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Microspheres of Human Serum Albumin with Barbiturates: Effect of Drug Partition Coefficient on Preparation and Drug Release

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Abstract—Human serum albumin microspheres were prepared with a series of barbiturates by the thermal denaturation method. The barbiturates were of similar general physicochemical properties but different partition coefficients. The total drug content of microspheres was dependent on the partition coefficient, and an increase in the partition coefficient caused a decrease in the drug content of finished microspheres due to drug migration to the outer organic phase during preparation. The ensemble drug release from microspheres was followed by the paddle method and by potentiometric titration with a pH-stat. Nonlinear regression analysis showed the best fit for the spherical Higuchi equation, especially first-order kinetics (bi-phasic). The drug partition coefficient affected the release in such a way that drugs with higher partition coefficients were released faster and to a greater extent than those with lower coefficients.

Biodegradable and biocompatible microcapsules and microspheres have been suggested as possible drug carriers for drug targeting and controlled release. Human serum albumin (HSA) microspheres have been investigated in-vitro and some preclinical results have been obtained (Deasy 1984). The studies of in-vitro drug release from albumin microspheres were performed to characterize the systems and to evaluate parameters important for the controlled release. Several mechanisms have been proposed to describe the release of various drugs from HSA microspheres. Some workers have found the release kinetics to be of the Higuchi type (Lee et al 1981; Ishizaka & Koishi 1983; Willmott et al 1985), while others have found first-order kinetics (Morimoto et al 1981) to be applicable. A bi-phasic (either zero- or first-order) release has often been found (Yapel 1985; Morimoto & Fujimoto 1985; Burger et al 1985; Willmott et al 1985; Gupta et al 1986a,b, 1988), and even a tri-phasic profile has been described (Willmott & Harrison 1988). More recently a new concept for interpretation of first-order release kinetics from albumin microspheres has been suggested (Okada et al 1991).

A variety of parameters connected with chemical and physicochemical properties of entrapped drugs, type of polymer used, microsphere characteristics and recipient media make it difficult to compare results obtained with different microsphere systems (Jalšenjak 1992). Among the various parameters influencing the permeation from microspheres which have been investigated, the influence of the partition coefficient has been little studied. The chemical nature of the drug molecule, as expressed by the partition coefficient, affects the drug release profile because it defines the tendency of drug distribution between the two major phases present. It is also partly responsible for governing membrane-controlled rather than matrix-controlled permeation (Roseman & Yalkowsky 1976).

Previously we have reported studies on drugs non-related structurally (Vidmar & Jalšenjak 1983). In this study, a series of barbiturates was chosen because they can be considered as structural analogues having similar general properties but different partition coefficients.

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Materials and Methods

Materials

Commercially available barbiturates (barbituric acid, phenobarbitone, pentobarbitone, secobarbitone) and their sodium salts (TCI, Tokyo) were of pharmacopoeial purity. The partition coefficients between chloroform and water for barbituric acid, phenobarbitone, pentobarbitone, and secobarbitone are 0·002, 0·05, 0·29 and 0·51, respectively. Lyophilized human serum albumin (Immunological Institute, Zagreb, Croatia) was used as received. Pepsin was of pharmaceutical purity and all other substances were of reagent grade purity. Pepsin solution was prepared by a modified procedure according to the British Pharmacopoeia 1973. Fifty milligrams of the substance was dissolved in 100 mL of 0·065 M HCl.

Microsphere preparation

Microspheres were prepared by thermal denaturation at elevated temperature by a modification of the methods of Yapel (1985), Maysinger et al (1989), and Morimoto & Fujimoto (1985). Human serum albumin (HSA) (1 g) was dissolved in 4 mL water and drug (300 mg) was added. The mixture was stirred in 200 mL sunflower oil and the water/oil emulsion formed was then added to another portion of sunflower oil (150 mL) and placed in a round-bottomed, three-necked bottle immersed in an oil bath. The mixture was warmed to 90 or 130°C for 45 min and cooled gradually to room temperature (21°C). The microspheres thus obtained were separated by vacuum filtration and washed with several portions of diethylether.

Release studies

An apparatus similar to the United States Pharmacopeia XX paddle method and potentiometric titration were used.

Table 1. Characteristics of HSA microspheres of barbiturates.

Sample	Drug	Denaturation temperature (°C)	M_o[a] (%)	d_g'[b] (μm)	σg[c]	d_{sv}[d] (μm)	d_{wm}[e] (μm)	M_m/M_o[f] (%)
A1	Barbituric acid	130	15·5	27·3	0·53	20·5	29·6	3·3
A2	Phenobarbitone	130	4·6	30·3	0·55	24·3	32·2	15·5
A3	Pentobarbitone	130	3·3	53·5	0·45	31·0	47·2	27·5
A4	Secobarbitone	130	5·0	38·5	0·50	26·7	38·9	38·3
B1	Barbituric acid	90	14·8	34·8	0·44	22·1	37·4	4·1
B2	Phenobarbitone	90	5·5	40·1	0·48	26·5	39·5	16·6
B3	Pentobarbitone	90	4·2	39·4	0·48	26·6	39·6	27·7
B4	Secobarbitone	90	4·5	58·6	0·44	32·0	48·6	46·9
C1	Barbiturate-Na	90	15·3	47·7	0·35	21·7	44·6	3·4
C2	Phenobarbitone-Na	90	5·3	35·4	0·46	23·1	37·7	15·6
C3	Pentobarbitone-Na	90	2·6	34·2	0·48	23·3	36·1	45·3
C4	Secobarbitone-Na	90	1·8	20·6	0·54	16·2	23·3	75·7

[a] Total drug content after a complete degradation of microspheres with pepsin. Mean value of three determinations; [b] geometric mean diameter; [c] geometric s.d.; [d] surface-volume mean diameter; [e] weight-moment mean diameter; [f] maximal drug release achieved in-vitro, M_m, per total drug content, M_o, of microspheres.

A dissolution bottle was filled with 100 mL water and rotated at 50 rev min^{-1} in a water bath at constant temperature (37°C). After the medium reached equilibrium temperature, a desired amount of microsphere product (100 mg) was added to the bottle. The dissolution samples (5 mL) were collected at the appropriate time intervals and a replacement of the sample with an equal volume of medium maintained at 37°C was made. The samples were filtered (0·45 μm Millipore) and after appropriate dilution with pH 10·0 borate buffer assayed by UV spectrophotometry. Experiments were carried out in triplicate. The data are presented as averages.

The instrumental arrangement for a continuous, automated, potentiometric titration procedure consisted of a pH-stat automatic recording titrator, a titration burette, a dissolution test apparatus, and a pulsating-type pump. The glass and calomel electrodes were calibrated with the standard buffer solution, and the pH-stat instrument was set to a desired end-point titration pH corresponding to a value at which the dissolution experiment was to be performed. The dissolution rate was followed by titration of the drug as it dissolved by means of automatic intermittent additions of small volumes of titrant solution (0·008 M NaOH and 0·008 M HCl for acids and salts, respectively) through the burette to the dissolution medium. The dissolution rates were estimated from the rate of consumption of titrant solution, the pH of the medium, the pK_a of the drug, and the normality of the titrant solution.

Total drug content

To obtain the total amount of drug in each preparation of HSA microspheres, a known weight of microspheres (100 mg) was digested with pepsin (5% w/w pepsin solution in 0·065 M HCl). The samples were filtered (0·2 μm Sartorius AG) and the total drug content was determined spectrophotometrically using a sample of digested HSA microspheres without incorporated drug as a blank.

Particle size analysis

Microspheres were sized in a 0·1% Tween 80 saline solution using a Coulter Counter (model Z_M).

Determination of swelling

Two separate samples of microspheres were prepared without any drug at 90 and 130°C as described previously. The microspheres were suspended in water and the diameters of ten microspheres (having the initial diameters similar to the surface-volume mean diameter (d_{sv}) of the sample) were measured directly under an optical microscope.

Results and Discussion

The characteristics of microspheres prepared are given in Table 1. Microspheres were spherical and of well defined shape. All the samples showed a tendency to form agglomerates after the drying step. While agglomeration was expressed more with the C samples, their redispersibility into separated entities was readily achieved in water. Redispersion of A and B samples in water was more difficult, and sonication or intensive agitation was needed to obtain separate microspheres. The appearance of barbituric acid microspheres was different from all other microspheres. Their surface was glossy and of slightly orange colour. This phenomenon was explained by denaturation of HSA, not only due to a temperature change but also because of a

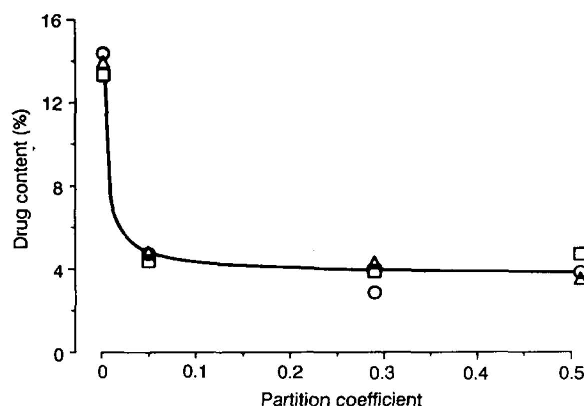

FIG. 1. Drug content of HSA microspheres, M_o, vs partition coefficient of barbiturates, K. ○ A samples; □ B samples; △ C samples.

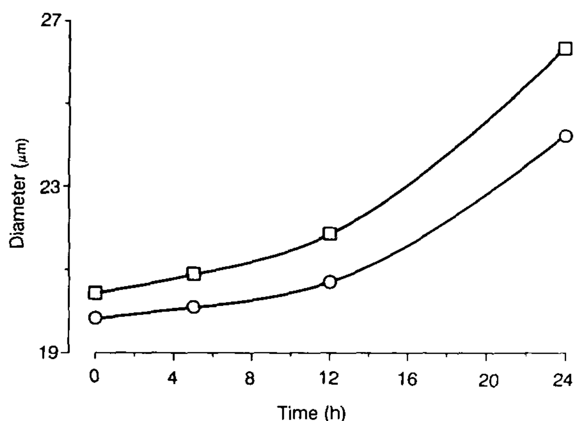

FIG. 2. Swellability of empty microspheres in water at 37°C. Points represent average value of ten measurements. Denaturation temperature: □ 90°C; ○ 130°C.

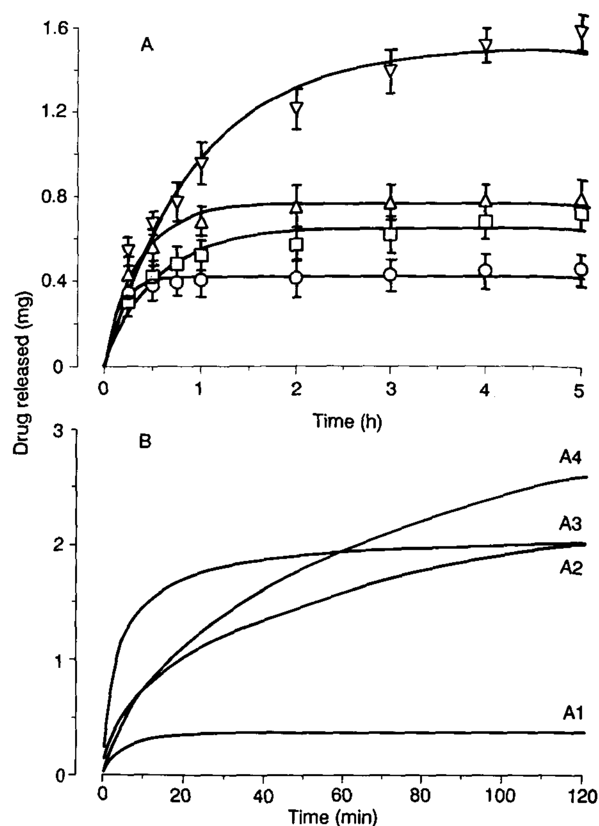

FIG. 3. A. Drug released vs time, from HSA microspheres into water at 37°C (paddle method). Points are mean values of three determinations; ○ A1; □ A2; △ A3; ▽ A4; lines are the best-fit curves for first-order kinetics. Bars represent s.e.m. B. Drug released vs time, for HSA microspheres into water at 37°C by potentiometric titration.

decrease of pH in the system. The value of the dissociation constant of barbituric acid is 4 and the dissociation of the acid in water caused a pH change that additionally denatured the HSA matrix.

It should be noted that the method of microsphere preparation once standardized was not modified, although the drug content in some cases was low. Denaturation temperature and the type of substance (acids vs salts) produce no significant (χ^2-test) difference in drug content. An increase in the drug partition coefficient caused a decrease in the amount of entrapped drug (Fig. 1). The drugs chosen for the study are structural analogues which differ little in general properties except for their partition coefficients (K). Starting with an initial emulsion/suspension system in which a drug is dissolved/dispersed in an inner aqueous phase surrounded by an outer oil phase, the drug is more efficiently entrapped in the developing albumin matrix if it has a low partition coefficient. During the process of preparation, HSA is denatured and the solvent (water) is evaporated so some of the drug is probably lost in the outer oily phase at the beginning of preparation before the solid albumin matrix is formed. Similar conclusions were reached by Tomlinson & Burger (1985) who have discussed the complex and interacting effects of drug solubility in water, drug partition between organic and aqueous phases, and drug protein binding during microsphere preparation.

Particle size analysis of polydisperse samples of microspheres indicated logarithmic-normal distribution. Microsphere sizes were between 5 and 80 μm. Polydispersivity of samples has been shown (Kondo 1978) for microspheres prepared by one of these techniques which include formation of a primary emulsion during the preparation. The samples were characterized by calculated mean diameters (Allen 1975).

Swelling of microspheres in aqueous medium has been shown previously (Zolle et al 1970; Gupta et al 1986a, 1988). Microspheres prepared at higher temperature (130°C) swell more slowly and to a lesser extent than microspheres prepared at 90°C (see Fig. 2). The degree of denaturation of HSA is an obvious reason for this behaviour. Microsphere diameters increased very slowly during the first few hours achieving their maximal value at about 24 h. Further swelling was not followed for two reasons; it had practically stopped and also it had no influence on the drug release, since this was complete after 5 h.

Two characteristic examples of drug release profiles from microspheres are shown in Fig. 3A and B, for the drug release measured with a more standard method and potentiometric titration, respectively. All the experiments were performed under the sink conditions in order to exclude the influence of already released drug in the recipient phase on general release profiles. This was especially important in the case of the A and B samples because of the low solubilities of substances employed. The general profile of drug release obtained was similar to previous results of other authors (Yapel 1985; Morimoto & Fujimoto 1985; Burger et al 1985). Concerning the possible mechanism of drug release, Khana et al (1969) showed that the drug solubility of poorly soluble drugs might be a dominant factor, if the drug dissolution from the surface or interior of microspheres (water filled pores) governs the mechanism of drug release. In such cases, Hixson-Crowell kinetics (1931) should be found for the release data. In the present study, the data did not conform to Hixson-Crowell kinetics, suggesting that drug solubility is not the mechanism of drug release.

It is possible that drug sorbed onto the surface of finished microspheres may influence the release profile. The sorbed

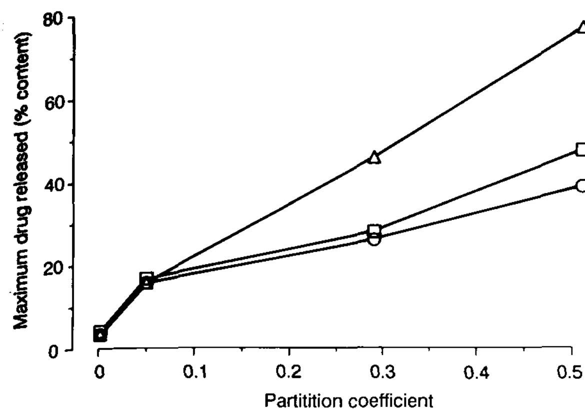

FIG. 4. Relationship between maximum drug released and drug content of HSA microspheres, M_o, vs partition coefficient of barbiturates, K. ○ A samples; □ B samples; △ C samples.

fraction of a drug is immediately released when microspheres are dispersed in aqueous medium giving an apparent burst-effect (Widder et al 1979; Gupta et al 1986b). Gupta et al (1986b) have shown that the sorbed portion of drug can be readily removed by sonication of microsphere dispersions. We subjected all the samples in this study to sonication during preliminary experiments but found that the two sets of data (i.e. with and without this treatment) were identical, indicating there was no sorbtion of drug onto the surface of the spheres.

The release in all cases was complete within 5 h and further prolongation of experiments, in some cases up to 100 h, produced no noticeable increase of released drug. Comparison of the maximal amount released and the drug content in microspheres shows that in no case was drug released completely (Table 1), and that the proportion between two amounts depended on the partition coefficient (see Fig. 4).

We propose that the total drug content of microspheres is dispersed into two fractions as follows. The available fraction for release in-vitro is physically bound or sorbed inside the polymer matrix or its pores; the fraction which is not available for release consists of drug molecules chemically bound to polymer. Only after total degradation of the microsphere structure can the complete release be achieved by the effect of proteolytic enzymes (as in total drug determination). It is clear that this portion of drug cannot be disregarded in-vivo.

Several mechanisms have been suggested as possible explanations for the drug release from microcapsules and microspheres (Jalšenjak 1992). The most commonly employed are as follows: zero- and first-order kinetics; Higuchi kinetics for planar and spherical systems; and modifications according to Baker & Lonsdale (1974), Jun & Lai (1983) and Brophy & Deasy (1987). They were used here to characterize the drug release from HSA microspheres. Also Lapidus & Lordi (1968) kinetics were tested because of swelling of microspheres. The preliminary calculations showed that the release data did not conform to Lapidus & Lordi (1968) kinetics. This, combined with the previously mentioned swelling (and its influence on mean diameter during the first 5 h after immersion of microspheres in aqueous medium) shows that swelling does not affect the drug release significantly, at least during the in-vitro testing needed to achieve the maximum of release. Evidently, in in-vivo investigations, swelling can not be disregarded because of longer periods of time during which microspheres might be in contact with their biological environment. Erosion of the swollen matrix by proteolytic enzymes should be considered in such a situation.

Typical examples of release data testing are shown in Figs 3A, B and 5A, B, C for first-order kinetics, planar and spherical Higuchi kinetics and Brophy & Deasy kinetics, respectively. Points represent the mean experimental values of three determinations and the lines of best-fit curves calculated by a curvefit computer program which consists of least-squares fit to a linear or nonlinear function with linearization of the fitting function. It should be noted that zero-order release was not found even at onset of the drug release. As the original Higuchi equation for planar systems includes a surface area term and the samples were not of equal d_{sv} (Table 1), the release data were corrected by a relevant total surface area, calculated from respective d_{sv} values for each sample. Sokoloski & Sheu (1987) suggested weight-moment mean diameter (d_{wm}) could be used when analysing the ensemble release kinetics; we found the best correlation was obtained with d_{sv}.

Consideration of all the results presented here, shows (Table 2) the spherical Higuchi model of release fits better than the planar model, while first-order kinetics is approximately the same or even better. The Brophy & Deasy

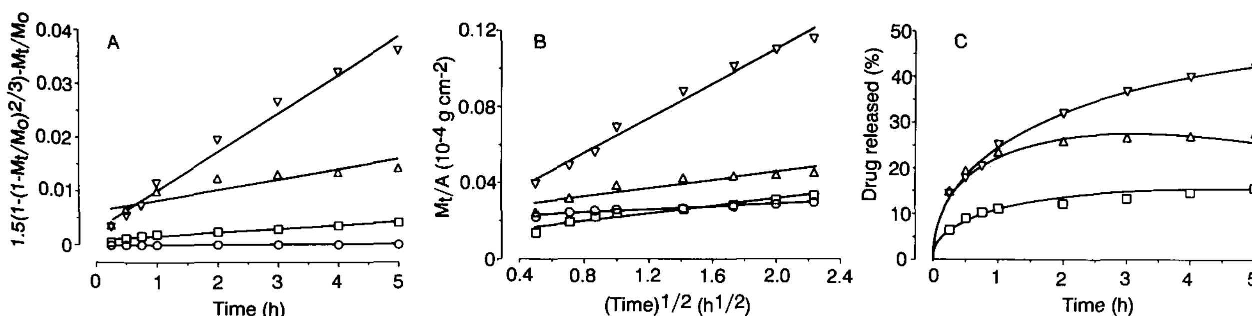

FIG. 5. A. Higuchi kinetics of drug release for spherical systems, notation according Jun & Lai (1983). Points are experimental, and lines are the best-fit curves. B. Higuchi kinetics of drug release for planar systems. Points are experimental but corrected for surface area, and lines are the best-fit curves. C. Drug release kinetics according to Brophy & Deasy (1987). Points are experimental, and lines are the best-fit curves. ○ A1; □ A2; △ A3; ▽ A4 samples.

Table 2. Correlation coefficients for various kinetics of barbiturate release from HSA microspheres obtained by the paddle method.

Sample	First-order[a]	Higuchi model			Brophy & Deasy[e] (1987)
		I[b]	II[c]	III[d]	
A1	0·973	0·969	0·955	0·969	
A2	0·959	0·970	0·973	0·970	0·970
A3	0·990	0·914	0·877	0·914	0·974
A4	0·973	0·992	0·990	0·992	0·998
B1	0·968	0·970	0·949	0·970	
B2	0·983	0·962	0·953	0·962	0·998
B3	0·981	0·962	0·937	0·962	0·997
B4	0·978	0·914	0·886	0·914	0·991
C1	0·997	0·893	0·823	0·893	
C2	0·964	0·938	0·924	0·938	0·962
C3	0·981	0·950	0·972	0·950	0·985
C4	0·987	0·978	0·998	0·978	0·989

[a] $M_t = M_0(1 - e^{-kt})$; [b] $M_t = k_H t^{1/2}$; [c] $(1{\cdot}5(1-(1-F)^{2/3}) - F = Kt$; [d] $M_t/A = k_H t^{1/2}$; [e] $M_t = B_1 t^{1/2} - B_2 t$.

Table 3. Correlation coefficients for first-order and bi-phasic kinetics of barbiturates release from HSA microspheres obtained by the potentiometric titration method.

Sample	First order[a]	Bi-phasic[b]
A1	0·993	0·998
A2	0·994	0·997
A3	0·949	0·990
A4	0·997	0·998
B1	0·982	0·995
B2	0·984	0·992
B3	0·988	0·999
B4	0·909	0·996
C1	0·996	0·998
C2	0·964	0·994
C3	0·980	0·992
C4	0·943	0·997

[a] $M_t = M_0(1 - e^{-kt})$; [b] $M_t = Ae^{-\alpha t} + Be^{-\beta t}$.

approach also gives a satisfactory fit. The fact that the latter equation was developed for matrix systems in which swelling or erosion of the matrix does not affect drug release, gives additional support to the view that these processes are not influencing the drug release from HSA microspheres studied in this work up to the time needed for the release of available drug.

A literature search failed to find comparative drug release studies for different methods (Jalšenjak 1992). Therefore, an additional method was used to assess drug release. Automatic and continuous potentiometric titration of released barbiturates with a pH-stat apparatus that enables identification of small changes of drug concentration, seemed an appropriate choice. The release profile shown in Fig. 3B conforms to the conclusions reached with the standard flask and paddle method. The first-order release was considered the best fit for all release data, but it was also found that a biphasic first-order release gives an even better correlation (Table 3). It would appear that the method of potentiometric titration distinguishes the release profiles better than the paddle method because more data is obtained during the experimental determination.

The effect of drug partition coefficient on drug release from microspheres is shown in Fig. 3. Besides the relative positions of release curves, the proportion of amounts released and contained in microspheres (Fig. 4) shows that the drugs with higher partition coefficients are released faster and to a greater extent then drugs with lower partition coefficients. The barbiturates used are members of a series of structural analogues having approximately the same mol. wt, dimension of molecules and diffusion coefficient in the polymer matrix. It should be recollected now that, for example, secobarbitone in comparison with barbituric acid has a greater tendency towards the organic phase during preparation, and its content in microspheres is lower. One would expect secobarbitone to be the slowest member of the series due to its preference for the polymer phase. However, from the theory of diffusion through polymeric films and matrices (Flynn 1974), as well as its adaptation to microspheres (Donbrow 1986), it follows that an apparent coefficient of diffusion governs the drug transport. The apparent coefficient of diffusion is a product of the real coefficient of diffusion and the drug partition coefficient between polymer and aqueous phase. While the diffusion coefficient of all barbiturates of the series can be considered of the same order of magnitude, the partition coefficients are quite different. Therefore, the apparent coefficient of diffusion is several orders of magnitude higher for secobarbitone and the transport of secobarbitone is the fastest.

We now speculate about what is happening after microsphere immersion into an aqueous medium. It was shown earlier that the drug release is not governed by a dissolution mechanism (Hixson-Crowell) and the diffusion mechanism is responsible for the drug release. The drug molecules near to the microsphere surface diffuse first into the receiving medium creating a drug-depleted layer in the matrix. Drug diffusion from the microsphere surface into water is approximately equal for all barbiturates because of the similar diffusion coefficient in water. However, the transport of drug from interior of microspheres to depleted regions depends on the apparent diffusion coefficient, which is higher for substances having higher values of the distribution coefficient. A stagnant aqueous layer around microspheres has not been studied but it seems appropriate to consider that its dimensions are equal for all microsphere types. It might be that this layer together with the layer depleted of drug but already filled with water, modulate the original Higuchi diffusion from the matrix, and HSA microspheres behave as reservoir systems having the drug release kinetics of first-order. Biphasic kinetics may be a result of two pools of available drug for release.

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