

Transport of Indomethacin from Kappa-Carrageenan based Nanogel

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Abstract

An effort has been made to prepare gel matrices using different concentrations of Carrageenan whose particle sizes could be exploited in the nanoscale. The Single emulsion technique has been employed for gel formulation. After formulation, gels were characterized for their particle size and zeta potential. Fourier Transform Infrared Spectroscopy (FTIR) was used in the analysis of nanogels, scanning electron microscopy (SEM) to envision the surface microstructures was also performed. It was found that average particle size was in the range of 2000- 4000 nm after drug entrapment and the particles were found to be spherical in shape. The entrapment efficiency of the model drug (Indomethacin) in gel matrix was found to be nearly 40%. Finally, the in vitro release profiles of indomethacin from the nanogel matrices were evaluated spectrophotometrically at 266 nm. Drug release from nanogel consisting of 4% carrageenan was found to be 68% for up to 24 h. Finally, the results have indicated that the Carrageenan nanogel carriers could be used for drug delivery applications.

Keywords: Nanogel; κ -Carrageenan; Indomethacin; Single emulsion solvent evaporation technique

Introduction

Over the past decades, there has been an increasing interest in the development of nanogel. Hydrogel nanoparticle is known as nanogel and can be used flexibly to provide new therapeutic strategies for autoimmune diseases and degenerative diseases [1-3]. Hydrogels are polymeric networks having three dimensional configurations and have the ability to imbibe and retain water within their structural moiety [4]. These gels at nanoscale level have gained considerable attention as drug delivery carriers [1]. Properties of nanogel such as bioavailability, large surface area for multivalent bioconjugation, diameter, mechanical property, size for specific purpose make it a good candidate for drug delivery systems [4]. Carrageenan is a biocompatible polysaccharides extracted from seaweeds [5]. The gelling properties of κ -carrageenan are higher than the other types, whereas its viscosity is lower [6]. Gel efficiency could be increased in the presence of K^+ ions at neutral or basic pH [7-10].

The present work focused on the design of nanogels by using different concentrations of carrageenan polymers. After preparation, the gels were characterized for their particle size and zeta potential. Fourier Transform Infrared Spectroscopy (FTIR) was used in the analysis of nanogels, scanning electron microscopy (SEM) to envision the surface microstructures was also performed. The release study was also evaluated using Indomethacin as a model drug.

Materials and Methods

Materials

Kappa - Carrageenan (KC) was a generous gift from SNAP, Natural & Alginate Product, Mumbai, India, Potassium Chloride GR, Cetyltrimethylammonium bromide (CTAB) were purchased from Himedia, Mumbai, Dichloromethane was purchased from SD Fine Chemicals, Mumbai, Indomethacin acquired from Sigma Aldrich, USA. All chemical were used as received and all aqueous phase solutions were freshly prepared using ultrapure water.

Methods

The nanogel carrier matrices were prepared by single emulsion solvent evaporation method. System consists of organic as well as

aqueous phase. Organic phase comprising of drug was dissolved in dichloromethane (DCM). Similarly aqueous phase comprising surfactant was dissolved in KCl solution. An aqueous phase was prepared by mixing kappa Carrageenan (1-4 wt.%) and surfactant in a homogenizer followed by sonication. The organic solvent was added drop wise during homogenization. The organic solvent present in the solution was evaporated by magnetic stirring for 12 h as shown in Figure 1. Centrifugation is done at 20,000 rpm for 30 min and the supernatant

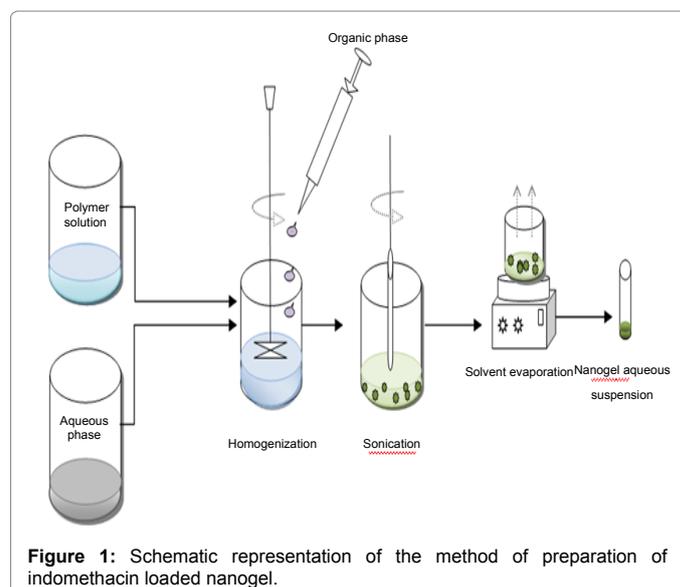


Figure 1: Schematic representation of the method of preparation of indomethacin loaded nanogel.

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was collected. The supernatant was again centrifuged with ethanol and the pellet formed was evaluated for particle size, zeta potential, and entrapment efficiency.

Characterization of carrageenan based nanogel

Particle size and zeta potential: The size of particles and zeta potential were determined using the Zetasizer (Nano ZS Malvern Instruments, UK). The samples were examined at 25°C to determine the mean particle size and zeta potential.

Drug entrapment efficiency: Indomethacin content was analyzed as described [11] by dissolving 20 mg of the pellet in 10 ml of phosphate buffer pH 7.4 solution. Prepared solution was then sonicated using probe sonicator for 15 min and in water bath sonicator (Oscar micro clean-103, Growell Instruments, Bangalore, India) for another 15 min. After sufficient dilution, the supernatant was collected through centrifugation for 30 min at 20,000 rpm. The amount of drug dissolved was determined spectrophotometrically at 266 nm (UV-1601PC, Shimadzu, Japan). Entrapment efficiency EE (%) was calculated using equation (1).

$$EE(\%) = \frac{\text{Experimental amount of drug}}{\text{Theoretical amount of drug}} \times 100 \quad (1)$$

Fourier transform infrared spectroscopy (FTIR): Infrared spectroscopy spectrum was obtained by using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded within the region of 4000 to 400 cm⁻¹.

Scanning electron microscopy (SEM): Shape and surface morphology of the freeze-dried nanogel or lyophilized nanogel were studied using SEM (ZEISS, EVO 18, Jena, Germany). Prepared samples were observed for morphology at an acceleration voltage of 5-20 KV.

In-vitro release study: The drug release kinetics of the prepared formulations was studied as described [11]. The batches of different formulations (2 mg dose equivalent) were placed in dialysis bag. Then prepared dialysis bag kept inside a 100 ml beaker containing 50 ml of dissolution medium (pH 7.4 PBS) and stirred with a magnetic bead on a magnetic stirrer at 10 rpm. Temperature of 37 ± 1°C was maintained throughout the study. Samples (1 mL) were withdrawn at regular time intervals and replaced with an equal volume of same medium. The amount of dissolved indomethacin at each time intervals were analyzed UV spectrophotometrically.

$$CR(\%) = \frac{\text{Experimental amount of drug release}}{\text{Theoretical amount of drug release}} \times 100 \quad (2)$$

Results are reported in all experiments as arithmetic means ± standard deviation. Triplicate analyses were performed on all measurements.

Results and Discussion

In the present study, an attempt was made to develop indomethacin loaded nanogel by single emulsion solvent evaporation method. Batches of nanogel with drug were formulated and comparative studies have been done based on particles size, zeta potential and entrapment efficiency (%). At high concentration of polymers (4 wt %) decrease in particle size was observed. Average particle size of KC3 was found to be 2091 nm which can permeate the epidermis layer and the same has been supported by the previous reports [12]. There was not much difference observed with entrapment efficiency among all the formulation.

The KC3 formulation showed a zeta potential of < -30 mV i.e.

-41.6, indicating good physical stability of these nanogel carrier. Table 1 shows all the results of different formulations. Formulation KC3 had the best average particle size among all the formulation. Therefore, KC 3 formulation was selected for the further studies and characterization.

FTIR spectral analysis for drug alone, physical mixture and formulated nanogel were analyzed as shown in the Figure 2. In Formulation maximum peaks were retained. The IR spectrum of Indomethacin alone is shown in Figure 2A. Indomethacin showed characteristic peaks 1725-1700 cm⁻¹ for presence of (C = O) carboxylic acid, small and medium stretch at 3400-2400 cm⁻¹ for (O-H) carboxylic acid, small stretching at 744-551 cm⁻¹ for (Cl) chlorine group (alkyle halides), strong bending at 1477 cm⁻¹ for (C-H) alkane stretching, 914-694 cm⁻¹ for aromatic stretch (out of plane bend) it has no aromatic ring, 1018-651.89 cm⁻¹ for (=C-H) alkene stretching(out of plane bend). These peaks are indicative values to elucidate functional groups of Indomethacin.

The IR spectrum of physical mixture of raw ingredients is shown in Figure 2B. IR spectra of carrier showed characteristic peaks at 3402-2511 cm⁻¹ for (O-H) stretch and it reveals free carboxylic acid group, small stretching at 702-578 cm⁻¹ for (Cl) chlorine alkyle halides group is present, strong bending at 1481 cm⁻¹ for (C-H) alkane shifted from 1477 cm⁻¹, 925-702 cm⁻¹ for (C-H)aromatic stretch(out of plane bend) was shifted, 1018-702 cm⁻¹ for (=C-H) alkenes stretch(out of plane bend).

The IR spectrum of nanogel carrier is shown in Figure 2C. Characteristic peak at 1725-1700 cm⁻¹ for the presence of (C = O) carboxylic acid was merged, small stretching at 718-624 cm⁻¹ for (Cl) chlorine group, strong bending at 1458 cm⁻¹ also for (C-H) alkane, 879-713 cm⁻¹ for (C-H)aromatic stretch(out of plane bend), 1022-713 cm⁻¹ for (=C-H) alkenes stretch(out of plane bend), 3500-3000 cm⁻¹ for the presence of alcohol and carboxylic groups. In Formulation maximum peaks were retained. The results indicated no considerable changes in the IR peaks of the drug when mixed with carrier, demonstrating the absence of any interaction between Indomethacin and nanogel carrier used.

The particles were found in the size range of about 2000- 4000 nm after encapsulation of drug, was observed with the help of scanning electron microscopy (SEM). The SEM micrographs are shown in Figure 4 majorities of the particles were found to be spherical in shape.

As shown in Figure 4 among the entire batches of formulation % cumulative drug release up to 24 hours is 64.14% for KC₃. However, % cumulative drug release in other batches KC₁ and KC₂ are 77.12, and 82.533 respectively. Hence results suggest that % cumulative drug release of other formulation is quite higher than the KC₃.

Conclusion

It was found that from all the formulations, formulation KC3 had the best average particle size of 2091 nm which also falls near the desired range. In addition formulation KC3 had the best % entrapment efficiency, drug release kinetics and zeta potential. However in other samples the particle size was found to be much greater. Studies have

Sample name	Wt. % KC	Particle size (nm)	Zeta potential (mV)	Entrapment Efficiency (%)
KC1	1	4185	-26.7	33.40
KC2	2	3379	-49.9	31.83
KC3	4	2091	-41.6	48.81

Table 1: Sample and composition of K- Carrageenan nanogels.

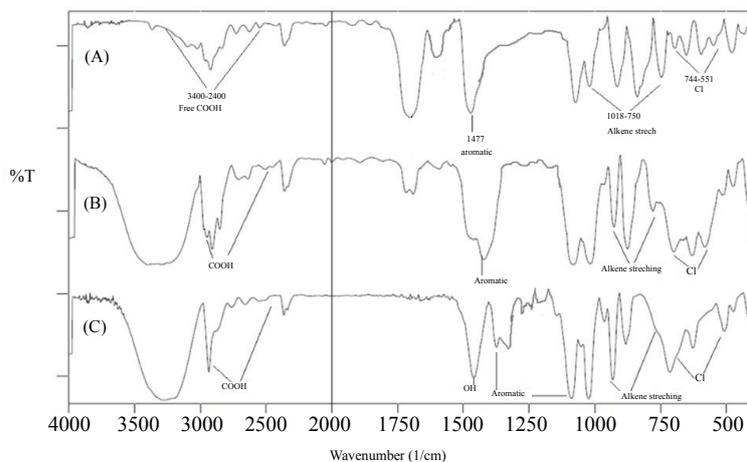


Figure 2: FTIR spectrums of (A), (B) and (C) of drug, physical mixture and optimized formulation (KC 3) respectively.

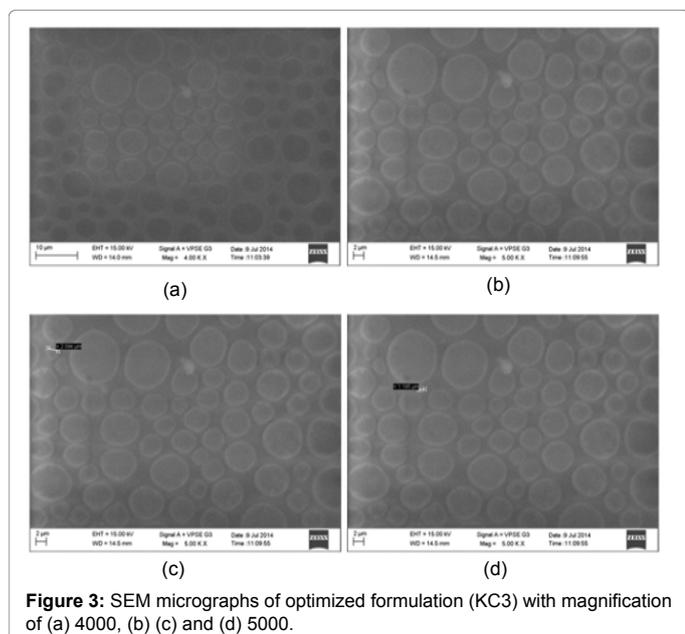


Figure 3: SEM micrographs of optimized formulation (KC3) with magnification of (a) 4000, (b) (c) and (d) 5000.

shown that nanogel carrier proves to be a good applicant for drug delivery. Overall, all the results prove the gels could be tuned to be a good candidate for biomedical applications.

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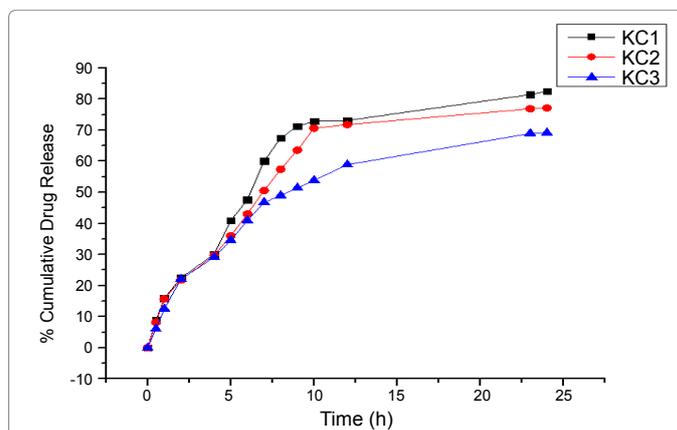


Figure 4: Experimental indomethacin release from KC 1, KC 2 and KC 3 drug loaded nanogels, Values are expressed as arithmetic means \pm SD (n = 3).

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