Pure & Appl. Chem., Vol. 70, No. 6, pp. 1283–1287, 1998.
Printed in Great Britain.

New concepts in controlled drug delivery

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NATURAL POLYMERS AS ADVANCED MATERIALS

A large number of both natural and synthetic polymers have been studied for possible application in drug delivery. The great advantage of synthetic polymers is their advantageous properties and wide choice availability. Two promising synthetic polymers which have been developed for biomedical applications are polyvinylpyrolidone and polyethylene glycol acrylate based hydrogels. Both of them are biodegradable and forms copolymers with natural macromolecules. On the other hand, natural polymers have the advantage of high biocompatibility and less immunogenicity. Among the natural polymers studied a special mention has to be made to collagen and gelatin. Other natural polymers include chitosan, alginate, starch, pectin, casein and cellulose derivatives. The composites of some the above natural polymers with synthetic polymers give added advantage as carriers for drug delivery by complimenting the properties of each other. Collagen poly-HEMA hydrogels have been prepared in our laboratory as an implant for delivering anticancer drugs such as 5-fluorouracil, mitomycin and bleomycin for solid fibrosarcoma in rat model. The same hydrogels have been used with some modifications for the delivery of model protein bovine serum albumin and vaccines such as tetanus and diphtheria toxoids in mice. Hybrid copolymers of collagen with biodegradable synthetic polymers polyethyleneglycol 6000 and polyvinylpyrolidone were developed for the controlled release of contraceptive steroids. The entrapped testosterone released 40% of steroid in first 10 days and there after the release was very slow. The total amount of drug released by 90 days was about 66%.

Gelatin-methotrexate (MTX) conjugate microspheres for chemotherapy

The anticancer drug methotrexate (MTX) was covalently linked to gelatin by the azide coupling-grafting method. Two gelatin-MTX conjugates were prepared by coupling (I) MTX azide to gelatin (GMC-I) and gelatin azide to MTX (GMC-II). The resulting conjugates were separated by gel filtration and characterized by UV and IR spectroscopy. The drug content of GMC-I and GMC-II was 205 μg MTX/mg gelatin and 203 μg MTX/mg gelatin respectively. GMC-I and GMC-II were used to prepare biodegradable hydrophilic gelatin microspheres (GMC-I and GMC-II) of different mean particle sizes (1-5, 5-10, 15-20 μm). The in vitro release of MTX from GMC-I and GMC-II was investigated in simulated intestinal fluid. GMCM-I released MTX in zero order manner for 9-11 days in intestinal medium and GMCM-II released MTX in a zero order manner for 7-10 days in intestinal medium (Fig.1a and 1b).

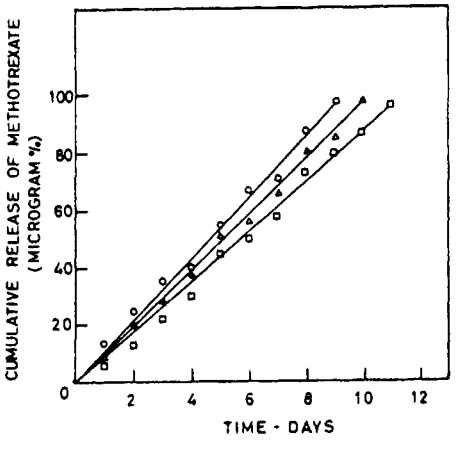

Fig.1a In vitro release of MTX in 0.01 M PB, pH 7.4 at 37°C from GMCM-I of: (○) mean particle size 1–5 μM; (Δ) mean particle size 5–10 μM; and (□) mean particle size 15–20 μM.

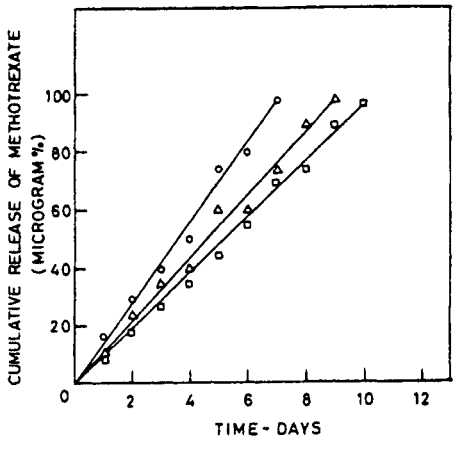

Fig.1b In vitro release of MTX in 0.01 M PB, pH 7.4 at 37°C from GMCM-II of: (○) mean particle size 1–5 μM; (Δ) mean particle size 5–10 μM; and (□) mean particle size 15–20 μM.

The release data also indicated that the rate of release of MTX decreased with increase in particle size of GMCM. Release of MTX was faster in gastric medium when compared to intestinal medium. The antitumor activity of gelatin methotrexate conjugate microspheres was evaluated for their efficacy towards the solid tumor fibrosarcoma in Wister rats. The results of the in vivo post inoculation and post transplantation studies and the [^{3}H] thymidine studies were established that conjugated MTX microspheres were very efficient in inhibiting tumor growth compared with the multiple high dose regimen of free MTX.

pH sensitive proteinoid microspheres

Prot A7, a polypeptide proteinoid containing 7 neutral and acidic aminoacids was synthesized by thermal condensation method. Using Prot A7, microsphere of 1-5μm size range were prepared by self-assembly process. These self-assembled microspheres were found to be pH sensitive in aqueous medium. The pH responsive dissolution behavior of the microspheres was verified by choosing methotroxate as a model drug. In the simulated gastric fluid (pH 1.2), only about 7% of the drug was released in the first 2h, whereas in the neutral medium (pH 7.0), 96% of the drug was released within 60 min. The release pattern clearly indicated their pH responsive dissolution behavior. Insulin was also entrapped in these microspheres as a model peptide drug. In vivo studies using the insulin entrapped microspheres with female albino Wister rats indicated appreciable reduction in the blood sugar level from 263 to 120 mg/100ml in the first 2h, in diabetic rats (Fig.2). The results indicates the effectiveness of the proteinoid microspheres for delivering insulin via oral route. These proteinoid microspheres will have wide applications for the oral delivery of protein and peptide drugs.

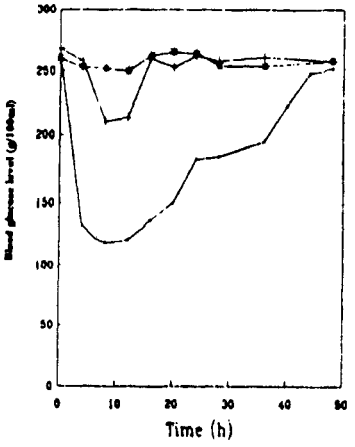

Fig.2 Blood glucose level after oral administration of insulin entrapped proteinoid microsphere (•) Proteinoid microsphere (◦) Raw insulin (▪) Placebo

Oral delivery of insulin and vaccines

Insulin containing gelatin microspheres (IGM) with and without soyabean trypsin inhibitor (TI) were prepared and coated with enteric polymers to protect them from degradation in stomach and to release the insulin upon reaching the intestine. Four types of coated IGM were prepared: (i) IGM coated with natural polymers (chitosan inner coat-alginate outer coat), (ii) IGM-TI coated with cellulose acetate phthalate, (iii) IGM-TI coated with cellulose acetate butyrate, and (iv) IGM-TI coated with natural polymers (chitosan inner coat-alginate outer coat). The protective efficiency of uncoated and four types of coated microspheres toward digestive enzymes such as pepsin and trypsin was evaluated under simulated physiological conditions. The microspheres ere characterized for their insulin content and particle size. The morphology of the microspheres was studied using scanning electron microscopy. The in vitro release studies of insulin from uncoated and coated microspheres indicated that the release followed a zero-order pattern, prolonging for 6 days from 2 days in the case of uncoated spheres. The uncoated and coated microspheres containing insulin (20

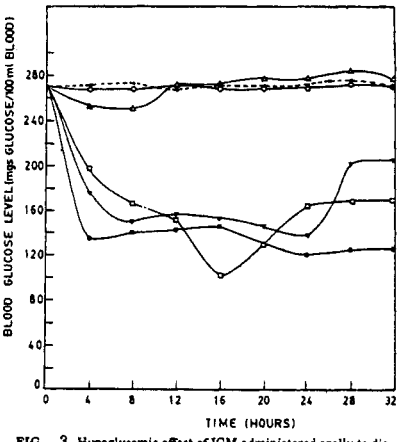

FIG. 3 Hypoglycemic effect of IGM administered orally to diabetic rats. (•) placebo microspheres; (○) IGM; (□) chitosan–alginate-coated IGM-TI; (△) chitosan–alginate-coated IGM; (■) CAP-coated IGM-TI; (▼) CAB-coated IGM-TI.

IU/kg) were orally administered to albino Wister rats by stomach tube, and insulin absorption was evaluated by assessing the hypoglycemic effect in normal and diabetic rats. A significant and continuous hypoglycemic effect was observed in diabetic rats (Fig.3) following oral administration of coated IGM containing TI when compared to the effect following administration of coated IGM without TI.

Immunization via oral administration presents several clear advantages over parentral vaccination. Both degradable and non degradable synthetic as well as natural polymers were used as delivery systems for vaccines. However, there are certain limitations for synthetic polymers because of an acidic environment created locally by the degradation of these polymers which may cause stability problems. An alternate to synthetic polymers are natural polymers, since they are biodegradable and body friendly. The work in Biomaterials laboratory found interesting alternatives to the synthetic polymers for vaccine delivery. The vaccines were entrapped in natural polymeric microsphers such as a gelatin (polypeptide), chitosan and alginate (poly saccharides). The microspheres were prepared by polymer dispersion technique developed in our laboratory in the size range of 1-10μm for targeting to payer's patches. A model protein, BSA was entrapped in all these systems and the in vitro release lasted for about 8-15 days. The release rate were extended by coating with a biodegradable polymer polycaprolactone (PCL). The PCL coated polymeric microspheres released 90-95% of the entrapped protein up to 55-60 days and followed a near zero order kinetics. The vaccines DT and TT were incorporated in the above microspheres and coated with PCL. In vitro release studies were carried out in simulated intestinal fluid. The release lasted about 60 days and followed a near zero order fashion. An optimally designed and formulated natural polymeric microsphere system should be capable of stimulating a mucosal antibody response (IgG, secretory IgA) as well as a systemic immune response. Orally administered antigens which are in a particulate formulations such as microspheres may be taken up by the M cells of the peyer's patches of the small intestine and high levels of secretory antibodies may be induced at diverse mucosal sites via the common mucosal immune systems (CMIS). Hence, these natural polymeric microsphere systems might be able to confer long lasting immunity for infectituous diseases with a single inoculation. These natural polymeric systems showed a marked potential for oral vaccine delivery systems.

Functional PMMA microspheres for orthopaedic applications

Poly(methyl methacrylate) [PMMA] as an adhesive in arthroplasty applications is well known. Some investigators have also attempted to use PMMA beads for hard tissue repair and regeneration. In the present paper, attempts were made to prepare microspheres of PMMA having carboxylic functionality by synthesizing PMMA using chain transfer agent, thioglycolic acid. By this process the molecular weight of PMMA can be reduced considerably compared to PMMA prepared conventionally. PMMA microspheres were prepared by using solvent evaporation technique and they were fully characterized. The microspheres were analyzed for particle size distribution and found to be 15 - 20 microns. The molecular weight of microspheres was found to be $1.3x10^4$ daltons by using Gel Permeation Chromatography. The presence of carboxylic groups in the microspheres was conformed using ^{13}C-NMR. Equilibrium swelling experiments of the microspheres were carried out and it was found that the microspheres. The SEM pictures of the microspheres indicated that they are spherical and porous. Experiments are in progress for their in-vitro release profile studies. It is aimed to use these microspheres in orthopaedics particularly in the repair and regeneration of bone. It is also planned to incorporate growth factors into the spheres to make them osteoinductive in addition to their osteoconductive property.

Responsive hydrogels for colon and stomach targeting

The development of pH sensitive, biocompatible and biodegradable hydrogels as novel drug delivery systems was carried out to achieve localized drug delivery. Two types of hydrogels sensitive to azoreductase secreted by micro flora of colon and acidic pH were prepared. Methacryloxy azobenzene poly (hydroxy ethyl methacrylate [poly(MAB-HEMA)] hydrogels were synthesized by copolymerization technique. Poly[N-vinyl pyrolidine-acrylic acid] - Polyethylene glycol interpolymer type hydrogel systems were prepared by free radical polymerization. Equilibrium swelling measurements of these two hydrogels which are pH sensitive, were carried out in simulated gastric and intestinal fluids. The anticancer drug 5-fluorouracil (5-FU) were entrapped in both these systems and the % entrapment and % loading were

calculated. The invitro release profiles of the incorporated 5-FU were carried out in simulated gastric and intestinal fluids and also in the presence of azoreductase. The results of this study suggest that combined delivery of Poly[NVP-AA]-PEG and poly(MAB-HEMA) hydrogels could be useful for localized delivery of 5-FU in the stomach as well as for colon. They can also be used individually for targeting to colon and stomach.

Polymeric liposomes with increased circulation time

Liposomes are proven as potential colloidal carriers for the delivery of drugs and therapeutic macromolecules. However biological instability remains a major problem. They are rapidly taken upon administration and this hinders its exploitation in drug delivery. In the present work an attempt was made to prepare polymeric liposomes using polyethylene glycol dimethacrylate(PEG-DM) and poly vinylpyrrolidone as one of the components to enhance their stability. Initially, PEG-DM containing monomeric liposomes and vinylpyrrolidone containing monomeric liposomes were separately prepared and they were subsequently polymerized using free radical initiator system. Both the monomeric and polymeric liposomes were fully characterized for FT-IR, FT-NMR, TEM, stability and plasma aggregability study. FT-IR and FT-NMR spectra of both polymeric liposomes confirmed the disappearance of vinyl unsaturation bond which indicated the actual polymerization of monomeric liposomes. The TEM of both monomeric and polymeric liposomes showed that the liposomes are spherical in shape and in the size range of 300-600 nm. The stability study was carried out based on the turbidity method and the data clearly showed that both the polymeric liposomes are quite stable as compared to the monomeric liposomes. Both the polymeric liposomes were able to resist the aggregation in human plasma when compared to monomeric liposomes (Fig. 4).

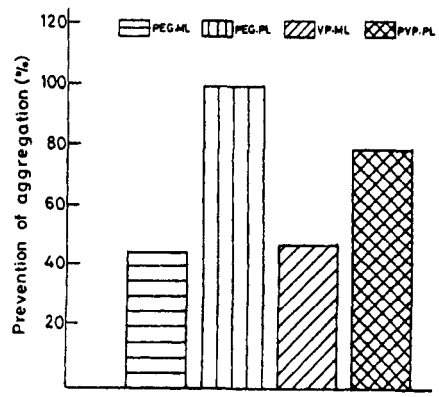

Fig.4 The histogram of the percentage prevention of liposome aggregation in human plasma.

Biopolymeric membrane controlled transdermal delivery systems

In most of the transdermal delivery systems fabricated, the rate controlling membranes are generally synthetic polymers. However no attempts were made to use natural polymer which are body friendly for this purpose. In the present investigation, an attempt was made to use natural polymeric films from collagen and chitosan as rate controlling membranes. Anti-hypertensive drugs such as propronolol hydrochloride and nifidifine were chosen for the fabrication of transdermal delivery systems. Transdermal devices were fabricated by adhesive sealing technique. In vitro drug release studies was carried out using modified Franz diffusion cells. Drug release was found to depends mainly on the type of membrane used to control the drug release suggesting that the rate kinetics of the drug release is efficiently controlled by the rate controlling membranes. By using collagen and chitosan membranes the permeability of the antihypertensive drugs can be programmed by chemical modifications of the natural polymers. By utilizing the highly biocompatible natural macromolecules for the fabrication of the transdermal devices the possibility of device associated adverse skin reactions can be completely avoided.

Biodegradable amino acid containing hydrogels for ophthalmic delivery

Hydrogels of copolymers of a biologically active glycinylmaleimide monomer with 2-hydroxyethyl methacrylic acid (HEMA) were prepared using different concentration of HEMA monomer (1:10 and 1:20 HEMA w/w). Synthesized Glycinylmaleimide, Poly(HEMA) and poly(Glycinylmaleimide-Co-HEMA) were characterized by IR and ^{1}H-NMR spectroscopies and thermogravimetric analysis. The equilibrium swelling

studies and in vitro degradation profiles were established in distilled water and media of various pH. The hydrogels showed about 95 to 98% entrapment of two ophthalmically active drugs namely gentamycin and 5-fluorouracil. The in vitro release profiles of the entrapped drugs determined in phosphate buffer, pH 7.4 showed that the copolymeric hydrogels had good potential for the controlled release of these drugs. These hydrogels hold promise as ocular inserts for the controlled delivery of ophthalmic drugs in eye treatment.

REFERENCES

1. R. Narayani and K. Panduranga Rao, *Int. J. Pharm.*, 128, 261 - 268 (1996)
2. A.B. Madhnan Kumar and K. Panduranga Rao, *Biomaterials*, (In Press)
3. R. Narayani and K. Panduranga Rao, *Drug Delivery*, 2, 29 - 38 (1995)
4. K.L. Shantha, P. Ravichandran and K. Panduranga Rao, *Biomaterials*, **16**, 1313 - 1318 (1995)