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Preparation of Bovine Serum Albumin Microspheres Containing Dexamethasone Sodium Phosphate and the in Vitro Evaluation

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Abstract: Dexamethasone sodium phosphate (DSP) is the most common corticosteroid used in the treatment of edema paired with brain tumors (1, 2). As with other corticosteroids, DSP has some adverse effects on the cardiovascular, immune and nervous systems. The objective of this study was to minimize the adverse effects of DSP and to extend the release time of the drug from microspheres by encapsulating with Bovine Serum Albumin (BSA). The microspheres were prepared by emulsion polymerization. An aqueous solution of

glutaraldehyde (25% w/v) was used as the crosslinking agent in two different amounts. The release time DSP was found to be extended in the series containing 15% DSP with the increase in the amount of glutaraldehyde used. Also it was observed that the release time is extended in series prepared using 0.5 mL glutaraldehyde with the amount of DSP.

Key Words: Dexamethasone Sodium Phosphate, BSA Microspheres, In vitro Evaluation.

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Introduction

Microparticulate carriers provide the localization of the drug at the site of action, lower the concentration of the drug and as a result reduce the side effects and increase patient compliance. Microspheres, that are monolytic microcarriers, can easily achieve the targetting of the drug to the site of action and the localization of the drug (1).

The blood-brain barrier (BBB), provides a control system that regulates the transport of the materials which is also affected by physical, humoral and neural changes. The BBB is present in all vertebrate brains and is laid down within the first trimester of human fetal life (2). Despite the endothelium in the peripheral tissues, in the cerebrovascular endothelium there is no penetration that allows the transport of the solute and the solvent but tight junctions exist. These tight junctions are as tight as any in biology and the electrical resistance across intraparenchymal endothelial cells may be as high as 8000 ohm-cm² (3); in this way the BBB has an important role in providing a stable extracellular environment in the central nervous system (4).

The degree of transporting of the drugs through the BBB depends on the solubility of the drug in lipids. The

other factors are the molecular weight, the charge of the drug and the capability of binding to proteins (5).

Dexamethasone sodium phosphate (DSP) is a white or slightly yellow crystalline powder. 1.3 mg of DSP is nearly equivalent to 1 mg of Dexamethasone. (DSP) is a corticosteroid which is widely used in the treatment of edema associated with brain tumors (6, 7). For the treatment of these cases, biodegradable PLGA implants are prepared and in vitro dissolution profiles are investigated. The release time of these implants were found to be 193 days. As with the other corticosteroids, DSP has some adverse effects on the immune, nervous and cardiovascular systems. In the treatment of edema generally an initial high dose of DSP is applied followed by lower doses (8).

The two most important factors in choosing the most suitable polymer are the biodegradability and the toxicity of the polymer. The polymer BSA (Bovine Serum Albumin) is biodegradable and non-toxic and it was chosen for the preparation of the microspheres.

The aim of this study was to reduce the side effects of DSP and to extend the release time using natural and synthetic polymers. In order to extend the release time of DSP, two different crosslinking times (30 min/60 min)

and two different quantities of glutaraldehyde (0.5 mL/0.75 mL) were used. The amounts of DSP were chosen as 5% and 15%.

Materials and Methods

Materials

DSP was purchased from Deva Holding A.Ş.; and the polymer was from the Armour Pharmaceutical Company. As the crosslinking agent, 25% aqueous solution of glutaraldehyde (Merck) was used.

Methods

Preparation

Microspheres containing DSP were prepared by emulsion polymerization (9, 10). 0.125 g of BSA was dissolved in distilled water and mixed with 100 mL of cotton seed oil. The homogenous mixture was added to 100 mL cotton seed oil continuously stirred at 1400 rpm and 25°C. The resulting microspheres were washed with diethyl ether (anhydrous) to remove the oil phase and the microspheres were stabilized using 0.5 mL and 0.75 mL glutaraldehyde solution (25% w/v) for 30 min. and 60 min. Afterwards the microspheres were dried in a vacuum oven overnight and stored at + 4°C.

Determination of Encapsulation Efficiency

10 mL of pH 7.4 phosphate buffer was added to accurately weighed 10 mg of DSP microspheres and this mixture was kept in an ultrasonic bath. After centrifugation, the supernatant was filtered through a 0.45 µm filter and absorbance was read at $\lambda=242$ nm. After drying, the residue was mixed with 5 mL 0.1 N glacial acetic acid and stored at + 4°C for one night. Later, it was centrifuged at 5000 rpm and the absorbance of the supernatant was read at $\lambda=242$ nm after filtration through a 0.45 µm filter.

Surface Morphology

SEM examination of the microspheres was carried out to study the surface morphology. Microspheres were mounted on metal stubs with conductive silver paint and then sputtered with a 150 Å thick layer of gold in a BIORAD apparatus. A scanning electron microscope (Jeol-SEM ASID-10 Device in 80 KV) was used to evaluate the surface characteristics of the microspheres.

In vitro Release

The in vitro release profiles of BSA microspheres containing DSP were investigated in pH 7.4 buffer

solution. Weighed amounts of microspheres were suspended in 25 mL of buffer solution at 35 ± 0.5 °C in horizontally shaken flasks. 1 mL samples were withdrawn and the appropriate volume fresh medium was added at regular time intervals. The amount of DSP released was calculated from the UV absorption measurements of samples at $\lambda=242$ nm.

Particle Size

The particle size distribution of BSA microspheres containing DSP were obtained with a Malvern Mastersizer (Malvern Inst., UK). Size analysis was performed after suspending the microspheres in an isotone solution containing 0.01% (w/v) Tween 80.

Results and Discussion

As seen in Figure 1 the surface of the BSA microspheres were found to be very smooth and the microspheres were spherical, but just after the release of the DSP was completed the smooth surface and the spherical outline were lost.

The particle size distribution, synthesis efficiency and encapsulation efficiency of the microspheres are given in Table 1.

The microspheres crosslinked using 0.5 mL glutaraldehyde showed an extension in the release time of DSP directly proportional with the increase in the amount of DSP. On the other hand, microspheres crosslinked using 0.75 mL glutaraldehyde showed an opposing profile and the release time of DSP decreased while DSP amount increased. When the series containing the same amount of DSP were compared, the release time of DSP was increased with the increasing amount of glutaraldehyde.

In the series containing 5% and 15% DSP, an extension in the release time of DSP was not observed for any of the eight series as the crosslinking time is increased, but in the series containing the same amount of DSP and with the same amount of crosslinking time, the release time is increased as the amount of glutaraldehyde is increased.

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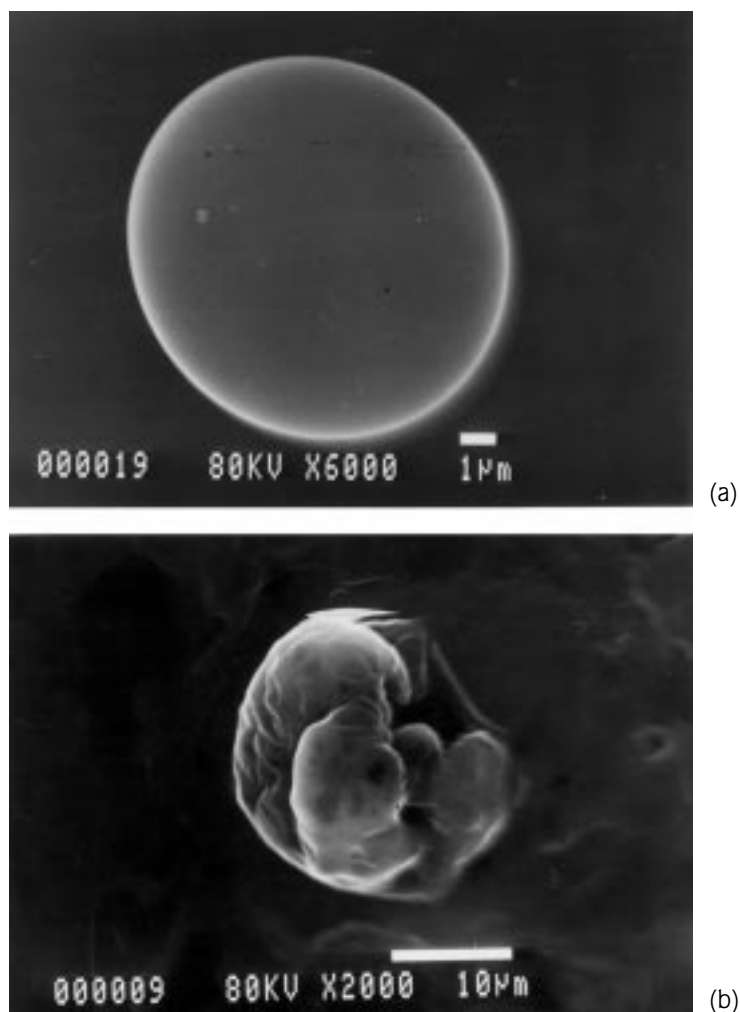
Figure 1. (a) Before Release
(b) After Release

Table 1. The particle size distribution, synthesis efficiency and encapsulation efficiency of the microspheres are given.

Series	Synthesis Efficiency (%)	Particle size (μm)	Encapsulation Efficiency (%)
A (5%DSP; 0.5mL Glutaraldehyde; 30 min)	74.74	32.70	38.4
B (5%DSP; 0.5mL Glutaraldehyde; 60 min)	69.25	23.41	44.24
C (15%DSP; 0.5mL Glutaraldehyde; 30 min)	78.33	26.94	49.6
D (15%DSP; 0.5mL Glutaraldehyde; 60 min)	76.52	20.02	53.67
E (5%DSP; 0.75mL Glutaraldehyde; 30 min)	55.47	22.24	70.91
F (5%DSP; 0.75mL Glutaraldehyde; 60 min)	88.76	15.44	71.38
G (15%DSP; 0.75mL Glutaraldehyde; 30 min)	69.7	21.28	65.36
H (15%DSP; 0.75mL Glutaraldehyde; 60 min)	81.18	29.79	51.87

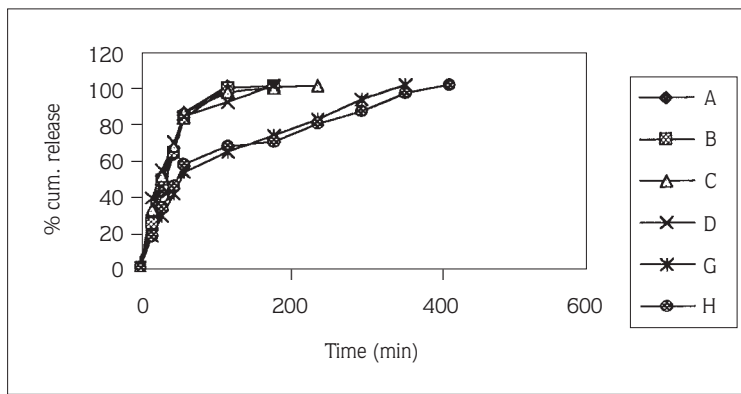
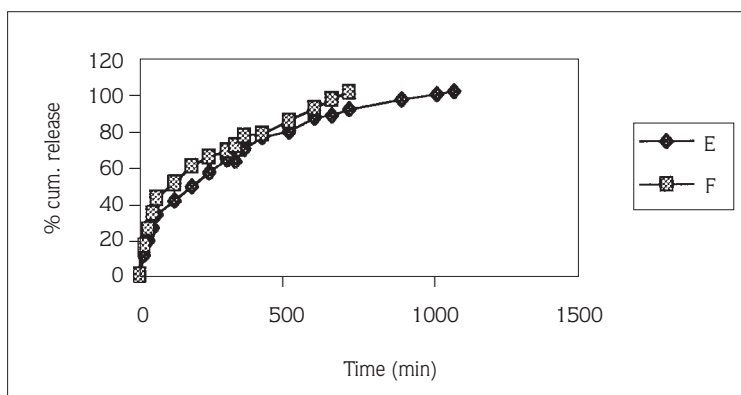


Figure 2. The in vitro release profiles of BSA microspheres containing DSP.



References

- Davis S.S., Illum L., McVie J.G., Tomlinson E., *Microspheres and Drug Therapy*, Amsterdam, 1984
- Mollgard K. and Saunders N. R., Complex tight junctions of epithelial and of endothelial cells in early foetal brain, *J. Neurocytol.*, 4:453-468, 1975.
- Butt A.M., Jones H.C., Abbott N.J., Electrical resistance across the blood-brain barrier in anaesthetized rats: A developmental study, *J. Physiol.*, 429:47-62, 1990.
- Brightman M.W., Morphology of blood-brain interfaces, *Exp. Eye Res.*, 25 (suppl):1-25, 1977.
- Pardridge W.M., Brain drug delivery and blood- brain transport, *Drug Delivery* 3: 99-115, 1996.
- Fallon P.A., Whateley, T. L., The development and evaluation of poly(lactic-glycolic acid) implants for sustained delivery of dexamethasone to the brain, *Proc. Int. Symp. Control. Release Bioact. Mater.*, 264-5, 1994.
- Crossan I.M., Whateley, T. L., Microspheres of dexamethasone as a biodegradable controlled release drug delivery system, *Proc. Int. Symp. Control. Release Bioact. Mater.*, 184-5, 1994.
- Martin Dale, *The Extra Pharmacopoeia*, 29th Edition, The Pharmaceutical Press, pp887-887, James E.F. Reynolds (Ed), London, 1989.
- Gürkan H., Kaş H.S., Hıncal A.A., Ercan M.T., Streptomycin sulphate microspheres formulation and in vitro distribution, *J. Microencapsulation*, 3, (2), 101-108, 1996.
- Vural I., Kaş H.S., Cave G., Cyclophosphamide loaded albumin microspheres. II. Release characteristics., *J. Microencapsulation*, 7: 511-516, 1990.