4. LITERATURE SURVEY

4.1. Alginate

Blandino et al. (Blandino et al., 1999) have investigated the influence of sodium alginate and $CaCl_2$ concentration on gelation kinetics. An increase in the concentration of alginate gives rise to a reduction in membrane thickness, while an increase in the concentration of calcium chloride leads to the formation of a thicker film. Experimental data were adjusted to the binomial diffusion equation.

Technology of mammalian cell encapsulation was reviewed by Uludag et al. (Uludag et al., 2000). Encapsulation physically isolates a cell mass from an outside environment and aims to maintain normal cellular physiology within a desired permeability barrier. The techniques include microencapsulation with polyelectrolyte complexation emphasizing alginate-polylysine capsules, thermoreversible gelation with agarose as a prototype system, interfacial precipitation and interfacial polymerization, as well as the technology of flat sheet and hollow fiber-based macroencapsulation They had singled out four aspects of encapsulated cells that are critical for the success of the technology, namely the capsule permeability, mechanical properties, immune protection and biocompatibility and also summarized methods to evaluate these properties .

Poncelet (Poncelet, 2001) have produced alginate microspheres by emulsification/internal gelation of an alginate sol dispersed within vegetable oil, followed by a reduction in pH to release calcium from an insoluble salt. Microspheres with mean diameters ranging from 50 to 1,000 microm were smooth, spherical beads with the narrowest size dispersion when using low guluronic and low viscosity alginate and a carbonate complex as calcium vector. The calcium salt must also be included within the alginate sol as a very fine powder to promote homogeneous gelation. Shrinking is more important, although the diffusion of large molecules is faster with internal versus external gelation.

Kikuchi and Okano (Kikuchi and Okano, 2002) have studied the pulsatile drug release control using hydrogels. They gave special attention to the thermally responsive poly(N-isopropylacrylamide) and its derivative hydrogels. Thermal stimuli-regulated pulsed drug release was established through the design of drug delivery devices, hydrogels, and micelles. Development of modified alginate gel beads with pulsed drug delivery characteristic is also described in this article.

Blandino et al (Blandino et al., 2003) have used encapsulation within calcium alginate gel capsules to produce a coimmobilized enzyme system. Glucose oxidase (GOD) and catalase (CAT) were chosen as model enzymes. The same values of Vmax and Km app for the GOD encapsulated system and for the GOD-CAT coencapsulated system were calculated. When gel beads and capsules were compared, the same catalyst deactivation sequence for the two enzymes was observed

A Plackett-Burman design was employed to develop and optimize a novel crosslinked calcium-aluminum-alginate-pectinate oilisphere complex as a potential system for the in vitro site-specific release of Mentha piperita by Sibanda et al. (Sibanda et al., 2004). The physicochemical and textural properties (dependent variables) of this complex were found to be highly sensitive to changes in the concentration of the polymers, crosslinkers, and crosslinking reaction times (independent variables). The Lagrangian technique produced no significant differences ($P > .05$) between the experimental and predicted total fracture energy values. Analysis of release data indicated that diffusion (Fickian constant k1 = 0.74 vs relaxation constant k2 = 0.02) was the predominant release mechanism.

Arica et al. (Arica et al., 2005) have investigated in vitro and in vivo studies of ibuprofen-loaded biodegradable alginate beads prepared by ionotropic gelation method The influence of various formulation factors on the encapsulation efficiency, as in vitro drug release and micromeritic properties, alginate concentration, percentage drug loading and stirring speed during the microencapsulation process.were studied. The yield of microspheres, as collected after drying, was generally 80-90. In vivo data showed that the administration of ibuprofen in alginate beads prevented the gastric lesions.

Benzoni et al. (Benzoni et al., 2005) have discussed Transient transfection of porcine granulosa cells after 3D culture in barium alginate capsules. The feasibility of two transient transfection techniques (liposome-mediated and electroporation) was assessed in primary porcine granulosa cells after a 6-day culture in an artificial extracellular matrix (barium alginate membrane). Human recombinant green fluorescent protein was chosen as a molecular readout, and protein expression was assessed after 48 hours from transfection. The results indicate that primary granulosa cell cultured in barium alginate capsules can be transfected by electroporation with high transfection yields.

Bhopatkar et al. (Bhopatkar et al., 2005) have studied ionotropic alginate beads for controlled intestinal protein delivery: effect of chitosan and barium counter-ions on entrapment and release.of haemoglobin (Hb). Coagulation with Ba^{2+}, Ca^{2+} and/or chitosan showed differences in the swelling index of the beads, in the encapsulation efficiency of Hb entrapment and in the release of the entrapped protein. Beads were stable in the gastric fluid but released their protein upon transfer to intestinal fluid. The release coincides with the burst and disintegration of beads.

Cao et al. (Cao et al., 2005) have used sodium alginate, as a model biomacromolecule to investigate the aggregation behaviors in aqueous solution after partial protonation of carboxylate groups in the alginate molecules. The alginate assemblies with core-shell structure can be generated by the partial protonation of carboxylate groups in sodium alginate chains using the protons released gradually from the reaction of K(2)S(2)O(8) with water at 70 degrees C in aqueous solution. The partial cross-linked alginate assemblies are pH sensitive and can change to hollow structure in the medium with relatively high pH value.

A new hypothesis on the role of alternating sequences in calcium-alginate gels was explained by Donati et al. (Donati et al., 2005). In view of the calcium binding properties of long alternating sequences revealed by circular dichroism studies which leads eventually to the formation of stable hydrogels, their direct involvement in the gel network is here suggested. The experimental curves, fitted by a model composed of a

Maxwell and a Voigt element in series, revealed an increase in the frictional forces between network chains with increasing length of the alternating sequences.

Propranolol-HCl-loaded calcium alginate (ALG) beads, propranolol-resin complex (resinate)-loaded calcium alginate (RALG) beads and polyethyleneimine (PEI)-treated RALG (RALG-PEI) beads were prepared by Halder et al. (Halder et al., 2005) using ionotropic gelation/polyelectrolyte complexation method. The beads were evaluated and compared in respect of drug entrapment efficiency (DEE) and release characteristics in SGF and SIF. The release of drug from all the beads was slow and incomplete in SGF owing to considerably less swelling of the beads. Kinetics of the drug release also confirmed the formation of physical barrier as anomalous transport type of release associated with. RALG beads tended to shift towards Fickian transport in case of RALG-PEI beads.

Liu et al. (Liu et al., 2005) have formed novel attrition-resistant and spherical enzyme granules encapsulating active subtilisin by emulsification of 2% alginate sol loaded with active enzyme, instantaneous gelation triggered through in situ release of Ca^{2+} (internal gelation), particle separation, and finally acetone extractive drying. The formulation and encapsulation conditions were optimized to maximize the resistance of the granule to compression and impact forces, consistent with enzyme release and particle dispersion in detergent solutions. The characteristics of the resulting microspheres, including their size and distribution, morphology, shrinkage, compression resistance, impact strength, solubility and encapsulation yield, were examined.

Lu et al. (Lu et al., 2005) have investigated the sol-gel transition in aqueous alginate solutions induced by chelation with calcium cations from in situ release with viscoelastic methods. Two alginate samples having different molecular weights (MW) were used over the concentration C(Alg) of 2 approximately 6 wt % with different mole ratio f of Ca2+ to the alginate repeat unit. With increasing C(Alg), f(gel) for the alginate with lower MW decreases dramatically while, f(gel) for the higher MW alginate with is almost a constant and n decreases with increasing C(Alg), indicating that the concentration dependence of n varies with MW of alginate in the starting solution.

Mi et al. (Mi et al., 2005) have studied two-component pH-sensitive hydrogel system composed of a water-soluble chitosan derivative (N,O-carboxymethyl chitosan, NOCC) and alginate cross-linked by genipin, glutaraldehyde or Ca2+. Preparation and structures of these hydrogels and their swelling characteristics and release profiles of a model protein drug (bovine serum albumin, BSA) SIF are reported. The amounts of BSA released at pH 1.2 from the genipin- and Ca(2+)-cross-linked hydrogels were relatively low (approx. 20%). The results indicated that the swelling behaviors and drug-release profiles of these test hydrogels are significantly different due to their distinct cross-linking structures.

Mukhopadhyay et al. (Mukhopadhyay et al., 2005) have studied reported the cross-linking of dried paracetamol alginate granules with calcium chloride solutions. The effect of calcium concentration, temperature of the treatment solution, stirring speed and time used during cross-linking of granules on water uptake by the granules during cross-linking and physical properties of the cross-linked and dried granules were studied. The variables most affected were water uptake, drug entrapment, early release, granule aggregation, calcium and sodium content.

Orive et al. (Orive et al., 2005) have presented a battery of in vitro techniques to assess the biocompatibility of alginates with different compositions and purities and alginate-based microcapsules. Study of the protein and polyphenol content of the alginates revealed clear differences between the nonpurified and the purified alginates. These results reinforce the idea of using the full battery of assays here reported to screen alginates and alginate-based microcapsules before implantation.

Setty et al. (Setty et al., 2005) have prepared furosemide-loaded calcium alginate (ALG), calcium alginate-polyethyleneimine (ALG-PEI) and alginate-coated ALG-PEI (ALG-PEI-ALG) beads by ionotropic/polyelectrolyte complexation method to achieve controlled release of the drug. Although variation in formulation factors did not influence the drug-loading efficiency (DLE) of ALG beads, rapid release of the drug in simulated intestinal fluid (SIF) could not be prevented. Alginate coating of ALG-PEI beads further prolonged the release of the drug by increasing membrane thickness and reducing swelling of the beads possibly by blocking the surface pores. The release data from ALG-PEI beads and ALG-PEI-ALG beads showed a good fit in power law expression and modified power law expression,respectively

Shi et al. (Shi et al., 2005) have prepared complex beads composed of alginate and carboxymethyl chitin (CMCT) by dropping aqueous alginate-CMCT into an iron(III) solution. The swelling behavior, encapsulation efficiency, and release behavior of bovine serum albumin (BSA) from the beads at different pHs were investigated. The BSA encapsulation efficiency was fairly high (>90%). It was found that CMCT disintegrated at pH 1.2 and alginate eroded at pH 7.4 while the complex beads could effectively retain BSA in acid (>85%) and reduce the BSA release at pH 7.4. The results suggested that the iron(III)-alginate-CMCT bead could be a suitable polymeric carrier for site-specific protein drug delivery in the intestine.

Wolf et al. (Wolf et al., 2005) have formulated microcapsules of high mechanical strength and optimum permeability by injection of BaCl2 crystals into alginate droplets before they come into contact with external Ba2+. A key requirement is that the system parameters (number of crystals, speed of the crystal stream etc.) are properly adjusted according to the mannuronic and guluronic acid ratio and the average molecular mass of the alginate as well as to the diameter of the microcapsules. The data also demonstrate that several steps of the alginate gelling process must be improved before such immunoisolation can be used in patients.

Yang et al. (Yang et al., 2005) have studied two new plate nozzles for the production of alginate microspheres which provide lower liquid resistance and yield well. Furthermore, the more uniform microsphere was produced within a wider range of frequency by plate nozzles. Experiments using multiple-nozzle synthetic red stone plate was easy to feasible.

Zhu et al. (Zhu et al., 2005) have presented a simple and high-efficiency approach to loading macromolecules into microscale carriers. Calcium-cross-linked alginate hydrogel microspheres were fabricated by an emulsification technique and then used as negatively charged templates to form polyelectrolyte multilayer coatings. A high loading efficiency of peroxidase in approximately 1.0 x 10(7) microcapsules (2.5 pg POx/capsule) was achieved with a low concentration of peroxidase loading solution (10 mug/mL).

4.2. Carrageenan

Tosa et al. have immobilized the enzymes and microbial cells using carrageenan as matrix (Tosa et al., 1979). They investigated conditions for the gelation κ-carrageenan in detail. κ-Carrageenan was easily induced to gel by contact with metal ions, amines, amino acid derivatives, and water-miscible organic solvents. By using this property of k-carrageenan, the immobilization of enzymes and microbial cells was investigated. Immobilized preparations were easily tailor-made to various shape such as cube, bead, and membrane. The obtained immobilized preparations were stable, and columns packed with them were used for continuous enzyme reaction for a long period. Their operational stabilities were enhanced by hardening with glutaraldehyde and hexamethylenediamine.

Malfait et al. have studied molecular structure of carrageenans and kappa oligomers using a Raman spectroscopic study (Malfait et al., 1989). They compared the Raman spectra of kappa and iota carrageenan in the region 700-1500 cm^{-1}. They described spectral differences depending on the amount and the location of the sulphate group on the ring, the chain length, the nature of the counterion and the conformation. They found that the ionic interactions in the Na^+ salts of the oligomers are different from those in K^+ and Rb-salts. On the macromolecular level, thy found that the vibrational movements of the skeleton are related to the chain flexibility and the conformation.

Moon and Parulekar have characterized kappa-carrageenan gels for immobilization of *Bacillus firmus,* a superior producer of an alkaline protease (Moon and Parulekar, 1991). They studied the effects of carrageenan concentration, gelation temperature, initial cell loading, and strength of the curing agent (KCl) on the properties of cell-free and cell-laden gels. For the range of carrageenan contents investigated [between 2% and 5% (w/v)], they observed that the mechanical strength of the gels with/without KCl curing was increased with an increase in carrageenan content of gels. The mechanical strength of each gel increased substantially upon extensive curing. Of cells that were viable prior to immobilization, 90-92% remained viable after formation and extensive curing of gels for cell-gel mixtures prepared at 45° C.

Theoretical and practical limitations of size and production rate for the large-scale production of kappa-carrageenan droplets for gel-bead production was studied by the Hunik and Tramper (Hunik and Tramper, 1993). Small scale batches of immobilized biocatalysts in kappa-carrageenan gel beads can be prepared by the conventional needle syringe arrangements. However, due to non-Newtonian fluid behavior of the kappa-carrageen solution, the droplet diameter can, within limits, can not be adjusted to the desired size. Hence they have scaled-up of the extrusion technique with the theory to predict the droplet diameters for non-Newtonian fluids. The emphasis was on the droplet formation, which is the rate-limiting step in this extrusion technique. Uniform droplets were formed by breaking up a capillary jet with a sinusoidal signal of a vibration exciter. They have utilized the theory of Newtonian fluids for the non-Newtonian kappa-carrageenan solution.

Quantitative determination of the spatial distribution of *Nitrosomonas europaea* and *Nitrobacter agilis* cells immobilized in kappa-carrageenan gel beads by a specific fluorescent-antibody labeling technique was carried out by Hunik et al. (Hunik et al., 1993). They combined the labeling and stereological methods, for the determination of spatial distribution of two microorganisms in a biofilm. Cells of *Nitrosomonas europaea* (ATCC 19718) and *Nitrobacter agilis* (ATCC 14123) were homogeneously distributed in a kappa-carrageenan gel during immobilization and allowed to grow out to colonies. The gel beads were sliced in thin cross sections after fixation and embedding. A two-step labelling method resulted in green fluorescent colonies in the cross sections. The positions and surface areas of the colonies of each species were determined. This technique will be useful for the validation of biofilm models, which predict such biomass distributions.

Ridout et al. have studied the gelation of mixtures of iota and kappa carrageenan using differential scanning calorimetry (DSC) (Ridout et al., 1996). They have observed that the iota and kappa components gel independently of each other. The DSC data conflict with evidence presented for the Domain model for carrageenan gelation (Morris, E.R., Rees, D.A. and Robinson, G.J. J. Mol. Biol. 1980; 138:349). Their study suggest that iota carrageenan gels show thermoreversible setting and melting behaviour associated with the coil-helix transition. Analysis of rheological data favours an interpenetrating network (IPN) model for gelation of the mixtures.

The specific role of co-ions and counter-ions on kappa-carrageenan conformation has been studied by Ciancia et al. (Ciancia et al., 1997). They described the role of iodide compared with chloride based on the results obtained from optical rotation, viscometry and microcalorimetry. Iodide gives a more stable helical conformation, with a larger degree of order; at the same time, it represses aggregation of the helices and the gelation ability. From the enthalpy of the conformational change, it seems that, even in the presence of iodide, the ordered conformation remains a double helix.

Michel et al. have studied the physico-chemical properties of carrageenan gels in presence of various cations (Michel et al., 1997). Phase separation and gelation induced by addition of monovalent and divalent cat-ions in iota and kappa carrageenan solutions were investigated as a function of the polymer and cation concentrations. The storage modulus (G') determined at a cation/polymer ratio was always higher for kappa- than for iota-carrageenan. For iota carrageenan, G' increased slowly with the monovalent salt concentration and more quickly with the divalent salt concentration. At the opposite, for kappa carrageenan, G' increased more rapidly in the presence of KCl than with calcium or copper. Nevertheless for large salt concentrations, G' became independent of the type and concentration of cations in the kappa carrageenan solution.

Organisation and association of kappa-carrageenan helices under different salt conditions has been observed by Piculell et al. (Piculell et al., 1997). Mixtures of the added salts NaI and CsI were used to gradually 'tune' the propensity of kappa-carrageenan (KC) helices to aggregate in solution. They showed that this method can be used to resolve the molecular events by which helix formation, under certain conditions, leads to gelation. They also present an overview of the various states of aggregation and organisation that appear for helical KC (non-degraded or ultrasonically degraded) when the NaI/CsI ratio and the concentration of KC are varied.

Mateus et al. have studied the diffusion into and from kappa-carrageenan gel beads in cell-free and cell immobilising kappa-carrageenan gel beads with and without chemical reaction (Mateus et al., 1999). The solutes were indole, L-serine, and L-tryptophan. The reaction was that of indole and L-serine to give L-tryptophan. Simultaneous diffusion of the three solutes resulted in lower diffusivities than those for individual solutes, suggesting the need to use multi-component diffusion theory. Diffusion with chemical reaction was reasonably well described by an effectiveness factor calculated using an effective diffusivity estimated from diffusion data without reaction.

Factorial analysis of the influence of dissolution medium on drug release from carrageenan-diltiazem complexes was studied by Bonferoni et al. (Bonferoni et al., 2000). They studied the influence of buffer composition, pH, and ionic strength on the release of diltiazem hydrochloride from a complex of the drug with lambda carrageenan. Two viscosity grades of carrageenan were also compared. The increase of ionic strength significantly increased complex solubility in all the buffer systems. Drug release rate decreased when high polymer grade was involved in the complex. At higher ionic strength drug release was no longer constant, but decreased with time, probably because of lower polymer solubility.

Jones et al. (2000) have investigated the interaction of kappa-carrageenan with nickel, cobalt, and iron hydroxides (Jones et al., 2000). It was shown that the functional polysaccharide kappa-carrageenan acts as an efficient stabilizer to prevent the precipitation of iron oxides and hydroxides up to very high pH values.

Thermodynamics of conformational ordering of iota-carrageenan in KCl solutions was discussed by Grinberg et al. using high-sensitivity differential scanning calorimetry (Grinberg et al., 2001). The polysaccharide was found to undergo two consecutive cooperative conformational transitions, which was represented by the scheme: $[H2]_2<2H2<4C$ where C is the random coil, H2 is the double helix, and $[H2]_2$ is the double helix dimer. The study suggests that the stacking effect in helices of iota-carrageenan is small.

Ikeda et al. have investigated microstructure of aggregated and nonaggregated kappa-carrageenan helices visualized by atomic force microscopy (Ikeda et al., 2001). Classically, gelation of kappa-carrageenan is believed to involve two steps: helix formation on cooling and a further specific cation (salt) induced side-by-side aggregation of helices. In the presence of an excessive amount of a gel-promoting salt, KCl, kappa-carrageenan appeared to form rigid rodlike structures considered as large aggregates of double helices. Even when the side-by-side interhelical aggregation was suppressed by diluting random coiled solutions prior to cooling, by adding an aggregation-impeding salt, NaI, or by transforming kappa-carrageenan into the tetramethylammonium (TMA) salt, branched rodlike structures were still evident, suggesting that the side-by-side aggregation of helices is not a prerequisite for kappa-carrageenan to form a network structure, at least locally.

Janaswamy and Chandrasekaran (2001) have studied the three-dimensional structure of the sodium salt of iota-carrageenan by using X-ray diffraction data collected from its polycrystalline and oriented fibers (Janaswamy and Chandrasekaran, 2001). The molecule forms a half-staggered, parallel, threefold, right-handed double helix that is stabilized by interchain hydrogen bonds from 2- and 6-hydroxyl groups in the galactosyl units. Both 2-

and 4-sulfate groups are essential in helix-helix interactions that are mediated only by sodium ions and water molecules.

Pelletier et al. have studied solution rheology of kappa-carrageenan in the ordered and disordered conformations induced by both electrolyte and temperature and was found to give rise to significantly different rheological properties under shear flow, extensional flow, and small deformation oscillation regimes (Pelletier et al., 2001). Shear flow displayed only shear thinning or Newtonian behavior, depending of the chain conformation. A larger range of properties was observed in elongational flow. Strain-thinning behavior was observed in the ordered conformation while strain thickening occurred in the disordered conformation.

Verapamil HCl and ibuprofen containing carrageenan beads were prepared and in vitro evaluation was carried out by Sipahigil and Dortunc (Sipahigil and Dortunc, 2001). Beads were prepared by ionotropic gelation method. The influence of formulation factors (drug content, polymer concentration, counterion type and concentration, outer phase volume) on the particle size, encapsulation efficiency and in vitro release characteristics of beads was investigated. The encapsulation efficiency of veraparnil HCl in the beads (34.8-71.1%) was higher than that of ibuprofen (23.6-58%). While about 30% of ibuprofen was released at 6 h, about 70% of verapamil HCl was released in 5 h from the carrageenan beads prepared.

van de Velde et al. have focused on the structure of kappa/iota-hybrid carrageenans (van de Velde et al., 2001). The coil-to-helix transition and temperature dependence of the viscosity of kappa/iota-hybrid carrageenans were studied using rheometry and optical rotation. The coil-to-helix transitions of the kappa/iota-hybrid carrageenans are significantly different from those of pure kappa- and iota-carrageenan, and from hand-made mixtures thereof. This provides evidence that the kappa/iota-hybrid carrageenans are mixed chains, containing both kappa- and iota-repeating units.

Effect of calcium ions on the organization of iota-carrageenan helices was investigate by Janaswamy and Chandrasekaran using an X-ray investigation (Janaswamy and Chandrasekaran, 2002). X-ray fiber diffraction analysis confirms that calcium iota-carrageenan forms a threefold, right-handed, half-staggered, parallel, double helix of pitch 26.42 Å stabilized by interchain hydrogen bonds. According to the detailed structural results, three helices are packed in a trigonal unit cell. Strong interactions between the sulfate groups of neighboring helices, mediated by calcium ions and water molecules, are responsible for stabilizing the three-dimensional structure.

Swelling behavior of kappa-carrageenan gels in water and KCl solutions was investigated by photon transmission experiments following the preparation of gels in the presence and absence of externally added K^+ ion as a gel promoting agent (Kara et al., 2003). Transmitted photon intensity, Itr, increased continuously during swelling depending on the carrageenan and ion content in the gel. It was confirmed that double helices in a swollen gel move much faster in pure water than in KCl solution during swelling.

Mangione et al. have studied, by optical rotation dispersion, light scattering and rheology, the thermoreversible gelation of kappa-carrageenan system to elucidate the processes involved in gel formation (on decreasing the temperature) and gel melting (on increasing the temperature) (Mangione et al., 2003). They showed that, on decreasing the

temperature, a conformational transition from coils to double helices first occurs, followed by aggregation of the double helices into domains and gel formation at appropriate polymer concentration. The helices can regain the coil conformation only when the aggregates melt at higher temperature, in full agreement with the old 'domain' model.

Tojo and Prado have determined the chemical composition of carrageenan blends by IR spectroscopy combined with a partial least-squares multivariate regression (PLS) multivariate calibration method (Tojo and Prado, 2003). This method allows the determination of the relative amounts of the different carrageenans in a rapid and accurate manner.

Investigation of the dynamic behavior of irradiated kappa carrageenan (in KCl) as a function of irradiation dose and temperature was carried out using dynamic light scattering (DLS) by Abad et al. (Abad et al., 2004). The intensity correlation function (ICF) shifted towards shorter relaxation times with increasing radiation dose as a result of radiolysis. The characteristic decay time distribution function, G(gamma), indicates the presence of fast and slow mode peaks respectively at around 0.1-10 ms and 100-1000 ms. A peak broadening of the fast mode peak in G(gamma) appeared with decreasing temperature, indicating that coil-to-helical conformational transition took place. The conformation transition temperature (CTT) decreased with increasing radiation dose.

Effect of potassium chloride and cationic drug on swelling, erosion and release from kappa-carrageenan matrices was investigated by Naim et al. (Naim et al., 2004). Erosion was not affected by concentration of KCl. Incorporation of drug favors water uptake, but in presence of KCl it was found to be reduced. Drug-containing matrices have shown higher release of KCl as compared with plain batches.

The fluorescence technique was employed to study gel-to-sol transitions in kappa-carrageenan systems with various carrageenan contents (Pekcan and Tari, 2004). Pyranine was used as a fluorescence probe for monitoring these transitions. It was observed that gel-to-sol transition temperatures, Tgs are found to be dependent to the carrageenan content.

Mangione et al. have studied K^+, Na^+ and their mixture on the conformational transition and macroscopic gel properties of kappa-carrageenan using different experimental techniques (Mangione et al., 2005). The macroscopic gelation properties of kappa-Carrageenan were found to be dependent upon co-solute type. Indeed, a more ordered and strong gel was obtained in the presence of K^+ with respect to Na^+ ions. The gel properties obtained using mixtures of two cosolutes are shown to depend on the $[K^+]/[Na^+]$ ratio.

Unperturbed dimensions of carrageenans in different salt solutions were studied by Marcelo et al. (Marcelo et al., 2005). The eluent was water containing 0.1 M concentration of different ionic salts, namely LiCl, NaCl, KCl and NaI, with the exception of kappa-carrageenan that aggregates in presence of KCl. Extrapolation to unperturbed conditions provides values of the characteristic ratio C(N)=56±1 and 40±5 respectively for lambda- and iota-carrageenans regardless of the ionic salt employed. However, kappa-carrageenan gives C(N)=31, 35 and 59, respectively, in presence of LiCl, NaCl and NaI, which clearly indicates that this polymer behaves on a different way in presence of NaI than with the other two salts.

4.3. Chitosan

Gaserod et al. (Gaserod et al., 1998) have studied studied the binding of chitosan to alginate beads quantitatively by using radioactive labelled fractions of chitosan. The alginate-chitosan capsules were made either by dropping a solution of sodium alginate into a solution containing chitosan or by incubating calcium alginate beads in a solution of chitosan. The binding of chitosan was markedly increased by reducing the number average molecular weight of chitosan below 20000 Da and by increasing the porosity of the alginate gel. The binding of chitosan was also found to increase with decreasing fraction of N-acetylations, FA, on chitosan in the range of FA = 0.3 to FA = 0, and with increasing pH in the range from pH 4 to 6.

Murata et al. (Murata et al., 1999) have prepared calcium-induced alginate gel beads containing chitosan salt (Alg-CS) using nicotinic acid (NA), and investigated its two functions in gastrointestinal tract, (a) NA release from Alg-CS, (b) uptake of bile acids into Alg-CS. NA was rapidly released from Alg-CS in diluted HCl solution (pH 1.2) or physiological saline without disintegration of the beads. When Alg-CS was placed in bile acid solution it took bile acid into itself. About 80% of taurocholic acid dissolved in the medium was taken into Alg-CS.

Sezer and Akbuga (Sezer and Akbuga, 1999) have investigated alginate and chitosan treated alginate beads and compared as an oral controlled release system for macromolecular drugs. Dextran containing beads were prepared by the ionotropic gelation method and the effect of various factors (alginate, chitosan, drug and calcium chloride concentrations, the volume of external and internal phases and drying methods) on bead properties were investigated. They proposed that chitosan treated alginate beads may be used as a potential controlled release system of such macromolecules.

Albarghouthi et al. (Albarghouthi et al., 2000) have immobilized an anti-hapten IgG on glutaraldehyde-activated alginate-chitosan gel beads. The antibody immobilization efficiency was influenced by glutaraldehyde-bead reaction time, IgG concentration and pH. In addition, immobilization conditions such as glutaraldehyde and antibody concentrations influenced antibody hapten binding affinity. The immobilized IgG on the beads was stable and no reduction in the percent binding to hapten was noticed. It was concluded that antibodies could be successfully immobilized on alginate-chitosan gel beads.

An oral formulation based on liposome encapsulated alginate-chitosan gel capsules was developed by Ramadas et al. (Ramadas et al., 2000) for insulin delivery for the treatment of diabetes. This formulation delivers insulin in the neutral environment of the intestine, by-passing the acidic media in the stomach, with increased drug absorption and bioavailability.

Yan, X. et al. (Yan et al., 2000) have studied chitosan-alginate polyelectrolyte complex (PEC) in situ in beads and microspheres. Coacervation between chitosan and alginate was rapid, but the rate may be controlled with the addition of water miscible organic solvents. Suspensions of fine, uniformly dispersed coacervates were produced by a dropwise addition of chitosan solution into sodium alginate solution in water under rapid agitation.

The PEC films were transparent and flexible. Microscopic heterogeneity in the films could be reduced by immersion in aqueous media, but this was accompanied by modifications in the thickness, permeability and mechanical property of the films.

A microsystem based on cross-linked alginate as the carrier of bovine serum albumin (BSA), used as a model protein, was proposed by Coppi et al. (Coppi et al., 2001). A spray-drying technique was applied to BSA/sodium alginate solutions to obtain spherical particles. The microparticles were hardened using solution of calcium chloride and chitosan (CS) to obtain stable microsystems. The evaluation of the interaction between BSA and alginate at different pH values by means rheological measurements confirmed the hypothesis. This approach may represent a promising way to devise a microcarrier system with appropriate size for targeting the Peyer's patches, with appropriate immobilization capacity, and suitable for the oral administration of peptidic drugs.

Vandenberg et al. (Vandenberg et al., 2001) have carried out a series of experiments to evaluate the influence of a number of physico-chemical factors on the diffusion of a model protein, bovine serum albumin (BSA), from dried chitosan-coated alginate microcapsules. Factors tested included alginate and chitosan concentration, calcium chloride (CaCl2) concentration in the gelation medium, loading rate, chitosan molecular mass and pH of the gelation medium. Alginate and chitosan concentration significantly influenced BSA retention during microcapsule manufacture and acid incubation, as did calcium chloride concentration in the gelation medium ($P<0.05$).

Yan, X. L. et al. (Yan et al., 2001) have prepared coherent CS-AL PEC films by casting and drying suspensions of chitosan-alginate coacervates. Films prepared with low-molecular-weight chitosan were twice as thin and transparent, as well as less permeable to water vapor, compared to films prepared with high-molecular-weight chitosan It may be inferred that the low-molecular-weight chitosan reacted more completely with the sodium alginate than chitosan of higher molecular weight. The PEC films exhibited good in vitro biocompatibility with mouse and human fibroblasts, suggesting that they can be further explored for biomedical applications.

Gonzalez-Rodriguez et al. (Gonzalez-Rodriguez et al., 2002) formulated alginate/chitosan particles by ionic gelation (Ca2+ and Al3+) for the sodium diclofenac release. The release of sodium diclofenac is prevented at acidic pH, while is complete in a few minutes when pH is raised up to 6.4 and 7.2. The alginate/chitosan ratio and the nature of the gelifying cation allow a control of the release rate of the drug. The release mechanism was briefly discussed.

Shu and Zhu (Shu and Zhu, 2002) explained how the release behavior of a model drug (brilliant blue, BB) from chitosan coating calcium-alginate gel beads (CCAGB) was influenced by the preparation methods. The CCAGB were prepared by dropping alginate solution into CaCl(2)/chitosan solution (method 1(a)), or into chitosan solution then gelled by CaCl(2) (method 1(b)), or into CaCl(2) solution then coated by chitosan (method 2). The influence on BB release from the beads by chitosan coating was not only related to the chitosan density on bead surface, but also preparation method and other factors. Drying process greatly influenced BB release profile due to the destroying of alginate-chitosan film.

Wang et al. (Wang et al., 2002) have studied chitosan-alginate PEC membrane as a wound dressing: Flexible, thin, transparent, novel chitosan-alginate polyelectrolyte complex (PEC) membranes, cast from aqueous suspensions of chitosan-alginate coacervates with CaCl(2), were evaluated as potential wound-dressing materials. Compared to conventional gauze dressing, the PEC membranes caused an accelerated healing of incision wounds in a rat model.

Chitosan-alginate beads loaded with a model protein, bovine serum albumin (BSA) were investigated by Anal et al. (Anal et al., 2003) to explore the temporary protection of protein against acidic and enzymatic degradation during gastric passage. The presence of chitosan in the coagulation bath during bead preparation resulted in increased entrapment of BSA. During incubation in simulated gastric fluid (SGF pH 1.2), the beads showed swelling and started to float but did not show any sign of erosion. After transfer to intestinal fluid, the beads were found to erode, burst, and release the protein. The enzymes pepsin and pancreatin did not change the characteristics of BSA-loaded chitosan-alginate beads..

Lai et al. (Lai et al., 2003) have prepared sponge by dissolving both polymers (either individually or mixed) in 1% acetic acid and freeze-drying the corresponding solutions. The dissolution of a model drug (paracetamol) from the sponges was assessed as a function of polysaccharide composition. It was noted that the sponges had a flexible yet strong texture, as assessed macroscopically. Dissolution studies indicated that systems containing chitosan alone showed the slowest release profile, with the mixed systems showing a relatively rapid dissolution profile. The use of chitosan and alginates together, therefore, appears to allow the formulator to manipulate both the mechanical properties and the drug release properties of the sponges.

Beads with enhanced stability acid media based on alginate and chitosan functionalised by succinylation (increasing the anionic charges able to retain protons) or by acylation (improving the hydrophobicity of matrix) were developed by Le-Tien et al. (Le-Tien et al., 2003) for immobilisation of bacterial cells. Beads formed by ionotropic gelation with CaCl 2 presented good mechanical characteristics.

The interpolyelectrolyte reaction between chitosan (CHI) and alginate (ALG) was followed by conductimetry and potentiometry.was studied by Becheran-Maron et al. (Becheran-Maron et al., 2004). The polyelectrolyte complex was formed using alginate samples with three different M/G values (0.44, 1.31 and 1.96). The composition of the complex, Z (Z = [CHI]/[ALG]) resulted 0.70 +/- 0.02, independently of the molecular weight of chitosan and the composition of the alginate used.

Cruz et al. (Cruz et al., 2004) have carried out diffusion studies of OTC (oxytetracycline) entrapped in microbeads of calcium alginate, calcium alginate coacervated with chitosan (of high, medium and low viscosity) and calcium alginate coacervated with chitosan of low viscosity, covered with PEG [poly(ethylene glycol) of molecular mass 2, 4.6 and 10 kDa, The diffusion coefficient, or diffusivity (D), of OTC was calculated by equations provided by Crank second law, considering the diffusion from the inner parts to the surface of the microbeads. It was possible to modulate the release rate of OTC in several types of microbeads. The presence of cracks formed during the process of drying the microbeads was observed by scanning electron microscopy.

Gotoh et al. (Gotoh et al., 2004) have used alginic acid and chitosan simultaneously, to form a rigid matrix structure of beads due to electrostatic interaction between carboxyl groups on alginic acid and amino groups on chitosan, and to prepare alginate-chitosan hybrid gel beads. The cross-linking reaction made the beads durable under acidic conditions. The adsorption of Cu(II), Co(II), and Cd(II) on the beads was significantly rapid and reached at equilibrium within 10 min at 25 degrees C. Adsorption isotherms of the metal ions on the beads exhibited Freundlich and/or Langmuir behavior, contrary to gel beads either of alginate or chitosan showing a step-wise shape of adsorption isotherm.

Lyu et al. (Lyu et al., 2004) have prepared alginate/chitosan microcapsules and enteric coated granules of mistletoe lectin. The results indicated that successful incorporation of VCA into alginate/chitosan microcapsules has been achieved and that the alginate/chitosan microcapsule protected the VCA from degradation at acidic pH values. And coating the VCA with polyacrylic polymers, Eudragit, produced outstanding results with ideal release profiles and only minimal losses of cytotoxicity after manufacturing step. The granules prepared with extract or whole plant produced the best results due to the stability in the extract or whole plant during manufacturing process.

Murata et al. (Murata et al., 2004) have investigated (a) CMP release from Alg-CS, and (b) uptake of bile acid into the Alg-CS, within the gastrointestinal tract. Dried Alg-CS gradually swelled in taurocholate solution, while releasing CMP and taking up bile acid. The amount of bile acid taken up into the Alg-CS increased incrementally according to the degree of deacetylation of CS. Furthermore, the molecular weight of CS also affected the properties of the Alg-CS. An approximately linear relationship was observed between CMP release and bile acid uptake of Alg-CS.

Taqieddin and Amiji (Taqieddin and Amiji, 2004) prepared alginate-chitosan core-shell microcapsules were prepared in order to develop a biocompatible matrix for enzyme immobilization, where the protein is retained either in a liquid or solid core and the shell allows permeability control over substrates and products. The microcapsule core was formed by crosslinking sodium alginate with either calcium or barium ions. The crosslinked alginate core was uniformly coated with a chitosan layer and crosslinked with Na-TPP. They illustrated a new method of enzyme immobilization for biotechnology applications using liquid or solid core and shell microcapsule technology.

Ionotropic gelation was applied to prepare single and multilayer beads using various combinations of chitosan and Ca^{2+} as cationic components and alginate and polyphosphate as anions by Anal and Stevens (Anal and Stevens, 2005). Beads prepared with higher concentrations of chitosan entrapped more ampicillin. The rate of release both in gastric and intestinal fluid and the kinetics of disintegration in intestinal fluid can be controlled by changing the chitosan concentration in the coagulation fluid. The release of the drug can also be controlled by the degree of cross-linking using polyphosphate. Theyconcluded that chitosan-alginate multilayer beads, cross-linked with polyphosphate offer an opportunity for controlled gastrointestinal passage of compounds with low molecular weight like ampicillin.

Bhopatkar et al. (Bhopatkar et al., 2005) prepared alginate beads containing the model protein haemoglobin (Hb) were prepared by coagulation with various counter-ions to improve the controlled release of the protein. The effect of Ba(2+) and Ca(2+) ions and of the polycationic polysaccharide chitosan was investigated. Coagulation with Ba(2+),

Ca(2+) and/or chitosan showed differences in the swelling index of the beads, in the encapsulation efficiency of Hb entrapment and in the release of the entrapped protein. The release coincides with the burst and disintegration of beads. Rate of protein release from the beads was affected by the Ba(2+) and chitosan concentration in coagulation fluid.

Haque et al. (Haque et al., 2005) have studied In vitro study of alginate-chitosan microcapsules: an alternative to liver cell transplants for the treatment of liver failure. They investigated the potential of AC microcapsules for the encapsulation of liver cells and show that the AC membrane supports the survival, proliferation and protein secretion by entrapped hepatocytes. The AC membrane provides cell immuno-isolation and has the potential for cell cryopreservation.

A complex composed of alginate blended with a water-soluble chitosan (N,O-carboxymethyl chitosan, NOCC) was prepared by Lin et al. (Lin et al., 2005) to form microencapsulated beads by dropping aqueous alginate-NOCC into a Ca^{2+} solution. These microencapsulated beads were evaluated as a pH-sensitive system for delivery of a model protein drug (bovine serum albumin, BSA). It was found that the test beads with an alginate-to-NOCC weight ratio of 1:1 had a better swelling characteristic among all studied groups. With increasing the total concentration of alginate-NOCC, the release of encapsulated proteins was slower. Thus, the calcium-alginate-NOCC beads with distinct total concentrations developed in the study may be used as a potential system for oral delivery of protein drugs to different regions of the intestinal tract.

Tapia et al. (Tapia et al., 2005) have established the diltiazem hydrochloride release mechanism from the chitosan-alginate matrix tablet (MCB/AS) and chitosan-carrageenan matrix tablet (MCS/CSI). The weight loss for MCS/CSI is mainly due to the weight loss of the matrix while for MCB/AS it is mainly due to the diltiazem hydrochloride released from the tablet.

Ye et al. (Ye et al., 2005) have studied deposition temperature effect on release rate of indomethacin microcrystals from microcapsules of layer-by-layer assembled chitosan and alginate multilayer films. The prolonged release of the encapsulated IDM was observed when the aqueous release solution containing 20 vol.% ethanol. It was very significant that increasing deposition temperature from 20 to 60 degrees C reduced the release rate efficiently, owing to the increase in multilayer thickness and formation of a more perfect multilayer film.

In order to obtain small microcapsules with high protein encapsulation efficiency and extended release characteristics various processing factors were studied by Zheng et al. (Zheng et al., 2005). Many process factors were tested including the concentration and molecular weight of alginate, the concentration and pH of chitosan, and surfactants, etc. Microcapsules were achieved with diameters less than 2 microm, high encapsulation efficiency (> 80%) and high loading rate (> 10% w/w). The results also showed that the initial BSA amount of 20%-30% loaded alginate microcapsules coated with 0.2%-0.5% chitosan solutions at pH 4 by the two-stage procedure present the best sustained releasing characteristics.

4.4. References

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