Alginate/k-carrageenan and Alginate/Gelatin Composite Hydrogel Beads for Controlled Drug Release of Curcumin

Sayeesh P M, Reshma Joy, Nibisha Pradeep, Franklin John, Jinu George*

Biotechnology Laboratory, Department of Chemistry, Sacred Heart College, Kochi, 682013, India

*Corresponding author: E-mail: jinujacob@shcollege.ac.in

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Abstract

Hydrogel beads based on natural polymers like alginate, κ-Carrageenan, and gelatin represent an efficient scaffold for controlled hydrophobic drug delivery. We report herein the development and characterization different formulations of hydrogel systems based on the above-mentioned polymers having adequate properties as drug delivery matrices. Different combinations of alginate/κ-Carrageenan and alginate/gelatin hydrogel beads were developed and drug release properties were compared using curcumin as a model drug. Alginate/κ-Carrageenan hydrogel beads with 50:50 weight ratio exhibited higher swelling and better drug release percentage than compared to other beads. Antibacterial activity of curcumin released from hydrogel beads against *B. cereus* was established by disc assay. Encapsulation efficiency and drug release behaviour of different formulations of alginate/κ-Carrageenan and alginate/gelatin indicates that the polymer blends synthesized possess considerable potential in pharmaceutical and medicinal applications. Copyright © VBRI Press.

Keywords: Hydrogel, curcumin encapsulation, antibacterial.

Introduction

Hydrophilic interpenetrating polymeric networks like hydrogels represent excellent carrier for hydrophobic drug encapsulation. In comparison to hydrophilic nano systems widely used in drug delivery, hydrogels show better swelling property and biocompatibility [**1-4**]. Hydrogels derived from natural polymers like polysaccharides, alginates, gelatin polysaccharide conjugates exhibit a gradual change in swelling properties upon variation in temperature, pH, and ionic strength. 3D polymeric network derived from hydrogels represent flexible tissue-like material for efficient delivery of hydrophobic drugs [**5**].

Alginates are naturally derived polysaccharide block copolymers composed of β-D-mannuronic acid monomers (M-blocks), α-L-guluronic acids (G-blocks) and regions of interspersed M and G units. Divalent cations such as Ca^{2+} can induce hydrogel formation by polysaccharide chain association in alginates [**6-10**]. Alginates have been extensively studied for drug delivery application due to their high encapsulation efficiency and they do not agglomerate in organs during drug delivery [**11**]. It is already established that blending is an effective and convenient method to enhance the performance of these polymer materials. Similarly, promising results were obtained using alginate-based scaffolds. Films [**12**], fibers [**13**], sheets [**14**] and beads [**15**] based on alginate-gelatin blends were prepared for drug delivery purposes and sponges [**16**] have been developed as gastro-retentive carriers. Previous research by Mi Li *et al.* [**17**] proved that gelatin core could be well protected by alginate shell keeping it intact in gastric juice and collapsing in the intestinal environment. Hence among the various synthetic and natural polymers that have been extensively studied for biomedical applications, gelatin represents a good choice for drug delivery applications [**18, 19**]. Gelatin modifications, as well as its combination with other biomaterials, find versatility in designing ideal carrier systems responding to the body stimuli. They play an important role in human adipose-derived stem cells encapsulation and thereby a good candidate for adipose tissue engineering and regenerative medicine [**20**]. Bio adhesive nature of alginate combined with the gel-forming ability of gelatin makes it a most extensively investigated material as tissue bio adhesives [**21**].

Various studies indicated that properties of hydrogel carriers and their responses to the body stimuli are in relation to biopolymers concentration, concentration effects.

Carrageenans represent a family of sulphated linear polysaccharides consisting of (1→3)-linked β-galactose

and (1→4)-linked α-D galactose units [**22**]. Three main types of Carrageenans are known: kappa-κ, lambda-λ and iota-i, depending on the number and the position of the ionic sulphate groups. The presence of a suitable cation, typically potassium, or calcium is an absolute requirement for gelation of the Carrageenans, especially kappa [**23**]. κ-Carrageenan (κ-Car) is a potent pharmaceutical material due to its excellent biocompatibility, low toxicity, and appropriate visco-elastic properties. Hydrogel formed from κ-Car is a suitable agent for drug delivery systems and it is capable of encapsulating drugs to prevent premature release and degradation in the localized environment. κ-Carrageenan/alginate hydrogels find industrial application for enzyme immobilization [**24**]. Introduction of magnetic nano particles into hydrogel produces significant scope in cell targeting as well [**25**]. Its considerable potential as carrier materials for cell delivery in tissue engineering and regenerative medicine has already been proved. Studies in the influence of pore size distribution on the drug release of gelatin/κ-Car hydrogel using thermoporometry points that presence of Carrageenan leads to increase in porosity of the hydrogels [**26**]. However, very high porosity does not improve the drug release significantly. The property of the carrier influences the behavior of drug delivery. In these scenarios, several investigations were done for curcumin delivery by different carrier molecules, such as polymeric micelles, nanoparticles, liposomes, lipid-based nanoparticles, hydrophilic polymers or hydrogels [**27-30**]. Naturally derived hydrogels are of particular interest as their macromolecular properties are similar to the natural extracellular matrix of human tissues [**31**]. Hydrogel beads based on alginate, gelatin, and κ-Car are suitable for the development of sustained and controlled release systems due to their biocompatibility, nontoxicity and hence extensively used for pharmaceutical applications [**32, 33**].

Natural polymer formulations (e.g., alginate, chitosan, Carrageenan, and fucoidan) have been widely used as drug carriers to deliver curcumin which is a natural polyphenolic compound isolated from the rhizome of turmeric (Curcuma longa) and several cancer drugs, such as doxorubicin and 5-fluorouracil (5-Fu) [**34-35**]. Curcumin has been extensively studied as a model drug to establish the efficiency of different encapsulation carriers. Although the therapeutic activity of curcumin is high, the bioavailability is a major concern. Several systems were developed so far to address the poor bioavailability of curcumin [**36-39**].

Several methods were established for hydrogel preparation consisting of conventional polymerization techniques [**40**]. Most of these methods including initiator mediated synthesis poses purity problem. Hydrogel beads were developed via ionotropic gelation using Ca^{2+} and K^{+} in this report. Physico chemical properties like swelling are monitored under different formulations. Swelling behavior of wet and dry alginate /κ-Car, alginate/gelatin hydrogel beads in various % (w/w) is compared. Results obtained indicate that under different formulations, of alginate/κ-Car and alginate/ gelatin, hydrogel beads have considerable potential as drug-carriers using curcumin as a model drug. Most importantly, the biological activity of released curcumin from the hydrogel bead is established by the antimicrobial assay.

Experimental

Materials and methods

Sodium alginate, κ-Carrageenan, (Sigma Aldrich), Gelatin, phosphate buffer saline (PBS, pH 7.4) and curcumin (Sigma Aldrich) were used without further purification. $CaCl_2$ and KCl were purchased from Merck (Germany). Distilled water was used in all experiments. Triplicate experiments were conducted for all.

Preparation of alginate/κ-Car and alginate/gelatin hydrogel beads

Alginate solution was prepared by mixing 1g in 100 mL of distilled water heated at 50°C. 1g of κ-Car were poured into 100mL of distilled water and the temperature was adjusted to 80°C, the prepared contents were stirred using magnetic stirrer until κ-Car was dissolved completely. Alginate and gelatin solutions were prepared separately by dissolving each of the biopolymers in distilled water and (for alginate) and 40°C (for gelatin) under constant stirring from 30 min to 1 h until complete dissolution. 50 mg of Curcumin was dissolved in 5 mL of ethanol (10mg/mL) and added to the above stirred solutions separately. The solutions were again re-stirred for 1 h at 80°C to obtain a clear, viscous and homogeneous solution without bubble [**41**]. Ionotropic method was used for the preparation of hydrogel beads [**42**]. Polysaccharide mixture composed of 50:50, 70:30, 80:20 (weight ratio) with concentrations varying from 1 to 2.5% (**Given in Table 1**) were prepared by mixing under constant stirring at 30°C for 30 min. 20 mL of the mixture was extruded in the form of droplets, using a 21-G needle to two different salt solutions, 100 mL of 2% (w/v) KCl and 100 mL of 2% (w/v) $CaCl_2$ for the preparation of alginate/κ-Car hydrogel beads and 200 mL of 2% $CaCl_2$ solution for alginate/gelatin hydrogel beads. To complete gelation, beads were maintained in the solution for 30 min then filtered, followed by washing with distilled water and then allowed to dry overnight at 37°C. It may be noted that the temperature of cross-linking solution was adjusted at 40°C. The beads were allowed to stir in salts solution for 1 h for hardening. Then, the beads were collected and immersed in excess water to remove un-participated ingredients. After purification, the beads were dried at 40°C for constant weight. The bead size was measured by taking 5±10 particles on a glass slide under polarized light. The mean diameter was calculated by measuring the number of divisions of the ocular micrometer covering the microspheres. The stage

micrometer was previously used to standardize the ocular micrometer.

Characterization and analysis

Curcumin-loaded and unloaded beads were characterized by Fourier Transform Infrared Spectrophotometer (Shimadzu, 8400) with 16 scans per sample. Scanning electron microscopy (SEM, LEO 1430 V) was used to analyze the morphology of hydrogel beads. UV-Vis spectroscopy was carried out on a Lambda 25-PerkinElmer UV-Vis spectrophotometer.

Swelling behavior

The swelling degree (SD) was determined gravimetrically as follows: Both the hydrogel beads were immersed in phosphate buffered saline (PBS, pH 7.4) at 37°C. The swelled samples were withdrawn from PBS at selected time intervals of 1, 2, 3, 4, and 10 up to 24h, wiped, weighed and placed again in PBS. The SD, in percentage, was calculated using equation (1).

$$SD = \frac{W_s - W_d}{W_d} * \ 100 \ (\%) \qquad (1)$$

where Ws and Wd are the weights of swollen and dry beads, respectively. The swelling capacities were measured in triplicate.

Release study of curcumin

Drug release profile from hydrogel was studied in phosphate buffer saline (PBS 0.01 M, pH=7.4) at 37 °C. Hydrogels beads (~30 mg) were immersed in 10 mL PBS solution and shaken in an incubator shaker at 120 rpm. 3 mL solution was taken out after regular time intervals and analyzed for the amount of drug released and then the same volume of fresh phosphate buffered saline solution was added. Drug release from a hydrogel mainly depends on the swelling behavior of the hydrogel, the interaction of the drug with the polymers and the solubility of the drug in the release media [**43**]. All release studies were performed in triplicate and the results were presented in terms of cumulative percentage release. The percentage drug release was calculated by using the following formula: Percentage drug release = (Amount of drug released /Amount of drug loaded) * 100

Antimicrobial activity

Antibacterial activity of the hydrogels beads was evaluated against Bacillus Cereus (*B. Cereus*) pathogens by agar diffusion method [**44**]. The agar plate was inoculated with 100 µL spore suspensions of bacteria. For each sample, one swelled bead (~10 mg) was placed on the agar plate away from the control (PBS) and incubated at 37°C for 24 h. After incubation, the zone of whole inhibition was measured. All tests were replicated three times.

Results and discussion

Characterization of hydrogel beads

FT-IR spectra

FTIR spectra of curcumin loaded and unloaded beads are shown in **Fig. 1.** Spectrum indicates the presence of alginate, κ-Car, gelatin, and curcumin in the loaded beads.

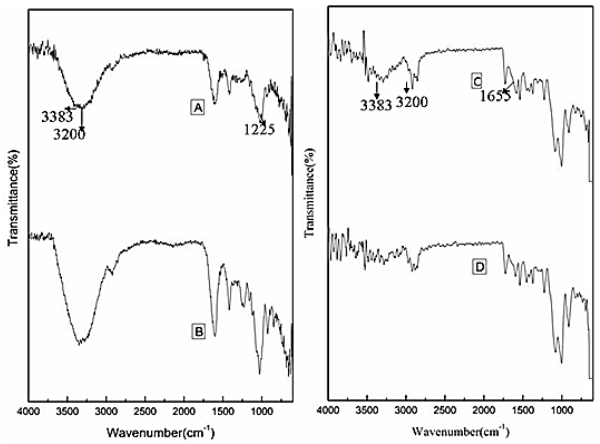

Fig. 1. FTIR spectra of (A) alginate/*κ-Car*, (B) curcumin loaded alginate/*κ-Car*, (C) alginate/gelatin, (D) curcumin loaded alginate/ gelatin hydrogel beads.

Evidence for the presence of alginate can be deduced from the peaks observed at 3383 cm^{-1} due to O-H stretching of hydroxyl and COO^- stretching of carboxylate ion. The spectra clearly show the presence of gelatin as confirmed by C=O stretching in carboxamide functional groups of substrate backbone at 1655 cm^{-1} and 3200–3600 cm^{-1} due to stretching of O-H groups. The presence of κ-Carrageenan was confirmed by the characteristic band at 1225 cm^{-1} due to sulphate stretching (S=O). Due to the low concentration of the drug in the hydrogel, drug peaks were not conspicuous in the drug-containing beads spectra. It seems that infrared spectroscopy is not sensitive enough to detect interactions between the drug and the polysaccharide network. Though confirmation can be made from a broad band at a range of 3200-3500 cm^{-1}, due to a hydroxyl group and band at 3079–3000 cm^{-1} from aromatic ν(C-H). The important absorption band at 1629 cm^{-1} and 1603 cm^{-1} correspond to the mixtures of stretching vibrations of (C=C) and (C=O).

Morphological analysis of hydrogel beads

Image of the swollen beads is shown in **Fig. 2** (supplementary). Beads obtained has a diameter of c.a. 0.50 cm. Morphological features of curcumin loaded and unloaded beads were analyzed through SEM images shown in **Fig. 3**.

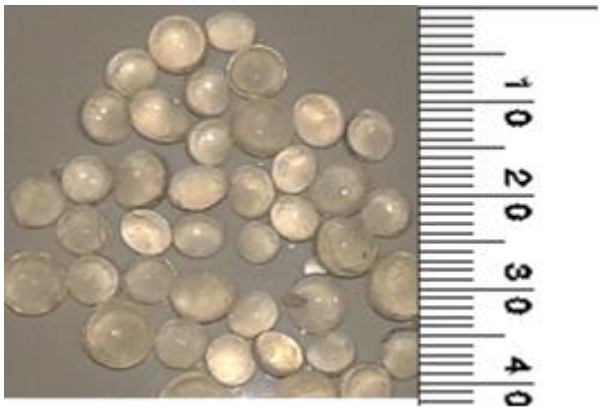

Fig. 2. Morphological analysis of hydrogel beads.

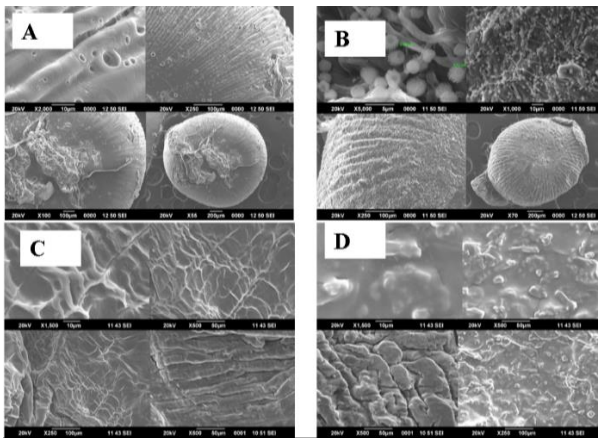

Fig. 3. SEM micrographs of unloaded (a) alginate/κ-Car, (b) alginate/gelatin hydrogel beads and curcumin loaded (c) alginate/κ-Car (d) alginate/gelatin hydrogel beads.

Alginate-gelatin hydrogel beads show a smooth and homogeneous morphology, suggesting component miscibility and blend homogeny. The morphological features of curcumin loaded and unloaded beads were analyzed by recording the SEM images. Beads produced using κ-Car has more pore structure on the surface than that of alginate/gelatin beads. Hydrogel beads containing gelatin appeared to be more polished and spherical compared to the beads consisting of alginate and κ-Car. This observation could be attributed to the higher cross-linking ability of alginate. Cross-linking takes place instantaneously upon dropping sodium alginate into the cross linking solution (consisting of Ca2+) leading to geometrically stable particles. Hydrogel beads containing alginate and gelatin appear to be smoother and more spherical compared to the beads consisting of alginate and carrageenan. This effect can be attributed to the higher cross-linking ability of alginate relative to κ-Car. Cross-linking takes place instantaneously upon dropping sodium alginate into the cross linking solution (consisting of Ca2+) leading to geometrically stable particles. Thus formulations containing κ-Car, beads were less spherical, possessing rough and folded surfaces. These images show that the pore size was homogenously distributed and increased with increasing κ-Car content indicating that κ-Car content is a key factor for controlling the pore size of the Alginate/κ-Car beads. Two different pore morphologies are obvious depending upon the presence of κ-Car/gelatin used.

According to our observations, the lower mechanically stabilized beads generally possess smoother morphologies. These images show that the pore size was homogenously distributed and increases with increasing carrageenan content. Morphological observation of the beads indicates that carrageenan content is a key factor for controlling the pore size. From the pore morphology, a formulation with 50:500 (1.5%) alginate/κ-Car, enabled the formation of uniform hydrogel with good miscibility and thus was found to be the most suitable hydrogel formulation.

Swelling degree

Effect of alginate, κ-Car and gelatin ratios on swelling

In the case of alginate/gelatin and alginate/κ-Car hydrogel beads, effect of sodium alginate on swelling behaviour has been studied by varying its amount in the polymer blend in the range 50-80% (w/w). Results shown in **Fig. 3** proves that initially at 50% of alginate content in hydrogel beads the swelling ratio is high; but above this (70-80%), a fall in swelling behaviour is observed. Initial increase in the swelling degree could be explained by the fact that alginate being a linear anionic hydrophilic polymer, its increasing amount in the hydrogel beads causes an increased hydrophilicity of the polymer network with fixed ionic charges and enhanced repulsion among polymer chains [**45**]. This leads to greater swelling. However the decrease in swelling degree observed beyond 50% can be reasoned to the fact that increase in alginate content results in a compact polymer network. This will form small pore sizes that slow down the diffusion of water molecules into the hydrogel beads and consequently the swelling degree decreases. The effect of gelatin on the swelling degree of the alginate/gelatin hydrogel beads has been studied by varying the amount of gelatin in the polymer blend in the range 20-50% (w/w). Hence it can be concluded that an increase in swelling degree is observed with increase in gelatin content. The effect of κ-Car on swelling degree of alginate/κ-Car hydrogel beads has been studied by varying the amount of κ-Car in the range 20-50% (w/w). Results shown in **Fig. 3** depicts that the swelling degree of alginate/κ-Car hydrogel beads increases with the increase in κ-Car content. With increase in κ-Car content, the beads become less compact in structure and large pores and surface cavities were observed in the hydrogel beads. The alginate/κ-Car (50:50) hydrogel beads show a higher degree of swelling than alginate/gelatin
(50:50) hydrogel beads. Swelling behaviour of hydrogel beads were examined by varying polymer concentration (1%, 2%, (figure not given) 1.5%, and 2.5%**.** From the swelling studies we conclude that increase in polymer concentration leads to enhancement

in the swelling degree of both hydrogel beads. In both cases, hydrogel beads with polymer concentration 2.5% show the highest swelling. Comparison of the swelling degree of alginate/κ-Car hydrogel beads against alginate/gelatin hydrogel beads shows that the former one with polymer concentration 2.5% and weight ratio 50:50 has higher degree of swelling than latter with polymer concentration 2.5% and weight ratio 50:50.

Release studies

Effect of alginate on curcumin release

Curcumin release studies were carried out in PBS (pH 7.4). Upon variation of sodium alginate ratio in the range 50-80% (w/w) in the polymer blend, the amount of curcumin released was found to diminish with time (**Fig. 4** and **5**). This observation could be explained on the basis of swelling behavior of hydrogel beads as discussed earlier. Moreover, by increasing the amount of alginate content, the volume fraction of alginate in the hydrogel beads increases leading to the fact that curcumin molecules have to travel a long path in order to diffuse out through the swollen beads because of its characteristic size [**46**]. This causes a slow release of curcumin from both the hydrogel beads.

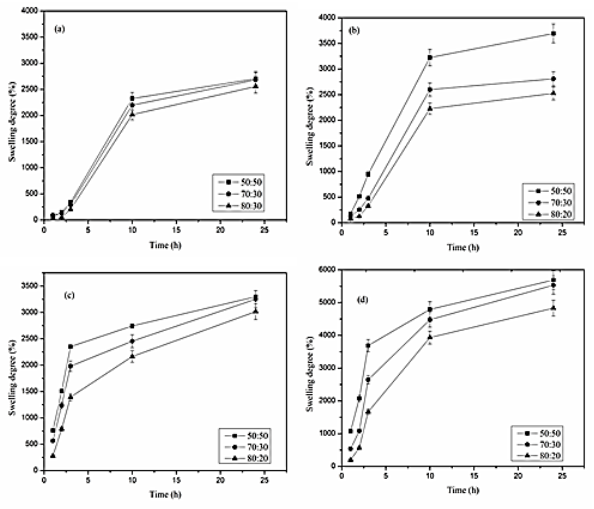

Fig. 4. Effect of alginate/gelatin weight ratio's on the swelling of the alginate/gelatin composite. Hydrogel beads at different polymer concentrations (a) 1.5% (b) 2.5% and effect of alginate/κ-*Car* weight ratio's on the swelling of the alginate/κ-*Car* hydrogel beads with polymer concentrations (a) 1.5% (b) 2.5%.

Effect of κ-Car and gelatin on curcumin release

κ-Car content on drug release has been studied by varying κ-Car in the range 20-50% (w/w). **Fig. 5**. clearly depicts that the amount of released curcumin increases with increasing κ-Car content. With the increase in κ-Car content in hydrogel beads, swelling increases resulting in enhanced drug release. Alginate/κ-Car beads with alginate/κ-Car weight ratio 50:50 showed highest curcumin release percentage (95.45%), owing to the fact that the matrices were built on weak entanglements driven by the presence of κ-Car.

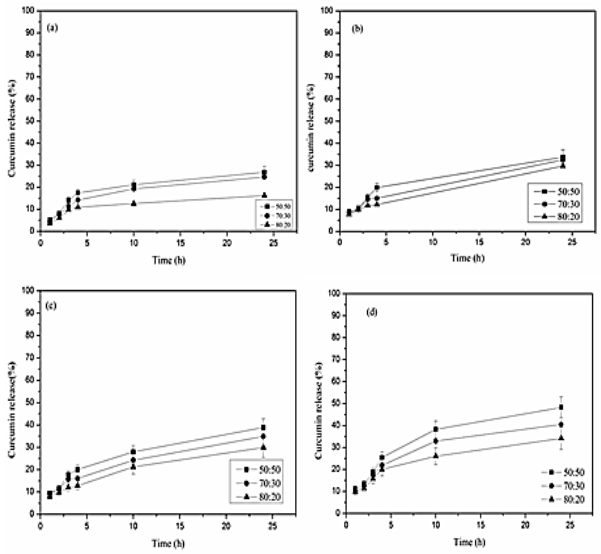

Fig. 5. Effect of alginate/κ-*Car* weight ratio's on fractional release of curcumin from the alginate/κ-*Car* hydrogel beads with polymer concentrations (a) 1% (b) 1.5% (c) 2% (d) 2.5 %.

Effect of gelatin on curcumin release

In the case of alginate/gelatin hydrogel beads, the influence of gelatin content on curcumin release was studied by varying its amount in the range 20-50% (w/w) (**Fig. 6**). Drug release investigations suggest that with increasing gelatin content, the release of curcumin from alginate/gelatin hydrogel beads increases and the observed curcumin release is due to the larger swelling. Alginate/gelatin hydrogel beads with 50:50 weight ratios showed 48.24% curcumin release.

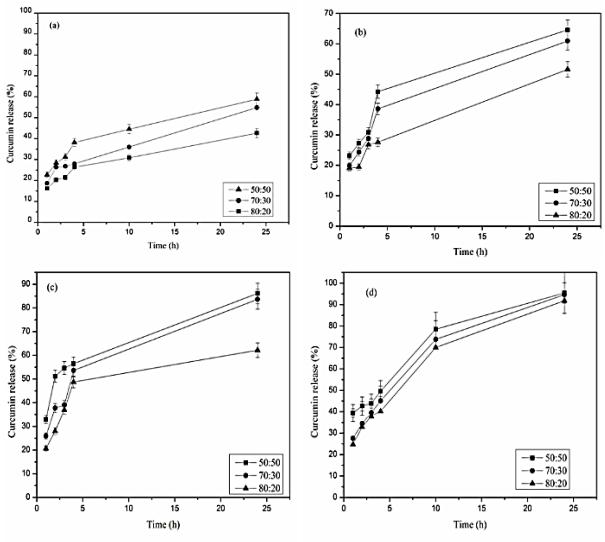

Fig. 6. Effect of alginate/gelatin weight ratio's on fractional release of curcumin from the hydrogel beads with polymer concentrations (a) 1% (b) 1.5% (c) 2% (d) 2.5%.

Table 1. The required amount of initial materials for the preparation of beads.

Materials	Weight ratio	Polymer conc.%	Salt Type	Salt conc.%
alginate/*κ-carrageenan*	50:50	1 1.5		
	70:30	2	$CaCl_2$·KCl	2
	80:20	2.5		
alginate/gelatin	50:50	1 1.5	$CaCl_2$	2
	70:30	2		
	80:20	2.5		

Effect of varying % composition of κ-Car and gelatin on curcumin release

Drug release from the hydrogel beads was also studied by varying polymer concentration in the range 1-2.5 %. In the case of both hydrogel beads, 2.5% is the best polymer concentration for curcumin release. Curcumin release from alginate/κ-Car hydrogel beads shows a higher release percentage than alginate/gelatin hydrogel beads. It can be concluded that higher swelling degree of the alginate/κ-Car hydrogel beads leads to greater curcumin release percentage.

Antimicrobial assay

Antimicrobial activity of curcumin released from alginate/κ-Car, alginate/gelatin hydrogel beads against *B. cereus* were assessed by *In vitro* disc diffusion method shown in **Fig. 7**. The disc was incubated at 37°C for 24h. R-700, R-400 indicates two concentrations (1 mg.mL-1, 1.5 mg.mL-1) respectively of alginate/κ-Car beads and D-700, D-400 indicates that of alginate/gelatin beads. The zone diameter of R-700 and D-700 were comparable. The inhibition zones observed were 30 mm and 27 mm respectively. The zone diameter of R-400 and D-400 were found to be 20 mm and 18 mm respectively. Disc assay confirmed that activity of curcumin is not lost after encapsulation in the hydrogel beads.

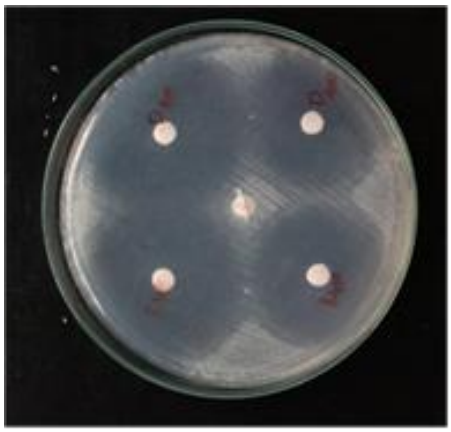

Fig. 7. Antimicrobial studies of hydrogel and its constituents against *B.cereus.*

Conclusions

The present study describes the development and evaluation of natural polymer-based hydrogel beads for drug encapsulation. Encapsulation of curcumin into alginate/κ-Car and alginate-gelatin hydrogel beads showed significant enhancement of curcumin dissolution in aqueous media. Alginate/κ-Car hydrogel beads with 50:50 weight ratio showed highest swelling degree having a higher of curcumin release percentage (95.45%) in PBS (pH 7.4). Swelling degree of alginate/gelatin hydrogel beads were lower than compared to the swelling behavior of alginate/κ-Car hydrogel beads. In the case of alginate/gelatin hydrogel beads, hydrogel beads with alginate/gelatin weight ratio of 50:50 showed highest swelling and 48.24% of curcumin release in PBS (pH 7.4). Curcumin release percentage of alginate/κ-Car hydrogel beads was higher in comparison to the drug release percentage of alginate/gelatin hydrogel beads. It was observed that κ-Car plays an important role in improving the swelling as well as release pattern of curcumin. Results obtained reiterate the increased efficacy of polymer blends with the incorporation of κ-Car for hydrophobic drug encapsulation. Effective drug release is also established. In fact, the drug release and biological activity of environment-friendly hydrogel beads based on natural polymers represent an innovative and adequate alternative for the development of novel therapeutic agents in drug discovery research.

Acknowledgments

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