globules larger than 5 μm increased from 0·0023 to 0·0035% as the admixtures were stored for one day at room temperature followed by two days at 5°C instead of by three days at 5°C. However, for the same storage conditions, admixtures formulated with 15% dextrose exhibited a change in larger globule weight percentage from 0·0033 to 0·0065%, a nearly twofold increase. These results clearly indicate that unnecessary exposure of inherently unstable 3-in-1 admixtures to room temperature should be avoided. Dextrose alone was shown to reduce the stability of the emulsion, mainly due to the lowering of pH, which results in a decrease (less negative) in the surface potential of the oil globules (Black & Popovich 1981). However, in 3-in-1 admixtures, the presence of amino acids prevents the dextrose-related pH drift so that the destabilization effect of dextrose does not occur. On the contary, the stabilization effect of dextrose on the fat emulsion in 3-in-1 admixtures, as demonstrated in this study, has been related to its viscosity-enhancing effect on the aqueous medium and the reduction of the attractive forces between oil globules, which result in a slower rate of globule coalescence (Washington et al 1990).

The lack of interaction between the dextrose concentration and the compounding method allows a more straightforward evaluation of the impact of this non-formulation variable on the stability of 3-in-1 admixtures. Admixtures prepared by simultaneous pumping yielded a mean weight percentage of 0·0045% for globules larger than 5 μm. For admixtures compounded using the sequential pumping method, the weight percentage of oil globules larger than 5 μm was 0·0033%. Although the analysis of variance indicates that admixtures compounded using the sequential pumping method exhibited a lower weight percentage of oil globules >5 μm, the impact shown by the method of compounding appears to be the least in comparison with those associated with the other two variables evaluated in this study.

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Albumin microspheres as a drug delivery system for dexamethasone: pharmaceutical and pharmacokinetic aspects

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Abstract—Four types of albumin microspheres containing dexamethasone prepared by varying cross-linking time—30, 60, 120 and 180 min—were tested to examine the influence of heat cross-linking degree on the in-vitro drug release. Release rate of dexamethasone associated with the microspheres was slower for samples with a longer time of high temperature denaturation. Encapsulation in heat-stabilized albumin microspheres for 60 min at 100°C significantly retards the in-vivo release of dexamethasone and hence retards absorption from the injection site.

Biodegradable albumin microspheres appear to be an exploitable delivery system for sustained and controlled release of drugs (Lee et al 1981; Morimoto et al 1985). Recently, albumin microspheres have received wide attention because of their specific, organ-targeting, biocompatability and other desirable characteristics of ideal drug carriers.

Formation of albumin beads under denaturing conditions has been reported by Soloway (1972). Dispersion of albumin solution into a hot organic phase results in stable microspheres. Scrambled disulphide bridges and lysinoalanine cross-links stabilize these structures (Royer et al 1983). Drug release from albumin microspheres may be controlled by their degree of cross-linking and drug/albumin ratio during preparation (Kim & Lee 1986). Drug that is associated with these carriers can be made available at the particle surface, may diffuse through the particle matrix, or may be available on disintegration of the particle due to enzymatic reactions (Gupta et al 1987). The first two processes can be evaluated by in-vitro dissolution studies.

Heat denaturation significantly sustained the in-vitro release of corticosteroids from microspheres; compared with release from microcrystalline drug suspensions (Burgess & Davis 1988).

The aim of this study was to evaluate the potential use of albumin microspheres as a drug delivery system for sustained release of dexamethasone.

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Materials and methods

Materials. Bovine serum albumin was purchased from IBBS (Vratza, Bulgaria), sunflower oil from Real Foods (St Zagora, Bulgaria), dexamethasone from Diosynth (Oss, Holland). Sodium chloride, diethylether, tris-(hydroxymethylmethylamine, acetic acid and chloroform were of AnalaR grade (Fluka).

Methods. Preparation of dexamethasone-associated albumin microspheres. Bovine serum albumin microspheres containing dexamethasone were prepared based on the principle of heat denaturation. Dexamethasone (0·1 g) was suspended in 10 mL sodium phosphate buffer. Bovine serum albumin (1 g) was dissolved in the suspension. The resulting suspension was added dropwise to 500 mL of preheated and constantly stirred (1500 rev min $^{-1}$) sunflower oil. Heating and stirring were maintained for 30, 60, 120 or 180 min at 100°C. The suspension was then allowed to cool to 25°C. After filtration and washing with diethylether the microspheres were stirred at 4°C until use.

Analysis of free dexamethasone. The total quantity of drug in solubilized microspheres or in samples from the release assay was determined spectrophotometrically at 239 nm (Gupta et al 1986).

Analysis of drug content in microspheres. Surface drug. To 5 mg microspheres, 5 mL 0·9% NaCl (saline) was added and the suspension was placed in an ultrasonic bath for 5 min. The suspension was then centrifuged at 4000 rev min^{-1} for 10 min. The microspheres were washed in the same manner three more times. The dexamethasone content in all supernatants was determined by spectrophotometry.

Entrapped drug. Microspheres obtained after four washings were digested overnight in 5 mL 0·5 M acetic acid. The digested homogenate was centrifuged at 4000 rev min $^{-1}$ for 10 min and the dexamethasone content was determined by spectrophotometry after extraction into chloroform. After evaporation to dryness the samples were all analysed in methanol. The digested microsphere residue was redigested to ensure total recovery of the entrapped drug.

Dissolution studies of dexamethasone-associated bovine serum albumin microspheres. About 100 mg microspheres was suspended in 900 mL Tris-buffer (pH 7·4), and saline in the dissolution apparatus, protected from light. The medium was maintained at 25°C and stirred at 100 rev min^{-1}. Five millilitres of dissolution medium was collected for 48 h at scheduled intervals and dexamethasone was determined by spectrophotometry.

Pharmacokinetic studies. Twelve healthy sheep, 40–60 kg, were used. Six sheep were injected intravenously with an aqueous methanol solution of dexamethasone (1 mg kg $^{-1}$); six were injected intramuscularly with dexamethasone albumin microspheres in suspension (1 mg kg^{-1}).

Blood samples were collected into heparinized tubes at 6, 9, 48, 72, 120, 144 and 150 h after administration and centrifuged within 4 h. Plasma thus prepared was stored at −20°C until HPLC analysis (Alvinerie & Tontain 1982). The mathematical method of Ritshel (1980) was used for pharmacokinetic analyses.

Results and discussion

Pharmaceutical aspects. It has been reported that in drug-associated albumin microspheres, about 40% of the total drug is released rapidly and the remaining 60% slowly. The mechanism of drug release from albumin microspheres depends on the location of the drug in the carrier as well as on the properties of the microsphere matrix. Drugs can be associated with albumin microspheres by either adsorption onto the particle surface or inclusion in the microsphere matrix (Gupta et al 1986).

The initial rapid release of drug is probably due to the desorption of the surface drug, or in the matrix channels; the release of drug from these sites will be diffusion controlled. The slow release rate is due to the release of the entrapped drug from the core of the microspheres after matrix degradation.

Table 1. Dexamethasone release (mg) into the washing media and the digested homogenate of dexamethasone–albumin microspheres.

	Stabilization time (min)			
	30	60	120	180
First washing	0·075	0·040	0·039	0·032
Second washing	0·025	0·022	0·027	0·020
Third washing	0·028	0·018	0·020	0·020
Fourth washing	0·014	0·017	0·015	0·0094
Digestion	0·11	0·13	0·126	0·174

The amount of drug recovered after each washing and after the digestion of the particles is presented in Table 1.

Four types of albumin microspheres with dexamethasone prepared by varying cross-linking time (30, 60, 120 and 180 min) were tested to examine the influence of heat cross-linking degree on the in-vitro drug release. The maximum temperature which dexamethasone would withstand without a significant level of degradation was 170°C.

For microspheres stabilized at longer cross-linking time, a greater content of drug was entrapped within the microspheres (Table 1).

Heat denaturation of albumin at high temperatures results in breakage and reformation of disulphide bonds and hence to considerable rearrangement of the molecular configuration through the formation of lysylalanine (*N*-(DL-2 amino-2-carboxyethyl)-L-lysine) (Sokolosky & Royer 1984). This results in a tightly woven structure with consequently reduced permeability to dexamethasone as shown by the slow drug release rates. The dexamethasone in the microsphere was stabilized at 100 C from 30 min to 3 h.

The release rate of dexamethasone associated with the microspheres in Tris-buffer was slower in the samples which were denatured by high temperatures for a longer time (Fig. 1). The model of the drug release is Higuchi's model (Higuchi 1963).

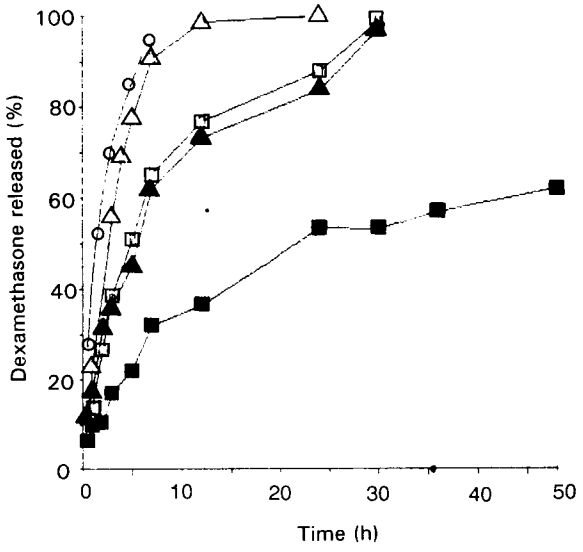

FIG. 1. The release rate of dexamethasone associated with albumin microspheres in Tris-buffer; △ stabilized for 30 min, □ stabilized for 60 min, ▲ stabilized for 120 min, ■ stabilized for 180 min, ○ pure compound.

Table 2. Dissolution parameters of the four preparations and of the pure drug substances.

		Stabilization time (min)			
	Pure drug	30	60	120	180
Time to 50% release (h)	1·34	2·73	4·89	4·80	2·44
Dissolution half-life (h)	2·14	3·94	8·02	7·98	4·59

The four preparations have an initial fast release phase lasting for less than 6 h, which appears to be a combination of the release of surface and possible entrapped dexamethasone as well as particle hydration. The terminal slow release phase is probably primarily due to the release of entrapped drug.

Table 2 shows in-vitro dissolution parameters of the four preparations. The microspheres stabilized during longer cross-linking time have similar dissolution parameters.

Pharmacokinetics in sheep. The dexamethasone–albumin preparation which released 50% of its drug content in 4·8 h in the dissolution test was used to study the pharmacokinetics in sheep.

The plasma concentration of dexamethasone from albumin microspheres increased up until 120 h (0·025 μg mL^{-1} post injection); at 72–120 h a plateau of plasma concentrations was observed and after 120 h the concentration decreased. The plasma concentration of dexamethasone from solution has the maximal values of 0·057 μg mL^{-1} at 3 h post injection (Fig. 2).

Table 3 presents the pharmacokinetic parameters of dexamethasone–albumin microspheres.

The results show that encapsulation in heat stabilized albumin microspheres significantly retards the in-vivo release of dexamethasone and hence retards absorption from the injection site. These results depend on both the properties of dexamethasone and the degradation of the albumin matrix at the site of injection.

This study demonstrates that substantial amount of drug associated with the albumin microspheres is present in the slowly released form.

This delivery device can be very well exploited for the purpose of a sustained or controlled release of other water-insoluble drugs. Release of a water-insoluble drug from bovine serum albumin microspheres can be controlled by adjusting the stabilization temperature of the carrier.

Table 3. Pharmacokinetic parameters of dexamethasone from albumin microspheres.

Absorption rate constant (h^{-1})	0·02
Absorption half-life (h)	34·6
Elimination rate constant (h^{-1})	0·1155
Elimination half-life (h)	6·0
C_{max} (μg mL^{-1})	0·025
Mean residence time (h)	85
t_{max} (h)	120
AUC (μg mL^{-1} h)	2568·72

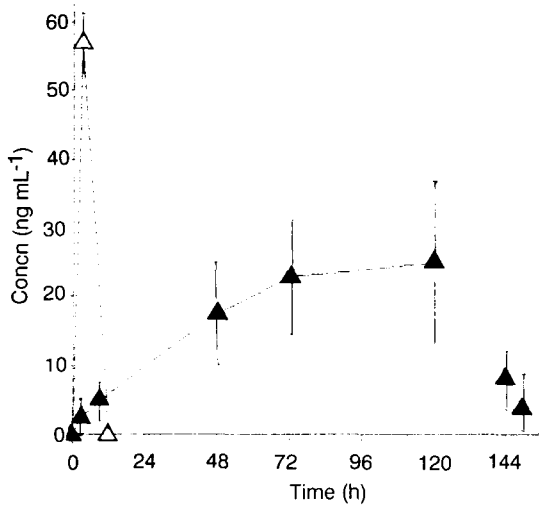

FIG. 2. The plasma concentration of dexamethasone after intramuscular administration of dexamethasone solution (△) and dexamethasone-albumin microspheres in suspension (▲). Bars represent the range.

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