August 2007 Volume 3 Issue 2

Trade Science Inc.

Macromolecules

An Indian Journal

Full Paper

MMAIJ, 3(2), 2007 [64-70]

Design And Synthesis Of A Polymeric Prodrug By Solid Phase Modifications Of Microspheres Prepared *Via* Precipitation Polymerization

Francesco Puoci*, Francesca Iemma, Umile Gianfranco Spizzirri, Giuseppe Cirillo, Mariarosaria Castiglione, Nevio Picci

Dipartimento Di Scienze Farmaceutiche, Universita Della Calabria, 87036 Arcavacata Di Rende (CS), (ITALY)

E-mail : francesco.puoci@unical.it

Received: 21th May, 2007 ; Accepted: 26th May, 2007

ABSTRACT

Recently, polymer conjugation with biologically active components was proposed to overcome pharmaceutical and pharmacokinetic barriers such as low oral absorption, lack of site specificity, chemical instability, toxicity and poor patient acceptance. This report describes the synthesis of new insoluble polymeric material as potential carrier for biomolecules. We prepared no-swellable microparticles to covalently link drug molecules by means of spacer containing functional groups able to undergo enzymatic or chemical degradation in a specific biological compartment. The microparticles were realized by precipitation polymerization of glycidyl methacrylate and divinylbenzene using 2,2'-azobis(isobutyronitrile) as initiator. Thus, the obtained beads have epoxy groups on surface which are susceptible of nucleophilic reactions. The microparticles were characterized by FT-IR spectrophotometer, scanning electron microscopy and dimensional analysis. One of the potential applications of synthesized microparticles is a colon specific release of 5-Amino Salicylic Acid(5-ASA) linked to polymer carrier by azo-bridge. The oxirane opening with 4-nitroaniline allows to introduce nitro groups on the particles surface. Reduction of nitro groups and subsequent copulation reaction of corrispodent diazonium chloride salt with salycilic acid carried out to conjugate. All reaction steps are based on solid phase transformations involving functional groups on the microspheres surface. This synthetic strategy produces solid intermediate easy to purify.

KEYWORDS

Drug delivery systems;
Polimerization of precipitation;
Functionalization of polymers;
Conjugated polymers;
FT-IR.

INTRODUCTION

A large number of therapeutic systems have undesirable properties that may generate pharmacological, pharmaceutical or pharmacokinetic barriers in clinical drug application. The chemical approach using reversible derivatives such as prodrugs, can be useful in the optimization of the clinical application of a drug. This approach offers high flexibility and improve drug efficacy. Prodrug reconversion(i.e. its conversion into its active form) occurs in the body inside a specific organ, tissue or cell and in most cases, normal metabolic processes such as the cleavage of a bond by specific cellular enzymes are utilized to release the therapeutic agent[1-3].

A polymeric prodrug, obtained by conjugation of a drug with a macromolecular system, shows a several advantages over low molecular weight agents such as protection of drug from deactivation and preservation of its activity during circulation and transport to targeted organ or tissue, improvement in pharmacokinetics and reduction in antigenic activity of the drug[4-8].

Our research was focused on design and synthesis of a new versatile material that can covalently bind biomolecules, such as drugs, antioxidants, etc. In this paper we describe the preparation and characteriza tion of polymeric microspheres bearing on the surface 5-Amino Salicylic Acid(5-ASA) molecules bounded by azo-bridges. This opportunity for reductive degradation of azo compounds by the intestinal microflora was exploited to prepare macromolecular prodrugs of the anti-inflammatory agent 5-ASA [9-12].

5-ASA is widely accepted in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease. When orally administered, 5-ASA is unstable in the gastric conditions and prone to be absorbed in the upper intestine, which causes low drug bioavailability and low efficiency for inflammatory colon disease. Therefore, the colon-specific delivery of 5-ASA is an important issue. Generally, the colon-specific delivery[13-15] of 5-ASA can be achieved by coating with pH-sensitive polymer[16,17], time-controlled formulation and device[18,19], coating with polymer which can be degraded by intestinal microflora[20-22], pressure controlled devices[23-25], and polymeric prodrug approaches[26].

Polymeric prodrugs with 5-ASA linked to the polymer backbones via spacers could successfully delivery 5-ASA to the colon[18-19].

We have realized no-swellable microparticles via a novel precipitation polymerization[29-31] containing epoxydic groups to link parent molecule of 5-ASA on surface.

The microparticles were realized by precipitation polymerization of glycidyl methacrylate (GMA) and divinylbenzene(DVB) in the presence of 2,2'-azobis(isobutyronitrile)(AIBN) as initiator. The microspheres were characterized by Fourier Trasform Infrared(FT-IR) spectrophotometer, scanning electron microscopy(SEM) and dimensional analysis. The number of epoxydic groups was estimatated by spectrophotometric determination of primary amino groups following the method of Gaur and Gupta[32] after oxirane ring opening with methanolic ammonia solution. The oxiranic rings on the material can undergo a several transformations in heterogeneous phase.

The opening of epoxy groups with 4-nitroaniline allows to introduce nitro groups on the particles surface. The azo-conjugate preparation consists of two steps: reduction of nitro groups and synthesis of diazonium chloride salt and in situ copulation reaction with salycilic acid. The amino groups obtained by reduction were spectrophotometrically determined[32]. All reaction steps involve solid phase chemical modifications producing intermediates easy to purify. Each materials modification by FT-IR spectrophotometer were confirmed.

EXPERIMENTAL

Materials

4-dimethylaminopirydine(DMAP), GMA, DVB, perchloric acid, p-nitroaniline, chromium chloride, sodium nitrite, salicylic acid were obtained from Sigma-Aldrich(Sigma Chemical Co, St. Louis, MO). AIBN was purchased from Sigma-Aldrich(Sigma Chemical Co, St. Louis, MO) and was recrystallized from methanol. Toluene, acetonitrile(CH_3CN), methanol (MeOH),

ethanol (EtOH), 2-propanol, tetrahydrofuran, ethylic ether, N,N-dimethylformammide(DMF), water, triethylamine(TEA), acetone, methylene chloride were reagent grade and they were provided by Carlo Erba Reagents(Milan, Italy). N-succinimidyl-4-O-(4,4'-dimethoxy triphenylmethyl)butyrate(SDTB) was prepared by known procedures[32] and it was purified by column chromatography(eluent diethyl ether/benzene=1/9). IR(KBr)υcm^{-1}: 2830(CH_3O); 1815-1785(C=O imide); 1739(C=O carboxyl); 703(trityl). ^{1}H-NMR($CDCl_3$, ppm) 2.00(m, 2H); 2.75 (m, 2H); 2.79 (s, 4H); 3.16 (m, 2H); 3.75 (s, 6H); 6.82(d, 4H, J_0=8.7Hz); 7.29(m, 9H).

Apparatus

Ultraviolet spectra were recorded with a U-2000 Hitachi spectrophotometer using 1cm quartz cells. FT-IR spectra were recorded as pellets in KBr in the range 4000-400cm^{-1} using a Perkin-Elmer PARAGON 1000PC spectrophotometer. The resolution was 1cm^{-1}. The number of scans was 100. ^{1}H-NMR spectra were run on Bruker VM-300 ACP. Particle size distribution was carried out using an image processing and analysis system, Leica DMRB equipped with a Leica Wild 3D stereomicroscope. This image processor calculates the particle area and converts it to an equivalent circle diameter. Scanning electron microscopy photographs were obtained with a Leo stereoscan 420; the sample surface was made conductive by the deposition of a layer of gold on the samples in a vacuum chamber.

Synthesis of copolymer (1)

0.14g(1.0mmol) of GMA were dissolved in a mixture of acetonitrile(45ml) and toluene(15ml), in a 100ml round bottom flask and then 2.25g(17.2mmol) of DVB and 0.080g(0.18mmol) AIBN were added. The polymerization mixture was degassed in a sonicating water bath, purged with nitrogen for 10 min cooling with an ice-bath. The flask was then gently agitated(40rpm) in an oil bath. The temperature was increased from room temperature to 60^{0}C within 2h, and then kept at 60^{0}C for 24 h. At the end of the reaction, the microspheres bearing epoxy rings **(1)** were filtered, washed with 100ml portions of acetone, methanol, 2-propanol and ethylic ether. Particles were successively dried under vacuum overnight at 40^{0}C. IR(KBr)υcm^{-1}: 3022 (Ph), 2922 and 2860 (CH_2), 1724 (C=O), 1595(Ph).

Synthesis of copolymer (6)

0.030g of**(1)** were suspended in 10.0 ml of methanolic solution of ammonia(6.8 N) and stirred for 72h at room temperature. The mixture was filtered and the polymer was washed with 25 ml portions of water, EtOH, 2-propanol, ethylic ether and then dried overnight under vacuum at 40^{0}C to yield the sample **(6)**. IR(KBr) υcm^{-1}: 3200-3550(O-H), 3200-3550(NH_2), 3018(Ph), 2923 and 2862(CH_2), 1723(C=O), 1597(Ph).

Synthesis of copolymer (2)

0.53g of **(1)** in 5.0ml of a mixture DMF/TEA(1:1 v/v) were suspended. At the suspension were then added 1.60g(11.6mmol) of p-nitroaniline and the mixture was heated at 50^{0}C for 72h. At the end of the reaction the microspheres were filtered, washed with 100ml portions of acetone, methanol, 2-propanol and ethylic ether and then dried overnight under vacuum at 40^{0}C to yield the sample **(2)**. IR(KBr)υcm^{-1}: 3200-3550(O-H), 3018(Ph), 2923 and 2862 (CH_2), 1723(C=O), 1597 (Ph), 1329 (NO_2).

Synthesis of copolymer (3)

0.15g of **(2)** in 2.0ml of DMF dry were suspended and 0.065g(0.53mmol) of chromium chloride[33] were added. The reaction mixture was stirred for 6 h at room temperature. The mixture was filtered and the polymer was washed with 100 ml portions of NaOH 0.1 N, DMF, methanol and methylene chloride and then dried overnight under vacuum at 40^{0}C to afford the sample**(3)**. IR(KBr)υcm^{-1}: 3200-3550(O-H), 3022(Ph), 2924 and 2862(CH_2), 1734(C=O), 1598 (Ph), 1243(C-N).

Synthesis of copolymer (5)

0.49g of **(3)** in 9.0ml of HCl(4 N, 36 mmol) were suspended and 0.17g(2.5mmol) of sodium nitrite were added. The suspension was stirred for 3 h at 0^{0}C to yield **(4)**. The so-formed diazonium chloride was consecutively coupled with 0.013g(0.094mmol) of salicylic acid and stirred for 24h at 0^{0}C. The mixture was filtered and the polymer was washed

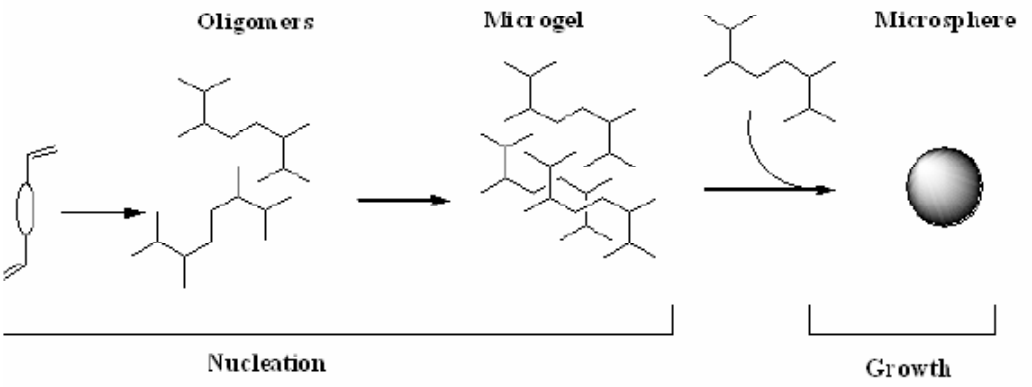

Figure 1: Proposed precipitation polymerization mechanism

with 100ml portions of acetone, methanol, 2-propanol and ethylic ether and dried overnight under vacuum to afford**(5)**. IR(KBr)υcm^{-1}: 3421(O-H), 3021(Ph), 2923 and 2866(CH_2), 1721(C=O), 1649(C=O), 1564(N=N), 1279(C-O).

Determination of primary amino groups on insoluble support (General Procedure)

0.010g of copolymers**(5)**and**(6)** were suspended in 250μl of SDTB solution in DMF(0.1 M), then 150μl of triethylamine dry and a catalytic amount of DMAP were added. The suspension was stirred for 2h at room temperature then the microspheres were filtered, washed with 20ml portions of ethylic ether and tetrahydrofuran. Finally, the functionalized particles were dried overnight under vacuum at 40^{0}C. In a volumetric flask an aliquot(1-3mg) of microparticles derivatized with SDTB was added of detrytilation solution(53% $HClO_4$ 16 N and 47% MeOH). After 1h the mixture was analyzed by UV-Vis spectrometry(λ=498nm).

RESULTS AND DISCUSSION

In this work, we have synthesized a novel polymeric prodrug to situ specific release of 5-ASA. For this purpose, salicylic acid was covalently linked to the microspheres by azo bridges, susceptible to enzymatic attack in the large intestine.

A material extremely versatile**(1)**, bearing epoxy groups on the surface, was prepared. This one is able to undergo nucleophilic attack to introduce convenient chemical residues covalently linked to the particles.

Microparticles were prepared according to procedure of Wang et al[29]. The proposed polymeriza tion mechanism is characterized by two parts: nucleation and growth of microspheres. The reaction begins as a usual solution polymerization where monomers and initiator were dissolved in the organic solvent and during the polymerization oligomers are formed. After a certain period of time the concentration of oligomers becomes sufficiently high to allow radical polymerization of oligomers to form a microgel(Nucleation). Each seed(Microgel) then grows by continuous capture of oligomers. This last effect prevents the occurrence of any further nucleation and hence uniformly-sized particles are produced (Figure 1).

During polymerization the growing polymer chains are separated from the continuous medium by enthalpic precipitation in cases of non favourable polymer-solvent interactions or entropic precipitation in cases where crosslinking prevents the polymer and the solvent from freely mixing. It is important to note that changing the rotation rate of the polymerization flask, the solvent and monomer and crosslinker concentrations, it is possible to obtain beads with different dimensions(micro or nanoparticles).

The material (1) was prepared by precipitation polymerization of GMA, as main functional monomer, and DVB, as comonomer/cross-linking agent (Figure 2).

FT-IR spectrum of microspheres confirms that

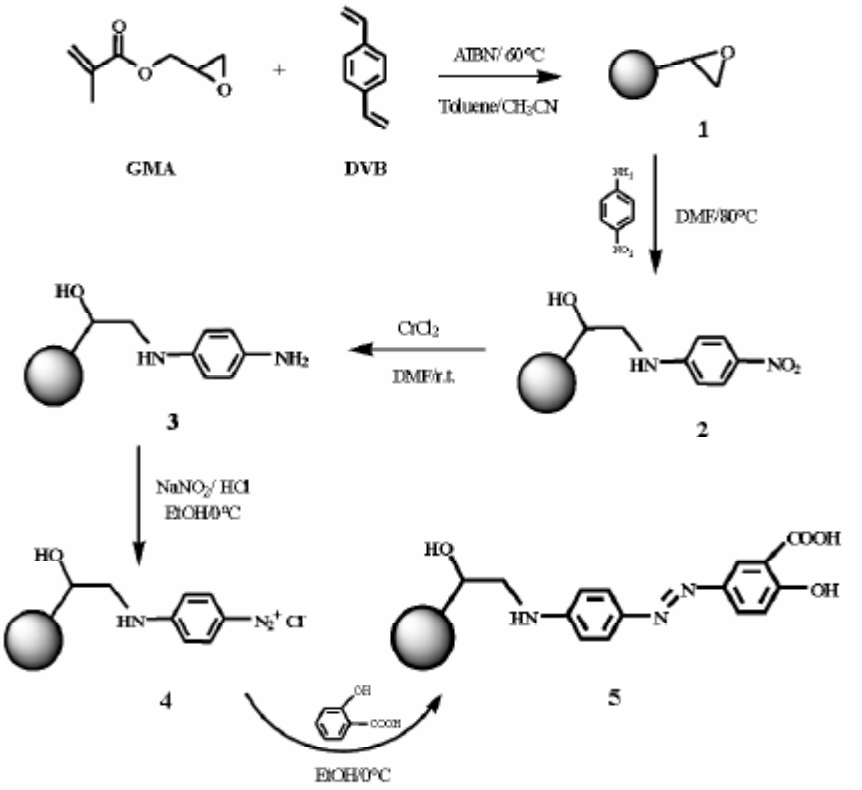

Figure 2: Synthesis of copolymer (6) by solid phase modifications of microspheres

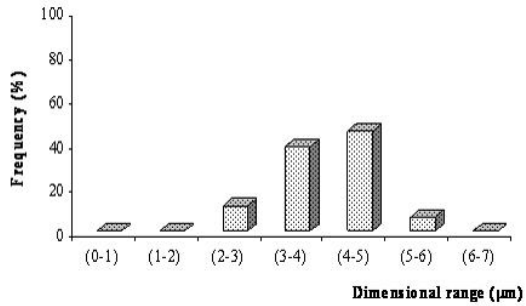

Figure 3 : Dimensional analysis of microspheres (1)

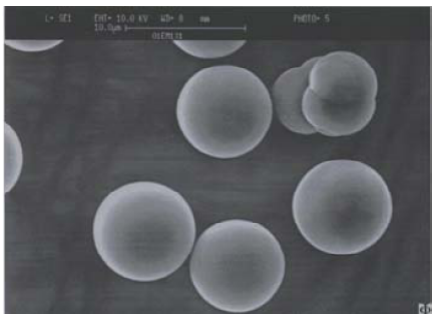

Figure 4 : Photomicrography of microspheres(1)

both monomers in the material appear. The matrix was also characterized by Scanning Electron

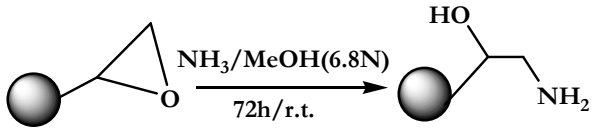

Figure 5: Derivatization of copolymer (1) with a solution of NH_3/MeOH

Micrographs(SEM)(Figure 3), where the spherical geometry and the practically monodispersion of prepared samples was confirmed. The dimensional range of the beads are 3-5nm.

In addition, the dimensional analysis(Figure 4), agrees with the SEM information, confirms a very narrow distribution size. This polymerization procedure can be consider as a methodology to prepare practically monodisperse micro and nanospheres.

To determine the epoxy rings concentration on the material**(1)**, oxirane groups, by ammonia methanolic solution, were opened (Figure 5).

The NH_2 content of**(6)** was estimated following the method of Gaur and Gupta[32] based on the labeling of the amino groups with 4-O-(4,4'-

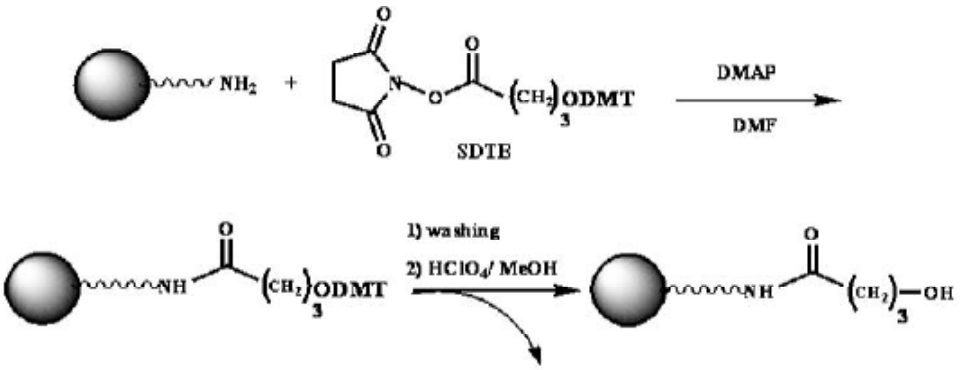

Figure 6: Estimation of NH_2 content in the resin in according to Gaur-Gupta method

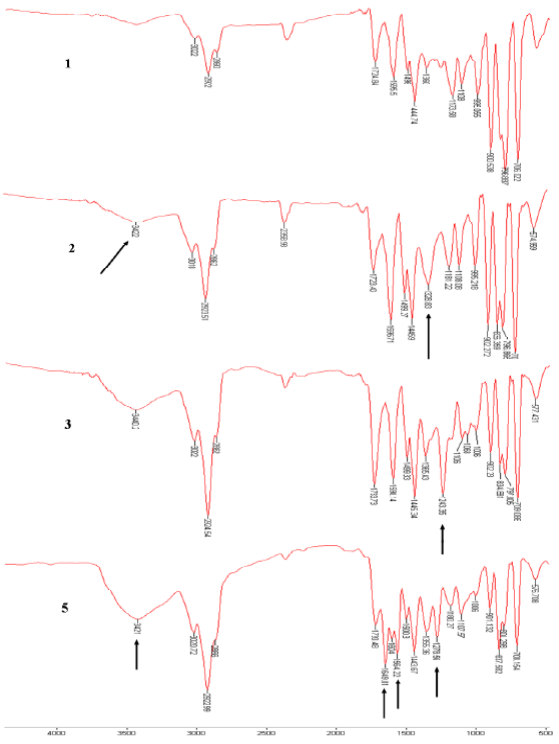

Figure 7: FT-IR spectra of microspheres after each solid phase modification

dimethoxytriphenylmethyl)-butyryl residues and the quantitative determination through UV-Vis spectroscopy of the 4,4'-dimethoxytriphenylmethyl cation(DMT^+; ε=70,000 at 498nm) released from the resin after treatment with $HClO_4$ (Figure 6).

The moles of primary amino groups per grams of polymer was determined, in according to equation 1:

$$\frac{\text{moles amino groups}}{\text{gresin}} = \frac{\text{A.V.}}{\text{P.70000}} \quad (1)$$

Where A, V and P are absorbance, detrytilation solution volume and microparticles amount respectively. We estimated for**(6)** a primary amino groups content of 142μmol/g. Epoxy rings opening reaction by ammonia was confirmed through FT-IR spectrum where a broad absorption band between 3200 and 3500cm^{-1}, ascribable to hydroxy and amino groups, appears.

To obtain**(2)**, epoxy groups of**(1)** underwent nucleophilic attack by 4-nitroaniline in DMF/TEA mixture (1:1v/v) at 80^{0}C for 72h. Typical bands of nitro and hydroxy groups, at 1329 and 3200-3500cm^{-1}, respectively, in the FT-IR spectrum, were detected.

In literature an efficient method for the solid phase reduction of aryl nitro groups to anilines is described. Among the several reduction conditions of nitro groups evaluated, optimal results by treating the resin with chromium chloride in DMF were achieved[33]. The reduction system, $CrCl_2$ in DMF, allows to obtain high yield without affecting others reducible functionality. Copolymer**(2)** nitro groups were successfully reduced with chromium chloride in DMF dry at room temperature for 6 h providing copolymer**(3)**. In the FT-IR spectra a strong band at 1243cm^{-1} can be assigned to C-N stretching absorption, characteristic of aromatic amines. At the same time the band at 1329 cm^{-1}, assigned to nitro groups, disappears(Figure 7). The NH_2 content of the resins**(3)** was equal to 25,1μmol/g.

Sodium nitrite in hydrochloric acid environmental converts primary amino groups of **(3)** in the correspondent diazonium chloride salt**(4)**. This one, coupling in situ with salicylic acid, forms the polymeric prodrug**(5)**. The transformation was confirmed by FT-IR spectra that shows the typical salicylic acid bands

at 1278, 1649 and 3421cm^{-1} and, at the same time, it is possible to note the characteristic absorption band at 1564cm^{-1}of azo groups present in the macromole cular system (Figure 7).

CONCLUSIONS

The precipitation polymerization represents a profitable method to obtain matrices of GMA and DVB. These matrices are insoluble and no water-swellable and they are characterized by a spherical shape and a narrow size distribution profile(4μm). The synthesized microspheres allow drug conjugation via designed linkages. In particular, our interest was focused on conjugates for colon-specific applications. For this aim we have bounded the salycilic acid, through azo-spacer, to spherical matrices. Heterogeneous reactions in the prodrug synthesis take place. In this way it is possible to obtain microparticle intermediates easy to purify. This prodrug have good potential to act as carriers for 5-ASA colon-specific drug delivery.

ACKNOWLEDGMENT

This work was financially supported by MIUR, (Programma di ricerca di rilevante interesse nazionale,2005) and the University funds.

REFERENCES

[1] A.Stanczak, A.Ferra; Pharmacol.Rep., **58(5)**, 599 **(2006)**.
[2] G.Van den Mooter; Exp.Opin.Drug.Del., **3(1)**, 111 **(2006)**.
[3] A.Tavora de Albuquerque Silva, M.C.Chung, L.F.Castro, R.V.Carvalho Guido, E.I.Ferreira, Rev.Med.Chem., **5(10),** 893 **(2005)**.
[4] J.Khandare, T.Minko; Progr..Pol.Sci., **31(4),** 359 **(2006)**.
[5] S.Borgeois, R.Harvey, E.Fattal; Amer.J.Drug.Del., **3(3),** 171 **(2005)**.
[6] A.W. Basit; Drugs., **65(14)**, 1991 **(2005)**.
[7] R.B.Greenwald; Exp.Op.Ther.Pat., **7(6)**, 601-609 **(1997)**.
[8] K.Hoste, K.De Winne, E.Schacht; Eur.J.Pharm. Biopharm., **58(1)**, 151 **(2004)**.
[9] S.Davaran, J.Hanaee, A.J.Khosravi; J.Contr.Rel., **58**, 279 **(1999)**.
[10] Q.X.Cai, K.J.Zhu, D.Chen, L.P.Gao; Eur.J.Pharm. Biopharm., **55**, 203 **(2003)**.
[11] S.Sakuma, Z.R.Lu; P.Kopeckova, J.Kopecek; J.Control.Rel., **75**, 365 **(2001)**.
[12] R.Rafii, W.Franklin, C.E.Cerniglia; Appl.Environ. Mivrobiol., **56,** 2146 **(1990)**.
[13] S.P.Baldwin, W.M.Saltzman; Adv.Drug.Deliv.Rev., **33**, 71 **(1998)**.
[14] H.Brøndsted, J.Kopecek; Biomaterials, **12**, 584 **(1991)**.
[15] M.K.Chourasia, S.K.Jain; J.Pharm.Pharma.Sci., **6**, 33 **(2003)**.
[16] A.Tromm, T.Griga, B.May; Hepato-Gastroenterology, **46**, 3124 **(1999)**.
[17] C.A.Sninsky, D.H.Cort, F.Shanahan, B.J.Bowers, J.T.Sessions, R.E.Pruitt, W.H.Jacobs, S.K.Lo, S. R.Targan, J.J.Cerda; Ann.Intern.Med., **115**, 350 **(1991)**.
[18] K.Niwa, T.Takaya, T.Morimoto, K.J.Takada; Drug. Target., **3**, 83 **(1995)**.
[19] M.E.MacNeil, H.N.E.Stevens; Patent, WO90-09168, **(1990)**.
[20] H.Brondsted, J.Kopecek; Biomaterials, **12**, 584 **(1991)**.
[21] H.Brondsted, J.J.Kopecek; Pharm.Res., **9**, 1540 **(1992)**.
[22] M.Saffran, G.S.Kumar, J.C.Savariar, C.Burnham, F.Williams, D.C.Neekers; Science, **233**, 1081 **(1986)**.
[23] Z.Hu, G.Kimura, Y.Ito, S.Mawatari, T.Shimokawa, T.H.Yoshikawa, Y.Yoshikawa, K.J.Takada; Drug. Target., **6**, 439 **(1999)**.
[24] M.Muraoka, G.Kimura, H.Zhaopeng, K.Takada, Nippon Rinsho; Jpn.J.Clin.Med., **56**, 788 **(1998)**.
[25] T.Takaya, C.Ikada, N.Imagawa, K.Niwa, K.Takada; J.Pharm.Pharmacol., **47**, 474 **(1995**).
[26] T.Takaya, K.Sawada, H.Suzuki, A.Funaoka, K.Matsuda, K.Takada; J.Drug.Target., **4**, 271 **(1997)**.
[27] M.R.Rashidi, J.Hanaee, A.Khani, M.Mahkam, M.J.Hashemi; Bioact.Com.Pol., **21(4)**, 315 **(2006)**.
[28] M.Mahkam, L.Doostie, S.O.R.Siadat; Inflammo pharmacology, **14**, 72 **(2006)**.
[29] J.Wang, P.A.G.Cormack, D.C.Sherrington, E.Khoshdel; Angew.Chem.Int.Ed., **42**, 5336 **(2003)**.
[30] E.Turiel, J.L.Tadeo, P.A.G.Cormack, A.Martin-Esteban; Analyst, **130**, 1601 **(2005)**.
[31] F.Puoci, F.Iemma, R.Muzzalupo, U.G.Spizzirri, S.Trombino, R.Cassano, N.Picci; Macromol.Biosci., **4**, 22 **(2004)**.
[32] R.K.Gaur, K.C.Gupta; Anal.Biochem., **180**, 253 **(1989)**.