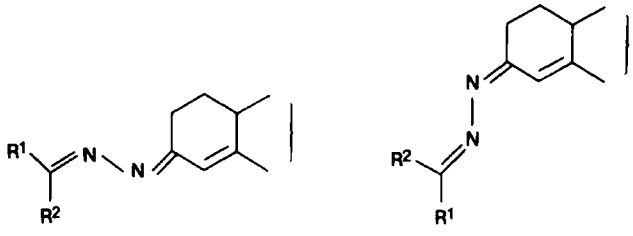
its low solubility in $CDCl_3$ prevented us from detecting all of the signals. It is also possible that the rates of interconversion of the two forms are such that while the proton chemical shifts are time-averaged in $CDCl_3$, although distinct in DMSO-$d_6$, certain of the $^{13}C$-NMR signals are broadened by these processes and hence obscured (8). Variable temperature studies that might answer these questions are beyond our present capabilities. The $^{13}C$-NMR spectra showed differences at C-3, C-4, and C-5 of ~2.5 and 7 ppm downfield and 1 ppm upfield, respectively, for V*a* in comparison with V*b*. Comparable differences were also found between the unseparated forms of III and VI and so have been assigned to *a* and *b* isomers in Tables I and II by comparison with V*a* and V*b* data. The resonances of carbons *anti* to hydrazones and oximes appear 6–12 ppm downfield from the positions of the corresponding *syn* carbons (9), again in agreement with assignment of the *anti* configuration to the *a* forms.

In further support, the order of elution (V*a* followed by V*b*) on high-performance liquid chromatography (HPLC) (3) is compatible with this assignment (6), and the UV data also suggest that the conjugated double bonds are more extended in V*a* than in V*b* (10, 11). Thus, the structures depicted are initially proposed for this series of compounds (the most stable rotational conformation about the N—N bond is shown).



V*a*,V*a*′ V*b*,V*b*′

V*a*,V*b*: $R^1$ = *p*-$(OCH_3)C_6H_4$, $R^2$ = H
V*a*′,V*b*′: $R^1$ = H, $R^2$ = *p*-$(OCH_3)C_6H_4$

A particularly interesting feature of the spectra of VI was that the aromatic carbon-13 signals were all doubled (except for C-3′ and C-5′ in $CDCl_3$). The signals, separated by 0.1–0.2 ppm, presumably arise from the *syn* and *anti* isomers, and were assigned (Table II) by comparison with the spectrum of the 2,4-dinitrophenylhydrazone of cyclohexanone in which they are not, of course, doubled. The additional possibility in azine V of *anti*/*syn* isomerism about the aldimino double bond (HC=N) leads to alternative structures V*a*′ and V*b*′, which are expected to have very similar NMR and UV absorption properties to those of V*a* and V*b*, respectively. It does not seem that mixtures are present, and it is very unlikely that the two forms of V are V*a* with V*b*′ or V*a*′ with V*b*, as neither the $^1H$-NMR nor the $^{13}C$-NMR shifts of the HC=N function differ between isomers (Tables I and II). The stable *anti* aldimino configuration (V*a* and V*b*) is favored.

It is evident that hydrazine-based derivatives of norethindrone can be produced *in vivo* by interaction with isoniazid. We have found that the metabolic disposition of the steroid is thereby altered[1]. Other pharmacologically important steroids and possibly other hydrazine-derived drugs may undergo similar interactions. *Syn* and *anti* isomers of norethindrone hydrazones arise and can be identified. They undergo rapid interconversion in some cases, but may be separated in others. Whether tissue enzymes, which further metabolize the hydrazones (3), are selective for *syn*/*anti* isomers remains to be determined. If such selectivity were to occur, one might expect that the rate of metabolism of various hydrazones would be dependent, *inter alia*, on the relative degree of interconvertibility of the isomers.

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# Effect of the Nonionic Surfactant Poloxamer 338 on the Fate and Deposition of Polystyrene Microspheres Following Intravenous Administration


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Received December 11, 1981, from the **Department of Pharmaceutics, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark*, and the ‡*Department of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.* Accepted for publication August 25, 1982.



**Abstract** □ The blood clearance and organ deposition of polystyrene microspheres in the rabbit following intravenous injection has been investigated using the technique of gamma scintigraphy, blood and organ level measurements, and histology. Uncoated microspheres of 1.27-μm diameter were cleared rapidly from the blood and were taken up primarily by the reticuloendothelial system in the liver. Coating of the microspheres with the nonionic surface-active agent poloxamer 338 reduced the uptake in the liver and gave a corresponding increase in the lungs.



**Keyphrases** □ Microspheres, polystyrene—effect of nonionic surfactants on blood clearance and deposition, intravenous administration □ Nonionic surfactants—effect on blood clearance and deposition of polystyrene microspheres, intravenous administration □ Deposition, tissue—polystyrene microspheres following intravenous administration, effect of nonionic surfactants □ Blood clearance—polystyrene microspheres following intravenous administration, effect of nonionic surfactants


Colloidal systems such as liposomes, microspheres, nanospheres, and emulsions have been investigated as potential drug-targeting devices (1–4). The fate of such particles in the body following administration is deter-




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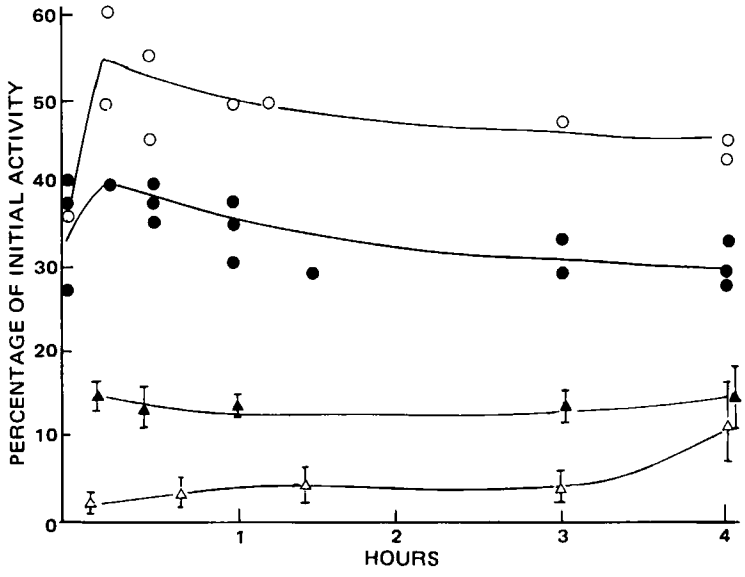


**Figure 1**—*Activity-time profiles for blood and liver following intravenous administration of $^{131}I$-labeled polystyrene microspheres. Key: uncoated, (○) liver, (△) blood; coated with poloxamer 338, (●) liver, (▲) blood.*

mined by the chemical nature of the colloid and its physical characteristics such as particle size and surface charge (5). The reticuloendothelial system plays a major role in clearing small particles from the circulation following intravenous administration, while larger particles are trapped in the capillary beds of the lungs. Studies on emulsion systems have indicated the important role of the surface layer of the emulsifier. The use of nonionic emulsifiers (*e.g.*, poloxamer) leads to slow clearance from the bloodstream and an altered distribution pattern to organ sites (6).

In this work such effects have been investigated further in rabbits using a model colloidal system, polystyrene microspheres. This material has been well characterized physically, including studies involving the adsorption of nonionic surfactants (7, 8). In addition, polystyrene microspheres labeled with a gamma ray-emitting radionuclide have been used to study the distribution, fate, and acute and semichronic effects of microspheres in animal models (9–11).

## EXPERIMENTAL

**Administration of Microspheres**—Polystyrene microspheres (1.27 μm) were obtained from a commercial supplier[1]. The particle size was verified using an electronic particle counter[2] with a 30-μm orifice tube. The particles were surface-labeled with iodine-131 by a process of irradiation using a cobalt-60 source with the microspheres suspended in a solution of 2-mCi $Na^{131}I$; the total irradiation dose was 5 Mrad. The particles were dialyzed free from $Na^{131}I$. Preliminary experiments *in vitro* and *in vivo* indicated that the integrity of the label was satisfactory, although some free iodide was detectable after dialysis for 120-hr against rabbit plasma.

In each experiment, 50–75 μCi of labeled material was administered. The particles ($\sim 2 \times 10^8$) were suspended in saline and rapidly injected. One group received microspheres that had been equilibrated for 24-hr in a saline solution containing 1% w/v poloxamer 338[3], molecular weight of poloxypropylene moiety 3250, polyoxyethylene content 80–90% (8).

The surface charge on the microspheres over the pH range 2–10 was determined using cell-microelectrophoresis[4] (12). The microspheres were also equilibrated at 25° at pH 7.4 with a 1% poloxamer 338 solution for 24 hr in the presence or absence of rabbit plasma (1:1 mixture with the microsphere suspension). All samples were diluted with $1.54 \times 10^{-4}$ *M* NaCl solution before measurement of the surface charge at pH 7.4. Any adjustment in pH was carried out using hydrochloric acid or sodium hydroxide. The state of aggregation of the microspheres was examined using the light microscope. The adsorption of poloxamer 338 on the surface of polystyrene microspheres of the same approximate size and the same surface characteristics has been investigated in detail by Kayes and Rawlins (5). Equilibration was reached in 24 hr. The isotherms were Langmuirian, and the thickness of the adsorbed layer was 26 nm.

**Animal Experiments**—New Zealand White rabbits 2–4 kg in weight were randomly divided into two groups of three. The microspheres were injected into the marginal ear vein. The distribution of the microspheres in various body organs was followed using external scintigraphic imaging[5]. Dynamic and static views were recorded at suitable times during a 10-day period and processed by computer[6]. Blood samples were removed at suitable intervals and were analyzed for radioactivity using a gamma counter[7]. At the end of 11 days the animals were sacrificed and the organs removed. Small samples were taken for histological investigation, and the total organ activity in the remainder was determined using a well-type gamma counter[8]. The histological samples were fixed, dehydrated, and mounted in wax. Microtome sections were stained using hematoxylin–eosin.

## RESULTS

**Properties of the Microspheres**—The electrophoretic mobility–pH relationship for the polystyrene microspheres showed that at a physiological pH value (7.4) in $1.54 \times 10^{-4}$ *M* NaCl, the particles carried a net negative charge (zeta potential) of −35 mV. In the presence of 1% poloxamer 338, the surface charge was reduced to −8 mV. This is due to the presence of an adsorbed layer of polymer on the surface of the particles (8). Incubation of polystyrene microspheres with rabbit plasma caused a reduction of surface charge to −15 mV. The presence of poloxamer on the surface of the particle prior to incubation with plasma caused the surface charge to be reduced to almost zero. The reduction in charge brought about by added plasma is thought to be due to the adsorption of plasma proteins (*e.g.*, albumin or globulin) or more specific substances such as fibronectin (13–15). Microscopic examination showed that added plasma caused aggregation of the microspheres, but this was prevented by the presence of poloxamer 338.

**Gamma Scintigraphy**—The processed computer images showed that the small microspheres were rapidly taken up by the liver of the rabbit (Fig. 1). No other body organs were visualized on the scintiscans except small quantities of activity that were observed in the thyroid and/or bladder, which can be attributed to the presence of small quantities of free iodide. There was no evidence of large numbers of particles being taken up by the lungs. The presence of an adsorbed layer of poloxamer 338 on the microspheres reduced the uptake in the liver. This difference,

[1] Dow Diagnostics, Dow Co., Indianapolis, Ind.
[2] Coulter Counter TAII, Coulter Electronics, Harpended, U.K.
[3] Pluronic F108 (poloxyethylene–polyoxypropylene copolymer), Ugine Kuhlmann Ltd., Bolton, U.K.
[4] Rank Mk. II Microelectrophoresis apparatus, Rank Bros., Cambridge, U.K.
[5] Maxi Camera II—gamma camera—General Electric, Milwaukee, Wis.
[6] Gammascope, Link Systems Ltd., High Wycombe, U.K.
[7] Intertechnique CG4000 gamma counter, Intertechnique, Uxbridge, U.K.
[8] Bucket Counter, Ortec, Bracknell, U.K.

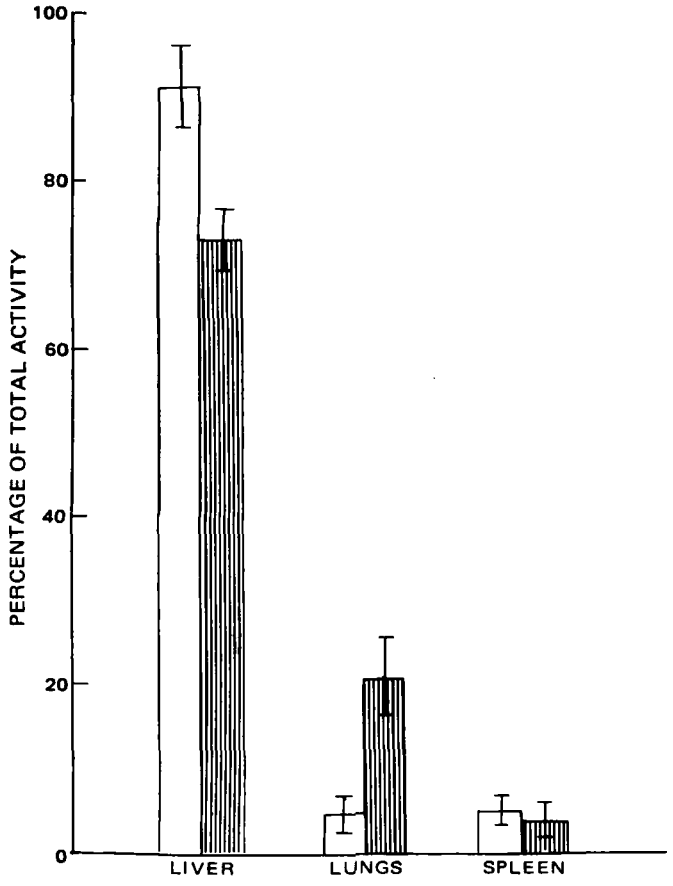


**Figure 2**—*Tissue distribution of iodine-131 11 days after administration of $^{131}I$-labeled polystyrene microspheres. Key: (□) uncoated; (■) coated with poloxamer 338.*

caused by the presence of the nonionic surfactant, was still evident after 11 days of imaging.

**Blood Levels**—The blood level–time profiles (Fig. 1) show that clearance of the microspheres from the blood is affected by the presence of poloxamer 338. In the absence of the surfactant, the particles are cleared rapidly and the blood levels are low. In contrast, in the presence of poloxamer 338 significant sustained levels of activity were observed over a period of many hours.

**Organ Levels**—The distribution of activity after 11 days postadministration is shown in Fig. 2 for the three major sites of uptake. The majority of the activity was in the liver, with much smaller quantities in the lung and spleen. The group that received microspheres coated with poloxamer had less activity in the liver and more activity in the lungs compared with the control group which received microspheres alone.

**Histology**—Conventional histological techniques would not be expected to show small individual microspheres of 1.27 μm in body tissues. Histological examinations were undertaken, therefore, to reveal aggregated particles. None were observed.

## DISCUSSION

**Effect of Poloxamer on the Deposition of Microspheres**—The results from scintigraphic imaging, blood level determinations, and organ uptake studies all show clearly that the adsorption of a layer of poloxamer 338 onto the surface of polystyrene microspheres affects their deposition in the rabbit following intravenous administration. Differential leaching of the radiolabel from the particles prior and subsequent to administration would be expected to alter the absolute values for deposition, but not their relative magnitudes. The uncoated particles were deposited mainly in the liver; it is well known that small colloidal particles will be cleared rapidly from the bloodstream by the reticuloendothelial system of this organ, *i.e.*, the Kupffer cells (16). Larger particles (normally those >7 μm) will be cleared by the lungs by a process of filtering or mechanical obstruction (10).

The coating of colloidal particles with polymers and macromolecules can alter organ uptake considerably. Wilkins and coworkers (17, 18) have demonstrated the importance of surface modification as well as surface charge. Negatively charged particles are removed from the circulatory system quite rapidly, whereas positive and neutral particles have different clearance patterns. Certain positively charged particles can be taken up by the lungs and then rapidly redistributed to the spleen rather than the liver (17). The important process controlling the fate and deposition of colloidal particles would seem to be one of the interaction of a foreign surface with the blood and the coating of that surface with various components such as albumin, globulin, *etc;* the exact coating material being determined by the nature of the particle itself. The coating then influences the interaction of the particle with cells of the reticuloendothelial system (5). Certain blood components (termed opsonins) can enhance phagocytic uptake (16). Van Oss *et al.* (19) have discussed the role of surface hydrophobicity in phagocytic engulfment and cell adhesiveness. Particles with low contact angles are taken up much less rapidly by phagocytes than particles with high contact angles. Poloxamer 338 causes a very significant reduction in the contact angle of the hydrophobic polystyrene particles.

Studies that have dealt specifically with nonionic surface-active agents have employed both polystyrene microspheres and emulsion systems. For example, Singer *et al.* (5) found that microspheres coated with polysorbate 80 showed the slowest decline in blood concentration. Similarly, Jeppsson and Rossner (20) using fat emulsions demonstrated the importance of the molecular weight of the adsorbed surfactant, poloxamer 338 (mol wt 3250) being much more effective at reducing the clearance rate of droplets than poloxamer 188 (mol wt 1750). Such differences could be due to the different thicknesses of the adsorbed layers (26 and 14 nm, respectively, for 338 and 188) and/or to differences in surface charge (8).

The increased uptake of the coated microspheres in the lungs (after 11 days) is more difficult to explain. One obvious reason would be aggregation, and the entrapment of the larger entities so created, in the lungs. Kanke *et al.* (10) have reported "clusters" of microspheres in histological preparations. However, no aggregates of a size that would be trapped in lung capillaries were seen in the histological specimens, and the *in vitro* studies showed that the nonionic surfactant brought about deaggregation even though the microsphere system with adsorbed poloxamer and plasma components had the lowest surface charge. It is recognized that a different type of aggregation–deaggregation process could occur *in vivo*, since the microspheres are exposed to different environments and the poloxamer could be modified by metabolic changes.

The critical minimum particle size for deposition in the lungs is usually regarded as being ~7–10 μm, depending on the nature of the particle and its shape (21). However, a recent report by Findler *et al.* (22) has shown that efficient localization of liposomes in the capillary bed of the lungs could be achieved using negatively charged multilamellar liposomes 1–2 μm in diameter. This size range covers the microspheres used in the present work. Furthermore, uptake of technetium-99m–sulfur colloid (particle size 400–600 nm) in the lungs has been reported in clinical diagnostic studies (23).

The pathophysiology of such increased uptake of colloid in the lung is poorly understood, but evidence from human and animal studies does not support the hypothesis that *in vivo* microaggregation results in the formation of microemboli that are trapped in the lung. The evidence does support the possibilities of increased phagocytic activity in the pulmonary capillary bed or adherence of colloid to altered endothelium in the pulmonary capillaries (23, 24). Thus, the modified surface properties of the microspheres coated by poloxamer could potentiate their uptake in the lungs.

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# Application of Postcolumn Ionization in the High-Performance Liquid Chromatographic Analysis of Butabarbital Sodium Elixir

**EDITH P. SCOTT**

Received February 16, 1982, from the *Food and Drug Administration, Atlanta, GA 30309.* Accepted for publication August 16, 1982.

**Abstract** □ A sensitive postcolumn ionization high-performance liquid chromatographic (HPLC) method for the quantitative determination of butabarbital sodium in butabarbital sodium elixir is described. The procedure employs a octadecylsilane column chemically bonded to porous silica microparticles. The mobile phase is a mixture of methanol and water (typically 35:65), adjusted to provide separation of butabarbital from two degradation compounds and other formulation ingredients. A buffer (pH 10) added between the column and detector provides for the primary ionization of the barbiturate necessary for optimum UV-detector sensitivity at ~240 nm. Determinations are made using the sodium salt; thus the need for extraction of the free base is eliminated. The procedure is linear over the 0.3–0.9-mg/ml concentration range of butabarbital sodium. Reproducibility values for 10 injections of a single reference standard range from 100.2 to 100.8% of theoretical with a mean of 100.5% and a coefficient of variation of 0.23%. An interlaboratory precision study for blind duplicates of one simulated product formulation and two commercial elixers produced coefficients of variation of 1.4, 1.3, and 1.1%, respectively. Recovery determinations for the drug in simulated product formulations ranged from 98.4 to 99.0%, intralaboratory, and 97.7 to 102.2%, interlaboratory. The HPLC procedure is stability indicating with respect to two decomposition products.

**Keyphrases** □ Butabarbital sodium—application of postcolumn ionization in higher-performance liquid chromatographic analysis, elixir □ Ionization—postcolumn, application in high-performance liquid chromatographic analysis of butabarbital sodium elixir □ High-performance liquid chromatography—application of postcolumn ionization, analysis of butabarbital sodium elixir

The chromaphoric characteristics of barbiturates establish them as prime compounds for high-performance liquid chromatographic (HPLC) analysis using a combination of reverse-phase chromatography and postcolumn ionization with a pH 10 buffer. Clark and Chan (1) have reported the advantages of combining conventional reverse-phase chromatography and postcolumn ionization into a single system. In this study, their analytical approach has been successfully employed in the assay of butabarbital sodium elixir. The procedure eliminates the need for extraction of butabarbital free acid. No sample cleanup was required because a suitable chromatographic system was found that would resolve the phenobarbital internal standard, butabarbital, placebo ingredients, and one decomposition product, capuride.

**Table I—Typical HPLC Standard Curve Data [a] for Butabarbital Sodium**

| Butabarbital Sodium Added, mg/50 ml | Butabarbital Sodium, Found, mg/50 ml | Percent of Theoretical |
|---|---|---|
| 16.73 | 16.54 | 98.9 |
| 23.24 | 23.19 | 99.8 |
| 30.36 | 30.27 | 99.7 |
| 38.00 | 38.00 | 100.0 |
| 46.00 | 45.82 | 99.6 |

[a] Correlation coefficient = 0.9999.

## EXPERIMENTAL

**Apparatus**—The liquid chromatograph[1] consisted of a solvent pump with flow controller; an injector with flowing-stream, valve-controlled

[1] Waters Liquid Chromatograph; Model 6000-A Solvent Delivery System, Model 720 System Controller, Model U6K Injector, Model 440 Absorbance Detector with 254 nm filter, Model 730 Data Module; Waters Associates, Milford, Mass.

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