Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2012; 37 (2) ; 120–128.
doi: 10.5505/tjb.2012.21931

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Mart, 2012 © TurkJBiochem.com

[Published online 30 March, 2012]

Preparation and characterization of ketoprofen loaded albumin microspheres

[Ketoprofen yüklü albumin mikrokürelerin hazırlanması ve karakterizasyonu]

Aydan Gülsu[1],
Hakan Ayhan[2],
Fatma Ayhan[1]

[1]Mugla University, Department of Chemistry, Biochemistry Division, Mugla, Turkey.
[2]Biochemist&Bioengeneer, P.B:48; 48000 Muğla, Turkey.

Yazışma Adresi
[Correspondence Address]

Professor Fatma Ayhan

Mugla University
Department of Chemistry,
Biochemistry Division
48000 Mugla, Turkey.
Tel: +90252 211 1495
Fax: +90252 2111472
E-mail: fayhan@mu.edu.tr

Registered: 14 April 2011; Accepted: 30 December 2011

[Kayıt Tarihi: 14 Nisan 2011; Kabul Tarihi: 30 Aralık 2011]

ABSTRACT

Aim: Albumin microspheres have found many applications in the diagnosis and treatment in recent years and more than 100 diagnostic agents and drugs have been incorporated into Albumin microspheres. The objective of this study was to investigate the effect of preparation parameters (Albumin concentration, stirring rate, crosslinker amount, crosslinking time) on the Albumin microsphere size and determine the release profile of ketoprofen loaded albumin microsphere.

Method: Albumin microspheres were prepared by an emulsion polymerization method using glutaraldehyde (GA) as the crosslinking agent. The prepared microspheres were then studied for their particle size, size distribution, release characteristics. The microspheres were characterized using an Optical Microscope.

Results: The microspheres had mean diameters between 2-25 µm of which more than 25 percent were below 10 µm. Drug release from the Albumin microspheres displayed a biphasic pattern characterized by an initial fast release, followed by a slower release. The total amount of drug released from microspheres in pH 7.4 phosphate buffer saline (PBS) at 37°C after 180 min was obtained as 33%.

Conclusion: In the present study the various parameters affecting the characteristics of the albumin microspheres were evaluated. According to these results optimal conditions were determined as: 0.1 mg.ml^{-1} Albumin concentration, 1000 rpm stirring rate, 1% GA amonut and 30 min crosslinking time. The drug release from Albumin microspheres was mainly controlled by diffusion and showed a biphasic pattern with initial release (burst effect), followed by a slow and controlled release phase resulting from controlled diffusion of the entrapped drug. Additionally, local gastrointestinal side effects are thought to be reduced by sustained release of ketoprofen and this must also be investigated with *in vivo* experiments.

Key Words: Microspheres, albumin, ketoprofen, biodegradable

Conflict of interest: Authors have no conflict of interest.

ÖZET

Amaç: Son yıllarda, Albumin mikroküreleri, teşhis ve tedavide birçok uygulama alanı bulmuş ve 100 den fazla teşhis ajanı ve ilaç içeren Albumin mikroküre hazırlanmıştır. Bu çalışmanın amacı hazırlama parametrelerinin (Albumin konsantrasyonu, karıştırma hızı, çapraz bağlayıcı miktarı, çapraz bağlanma süresi) Albumin mikroküre boyutu üzerine etkisini incelemek, ilaç yükleme ve ketoprofen yüklü Albumin mikrokürelerin ilaç salım profilini belirlemektir.

Yöntem: Albumin mikroküreler, glutaraldehid (GA) çapraz bağlayıcı ajan kullanılarak emülsiyon polimerizasyonu yöntemi ile hazırlanmıştır. Hazırlanan mikrokürelerin ortalama partikül boyutu, boyut dağılımı ve salım karakteristiği incelenmiştir. Mikroküreler optik mikroskop yardımıyla karakterize edilmişlerdir.

Bulgular: Mikroküreler %25 inden fazlası 10 µm olmak üzere 2-25 µm aralığında boy dağılımı göstermiştir. Albumin mikrokürelerden ilaç salımı, başlangıçta ani salımı (burst effect) izleyen yavaş ve kontrollü salım ile, bifazik model sergilemesi, hapsolan ilacın kontrollü difüzyonu ile sonuçlanmıştır. kontrollü pH 7.4 fosfat tamponunda (PBS) 37 °C de 180 dakika sonunda mikrokürelerden toplam ilaç miktarının % 33' ünün salındığı gözlenmiştir.

Sonuç: Bu çalışmada albümin mikrokürelerin karakteristiğine etki eden çeşitli parametreler değerlendirilmiştir. Bu sonuçlara göre optimum koşullar: 0.1 mg.ml^{-1} Albumin derişimi, 1000 rpm karıştırma hızı, %1 GA miktarı, 30 dk çapraz bağlanma süresi olarak belirlenmiştir. Albumin mikrokürelerden ilaç salımının temel olarak, difüzyon kontrollü olduğu, tutuklanan ilacın, difüzyon sonucu, başlangıçta ani salımı (burst effect) izleyen yavaş ve kontrollü salımı ile bifazik model sergilediği görülmüştür. Ayrıca ketoprofenin kontrollü salımı sayesinde lokal gastrointestinal yan etkilerinin azalabileceği düşünülmekte, bunun *in vivo* deneylerle de incelenmesi gerekmektedir.

Anahtar Kelimeler: mikroküre, Albumin, ketoprofen, biyoparçalanma.

Çıkar çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction

Microspheres can be defined as solid, spherical particles ranging in size from 1 to 1000 μm [1]. They are made of polymeric, waxy or other protective materials such as starches, gums, proteins, fats and waxes and used as drug carrier matrices for drug delivery. Albumin and gelatin are among the natural polymers which are used in preparation of microspheres. The synthesis and use of Albumin microspheres to improve the efficiency of therapeutic drugs has been the topic of many reviews [2-5].

Albumin is a major plasma protein constituent, accounting for ~ 50 %of the total protein in human plasma [6]. Since they were first described by Kramer [7], Albumin microspheres have been extensively investigated in controlled release systems as vehicles for the delivery of therapeutic agents to local sites. The exploitable features of Albumin include its reported biodegradation into natural products [8], its lack of toxicity [9,10] and its nonantigenicity. Albumin microspheres are metabolized in the body, and the size of particles, degree of stabilization and site of metabolism are the main factors influencing the extent of metabolism [8]. Drug release from the microspheres can be widely modulated by the extent and nature of crosslinking, size, the position of the drug and its incorporation amount in the microspheres [11]. Colloidal forms of Albumin have been considered as potential carriers of drugs for their site-specific localization or their local application to anatomically discrete sites [12].

Albumin has been used as a carrier for targeting drugs to tumors and since the synovium of the rheumatoid arthritic patients shares various features observed in tumors. Albumin-based delivery systems can be used to target drugs to the inflammated joint. Intravenous administration of the drugs coupled with Albumin has been reported to improve the targeting efficiency of the drug to arthritic regions [13]. The circulation half-lives of the drugs have been reported to dramatically increase when the drug is conjugated with albümin [14]. Increasing the circulation half-life of the formulation by reducing its uptake by the reticuloendothelial system has been shown to improve the targeting efficiency of the formulation to the arthritic paws of rats [15]. There are several reports on the use of long circulating liposomes to target the drugs to the arthritic joints [15,17]. However, there are only a few reports on the use of microspheres for targeting the drugs to the arthritic joints.

More than 100 therapeutic and diagnostic agents have been incorporated into Albumin microspheres and drugs of various therapeutic categories such as nifedipine [18,19], mitoxantrone [20], dexamethasone [21], salbutamol sulfate [22] have been prepared and characterized as Albumin microsphere delivery systems. Two methods have been developed for the preparation of Albumin microspheres which include heat stabilization and chemical cross-linking by using GA [23].

GA is one of the most common dialdehydes used to crosslink bioprostheses and proteins. The aldehyde group reacts with a primary amine to form a Schiff base. In comparison to other aldehydes, GA reacts relatively quicker and is able to span various distances between amino groups both intra- and intermolecularly. In addition, it yields chemically, biologically, and thermally more stable cross-links [24].

Ketoprofen, 2- (3-benzoylphenyl) propionic acid, is a widely used non-steroidal anti-inflammatory drug (NSAID) for the treatment of rheumatoid arthritis and osteoarthritis as well as in the treatment of various other painful inflammatory states. After peroral administration ketoprofen is readily absorbed from the gastrointestinal tract, exhibiting a short biological half-life of 1-2 h. Like other NSAIDs ketoprofen has been found to cause local adverse effects in the gastrointestinal tract. Therefore, ketoprofen is a candidate for controlled drug delivery.

Sustained release of ketoprofen has been achieved by cellulose-acetate butyrate- polystyrene microcapsules using a complex emulsion technique [25] and encapsulation with ethylcellulose by o/w solvent evaporation method [26]. Several types of Eudragit also were used in solvent evaporation process to yield ketoprofen-containing microcapsules [27,28].

In the present study, Albumin microspheres were prepared by the emulsion polymerization technique in order to study the influence of varying preparation parameters and the release profile of the ketoprofen loaded Albumin microspheres. After examination of all formulation parameters on the microsphere properties, the optimized batch was selected and in vitro study was performed.

Materials and Methods

1. Chemicals

BSA (Bovine Serum Albumin) (Sigma), GA (J.T.Baker), Diethylether (Merck), Ketoprofen, Olive oil (Komili).

2. Preparation of Unloaded Microspheres

Albumin microspheres were prepared by using emulsion polymerization technique. A number of variables were studied affecting size and shape.

Briefly, a weighed amount of BSA (25-75 mg) was dissolved in distilled water (0.25 ml). This solution was then added to 25 ml olive oil which was continuously stirred at various stirring rates (150-14000 rpm) at room temperature of 25°C. Stirring was continued for 5 min to obtain a water/oil (1:100) emulsion. Different ratios (0.1-3%) of GA (25%, w/v) were added into the emulsion to crosslink the Albumin present in the internal phase of the emulsion. Stabilization process was continued at various stabilization times (30-180 min). Microspheres were formed then separated by centrifugation and washed with 30 ml of diethyl ether to remove excess GA and the olive oil. The percentage yield of microspheres were calculated using the below mentioned formula.

Percentage Yield = (Practical Yield / Theoretical Yield) × 100 (Eq. 1)

The prepared microspheres were then characterized for their various properties.

3. Drug Loaded Microsphere Preparation

The Albumin microspheres containing ketoprofen were prepared by the same method which was described above. After determining of optimal conditions, BSA (25 mg) and ketoprofen (0.25 mg) were dissolved in PBS buffer (0.25 ml, pH 7.4). This solution was added to 25 ml olive oil which was continuously stirred at 1000 rpm at the room temperature. The resulting microspheres were stabilized using 0.25 ml GA solution (25%, w/v) for 30 min. After the stabilization process, the microspheres were centrifugated and washed with anhydrous diethylether in order to remove excess GA and the olive oil.

4. Characterization of microspheres

4.1. Surface Morphology of Microspheres

Microspheres were charactherized firstly by inverted optical microscope (Leica microsystems, DFC 295). Scanning electron microscopy (SEM) of the Albumin microspheres was performed to examine the surface morphology. The microspheres were mounted on metal stubs and then coated with gold. Photomicrographs were taken using a Jeol scanning electron microscope (SEM JEOL JSM-5910 LV).

4.2. In Vitro Release

An incubation method was used for investigation of in vitro ketoprofen release from the Albumin microspheres. Ketoprofen loaded microspheres (25 mg) were suspended in 10 ml of PBS (pH 7.4) and then immersed with agitation in a laboratory shaker. At various time intervals, samples (1 ml) were withdrawn and replaced by an equal volume of fresh medium. The absorbance values of the samples were measured (LABOMED, Inc. Spectro UV-vis double) at 260 nm [29]. The absorbance of ketoprofen in phosphate buffer (PBS) shows a maximum at 260 nm [30,31]. The concentration of ketoprofen released from the networks was expressed as a percentage of the total ketoprofen available and plotted as a function of time. Each value was given as the average of the three experimental results.

4.3. Determination of Unloaded Microsphere Degradation

The hydrolytic degradation of Albumin microspheres was investigated PBS (pH 7.4 at 37°C) by weight losing method. 25 mg unloaded Albumin microspheres prepared at optimum conditions were incubated in 10 ml of PBS. Samples were collected every 24 hours for a period of 30 days. Microsphere degradation was measured by centrifugation of suspension and weighing the precipitate.

The degradation percentage was calculated by the equation 2:

Degradation %= 100 (W_0 -W) / W_0 (Eq. 2)

where W_0 and W are, the initial weight of unloaded microsphere and the weight of unloaded microsphere after incubation in PBS, respectively.

Results and Discussion

1. Synthesis of Microspheres

An emulsion crosslinking method was used to prepare the microspheres. Albumin was dissolved in distilled water. The method involved the formation of small droplets of aqueous Albumin in natural oil. Being a soluble polymer, Albumin has to be chemically crosslinked to become insoluble at 37°C. GA is used as the crosslinking agent and sabilizer to obtain rigid and stable microspheres.

Albumin was selected as a natural matrix material for its biocompatibility, lack of toxicity, nonantigenicity and ability to control the physicochemical characteristics of microspheres produced, depending on the crosslinking method and characteristics of crosslinking agent [32]. The yield value of the microspheres ranged from 90%to 99%. The yield depends on the stabilization period and the concentration of crosslinking agent used for the preparation process [33]. At high GA concentrations, a slight decrease was observed. In the presence of low GA concentration, the yield value increased.

2. Morphology and Size Distribution

2.1. Albumin Concentration Effect

As the concentration of Albumin increases, the viscosity of the aqueous phase increases as the resistance to deformation and disintegration under shear increases. Thus, for a given shear rate, the droplet size is expected to increase as the viscosity of dispersed phase increases. In addition, as the concentration of Albumin increases, more particle forming material is available.

The particle size of the Albumin microspheres at optimum conditions ranged from 2 to 25 μm. The microspheres were photographed by an optical camera (Fig 1-4) and as it is shown microspheres are spherical with quite smooth surfaces**.**

At low concentration of Albumin (below 0.1 $mg.ml^{-1}$) no microsphere product was obtained. By increasing the Albumin concentration (Fig 1) the mean particle size of microspheres increased. This finding is in accordance with other researcher group [34]. This observation may be attributed to an increase in the viscosity of the dispersed phase, making the coalescence of emulsified dispersed droplets easier.

In this study 0.1 $mg.ml^{-1}$ Albumin concentration was selected. Because, Albumin amount was found satisfactorily enough and economical for the preparation viewpoint.

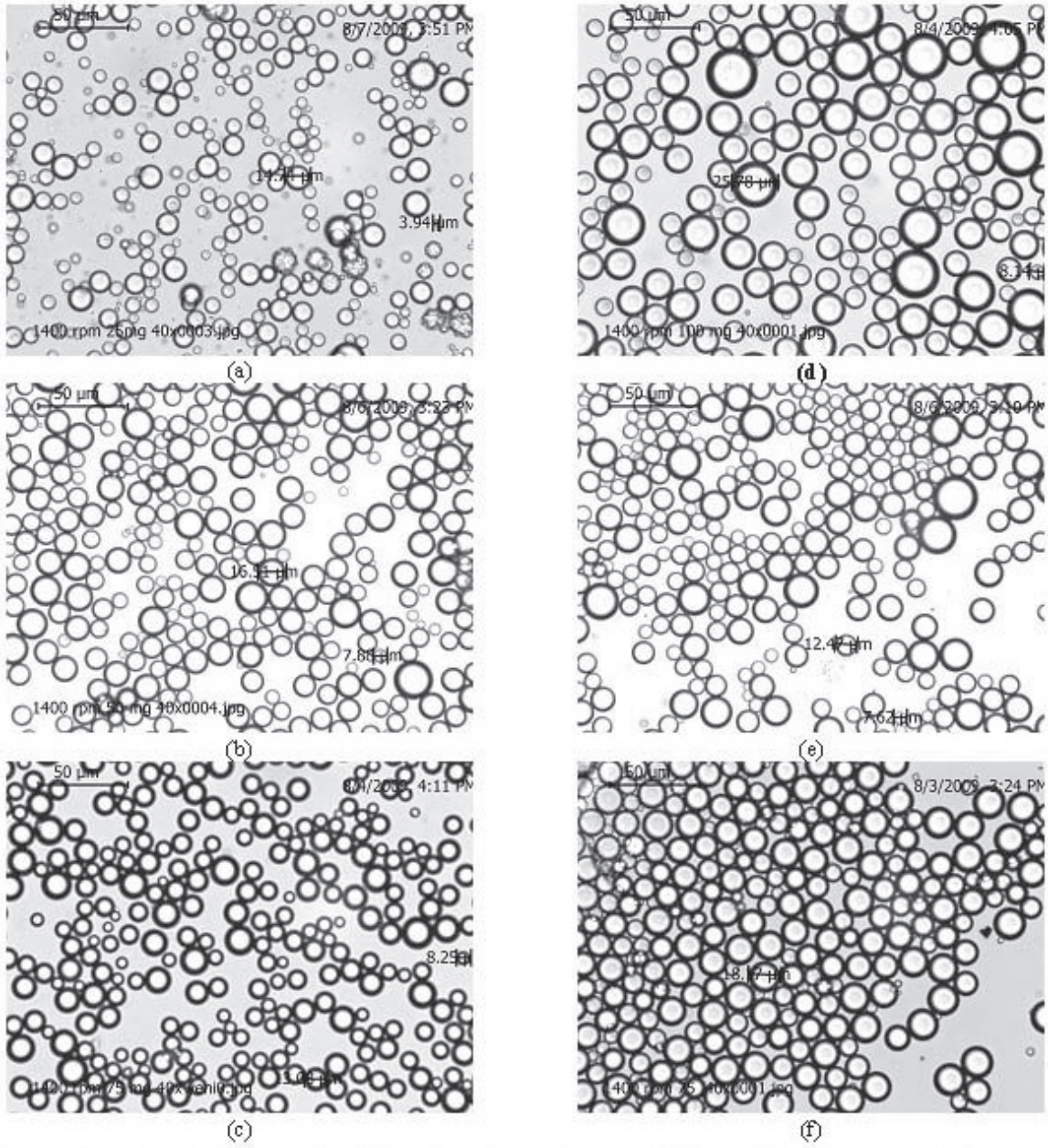

Figure 1. Optical micrographs of Albumin microspheres at different Albumin concentrations (mg.ml-1); a) 0.1; b) 0.15; c) 0.2; d) 0.25; e) 0.3; f) 0.35.

2.2. Stirring Rate Effect

The effect of stirring rate on 0.1 mg.ml^{-1} was evaluated. The results of stirring rate on the particle size are shown in Fig. 2. It can be seen that by increasing the rate of stirring, the mean size of microspheres decreased. A similar result has been reported elsewhere [34]. This was expected, because high stirring rates provide the sheering force needed to separate the oil phase into smaller droplets.

As the stirring rate decreased below 1000 rpm (150-850 rpm) it is seen that size distribution becomes wider. As the stirring rate increased above 1000 rpm no significant effect on particle size and size distribution was seen. The particle size of the Albumin microspheres ranged from 7 to 21 μm at 1000 rpm stirring rate. Comparatively narrow size distribution may be due to the lower viscosity of the external phase, which offered less resistance to the spheres formed. The maximum particle size at 1000 rpm stirring rate was below 30 μm. The particle size distribution observed is similar to that required for intramuscularly administered products [35].

1000 rpm stirring rate was selected as the optimum stirring rate and studies were maintained with this parameter.

2.3. Crosslinker Effect

In order to evaluate the effect of the crosslinking agent GA concentration to the particle size and also to enhance

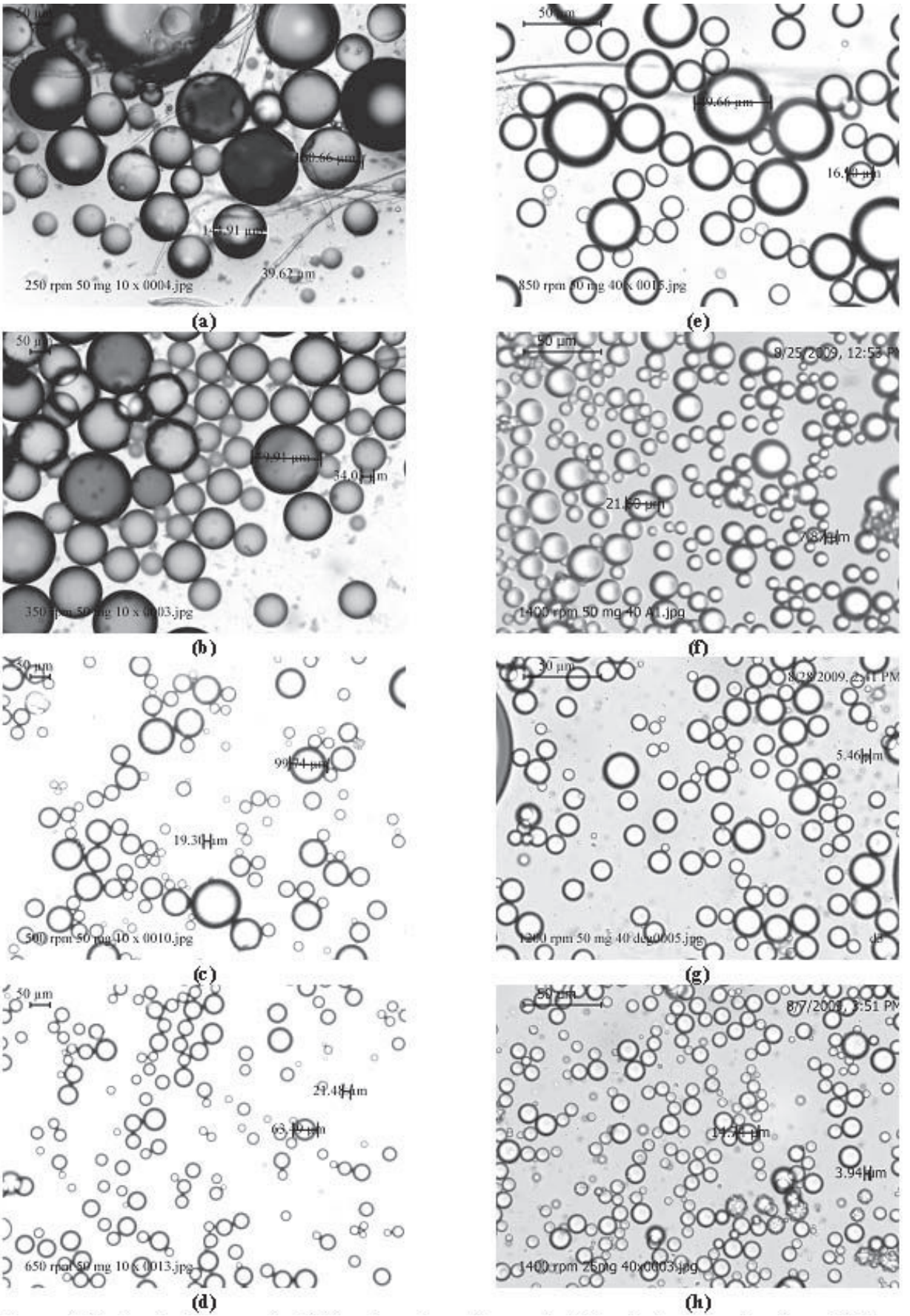

Figure 2. Optical micrographs of Albumin microspheres at different stirring rates (rpm) (10X and 40X Magn.)
a) 250 (10X), b) 350 (10X), c) 500 (10X), d) 650 (10X), e) 850 (10X), f) 1000 (40X), g) 1200 (40X), h) 1400 (40X).

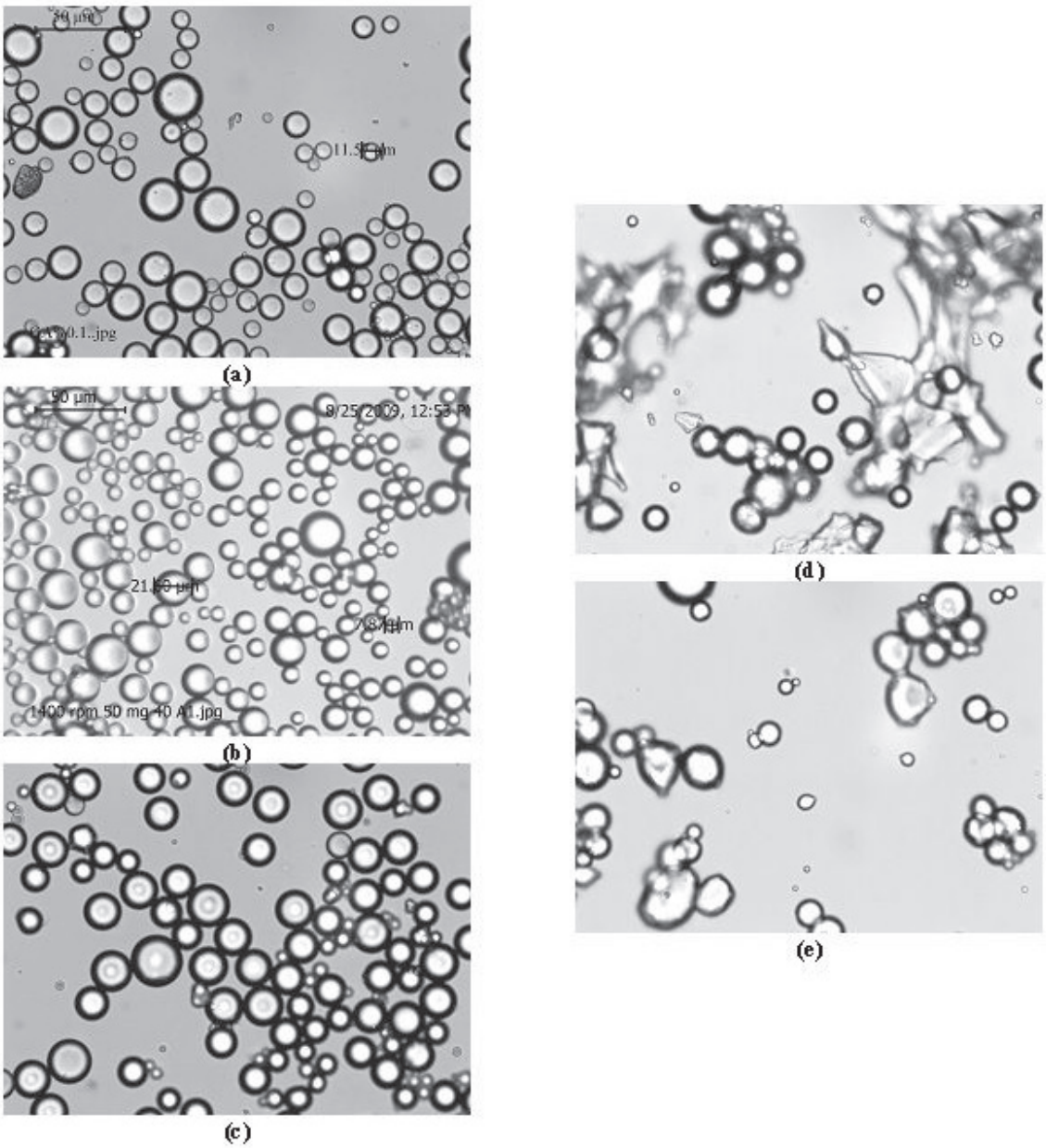

Figure 3. Optical micrographs of Albumin microspheres at different crosslinking amounts.
a) 0.1%, b) 0.2%, c) 1%, d) 2%, e) 3%

the mechanical properties of the microspheres GA was used in different concentrations. Albumin microspheres were prepared at 0.1 mg.ml^{-1} Albumin concentration, 1000 rpm stirring rate and GA concentrations were ranged srom 0.1 to 3%.

GA which has a wide spectrum of industrial, scientific and biomedical applications, was used as the crosslinking agent in this study. GA has two potential sites for crosslinking action. GA could create links between Albumin chains. However, some toxicity problems may be associated with its use. It is irritant to skin, eye and the respiratory tract [36]. Longterm exposure to 100 ppb GA vapor (5 days a week for 78 days) causes respiratory tract lesions including hyperplasia of squamous epithelium, necrosis and exfoliation of epithelial cells and granulocytes [37]. Therefore, it is important to use GA at minimum concentration to avoid health problems.

The increase in particle size as the stabilizing agent concentration decreases is documented and generally accepted in the literatüre [38,39]. The particle size is directly related to the viscosity of the dispersed phase and inversely proportional to the viscosity of the continuous phase and the concentration of stabilizing agent [40]. The GA concentration controls microsphere crosslink density and therefore swelling, drug entrapment, and release characteristics [41].

The crosslinking reaction with GA occurred at room temperature in aqueous media without requiring another chemical compound to activate the reaction. It was observed that Albumin microspheres exhibited wide

size distribution at low GA concentrations (0.1-0.2%). In the precence of higher GA concentrations above 1%, microspheres lost their smoothness. It was seen that the surface morphology of the microsphere becomes rougher. This finding is in agreement with the other research group [42]. Therefore, 1% GA concentration was selected to obtain more stabile and with narrow size distribution as optimum crosslinker amount.

2.4. Crosslinking Time Effect

It has been reported that the particle size and release characteristics of the microspheres could be controlled by changing the type and concentration of crosslinking agent as well as crosslinking time. Since, GA is responsible for the formation of crosslinks, increasing the amount of GA and the crosslinking time will increase the polymer density.

It was observed that the size of the microspheres increased with the increase in crosslinking time. The similar results has been reported by another researchers [33].

As it can be seen from Fig 4, size of the microspheres increased with the increase in crosslinking time from 30 min to 180 min The microspheres had mean diameters in between 7-30 µm which more than 75 % were above 25 µm at 60 min, 120 min and 180 min. At 30 min microsphere size ranged from 2-25µm.

2.5. Surface Morphology of Microspheres

Fig. 5. shows the morphological characteristics of Albumin microspheres. The SEM photomicrographs of the microspheres reveal that they are spherical, nonporous and uniform with a smooth surface. It was reported that microspheres obtained from natural polymers are not perfectly spherical because of the variations in molecular weight and other properties of the polymer [43]. It was observed that prepared microspheres are spherical with quite smooth surfaces. This result may be because of the low viscosity of olive oil, which was the external phase. The microspheres formed may not have experienced much resistance from the dispersion medium due to the low viscosity.

The surface morphology of the microspheres prepared by using 0.1 mg.ml^{-1} Albumin concentration, 1000 rpm stirring rate, 1%GA and 30 min crosslinking time, was smooth and having a particle size distrbution in the range 2-25 µm.

All these results indicate that the particle size of microspheres can be modified by varying the factors such as Albumin concentration, stirring rate, crosslinking amount and duration of stabilization.

3. In Vitro Release Profile

Ketoprofen release from the Albumin microspheres is depicted in Fig.6. The profile displayed a biphasic

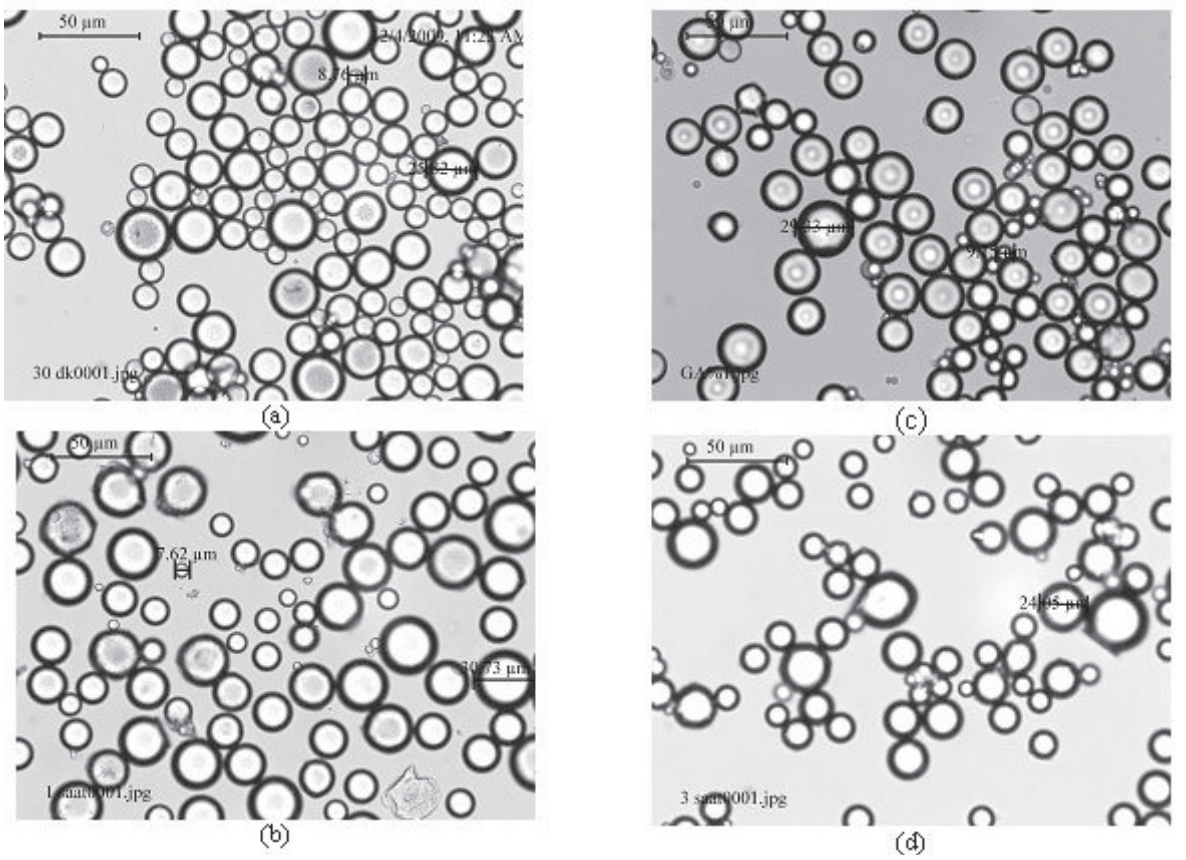

Figure 4. Optical micrographs of Albumin microspheres at different crosslinking times. a) 30 min; b) 60 min; c) 120 min; d) 180 min.

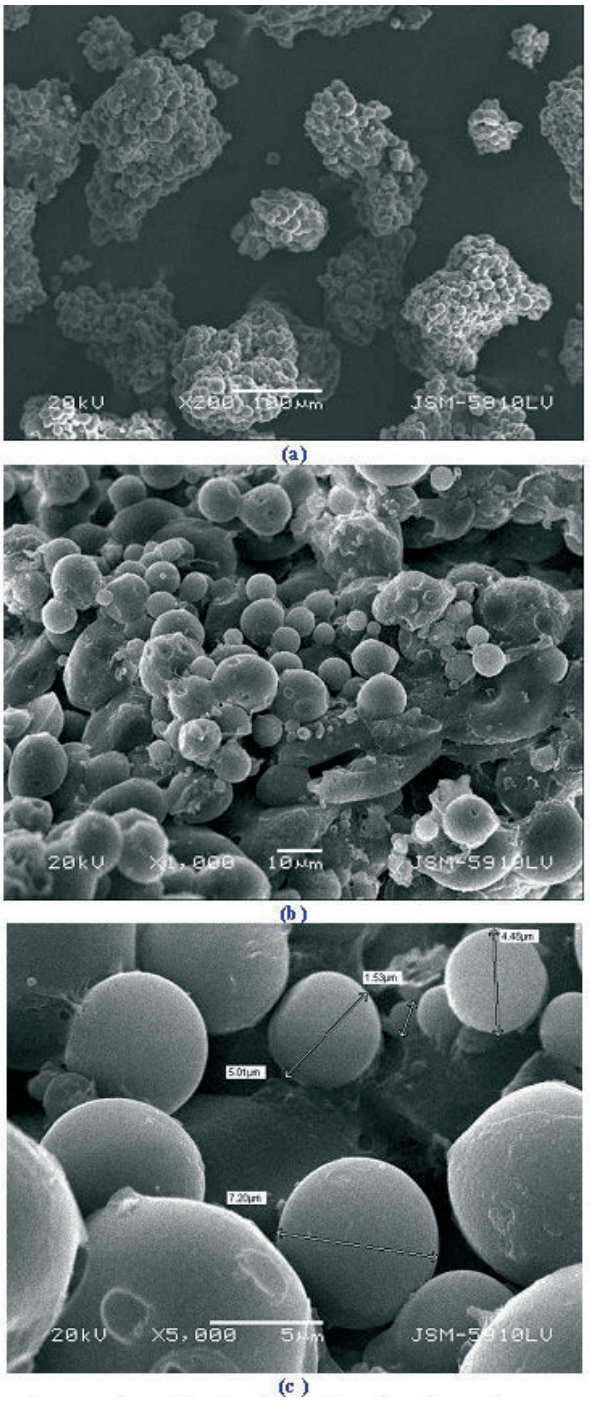

Figure 5. SEM micrographs of unloaded Albumin microspheres obtained at different magnifications. a) 200X; b) 1000X; c) 5000X.

release pattern characterized by an initial fast release (burst effect), followed by a slower release. The initial phase was completed within 60 min. The first burst effect is due to dissolution of ketoprofen incorporated near the surface of the particles. Besides, small size microspheres (those in the size range of 1-5 µm) which might be due to the fact that smaller particles offered more surface area to release the drug. The amount of drug released from microspheres after 180 min was 33%. The total content of ketoprofen encapsulated (100%) was released within 24 h.

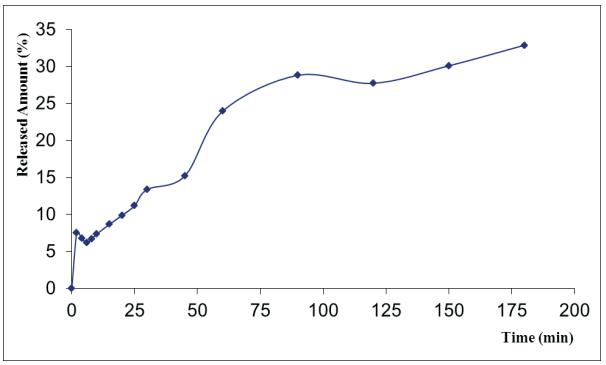

Figure 6. In vitro release profile of ketoprofen from Albumin microspheres.

Conclusion

Albumin microspheres could be prepared with various particle sizes. Their characteristics could be controlled by applying different parameters such as Albumin concentration, stirring rate, crosslinking amount and crosslinking time. The size of microspheres and their drug content determine the rate and pattern of drug release. Desired drug release rate could be obtained by varying the compression force and the amount of drug loading.

In conclusion, with the use of Albumin, it can be possible to design a biodegradable controlled drug delivery system. By variation of the preparation parameters, the release pattern might be modified in such away that a better mode of ketoprofen therapy can be achieved. Additionally, local gastrointestinal side effects are thought to be reduced by sustained release of ketoprofen and also *in vivo* experiments have to be studied.

Conflict of interest: Authors have no conflict of interest.

References

[1] Burgess DJ, Hickey AJ. (1995) Microsphere technology and applications. In: Swarbrick, J., Boylan, J.C. (eds.) Encyclopedia of pharmaceutical technology, Marcel Dekker, New York, pp: 1-29.

[2] Widder KJ, Senyei AE, Ranney DF. (1979) Magnetically responsive microspheres and other carriers for the biophysical targeting of antitumor agents. Adv Pharmacol Chemother 16: 213-271.

[3] Kreuter J. (1983) Evaluation of nanoparticles as drug-delivery systems I. Preparation methods. Pharm Acta Helv 58: 196-208.

[4] Gupta PK, Hung CT. (1989). Albumin microspheres II: Applications in drug delivery. J Microencapsul 6:463-472.

[5] Arshady R. (1990) Albumin microspheres and microcapsules: Methodology of manufacturing techniques. J Control Release 14:111-131.

[6] Bogdansky S. (1990) Natural polymers as drug delivery systems. In: Chasin M, Langer R, (eds.) Biodegradable Polymers as Drug Delivery Systems,New York, NY: Marcel Dekker Inc. 231-259.

[7] Kramer PA. (1974) Albumin microspheres as vehicles for achieving specificity in drug delivery. J Pharm Sci 63:1646-1647.

[8] Bernard NG, Shaw SM, Kessler WV, Landolt RR, Peck GE, Dockerty GH. (1980) Distribution and degradation of I-125 Albumin microspheres and technetium.99m sulphur colloid. J Pharm Sci., 15:30-34.

[9] Schäfer V, von Briesen H, Rübsamen-Waigmann H, Steffan AM, Royer C, Kreuter J. (1994) Phagocytosis and degradation of human serum Albumin microspheres and nanoparticles in human macrophages. J Microencapsul.11:261-269.

[10] Müller BG, Leuenberger H, Kissel T. (1996). Albumin nanospheres as carriers for passive drug targeting: an optimized manufacturing technique. Pharm Res 13:32-37.

[11] Tomilson E, Burger JJ, Schoolderwoerd EMA, McVie JG. (1984). Human serum Albumin microspheres for intra-arterial drug targeting of cytostatic compounds. In: Davis SS, Ilium JG, (eds.) Microspheres and Drug Therapy. Amsterdam, The Netherlands: Elsevier, 75-91.

[12] Tomilson E, Burger JJ. (1987) Monolithic Albumin particles as drug carriers. In: Ilium L, Davis SS (eds.) Polymers in Controlled Drug Delivery. Bristol, UK: IOP Publishing Ltd, 25-488.

[13] Wunder A, Muller-Ladner U, Stelzer EH. (2003) Albumin-based drug delivery as novel therapeutic approach for rheumatoid arthritis. J Immunol 170: 4793-4801.

[14] Breton J, Pezzi N, Molinari A. (1995) Prolonged half-life in the circulation of a chemical conjugate between a pro-urokinase derivative and human serum Albumin. Eur J Biochem 231: 563-569.

[15] Srinath P, Chary MG, Vyas SP, Diwan PV. (2000). Long-circulating liposomes of indomethacin in arthritic rats: a biodisposition study. Pharm Acta Helv 74: 399-404.

[16] Metselaar JM, Wauben MH, Wagenaar Hilbers JP, Boerman OC, Storm G. (2003) Complete remission of experimental arthritis by joint targeting of glucocorticoids with long circulating liposomes. Arthritis Rheum 48: 2059-2066.

[17] Corvo ML, Boerman OC, Oyen WJ. (1999) Intravenous administration of superoxide dismutase entrapped in long circulating liposomes. II. *In vivo* fate in a rat model of adjuvant arthritis. Biochim Biophys Acta 1419: 325-334.

[18] Chuo WH, Tsai TR, Hsu SH, Cham TM. (1996a) Preparation and in-vitro evaluation of nifedipin-loaded Albumin microspheres crosslinked by different glutaraldehyde concentrations. Int J Pharmaceut 144: 241-245.

[19] Chuo WH, Tsai TR, Hsu SH, Cham TM. (1996b) Development of nifedipin-loaded Albumin microspheres using a statistical factorial design. Int J Pharmaceut 134: 247-251.

[20] Luftensteiner CP, Schwedenwien I, Paul B, Eichler HG, Viernstein H. (1999) Evaluation of mitoxantrone-loaded Albumin microspheres following intra-peritoneal administration to rats. J Control Release 57: 35-44.

[21] Ghassabian S, Ehtezazi T, Foroutan SM, Mortazavi SA. (1996). Dexamethasone-loaded magnetic Albumin microspheres: Preparation and in vitro release. Int J Pharmaceut 130: 49-55.

[22] Karunakar S, Singh J. (1994) Preparation, characterization and in-vitro release kinetics of salbutamol sulfate loaded Albumin microspheres. Drug Dev Ind Pharm 20: 1377-1399.

[23] Chatterjee J, Haik Y, Chen CJ. (2001). Synthesis and characterization of heat-stabilized Albumin magnetic microspheres. Colloid Polym Sci, 279: 1073-1081.

[24] Jayakrishnan A, Jameela SR. (1996) Glutaraldehyde as a fixative in bioprostheses and drug delivery matrices. Biomaterials 17: 471-484.

[25] El-Gibaly I, Safwat SM, Ahmed MO. (1996) Microencapsulation of ketoprofen using w/o/w complex emulsion technique. J Microencapsul 13: 67-97.

[26] Bodmeier R, Chen H. (1989). Preparation and characterization of microspheres containing the anti-inflammatory agents indomathacin, ibuprofen and ketoprofen. J Control Release 10: 167-175.

[27] Goto S, Kawata M, Nakamura M, Maekawa K, Aoyama T. (1986a), Eudragit RS and RL (acrylic resins) microcapsules as pH insensitive and sustained release preparations of ketoprofen. J Microencapsul 3: 293- 304

[28] Goto S, Kawata M, Nakamura M, Maekawa K, Aoyama T. (1986b) Eudragit E, L and S (acrylic resins) microcapsules as pH sensitive release preparations of ketoprofen. J Microencapsul 3: 305-316.

[29] Sökmen N, Uysal S, Ayhan F, Özyazıcı M, Ayhan H. (2009), Photopolymerized Poly (ethylene Glycol) Diacrylate Hydogels for Controlled Release of Ketoprofen. Hacettepe J Biol and Chem 37 (4): 337-343.

[30] Lhiaubert V, Gutierrez F, Penaud-Berruyer F, Amouyal E, Daudey JP, Poteau R, Chouini-Lalanne N, Paillous N. (2000). Spectroscopic and theoretical studies of the states of fenofibric acid and ketorofen in relation with their photosensitizing properties. New J Chem 24: 403-410.

[31] Zgolak K, Jeszka A, Kabzinski T. (2007) The study of the interactions between ketoprofen and N-acetyl L-tyrosine ethyl ester. Food Chem and Biotechnol 71: 49-58.

[32] Gupta PK, Hung CT. (1989). Albumin microspheres I: Physicochemical characteristics. J Microencapsul 6: 427-462.

[33] Şahin S, Selek H, Ponchel G, Ercan MT, Sargon M, Hincal AA, Kas HS. (2002) Preparation, characterization and *in vivo* distribution of terbutaline sulfate loaded Albumin microspheres. J Control Release 82: 345–358.

[34] Thakkar H, Kumar R, Sharma, Mishra, A.K., Chuttani, K., Murthy, R.R. (2005).Albumin Microspheres as Carriers for the Antiarthritic Drug Celecoxib. AAPS PharmSciTech 6 (1): E65–E73.

[35] Morimoto K, Katsumata H, Yabuta T. (2001). Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. Eur J Pharm Sci 13:179-185.

[36] Ballantyne B, Jordan SLP. (2001) Toxicological, medical and industrial hygiene aspects of glutaraldehyde with particular reference to its biological use in cold sterilization procedures. J Appl Toxicol 21: 131–151.

[37] Zisshu D, Bonnet P, Binet S. (1998). Histopathological study in B6C3F mice chronically exposed by inhalation to glutaraldehyde. Toxicol Lett 95: 131–139.

[38] Luftensteiner CP, Viernstein H. (1998) Statistical experimental design based studies on placebo and mitoxantrone-loaded Albumin microspheres. Int J Pharmaceut 171:87-99.

[39] El-Mahdy M, Ibrahim ES, Safwat S, El-Sayed A, Ohshima H, Makino K, Muramatsu N, Kondo T. (1998). Effects of preparation conditions on the monodispersity of Albumin microspheres. J Microencapsul 15: 661-673.

[40] Arshady R. (1990). Albumin microspheres and microcapsules: Methodology of manufacturing techniques. J Control Release 14:111-131.

[41] Hadba, A.R., (2001). Synthesis, Properties and *in vivo* evaluation of sustained release Albumin-mitoxantrone microsphere formulations for nonsystemic treatment of breast cancer and other high mortality cancers, Ph.D Thesis, University of Florida,USA.

[42] Eroglu, H., Kas, H.S., Oner, L., Turkoglu, O.F., Akalan, N., Sargon, M.F., Ozer, N., Hıncal, A.A. (2000). *In vitro / in vivo* characterisation of bovine serum Albumin microspheres containing dexamethasone sodium phosphate, S.T.P. Pharma Sci. 10, 303–308.

[43] Mathew,S.T., Devi, S.G., Sandhya, K.V., (2007) Formulation and Evaluation of Ketorolac Tromethamine-loaded Albumin Microspheres for Potential Intramuscular Administration, AAPS PharmSciTech., 8 (1): Article 14.

[44] Tabassi, S.A.S, Razavi, N. (2003). Preparation and characterization of albümin microspheres encapsulated with propanolol HCl, DARU, J Pharma Sci, 11 (4): 137-141.