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# PREPARATION AND CHARACTERIZATION OF ALBUMIN MICROSPHERES ENCAPSULATED WITH PROPRANOLOL HCl

SAYYED ABOLGHASSEM SAJADI TABASSI, NAHEED RAZAVI

School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

**ABSTRACT**

Albumin microspheres (AMS) have found many applications in the diagnosis and treatment in recent years and more than 100 diagnostic agents and drugs have been incorporated into AMS. In the present study Bovine Serum Albumin (BSA) based microspheres bearing propranolol hydrochloride were prepared by an emulsion-internal phase stabilization technique. The prepared microspheres were studied for particle size distribution, drug loading, release characteristics, bioadhesion and in-vitro controlled diffusion across the rat intestine. The microspheres had mean diameters between 1-25 μm of which more than 50 percent were below 5 μm. The encapsulated drug was found to be about 9% w/w of that initially added to microspheres and the superficial drug was 25% of the total amount of the encapsulated drug. Also AMS were noted to possess good bioadhesion in such a way that about 70% of microspheres remained adherent on the surface mucosa of rat jejunum.The drug release from albumin microspheres was mainly controlled by diffusion and showed a biphasic pattern with a high initial release (burst effect), followed by a more gradual terminal release. The total amount of drug released from microspheres after 12h was 70%. In vitro experiments on the rat intestinal segments revealed that the microspheres could effectively pass their content through intestinal membrane.

**Key words:** Albumin microspheres, Propranolol, Bioadhesion, Release, Size distribution

## INTRODUCTION

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm (1). They are made from 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. Preparation of uniformly sized AMS was first reported in the late 60's and early 70's (2, 3, 4). AMS have received wide attention during the recent decades due to their specificity (5), biodegradability (6) and other desirable characteristics such as non-toxicity and biocompatibility (7) as an ideal drug carrier. More than 100 therapeutic and diagnostic agents have been incorporated into albumin microspheres and drugs of various therapeutic categories such as nifedipine (8, 9), mitoxantrone (10), dexamethasone (11), salbutamol sulfate (12) have been prepared and characterized as AMS delivery systems. Two methods have been developed for the preparation of albumin microspheres which include heat stabilization and chemical cross-linking by using glutaraldehyde (13).

Propranolol is a beta adrenergic blocking agent which is widely used in the treatment of angina pectoris, high blood pressure and migraine prophylaxis. The need for controlled systemic delivery of propranolol by some convenient delivery system is well established and a microsphere prepared from Human Serum Albumin (HSA) was one of those systems which were studied for its nasal delivery (14). Due to variable absorption rates and high first pass metabolism of the oral route, several other researchers have also attempted to deliver propranolol via nasal route (15, 16).

The aim of the present study was to prepare and characterize Bovine Serum Albumin (BSA) microspheres loaded with propranolol HCl. BSA microspheres were prepared by an emulsification technique and stabilized by heat denaturation. The prepared microspheres were then studied for their particle size, size distribution, release characteristics, bioadhesion and drug diffusion profile across the rat intestine.

## MATERIALS AND METHODS

*Chemicals*

Bovine Serum Albumin and sorbitan mono-

*Correspondence:* author: S. A. Sajadi Tabassi, Dept. Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, P O Box 91775-1365 Mashhad, Iran. Fax: +98511 8623251- Email: s-sajjadi@mums.ac.ir

oleate (Span® 80) were from Fluka (Switzerland), Propranolol HCl was obtained from Tolidarou (Iran), Polyoxyethylene sorbitan mono-oleate (Tween® 80), glacial acetic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, disodium hydrogen phosphate and sodium chloride were from Merck (Germany). Pure maize oil was supplied by Emirates Refining Company (Dubai) and petroleum ether by Jahad Chimi (Iran). All chemicals were of analytical grade.

*Preparation of BSA microspheres*

BSA microspheres containing propranolol HCl were prepared by emulsification-heat stabilization technique described previously (12) with slight modifications. Briefly, a 5% solution of BSA containing 0.1% Tween® 80 was made to which 4% propranolol HCl was added and used as the aqueous phase. The oil phase composed of 30 ml maize oil and 10 ml petroleum ether with 1% Span® 80 as emulsifier were mixed together and allowed to stir for 10 min at 1000 rpm. The aqueous phase was added dropwise to the oil phase and stirred on a magnet stirrer at 1000 rpm for 30 min to form the initial emulsion. This emulsion was then added to 40 ml of maize oil preheated to 120° C and stirred at 1000 rpm for 15 min to allow the formation and solidification of microspheres. The microsphere suspension was centrifuged at 3500 rpm for 30 min and the settled microspheres were washed three times with ether to remove traces of oil on microsphere surfaces. The microspheres were vacuum dried in a desiccator overnight and stored at 4ºC in dark. Three batches of microspheres were prepared by the above mentioned method and marked as MC1, MC2 and MC3.

*Analytical Method*

To determine the loading capacity, drug release and diffusion, the analysis of propranolol was carried out by UV-spectroscopy (Shimadzu uv/vis 160A, Japan). The absorption spectra of propranolol were obtained in distilled water, phosphate buffer saline (PBS, pH 6.8) and 0.1 M acetic acid and its absorption maxima ($\lambda_{max}$) were found at wavelengths of 215, 216 and 230 nm in the above three media respectively.

*Determination of the drug content*

Ten mg of the dispersion of microspheres in distilled water was centrifuged at 5000 rpm for 15 min. In order to determine surface drug, the supernatant was assayed for propranolol content by a spectrophotometric method at 216 nm. The settled microspheres were completely hydrolyzed in 0.1 M acetic acid and assayed for determination of bulk drug content as mentioned above.

*Determination of mucoadhesion*

BSA microspheres were tested for mucoadhesion using the method described by Ranga Rao and Buri (17). Five cm length rat jejunum was cut longitudinally and placed on a polyethylene support (a tube with 2 cm diameter cut longitudinally at its center) and held in position with the aid of pins. A suspension containing 5 mg of BSA microspheres was poured dropwise at the mucosal site of the rat jejunum and placed in a desiccator maintained at 80% relative humidity for 20 min to allow the microspheres to hydrate and to interact with the mucus glycoprotein and also to prevent drying of mucus. After 20 min, the polyethylene support was introduced into a plastic tube cut in a similar manner and held in inclined position. The mucosal lumen was thoroughly rinsed with PBS pH 6.8 at the rate of 5ml/min with the aid of a burette tube the tip of which was placed 2-3 cm over the microsphere bearing tissue so that the liquid was able to flow evenly over the mucosa. The washings were collected into a beaker and the microspheres in the perfusate were settled by centrifugation, dried at 50°C and weighed. The ratio of adhered to applied microspheres was computed as percent adhesion.

*Determination of size distribution of microspheres*

The microspheres were sized and photographed in normal saline containing 0.1% Tween 80 to prevent aggregation under a light microscope (Olympus C 011, Japan) equipped with an ocular micrometer and a light camera (Seagul DF-1, China). Two hundred microspheres were sized by the above mentioned method and the mean diameter as well as size distribution of microspheres were determined.

*Release studies*

In vitro release of propranolol from BSA microspheres was determined in a USP dissolution apparatus (Pharma Test PTWS, D-63512, Germany) using paddle method with six flasks in PBS pH 6.8 at 37±0.5ºC. The contents of the flasks were stirred at 100 rpm. Samples were taken at 15 min intervals for the first two hours and every one hour afterwards up to 12 hours and assayed spectrophotometrically at 216 nm wavelength as mentioned above.

*In vitro diffusion studies*

Diffusion of propranolol HCl from BSA microspheres across the rat duodenum was

studied using a Franz diffusion cell (Scientific and Industrial Research Organization, Iran). Rat duodenum was removed and the mucosal side was washed with PBS pH 6.8 in order to remove any debris and cut into equal segments with about 1 $cm^2$ surface area. Ten mg of BSA-propranolol microspheres was placed on the mucosal side of an intestinal segment and kept for 30 min to allow complete mucoadhesion. An intestinal segment bearing BSA-propranolol microspheres was placed between the donor and receptor compartments of the diffusion cell which contained 5 and 26 ml PBS pH 6.8 respectively and maintained at 37ºC. Samples were withdrawn at 1 hour intervals for 12 hours and their drug content was determined by the spectrophotometer at 216 nm wavelength as mentioned before.

## RESULTS AND DISCUSSION

In the present study BSA microspheres encapsulated with propranolol HCl were prepared by an emulsification technique. Two hundred microspheres of each batch were sized by a light microscope equipped with an optical micrometer and the average percentage frequency was plotted against size ranges (fig 1).

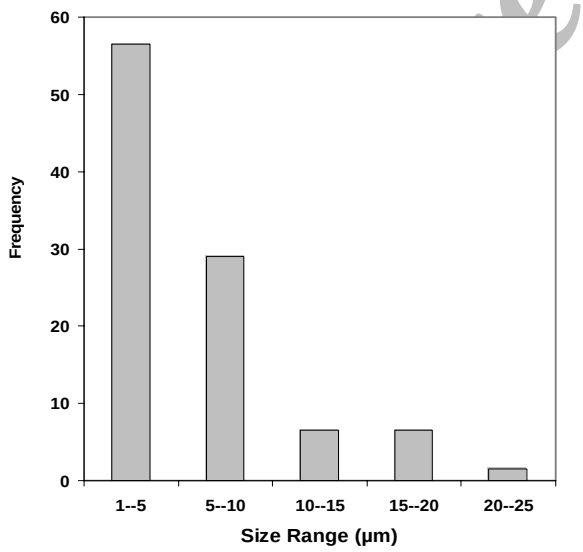


**Figure 1** Size range vs. average frequency of three batches of BSA-propranolol microspheres prepared by heat denaturation method. Two hundred microspheres of each batch were sized in a light microscope equipped with an optical micrometer and plotted as the mean percentage of total microspheres present in each size range

The mean size range of the three batches of microspheres was estimated between 1-25 µm with nearly 60% lying between 1-5 µm. It has earlier been reported that BSA-salbutamol microspheres prepared with various albumin concentrations for pulmonary delivery showed almost the same size range for microspheres prepared with 5% albumin (12).

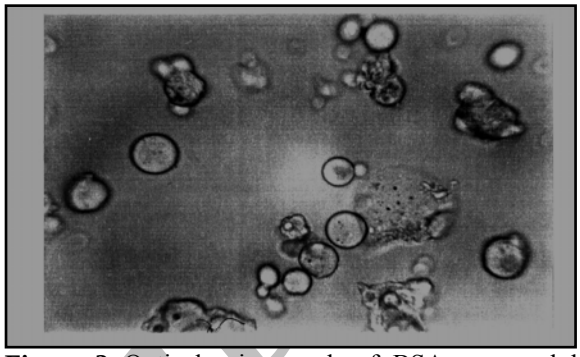

**Figure 2** Optical micrograph of BSA-propranolol microspheres stabilized by heat denaturation method with an average size range of 1-25 µm, taken by a camera mounted light microscope (x1600). As it indicates microspheres have spherical shape

The microspheres were also photographed by an optical camera (Fig 2) and as it shows microspheres are spherical with quite smooth surfaces. It has previously been suggested that heat stabilized albumin microspheres exhibit better size distribution and surface characteristics (13).

Drug loading was found to be between 7.5-10.4% for three batches with an average value of about 9%. It was also found that about 25% of the total encapsulated drug was present on the surface of microspheres. In a study on mitoxantrone-albumin microspheres prepared by chemical cross-linking with glutaraldehyde, 8.2% drug loading was achieved (10). Another group of researchers reported that various factors such as drug to albumin ratio, concentration of surfactant, stirring rate of the emulsion and average size of microspheres could affect drug loading (8). They found that drug loading could be increased by increasing drug to albumin ratio, decreasing surfactant concentration and increasing the stirring rate. In another study albumin microspheres loaded with terbutalin sulfate were prepared by a chemical cross linking method using glutaraldehyde and achieved 60% drug loading (18). Since the size range reported by this group was between 21-48 µm, it can be said that drug loading could be increased by increasing microsphere size. Stabilization method of microspheres (chemical vs. heat) could also be considered as another

contributing factor in drug loading. In the preparation of salbutamol loaded albumin microspheres it was found that drug loading decreased by increasing both the time and temperature of denaturation condition (12). In the present study it was noted that drug loading considerably increased when the percentage of the incorporated drug was increased from 2 (data not shown) to 4. However drug loading was still low which might be attributed to the high temperatures used for microsphere stabilization and also to the high partition coefficient of the model drug which could lead to the loss of the drug due to its migration to the organic phase. Mucoadhesion of BSA-propranolol micro-spheres was estimated to be on average 75% which is in accordance with the previous report (14). The high degree of mucoadhesion makes this system a good candidate for nasal delivery of the entrapped drug as it has previously been reported (16).

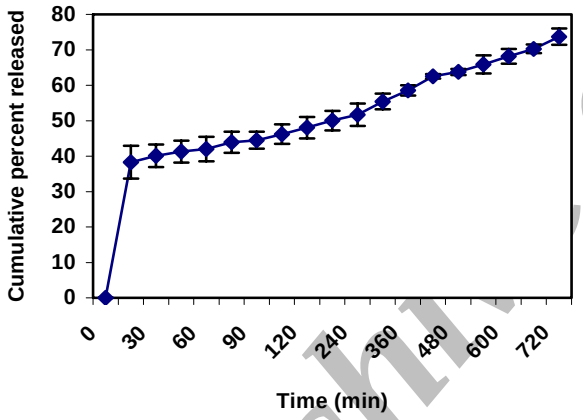


**Figure 3** Plot of average percentage propranolol HCl released from BSA microspheres in PBS pH 6.8 at 37°C as a function of time, each data point represents a value of mean±SD from three batches of microspheres

Release studies were carried out on three batches of microspheres and showed a biphasic pattern in a way that about 40% of the drug released during the first 15 min followed by a more gradual release pattern reaching 70% after 12 hr. Figure 3 shows release profile of three batches as mean±sd values. The initial burst release could be related to the surfacial drug as well as 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. A similar release pattern has been reported elsewhere in which about 50% of the loaded drug released within 1 h which was attributed to the amount of drug adsorbed on the surface of microspheres (18).

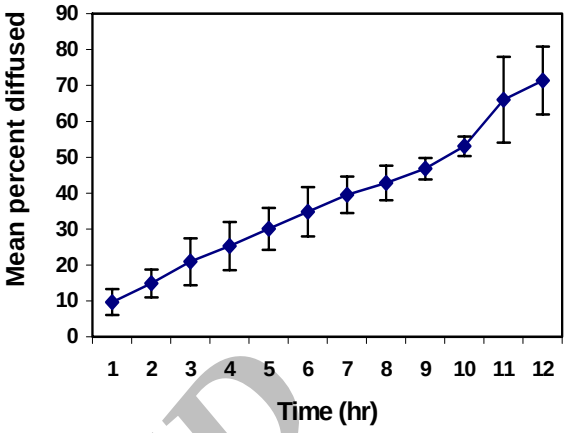


**Figure 4** Plot of percentage propranolol HCl loaded in BSA microspheres diffused across rat duodenum at 37°C as a function of time, each data point represents a value of mean±SD from three batches of BSA-propranolol microspheres

In another study a sustained release parenteral form of mitoxantrone loaded albumin microspheres was prepared for intraperitoneal administration to rats and found that the system released 75% of its drug contents in 10 hrs (10). Another group reported 30% initial release during the first 5 min from BSA-salbutamol microspheres followed by a very gradual release pattern which finally reached 100% after 24 hr (12). They also found that drug release from microspheres was remarkably slower than that of the plain drug. These authors suggested that various factors such as particle size, amount of loaded drug and denaturation conditions might affect release characteristics of microspheres, and it is possible to modulate the drug release rates by variation in the size as well as preparation conditions of the microspheres.

In vitro diffusion studies across rat intestine (duodenum) were also carried out on three batches of microspheres and reported as average percentages of the diffused drug (Fig 4). This experiment revealed that drug diffusion took place in a gradual pattern such that after 1 hour only about 10% of the drug diffused and drug diffusion reached 80% after 12 hours. The gradual diffusion may be attributed to the mucoadhesive properties of BSA microspheres which provide sustained and continuous release and diffusion of the drug from microspheric matrices. This makes the microsphere system of propranolol HCl as a sustained release (SR)

delivery system which have advantages of reducing the number of dosing, providing better patient compliance, reducing drug loss due to the first pass metabolism, and finally increasing drug bioavailability due to longer contact time of the delivery system with the absorption site.

## CONCLUSION

Both release and diffusion patterns show that a sustained release form of the drug can be obtained by utilizing microsphere system of propranolol. Such a system has several advantages for drug delivery. In the case of propranolol which possesses low and variable bioavailability with a high percentage of first pass metabolism via the oral route, this system can provide a more steady plasma level of the drug. Having a high degree of mucoadhesion, this system can be used for the delivery of propranolol via nasal route which could eventually eliminate the first pass effect and thus improving drug's bioavailability.

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