produced at 1635.64 cm^{-1} in κ-CG integrated smart liposome and 1639.49 cm^{-1} in the liposome vehicle was due to cyclic alkene compound in cholesterol in the liposome vehicle. The 2000.18 and 1994.40 cm^{-1} peaks formed from aromatic compounds in κ-CG. From these results, we can say that the κ-CG has been integrated with the smart liposomes.

Entrapment Efficiency

Integrating κ-CG (drug) into the liposomes is crucial during the generation of smart liposomes. The EE% refers to the amount of the κ-CG (drug) successfully integrated into the smart

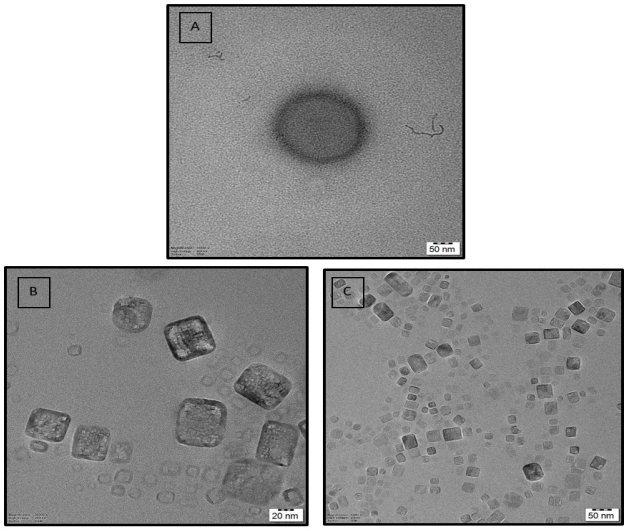

Figure 2: Representative images of TEM for liposome (A) and κ-CG integrated smart liposomes (B & C)

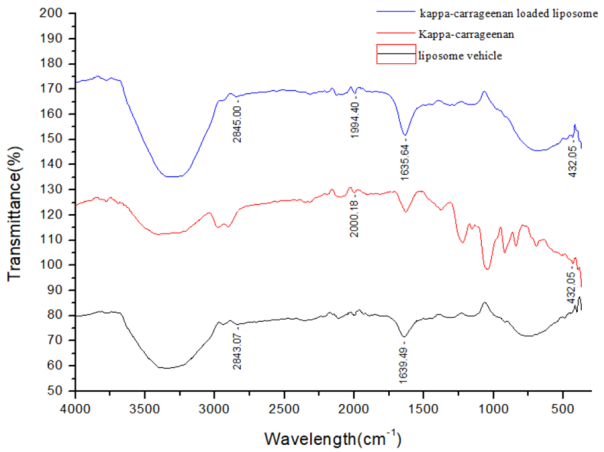

Figure 3: Combined FTIR analysis of κ-CG integrated smart liposomes, κ-CG, and Liposome.

liposomes. The higher the EE%, the more influential the smart liposomes will be in delivering the drug. In this case, the EE% was found to be 68% based on applying the below-mentioned formula, which indicates that a significant amount of the κ-CG (drug) was successfully integrated into the liposomes. Thereby enabling the conversion to the biphasic liquid crystalline smart liposomes. This was determined using a specific formula that accurately calculates the entrapment efficiency.

EE% = (Total drug - Free drug)/ Total drug * 100 (1)

In-vitro Drug Release Studies

In (Figure 4) it is evident that there is a notable contrast between the control and sample groups concerning the release of the drug. The control group containing free-drug (κ-CG) displayed considerably elevated drug release levels in short duration compared to the smart liposome group. The smart liposomes exhibited a gradual, time-dependent release trend

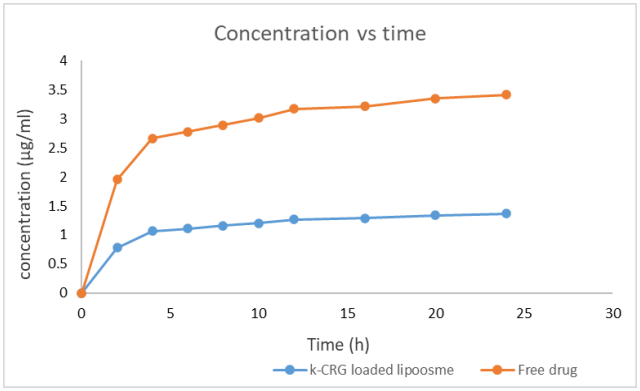

Figure 4: Comparative drug release analysis between κ-CG integrated smart liposomes and κ-CG alone.

Table 1: Salient peaks of FTIR analysis

Sample	*C-H stretching (cm^{-1})*	*C=C stretching (cm^{-1})*	*C-H bending (cm^{-1})*	*Fingerprint region (cm^{-1})*
κ-CG loaded liposome	2845.00	1635.64	1994.40	432.05
κ-CG	-	-	2000.18	432.05
Liposome control	2843.07	1639.49	-	-

of κ-CG, indicating a sustained release. These findings suggest that the smart liposome formulation is efficacious in achieving sustained κ-CG release, thus holding the potential for prolonged and controlled therapeutic outcomes.

CONCLUSION

Liposomes typically comprise amphiphilic molecules with a hydrophilic head and hydrophobic tail. The specific conditions, such as the lipid composition, concentration of sulfated polysaccharide, and preparation methods, play a crucial role in determining the exact properties and nanostructure of the resulting smart liposomes. This study observed that the highly sulfated polysaccharide κ-CG can induce such cubic phase tendency in the liposomes. Sulfated polysaccharides like κ-CG, when introduced to the liposome formulation, interacted with the phospholipid bilayer to generate the LPHS-mediated drug delivery system. Overall, the interaction between sulfated polysaccharides and lipids transforms the typical bilayer structure of nano-liposomes and induces the formation of smart liposome structures, which can be much smaller in size and advantageous for drug delivery. Such smart liposome development offers numerous other applications where sustained release and stability are essential. By controlling drug release, κ-CG-based delivery systems may reduce the systemic toxicity of specific cancer treatments. K-CG is biocompatible and non-toxic, which suggests that it could be a viable option for drug delivery systems. The development of such a promising drug delivery system as a nano platform and entrusting its applications in the clinical industry has the potential to transform the medical industry and benefit society.

Despite the above results, further studies are warranted to determine the drug-loading capacity, bioavailability, stimuli responsiveness, and pharmacokinetics potential of the smart liposomes alone or in combination with other therapeutic agents through more preclinical and clinical studies.

AUTHOR CONTRIBUTIONS

VSRK contributed to the design, conceptualizing of the idea, providing resources and design of this manuscript. SP, KK and VSRK contributed to the manuscript's analysis, interpretation, drafting and editing. All the authors have confirmed the integrity of the manuscript.

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