CHAPTER 2

STUDY ON THE RELATED LITERATURES

A STUDY ON THE RELATED LITERATURE ON IONOTROPIC GELATION TECHNIQUE

2.1 Introduction to the method

Ionotropic gelation method is one of the most economical methods in the preparation of microparticulate oral drug delivery system. The author prepared calcium alginate micropellets using sodium alginate as the primary polymer gelled in calcium chloride solution, which acts as a counterion. The drug was uniformly dispersed in the sodium alginate mucilage. The mucilage was extruded through needle in the counterion solution. The gelled micropellets, which formed instantly were cured, separated and washed with distilled water and dried to get micropellets that can sustain the release of the drug, *in vitro,* till 6 hour. The release was further retarded by the use of aqueous colloidal polymeric dispersion which formed a strong gel network matrix in the micropellets along with alginate moiety. Several literatures were consulted for the work whose abstracts are discussed below.

2.2 Brief Review of the Related Literatures

❖ **Francis V. Lamberti *et al.*[1]** prepared microspheres of erythrocyte using Eudragit- RL as polymer. Erythrocytes were suspended in a 3 %(w/v) sodium alginate solution in isotonic phosphate buffered saline (pH 7.4). The concentration of packed cells was 0.3 w/w; and were added drop wise to100ml of gently stirring buffer (pH 7.4). A vertically mounted syringe pump used to extrude the alginate / erythrocyte suspension at a rate of 0.1ml/minute. Droplets formed on the tip of a 22 gauge needle were sprayed into the stirring buffer solution by a co-axial air stream in a similar manner to that of **Lim and Sum**[2]. The cross linked micropellets were decanted and washed in fresh buffer to ensure that cross linking was complete prior to subsequent treatment. The alginate immobilized erythrocytes from the erythrocyte/ alginate suspension were coated with Eudragit - RL by gently shaking the alginate micropellets for 30 min in the 0.5% w/v Eudragit emulsion. The capsules were removed and washed to remove excess polymer and stored in buffer at 40°C for up to 3 weeks.

❖ **Toshihisa yotsuyanagi *et al.*[3]** prepared blank calcium alginate micropellets. Gel micropellets were prepared by dropping sodium alginate solution (1, 2, 3 and 4% w/v in distilled water) with a polythene tubing nozzle (0.85mm i.d. and 1.67mm o.d.). The pumping rate was 0.11m per minute. The falling distance was 3.5cm. The weights of one droplet of various alginate solutions were almost equal to each other, being 35.2+0.2 (S.D) mg. The gel micropellets which were allowed to stand in the Calcium Chloride solution for more than 300 hr were assumed to be fully cured.

❖ **Naoki Segi *et al*** [4] prepared calcium alginate micropellets with propranolol. Alginate gel micropellets were prepared by dropping sodium alginate solution (2, 3, 4 and 5% w/v in distilled water) into 0.1M $CaCl_2$ solution, using a peristaltic pump with a polythene tubing nozzle (0.5 mm i.d. and 0.08 mm o.d.). The pumping rate was 10 micropellets per minute. The falling distance was 3.5cm. The gel micropellets which were allowed to stand in the calcium chloride solution for more than 3 days were assumed to be fully cured. Washed gelled micropellets were placed in distilled water or buffer, (pH 3-4, acetate buffer solution) containing various concentrations (30-150 mM) of propranolol. Loading of fully cured micropellets was done in the drug solution (35 mM) containing calcium chloride (0.1 M). The gelled micropellets were allowed to stand in the drug solution for 24 hours at 25°C.

❖ **Bodmeier *et.al*[5]** prepared spherical agglomerates of water insoluble drug (Griseofulvin, Tolbutamide, Sulfadiazine, Ibuprofen and Indomethacin) by ionotropic gelation. The drugs (90-98% w/w) of total solid were dispersed into aqueous solution of sodium alginate [1% w/v in deionised water]. The micropellets were formed by dropping the dispersions (8 ml) through a disposable syringe into gently agitated aqueous solution of counterions (30 ml, 1%w/v). The gelled micropellets were separated after 5 minutes and rinsed with deionised water, and either freeze or air dried for 24 hrs followed by oven drying at 60°C for 6 hrs. Smaller micropellets were prepared in forcing the drug dispersion with compressed air through a needle on the solution of counterions. The particle size could be varied by adjusting the air pressure.

❖ **Toshihisa Yotsuyanagi *et al*** [6] prepared alginate gel micropellets by dropping the polymer solution (4% w/w) into a 0.1M calcium chloride solution, using a peristaltic

pump with a polythene tubing nozzle (0.5 mm i.d. and 0.8 mm o.d.). The pumping rate was 4 micropellets per minute. The falling height was 3.5 cm. The gel micropellets were allowed to cure in calcium chloride solution for more than 3 days.

❖ **Ronald Bodmeier *et al*** [7] prepared drug loaded (Ibuprofen, Theophyline, Guaphenesin and Pseudoephedrine hydrochloride) calcium alginate micropellets with aqueous colloidal polymer dispersions (Aquacoat, Surelease, Eudragit NE3OD, Eudragit L 30 D, RL30D, RS30D). The drug (2g) was dissolved or dispersed in an aqueous solution of sodium alginate (2%w/w) and added to the polymer dispersion (30% w/w including 20% w/w plasticizers). The drug containing particles were formed by dropping the bubble-free dispersions through a disposable syringe into gently agitated calcium chloride (1% w/v) solutions (40ml). The gelled micropellets were separated after 1-2 minutes by filtration, rinsed with distilled water and dried under vacuum at 60°C for 12 hours.

❖ **L.Y.Lim *et al*** [8] prepared drug loaded calcium alginate microspheres by two ways. 50ml of 2% w/v aqueous solution of sodium alginate was introduced drop-wise from a blunt size 14 needle into 100ml of an aqueous solution of calcium chloride being stirred at 300 revolutions per min. The concentration of calcium chloride in the solution ranged from 0.25% w/v to 7.5% w/v. One hour after the first drop of alginate was added to the counterion solution, the calcium alginate micropellets were harvested by filtration, washed with distilled water and dried at 60°C for 10 hrs in an oven. Drug loading carried out by two methods designated as the sequential and simultaneous methods. In the sequential method, calcium alginate micropellets were prepared as described in the previous paragraph. The wet micropellets were then immersed and stirred for 2 hrs in a solution containing 5% w/v propranolol hydrochloride. In the simultaneous method, the gelation of micropellets by calcium ions occurred simultaneously with the drug loaded into the micropellets. The sodium alginate solution was introduced drop-wise into calcium chloride solutions (concentration ranging from 0.5 to 7.5% w/w), which also contained 5% w/v, propranolol hydrochloride. After 2hrs of interaction, the micropellets were removed from the counterion solutions. The drug loaded micropellets were washed and dried at 60°C for 10 hrs in an oven.

❖ An attempt was made by **Lim *et al.***[9] to prepare chitosan microspheres by an emulsion-phase separation technique but without the usual use of glutaraldehyde as a cross-

linking agent. Instead ionotropic gelation was employed in a W/O emulsion. The effect of formulation factors was examined. The results showed that microspheres so formed were spherical, free-flowing and had smooth surfaces. The rate of addition of counter-ions was important. Gelation of chitosan droplets should take place before the destabilizing effect of the counter-ions occurred. This effect is associated with the increase in aqueous phase volume when the counter-ions solution is incorporated.

- Gellan gum micropellets of propranolol hydrochloride, a hydrophilic model drug, were prepared by **Kedzierewicz *et al.*[10]** by solubilising the drug in a dispersion of gellan gum and then dropping the dispersion into calcium chloride solution. The droplets formed gelled micropellets instantaneously by ionotropic gelation. Major formulation and process variables, which might influence the preparation of the micropellets and the drug release from gellan gum micropellets, were studied. Very high entrapment efficiencies were obtained (92%) after modifying the pH of both the gellan gum dispersion and the calcium chloride solution. The micropellets could be stored for 3 weeks in a wet or dried state without modification of the drug release. Oven-dried micropellets released the drug somewhat more slowly than the wet or freeze-dried micropellets. The drug release from oven-dried micropellets was slightly affected by the pH of the dissolution medium. Gellan gum could be a useful carrier for the encapsulation of fragile drugs and provides new opportunities in the field of bioencapsulation.

- **A.D.Sezer[11] and J.Akbuga** have investigated the possible applicability of chitosan treated alginate micropellets as a controlled release system of small molecular drugs with high solubility. Timolol maleate (MW 432.49) was used as a model drug. The micropellets were prepared by the ionotropic gelation method and the effect of various factors (alginate, chitosan,drug and calcium chloride conc., the volume of external and internal phases and drying methods) on bead properties were also investigated. Spherical micropellets with 0.78 – 1.16 mm diameter range and 10.8 – 66.5% encapsulation efficiencies were produced. Higher encapsulation efficiencies and retarded drug release were obtained with chitosan treated alginate micropellets. Among the different factors investigated such as alginate, drug, chitosan and $CaCl_2$ concentrations, the volume of the external and internal phases affected bead properties. The drying technique has an

importance on the bead properties. The release data was kinetically evaluated. It appeared that chitosan treated alginate micropellets may be used for a potential controlled release system of small molecular drugs with solubility, instead of alginate micropellets.

❖ **O.Sipahigil[12] and B.Dortune** have studied the preparation and evaluation of carrageenan micropellets as a controlled release system for a freely water soluble drug verapamil hydrochloride and a slightly water soluble drug, Ibuprofen. Micropellets were prepared by ionotropic gelation method. The influence of formulation factors (drug content, polymer concentration, counter-ion type and concentration, outer phase volume) on the particle size, encapsulation efficiency and *in vitro* release characteristics of micropellets was investigated. The encapsulation efficiency of Verapamil HCl in the micropellets (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 micropellets prepared.

❖ Multiple unit dosage forms for oral delivery of bioactive agents offer many advantages over single unit products (e.g., site-specific delivery, predictable gastrointestinal transit time and less localized adverse effects). In view of such benefits, **Pillay *et al.*[13]** investigated the cross-linking of sodium alginate, low methoxylated pectin and their novel binary mixture with calcium ions through ionotropic gelation to palletize the model drug, diclofenac sodium, using "environmentally benign" solvents and processing techniques. Cross-linked pellets of the above polymers in 2% w/v aqueous solution of calcium chloride were prepared and evaluated for their structural and release behavior. The average size of the different pellets was 1.3 mm and drug entrapment capacity was optimized by reducing the pH value of calcium chloride solution to 1.6. Three types of pellet formulations were subjected to dissolution studies using the USP XXIII apparatus over a pH range simulating the gastrointestinal tract. Negligible drug release occurred in pH 1-4. However, rate of drug release in pH 6.6 ranged from rapid to slow (i.e., 100% drug release in 4 to 10 h, respectively) but always in a controlled manner. Weight change/erosion studies and swelling measurements were used to provide experimental correlation of kinetic model analysis for each of the three pellet systems. It is concluded that the proper selection of rate-controlling polymers and their interactive potential for cross-linking is important, and will

determine the overall size and shape of pellets, the duration and pattern of dissolution profiles, pH sensitivity, drug loading capacity and mechanism of drug release.

- A new process is described for the preparation of chitosan gel micropellets using molybdate as the gelling agent by **Dambies *et al.*[14]** This new gelation technique leads to a structure different from that produced during alkaline coagulation of a chitosan solution. Instead of a morphology characterized by large open pores, gel micropellets produced in a molybdate solution, under optimum conditions (pH 6; molybdate concentration, 7 g/l), have a double layer structure corresponding to a very compact 100-micron thick external layer and an internal structure of small pores. Experimental conditions, especially pH and molybdate concentration, were selected to optimize molybdate content and the stability of the bead shape.

- A novel oral multiple-unit dosage form, which overcame many of the problems commonly observed during the compression of micro particles into tablets, was developed in the study of **Bodmeier *et al.*[15]** Micro or nanoparticles were entrapped in micropellets formed by ionotropic gelation of the charged polysaccharide, chitosan or sodium alginate, in solutions of the counter-ion, tripolyphosphate (TPP) or calcium chloride ($CaCl_2$), respectively. The described technique did not change the physical properties of the microparticles, and it allowed a high micro particle loading (upto 98%). The ionic character of the polymers allowed pH-dependent release of the micro particles. Chitosan micropellets disintegrated and released the micro particles in 0.1N HCl, while calcium alginate micropellets stayed intact in 0.1 N HCl but rapidly disintegrated in simulated intestinal fluids. Coating the calcium alginate micropellets with cellulose acetate phthalate resulted in an enteric drug delivery system. Scanning electron microscopy and dissolution and disintegration tests were used to characterize the micro particle containing micropellets. The disintegration time of the micropellets was studied as a function of the solution viscosity of the polysaccharide, gelation time, counter-ion concentration and method of drying.

- An attempt was made by **Jain *et. al.*[16]** to improve the pharmacokinetic behavior of 5-fluorouracil (5-FU) by incorporating it into lipoprotein imitating synthetic carrier supramolecular biovector (SMBV) which is an important prerequisite for achieving its better therapeutic performance against cancer. The polysaccharide core of SMBVs was

prepared by ionotropic gelation technique by cross-linking polyguluronate units in the alginate molecules with calcium ions to form so called "egg-box structure". The formulation and process variables were optimized to obtain particles of nanometer size range. Hydrophobization was carried out by fatty-acylation on the surface followed by phospholipid coating. Palmitoyl polyethylene glycol (p-PEG) was anchored to impart stealth behavior. The scanning electron microscopy showed discrete spheres of average diameter 748 nm. Polydispersity was estimated to be 0.37. Overall zeta potential was -21.3 mV. The drug loading capacity and encapsulation efficiency was found to be 10.0% and 97.9% respectively. The release from drug solution (AP) followed zero-order kinetics. Higuchi release pattern was obtained for egg-box complex cores (API) while first-order pattern was followed for fatty acylated (AP2) and lipid coated cores before (AP3) and after p-PEG anchoring (AP4). The amount of drug liberated in 24 h was in the order AP > AP1 > AP2 >AP4 > AP3.

The release pattern obtained was a combined effect of drug diffusion through egg-box matrix as well as partitioning in hydrophobic layer and p-PEG layer around the SMBV. The stability study showed negligible leakage and no appreciable change in particle size upon storage at different temperatures which is an indication of good stability of SMBV formulation. The plasma clearance data revealed increase in circulation half-life of drug and bioavailability. Tissue distribution data obtained was a result of competitive uptake of formulations from tissue macrophages and lymphatics depending upon its surface characteristics and residence period in vascular system. The enhanced delivery of the drug to lymphatics and improvement in its half-life render SMBVs useful for control of metastasis and tumor growth.

❖ A micro particle carrier based on alginate and poly-L-lysine was developed and evaluated for the delivery of antisense oligonucleotides at the intestinal site by **Gonzalez *et al.***[17] Formulations of oligonucleotide-loaded micro particles having differences in the carrier molecular weight and composition were characterized *in vitro* and *in vivo*.

Polymeric micro particles were prepared by ionotropic gelation and cross-linking of alginate with calcium ions and poly-L-lysine. The loading of the antisense oligonucleotide into the micro particles was achieved by absorption in aqueous medium. The association capacity, loading and particle size of the micro particles were characterized. The *in vivo* performances of various formulations after intrajejunal administration were studied in rat and in dog models.

Micro particles had a sponge-like structure and an oligonucleotide loading of 27-35%. The composition of the medium affected the particle size and the *in vitro* release profiles. The oligonucleotide bioavailability after intrajejunal administration to rats in the presence of permeation enhancers was good for most of the tested systems. The application of micro particles in powder form compared to an equivalent suspension improved the intrajejunal bioavailability of the oligonucleotide (25% and 10% respectively) in rats. On the contrary, the intrajejunal administration to dogs resulted in poor oligonucleotide bioavailability (0.42%).The formulation of antisense oligonucleotides within alginate and poly-L-lysine micro particles is a promising strategy for the oral application.

❖ **Shu *et al.*[18]** prepared chitosan microsoheres by an emulsion-phase technique without the use of chemical cross-linking agents, alternatively, ionotropic gelation was employed in a W/O emulsion. The possibility of three kinds of anions (tripolyphosphate, citrate and sulphate) to interact with chitosan was investigated by turbidimetric titration. The results indicate that there are electrostatic interactions between the above anions and chitosan in a certain region of solution pH (1.0-7.5 for sulphate/chitosan, 4.5-7.5 for citrate/chitosan and 1.9-7.5 for tripolyphosphate/chitosan), that is related to the natural characteristics of the anions. Out of the pH region where anions can interact with chitosan, no microspheres were formed. However, even in the pH region where anions can interact with chitosan, only irregular micro particles were obtained in the case of the conventional emulsification and ionotropic gelation method, while spherical microspheres with diameters in the range of tens of microns were obtained when a modified process was employed. The key point of the modified process is the introduction of gelatin and allowing the ionic cross-linking process of chitosan/gelatin W/O emulsions to take place under coagulation conditions at a low temperature. The surface of sodium sulphate cross-linked chitosan/gelatin and sodium citrate cross-linked chitosan/gelatin microspheres was very smooth, but large gaps were observed on the surface of tripolyphosphate/chitosan microspheres. The increase of stirring speed led to a decrease in diameter and a narrowing in size distribution.

❖ Ionotropic gelation by divalent metal interaction was employed as an approach to design a modified release multiple-unit oral drug-delivery system by **Pillay *et al.*[19]** This process was achieved by cross-linking indomethacin-sodium alginate dispersion with

calcium ions to induce the spontaneous formation of indomethacin-calcium alginate gel discs. A significant part in the validation of the integrity of the system, involved a preformulatory stage for the optimization of the curing conditions and potency determination of the gel discs. A three-phase approach was developed to establish the critical curing parameters. Since curing involved cross-linking of the sodium alginate with calcium ions, an optimal concentration of calcium chloride (phase one) and cross-linking reaction-time (phase two) had to be determined. Further more; the third phase involved the optimization of the air-drying time of the gel discs. In phases one and two, stabilization of *in vitro* drug-release characteristics was used as the marker of optimal cross-linking efficiency. Phase three was based on achieving fully dried gel discs by drying to constant weight at 21°C under an extractor. The study revealed that optimal cross-linking efficiency was achieved in 1% w/v calcium chloride solution for 24h and air-dried at 21°C under an extractor for 48 h. the three solvent/solution systems investigated for their ability to liberate completely the drug from the matrix system were methanol, sodium citrate (1% w/v) and phosphate buffer pH 6.2. Phosphate buffer provided optimal drug removal, in addition to its ability to induce swelling of the calcium alginate gel discs. Furthermore; drug loading also increased with the use of increasing concentrations of sodium alginate in the formulations.

- Microspheres of *Bacillus subtilis* were prepared using sodium alginate by **Lamas *et al.***[20] Some typical properties of micro-encapsulated systems, such as microorganism content, particle size and germination time, were studied. Calcium alginate microspheres were obtained by the emulsification method, dropping into a solution of calcium salt. The conditions of the preparation steps were very soft to produce calcium alginate microspheres containing cells with no apparent changes in general biological properties. The hydogel matrix provides protection without preventing communication with the surrounding medium.

- To investigate whether the widely accepted advantages associated with the use of chitosan as a nasal drug delivery system might be further improved by application of chitosan formulated nano-particles; a study was carried out by **Dyer *et al.***[21]

Insulin-chitosan nano-particles were prepared by the ionotropic gelation of chitosan glutamate and tripolyphosphate pentasodium and by simple complexation of insulin and chitosan. The nasal absorption of insulin after administration in chitosan nano-particles

formulations and in chitosan solution and powder formulations was evaluated in anaesthetized rats and/or in conscious sheep.

Insulin-chitosan nano-particles formulation produced a pharmacological response in the two animal models, although in both cases the response in terms of lowering the blood glucose levels was less (52.9 or 59.7% of basal level in the rat, 72.6% in the sheep) than that of the nasal insulin chitosan solution formulation (40.1% in the rat, 53.0% in the sheep). The insulin-chitosan solution formulation was found to be significantly more effective than the complex and nano-particles formulations. The hypoglycemic response of the rat to the administration of post-loaded insulin-chitosan nano-particles and insulin-loaded chitosan nano-particles was comparable. As shown in the sheep model, the most effective chitosan formulation for nasal insulin absorption was a chitosan powder delivery system with a bioavailability of 17.0% as compared to 1.3% and 3.6% for the chitosan nano-particles and chitosan solution formulations, respectively.

It was shown conclusively that chitosan nano-particles did not improve the absorption enhancing effect of chitosan in solution or powder form and that chitosan powder was the most effective formulation for nasal delivery of insulin in the sheep model.

- Approaches using immobilized biological materials are very promising for application in different branches of the food industry, especially in the production of fermented beverages. Materials tested by **Navratil *et al.*[22]** for the process of entrapment belong to the family of charged polysaccharides able to form beaded hydrogels by ionotropic gelation (e.g. alginate, pectate, kappa-carrageenan) and synthetic polymers (e.g. polyvinyl alcohol) forming bead and lens-shaped hydrogels by thermal sol./gel transition. Concentration of a gel, conditions and instrumentations of gelation process, bead and size distribution, porosity, diffusion properties, mechanical strength, storage and operational stability and many other parameters were followed and optimized. Research has been oriented especially to practical applications of immobilized cells. Brewing yeast cells were successfully immobilized by entrapment materials and used in a process of batch and continual production of beer, including primary and secondary fermentation of wort. Other applications include continual production of ethanol by fermentation of different saccharide substrates (molasses, glucose syrup, and wheat hydrolysate), breads and non-alcoholic beverages production.

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