Studies on the development of ^{99m}Tc-labeled biphosphonate alginate beads

Maya Vishwakarma,
Guru Pad Bandopadhyaya

Department of Nuclear Medicine, All India Institute of Medical Sciences, New Delhi-110029-India

***

Keywords: Drug delivery − Biphosphonate −Osteoporosis −EDTMP −Sodium Alginate

Correspondence address:
Dr. G.P. Bandopadhyaya,
Department of Nuclear Medicine,
All India Institute of
Medical Sciences (AIIMS),
New Delhi -110029, India.
E-mail: guru47@gmail.com

Received:
16 February 2010
Accepted revised:
5 May 2010

Abstract

The preparation of alginate-dextran pyrophosphate/biphosphonate loaded beads, were labeled with technetium-99m (^{99m}Tc) and rhenium-188 (^{188} Re). The radiolabelled phosphonic acid derivatives, are well-known bone imaging agents and have also been used for bone pain palliation treatment. The alginates have been used extensively as an excipient in drug products due to their thickening, gel forming and stabilizing behavior. *The aim* of this study was the preparation of alginate polymeric beads with and without dextran coating, to be used for imaging and possible treatment. We studied ^{99m}Tc-labelled biphosphonate alginates. We reported *our results* on the basis of size, swelling capacity and the coating material. The size effect of loading, decreases size and increases loading capacity of alginate beads. Pyrophosphate (PYP) loaded beads had 95% swelling, while ethylene diamine tetramethylene phosphonic acid (EDTMP) loaded beads had 90% (swelling). However combination of both (PYP+EDTMP) loaded beads had 95% swelling. Sustained drug release study indicated different ratios of EDTMP, PYP and EDTMP+PYP loaded beads on different days. Total drug extracted from 30g beads was 1365.45μg, 5352.86μg and 711.8μg, from EDTMP, PYP and EDTMP+PYP respectively during 15 days of studies. Binding with PYP and EDTMP was 98% and 99% respectively. *In conclusion*, (chemical and physical characteristics of ^{99m}Tc-biphosphonate and other alginate beads that we have prepared suggest that the alginate beads could be used for diagnostic and therapeutic purposes.)

Hell J Nucl Med 2010; 13(3):224-228 • Published on line: 25-11-10

Introduction

Controlled drug delivery technology involves multidisciplinary scientific approach and represents one of the frontier areas of science, contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, including improved efficacy, reduced toxicity and improved patient compliance. Such systems often use macromolecules as carriers for the drugs. By doing so, treatments that would not otherwise be possible are now in conventional use. The drug delivery system (DDS) concept is not new, but great progress has recently been made in the treatment of a variety of diseases [1, 2]. Targeting delivery of drugs to the diseased osteoporosis is one of the most important aspects of DDS [3].

Alginate polymers have been widely used in numerous biomedical applications, including drug delivery systems, as they are biodegradable biocompatible, and mucoadhesive. These delivery systems are formed when monovalent, water-soluble alginate salts undergo an aqueous sol-gel transformation to water-insoluble salts due to the addition of divalent ions such as, calcium, strontium, and barium [4]. Alginates are random, anionic, linear, polymers consisting of varying concentrations of guluronic or mannuronic acid. Salts of alginate are formed when metal ions react with the guluronic or mannuronic acid residues. Although strontium and barium alginate forms stronger insoluble matrices, calcium alginate is commonly used and forms a matrix for various delivery systems including gels, films, beads, micro particles and sponges [5-8]. Calcium ions have unequal affinity for the guluronic and mannuronic acid units of alginate [4]. Alginate as a matrix is drawing increasing interest on account of its biocompatibility, low toxicity [9, 10] and easy bead formation by ionotropic geletion [11]. An important feature of alginate is its ionotropic gelation induced by bivalent (i.e. Ca^{2+}) or polyvalent cations, which ionically cross-link carboxylate groups in the uronate blocks of alginate, giving an insoluble gel at low pH, but becoming soluble at a neutral or higher pH. This behavior affords interesting entrapment, which prevents the solubilization of beads in the stomach and gives certain moderate protection of cells against acid shock. In addition, its great solubility at intestinal pH allows the release of viable cells into the intestinal tract [12].

The aim of this study was the preparation of alginate polymeric beads with and without dextran coating, the loading of a radionuclide

drug under various experimental conditions and the (study of their chemical and physical characteristics.) development of alginate beads to be used for diagnosis and treatment.

Materials

Sodium alginates (75,000-100,000 Dalton in size) extracted from *Macrocystis pyrifera,* were purchased from Marine Brown Algae, Central drug house **(**CDH) chemicals, New Delhi. Sodium pyrophosphate (anhydrous) $Na_4P_2O_7$, formula weights (FW) 265.9, stannous chloride (anhydrous) $SnCl_2$ FW 189.6, dextran (75,000-100,000 Dalton) extracted from *L. mesenteroides* purchased from Sigma Chemical Co. USA. Calcium chloride dehydrate ($CaCl_2$) mole Wt.147.02 was purchased from Qualigens Fine Chemicals, Mumbai. Technetium-99m-pertechnetate (^{99m}Tc-P) was freshly eluted from a molyvdenum-99 (^{99}Mo)/^{99m}Tc column generator supplied by Amrol, Turkey.

Methods

Preparation of solutions

a) Alginate beads were obtained by gelification of a sodium alginate solution with calcium chloride. Dextran (0.013mg) was added in the alginate solution as a coating material for inside coating of the beads. Calcium chloride solution was used as a polyelectrolyte (cationic) complex. Alginate solution (1gm/10ml) was prepared with distilled water. As we know, alginate comes in jelly form so it must be properly stirred magnetically. b) Calcium chloride (25gm/ml) solution was prepared as a stock in distilled water. c) Ethylene diamine tetramethylene phosphonic acid (EDTMP) solution (5mg/ml) was also prepared. The (chemical) EDTMP is semi-soluble in water so the solution was made after heating it gently lukewarm in warm water and pH was adjusted between 6.5 to 7 using 1N NaOH drop wise. d) Pyrophosphonate (PYP) solution containing 5mg/ml was made and its pH was also adjusted between 6.5 to 7 using stannous chloride (dissolved in 5mg/ml 1N HCl).

Preparation of alginate beads

Preparations of simple alginate beads were made using alginate solution only. Three different sizes of beads were made using a 5ml syringe with three different size needles (21G, 25G and 26G). In a beaker containing 50ml $CaCl_2$ solution, various graded solutions of alginate were dropped through needles of graded gauges thereby round shaped transparent beads were formed. After 2h all calcium chloride containing solution was removed from the beaker and beads were kept for drying after taking the initial weights. The polymeric beads were dried for two days at room temperature in Petri dishes. The initial and the dry weight of the total micropellets prepared was recorded. This preparation referred to alginate-dextran beads, to alginate-dextran-EDTMP beads, to alginate-dextran-PYP and to alginate-dextran-PYP-EDTMP beads.

Labeling of EDTMP with ^{99m}Tc-P was as follows: a) Five mg of EDTMP were dissolved in 10ml lukewarm injectable sterile water. . b) The $SnCl_2$ solution as prepared earlier was added to EDTMP solution and pH was adjusted to 7. c) A dose of 240MBq of ^{99m}Tc-P was added and mixed with the EDTMP solution. e) The whole solution was boiled for 30min. in a water bath. f) The solution was run on thin layer chromatography (ITLC) to see the labeling efficiency. g) If labeling efficiency was more then 90% we added the whole solution to the alginate solution, which was prepared before. h) Syringe shield should be used while making the pellets. i) The calcium chloride solution should be lead cover protected.

Table 1. *Alginate beads of different size*
pH was in all cases: 7

Serial No.	Sample	Wet weight (gm)	Dry weight (mg)	No.of beads	Swe-lling %
1	Alginate simple 21G	0.017	0.002	614	88
2	Alginate simple 25G	0.014	0.0016	676	89
3	Alginate simple 26G	0.010	0.001	245	90
4	Alginate+ Dextran beads 21G	0.013	0.001	294	92
5	Alginate+ Dextran beads 25G	0.013	0.001	406	91
6	Alginate+ Dextran beads 26G	0.010	0.0009	300	90
7	Alginate+ EDTMP beads 21G	0.016	0.001	220	94
8	Alginate+ EDTMP beads 25G	0.130	0.001	222	92
9	Alginate+ EDTMP beads 26G	0.008	0.006	450	92

Table 2. *Preparation of radiolabeled and dextran coated alginate beads. These preparations are shown in Tables 1 also*

Serial No.	Properties	Alignate+ ^{99m}Tc - EDTMP	Alginate+ Dextran+ ^{99m}Tc- EDTMP	Alginate+Dextran+ EDTMP+ ^{99m}Tc- PYP
1	Wet weight beads (mg)	0.11	0.018	0.011
2	Dry weight beads(mg)	0.001	0.002	0.001
3	No. of beads	655	895	1420
4	Swelling %	91	89	91

Quality control

Labeling efficiency and stability study

The ratio of distance travelled by the solute to the distance travelled by the solvent front was calculated as an Rf value for reduced/hydrolyzed (R/H**)** technetium**.** The free ^{99m}Tc pertechnetate that moved with the solvent front (rf=0.9) was estimated as 1.5%-5% of the total radioactivity added. However when paper chromatography was done using saline as solvent front, we did not found any hydrolyzed form of technetium. Moreover, the labeling efficiency of ^{99m}Tc to alginate-dextran beads by stannous chloride method was assessed by instant thin layer chromatography (ITLC) using silica gel coated fiber sheets (1x8cm strips, Gelman Science Inc., Ann Arbor, MI, USA) [13]. The ITLC was performed using methyl ethyl ketone as the mobile

phase. The contaminants were identified as bound and free activity. The retardation factor (RF) ratio in planar chromatography is the distance travelled by the centre of the spot (b) to the distance simultaneously travelled by the mobile phase (a): *RF = b/a*

By definition the RF values are always less than unity. They are usually given to two decimal places. In order to simplify this presentation the hRF values may be used: they correspond to the RF values multiplied by 100.

The labeling efficiency was more then 95% in all cases. The labeling efficiency was also calculated by placing the chromatograms under the gamma camera (Siemens, USA) using the region of interest programme. Labeling % was further verified after cutting the same chromatogram into pieces and counting them in a well type (Atom Lab. Biodex Medical Systems, USA) using the following equation:

$$\text{Labeling efficiency \%} = \frac{\text{Total counts- counts of free pertechnetate}}{\text{Total counts}} X100 \quad (1)$$

(IUPAC Compendium of Chemical Terminology 2nd edition 1997)

Table 3. *Preparation of alginate dextran coated beads(Alg+Dextr) plus PYP or and EDTMP*

Serial No.	Sample of Alginate + Dextran	Wet. weight (gr)	Dry weight (mg)	No.of beads	Swe-lling %
1	Pyp (A1) 21	7.054	0.751	495	93
2	Pyp (B1) 24	6.195	0.672	580	90
3	Pyp (C1) 26	7.622	0.655	395	95
4	EDTMP (D1) 21	6.406	0.698	333	89
5	EDTMP (E1) 24	5.791	0.697	480	92
6	EDTMP (F1) 26	6.621	0.675	655	90
7	EDTMP+PYP (G1) 21	8.836	0.862	425	95
8	EDTMP+PYP (H1) 24	7.841	0.768	560	93
9	EDTMP+ PYP (I1) 26	8.367	0.818	435	95

pH was in all cases: 7

Characterization of beads
Size and morphology studies

The particle size and the particle size distribution of beads were analysed by scanning electron microscopy [14]. The average particle size of the beads was 100 - 200μm expressed as the volume-surface diameter (d_{vs}). Results were the means of triplicate experiments [15].

In vitro swelling studies

In vitro swelling studies (S%) were calculated by this formula:

$$S\ (\%) = [(Mt–Mo)/Mo] \times 100 \quad (2)$$

Where, Mo is the dry/initial weight of beads, Mt is the weight of swollen beads at given time (t), in water.

Determination of drug content in beads

Accurately weighed and counted samples of drug-loaded beads (30mg) were mixed with 1ml of distilled water until their dissolution, which occurred in about 1h under magnetic stirring. The insolubility of beads drug was extracted with distilled water. This was sufficient to ensure complete drug recovery. The concentration of the drug in water was determined using an UV spectrophotometer at a wavelength of 350nm, (Shimadzu UV-2101PC-UV-VIS scanning spectrophotometer). Preliminary UV scanning showed that the presence of the polymers did not interfere with the absorbance of drug at 350nm. Drug content was calculated as the detected amount of drug with respect to the theoretical amount of the drug used for the preparation of beads and expressed as a percentage. Each determination was carried out in triplicate.

In vitro drug release study

In vitro release rate studies were conducted in PYP and EDTMP loaded alginate beads. Approximately % swellings were recorded in alginate and in alginate plus dextran coated beads. The release rate studies indicated that the release of drug was proportional to the swelling behavior of the alginate gels. However after coating alginate with dextran it behaved differentially.

In-vitro release of the drug from the beads was studied in sterile distilled water. A fixed amount of beads equivalent to about 50mg of drug were added to the medium. About 500μl of the sample was withdrawn at different time intervals and the drug released from the beads was measured using a spectrometer at 350nm after adding an equal volume of fresh water medium. Each experiment was performed in triplicate.

Results

The results were compared and interpreted on the basis of their size, swelling capacity and the coating material (dextran). Release rate studies, labeling with ^{99m}Tc and electron microscopic analysis were also evaluated. As for size effect, the loading capacity was high in small sized beads compared to higher size. The 21G, 25G, 26G-size needles were used for preparation of beads. The beads prepared through 26G sized needles had the highest release. Swelling was also high in small sized polymer beads. As for swelling capacity, small sized alginate dextran pyrophosphate gives 95% swelling whereas alginate dextran EDTMP had the lowest swelling, 90%. However with the combination of both EDTMP and PYP, swelling capacity increased to 95%.

Effect on coating

The electron microscopic analysis showed the different morphology on the coating of alginate beads to different structure. The alginate beads had rough surface while on coating the beads with dextran, smooth surfaced beads were formed. After loading the drugs in dextran coated beads the smoothness further increased. After labeling dextran alginate beads with ^{99m}Tc, their porosity was increased. The swelling of the coated alginate beads was also increased to 94%.

Drug release

The drug release studies-curves were plotted and calculated with the help of a spectrophotometer using a standard curve of EDTMP (at 350nm), PYP (at 280nm) and both EDTMP+PYP (at 225nm). As such release rate depends on so many factors such as pH, temperature,

properties of polymers etc., cumulative release rate curves were plotted. The sustained release rate of EDTMP was calculated continuously for 15 days. A total of 1365.45µg of drugs from 30mg beads was extracted during the 15 days (Fig.1).

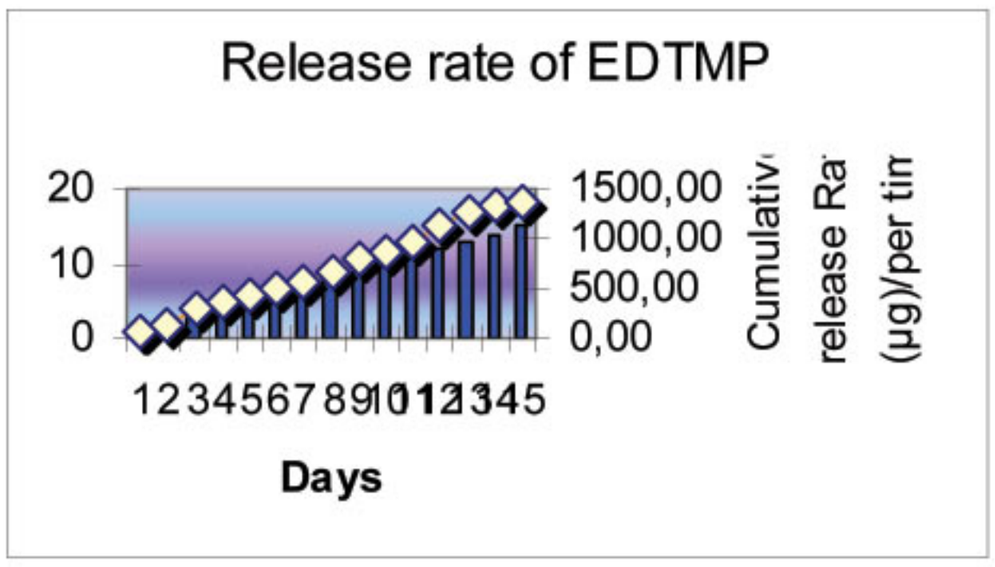

Figure 1. Cumulative release rate of EDTMP

The release rate of pyrophosphate indicated the same trend till the 15th day; however the initial burst was high. After 3rd day of release, the drug release was decreased significantly. The highest amount was released on the 1st, 2nd, and 3rd days. The total drug released was approximately 5352.86µg. from 30mg beads. (Fig. 2)

However in cases where combination of EDTMP+PYP was used, the release rate studies were conducted till the 10th day. The total amount of drug released was approximately 711.8µg. and that was the lowest as compared to EDTMP and PYP from the 30mg beads (Fig.1 and 3).

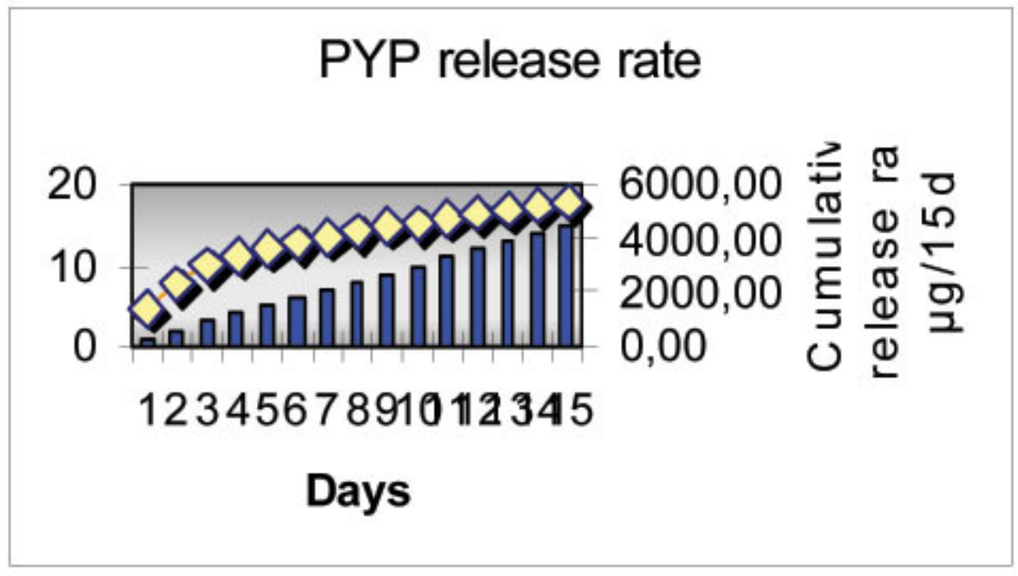

Figure 2. Cumulative release rate of PYP drug.

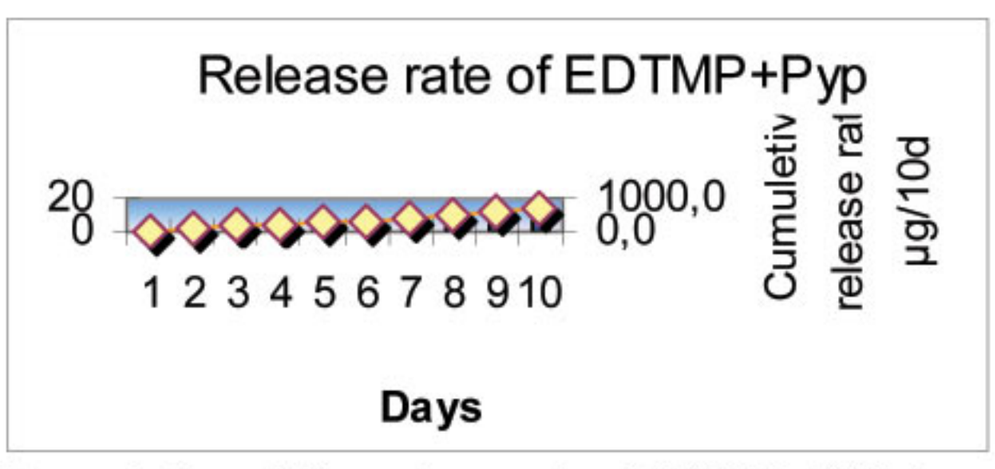

Figure 3. Cumulative release rate of EDTMP+PYP drug.

Labeling with technetium-99m

Technetium-99m pyrophosphate and EDTMP are bone-seeking agents. In our studies we labeled pyrophosphate alginate beads with ^{99m}Tc. The physical half-life of ^{99m}Tc-P is only 6h, and it gets easily converted into a stable ^{99}Tc after emitting gamma rays. We found that the swelling rate of the alginate beads up to 91% increases the labeling with ^{99m}Tc due to increase in the surface area. Binding efficiency of ^{99m}Tc with EDTMP and PYP were also high i.e. 98% and 99% respectively. The ITLC strips were scanned under the gamma camera and the binding efficiencies were calculated using the region of interest programme. Chromatography was run using methyl ethyl ketone as solvent phase and the silica as solid phase. We further evaluated our studies using saline in lieu of methyl ethyl ketone. In case of normal saline, total activity moves towards the solvent front and appears as a round spot at the top of the paper. The paper was cut into pieces and counts were measured in the well type counter.

Electron microscopic analysis

The morphological analysis of alginate beads depends on the concentration of the solution, the coating with dextran, the loading of drug and labeling with ^{99m}Tc inside the alginate beads. The ^{99m}Tc labeled with EDTMP has shown a spotted rough surface. However, after coating with dextran the rough surface was modified to smooth surface. Further loading with PYP in alginate beads demonstrated a very smooth surface (Fig. 4).

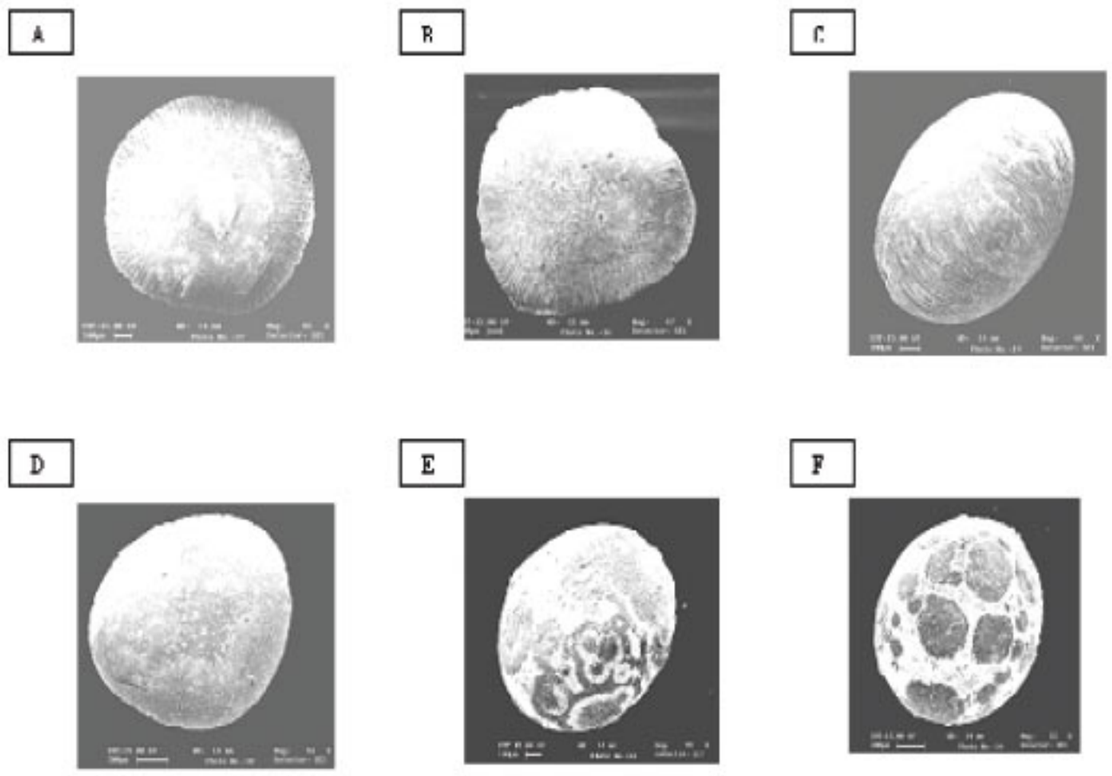

Figure 4. [A] Alginate+Dextran+EDTMP-^{99m}Tc [B] Alginate + Dextran + EDTMP [C] Alginate+Dextran+PYP-^{99m}Tc [D] Alginate + Dextran + PYP [E] Alginate + Dextran + EDTMP + PYP-^{99m}Tc [F] Alginate + Dextran + EDTMP + PYP.

Discussion

Designing alginate polymeric beads for sustained drug delivery system demonstrated an excellent tool for the controlled and targeted drug delivery on the basis of various experiments conducted. The targeted sustained release rate as calculated in our water system, demonstrated that release of the drug can be manipulated by changing the size of the polymer, drug loading and the surfactant used in our studies, as well as demonstrated by others. The drug release could be continuous acting through diffusion alone, as occurs for smaller molecules or it could be triphasic, as occurs for most high molecular weight peptides and proteins. The degradation occurs only at the surface of the polymer and the release rate is proportional to the surface of the drug. In our studies we were successful in loading the phosphonate compound (EDTMP) that can prevent

osteoporosis and may be utilized for radiolabeled drug cancer treatment. Technetium-99m labeled PYP could be used diagnostically. In our studies the small size beads had high loading capacity, suggesting the possibility that small partial size drug released through pellets could penetrate the cell. High swelling rate increases the release rate of the alginate beads. The release rate was effective for a long time. The combined preparation of alginate beads (EDTMP+PYP) made a complex that reduced the release rate of beads.

In conclusion, we have developed ^{99m}Tc-biphosphonate alginate beads and studied their chemical and physical characteristics. Further studies may suggest that the alginate beads we have prepared could be used in diagnostic and therapeutic purposes.

Bibliography

1. Liwis G, Olbricht W. Development of a portable therapeutic ultrasound system for military, medical and research use. *Rev Sci Instrum* 2008; 79: 114302-16.
2. Canh Le Tien, M. Lacroix, P. Ispas-Szabo et al. *N*-acylated chitosan: hydrophobic matrices for controlled drug release. *J. of Controlled Release* 2003; 93: 1-13.
3. Ezra A,Golomb G: Administration routes and delivery system of biophosphonates for the treatment. *Adv Drug Del Rev* 2000; 42: 175-95.
4. Gombotz WR. Wee SW. Protein release from alginate matrices. *Adv Drug Delivery Review* 1998; 31: 267-85.
5. Al-Musa S, Abu Fara D, Badwan AA. Evaluation of parameters involved in preparation and release of drug loaded in cross-linked matrices of alginate. *J Controlled Release* 1999; 57: 223-32.
6. Dornish M, Aarnold M, Skaugrud O. Alginate and chitosan: Biodegradable biopolymers in drug delivery system. *Eur J Pharm Sci* 1996; 4: S153.
7. Rajaonarivony M, Vauthier C, Couarraze G et al. Development of a new carrier made from alginate. *J Pharm Sci* 1993; 82: 912-7.
8. Polk A, Amsden B, De Yao K, Peng T, MFA. Goosen. Controlled release of Albumin from chitosan-alginate microcapsules. *J Pharm Sci* 1994; 83: 178-85.
9. Shapiro, L. and Cohen, S. Novel alginate sponges for cell culture and transplantation. *Biomaterials* 1997; 18: 583-90.
10. Lee KY, Bouhadir KH, Mooney D J. Degradation Behavior of Covalently Cross-linked Poly (aldehyde guluronate) Hydrogels, *Macromolecules* 2000; 33: 97-101.
11. Grant GT, Morris ER, Rees DA et al. Biological interactions between polysaccharides and divalent cations: *FEBS Lett.* 1973; 32: 195-8.
12. C Le-Tien, M Millette, MA Mateesu. Modified alginate and chitosan for lactic acid bacteria immobilization. *Biotechnol Appl Biochem* 2004; 39: 347-54.
13 R. Van Grieken, M. De Bruin. Nomenclature for radioanalytical chemistry. *Pure and Appl. Chem* 1994; 66: 2513-26.
14. Gavini E, Rassu G. Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: in vitro/ex-vivo studies. *J of Pharmacy and Pharmacology* 2005; 57: 287-94.
15. Arica B, Calts S, Kas HS et al. A.A. 5-Fluorouracil encapsulated alginate beads for the treatment of breast cancer. *Int J Pharm* 2002; 242: 267-9.