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COMMUNICATIONS

A factorial design study on the physical stability of 3-in-1 admixtures

L. C. LI, T. P. SAMPOGNA, *Hospital Products Division, Abbott Laboratories, Abbott Park, IL 60064, USA*

Abstract—The effects of dextrose concentration, the compounding method, and storage conditions, on the physical stability of 3-in-1 admixtures were investigated using a 2^n factorial design. The main effect of these three variables on the weight percent of oil globules larger than 5 μm (by HIAC) was found to be statistically significant. However, the effects of interaction amongst these variables, except the two-way interaction between dextrose concentration and storage conditions, were found to be statistically insignificant. A higher dextrose concentration was shown to enhance the physical stability of the admixtures, while low-temperature storage (three days at 5 C) was more favourable for maintaining the physical stability of the admixtures with a low dextrose concentration. Although sequential pumping produced admixtures with a slightly lower final weight percentage of larger oil globules (> 5 μm), the method of compounding has the least impact on the physical stability of the admixtures in comparison with the other two variables evaluated in this study. The storage of the admixtures at room temperature for one day was shown to have a greater adverse effect on admixtures with a low dextrose concentration.

Total parenteral nutrition (TPN) or 3-in-1 admixtures are dilute dispersions of fat emulsion in an aqueous medium containing amino acids, dextrose, water-soluble vitamins, and electrolytes. These admixtures are administered through either a central venous catheter or a peripheral vein to patients who have been compromised by disease, surgery, or trauma. Pharmaceutically, these admixtures are very delicate systems which suffer from an inherent physical instability of their fat emulsions. Creaming, coalescence, and phase separation are some of the problems creating a concern for the clinical use of unstable 3-in-1 admixtures. Oil globules in the emulsion with sizes larger than 6 μm have been shown to be responsible for the adverse reactions associated with the infusion of 3-in-1 admixtures (Atik et al 1965).

The determination of variables affecting the stability of the fat emulsion in 3-in-1 admixtures is of paramount importance in the preparation of stable admixtures. The effects on the physical stability of the fat emulsion by formulation components such as dextrose, amino acids, and electrolytes in a 3-in-1 admixture have been well-documented (Black & Popovich 1981; Washington 1990; Washington et al 1990, 1991). In addition to these formulation variables, the impact of some non-formulation variables such as compounding sequence and storage temperature, on the stability of 3-in-1 admixtures may be significant depending on the inherent physical stability of the formulation; a non-formulation variable may not show any discernible effect on some inherently stable 3-in-1 admixtures, but its effect may be manifested in some unstable formulations. Therefore, the influence of non-formulation variables on the stability of 3-in-1 admixtures should be determined using admixture formulations with different degrees of inherent physical stability. The influence of dextrose concentration in a 3-in-1 admixture has been shown to be significant; an increase in dextrose concentration enhances the physical stability of a 3-in-1 admixture (Washington et al 1990). In the present study, two non-formulation variables, the compounding method and storage conditions, were evaluated with respect to their influence on the physical stability of 3-in-1 admixtures containing two levels (high and low) of dextrose (Table 1). The weight percentage of oil globules larger than 5 μm was used as the measure of the physical stability of the admixtures. The study was conducted using a factorial design which allowed the simultaneous determination of the main effect as well as the effects of interaction amongst these three variables. The significance of these effects was tested by analysis of variance.

Correspondence: L. C. Li, Abbott Laboratories, 97d, AP4, Abbott Park, IL 60064, USA.

Materials and methods

Experimental design. Factorial designs have been widely used to determine the simultaneous effects of several variables and their interactions (Anderson & McLean 1974; Bolton 1990). In this study, a factorial design was used to determine the impact of dextrose concentration, the compounding method, and storage conditions on the physical stability of 3-in-1 admixtures. The entire experiment was conducted in duplicate and the experiment replication is considered as an additional variable. Therefore, this study is actually a 2^4 factorial design. The experimental results were analysed statistically using analysis of variance.

Materials. Details of the formulations are shown in Table 2.

Procedures. The admixtures were compounded by two different pumping methods using the Nutrimix Macro Automated TPN compounder (Abbott Laboratories). In the simultaneous pumping mode, the amino acids, dextrose, lipids, and Sterile Water for Injection were simultaneously added to a 1 L PVC phthalate-free container. The electrolytes were sequentially added in the following order: potassium phosphate, sodium chloride, potassium chloride, magnesium sulphate, calcium gluconate, trace metals. After the addition of each electrolyte, the container was inverted 12–14 times to ensure adequate mixing. In the sequential pumping mode, the core was prepared by sequentially adding the base substrates in the following order: lipids, amino acids, sterile water, dextrose. The electrolytes were added to the core in the same order as they were added for the simultaneous mode. M.V.I.-12 was added to the admixtures before the final sampling of the stored samples.

Admixtures containing 15% and 30% dextrose were prepared using these two different compounding methods. For each combination of dextrose concentration and compounding method, the samples were divided into two groups. One group was stored for two days at 5 C, followed by one day at room temperature (21 C) before final testing. The other group was placed at 5 C for three days. At the conclusion of storage, the visual appearance of each admixture was evaluated. A 10 mL sample of the admixture was sent for HIAC measurement of oil globules larger than 5 μm. The detailed testing procedures for determining the oil-globule size distribution using HIAC have been reported elsewhere (Tripp 1990). The physical instability of the fat emulsion in a 3-in-1 admixture is usually manifested by a

Table 1. The variables evaluated in this study.

Variable	Number of levels	Actual levels
Replication (R)	2	1 (R1) vs 2 (R2)
Dextrose concn (D)	2	15% (D1) vs 30% (D2)
Compounding method (M)	2	Simultaneous pumping (M1) vs sequential pumping (M2)
Storage condition (S)	2	Two days at 5°C and one day at room temperature (S1) vs three days at 5°C (S2)

Table 2. Sources of products used in this investigation.

Product	Lot no.	Manufacturer	Formulation	
			15% Dextrose	30% Dextrose
Aminosyn II 10%	55626 DM	Abbott	350 mL	335 mL
Dextrose 70% Injection	54548 DM	Abbott	225 mL	925 mL
Liposyn II 20%	47101 DE	Abbott	240 mL	230 mL
Sterile Water for Injection	48903 DM	Abbott	185 mL	—
Sodium Chloride Injection	41259 DK	Abbott	40 mEq L^{-1}	40 mEq L^{-1}
Potassium Phosphate Injection	47145 G7	Abbott	9 mM	9 mM
Potassium Chloride Injection	50589 DK	Abbott	16 mEq L^{-1}	16 mEq L^{-1}
Magnesium Sulfate Injection	50361 DK	Abbott	4 mEq L^{-1}	4 mEq L^{-1}
Calcium Gluconate Injection	390850	Lyphomed	4·7 mEq L^{-1}	4·7 mEq L^{-1}
Trace-Metal Additive*	51260 DK	Abbott	2 mL L^{-1}	2 mL L^{-1}
M.V.I.-12**	H85805	Rorer	4 mL L^{-1}	4 mL L^{-1}

* Trace-Metal Additive contains zinc, chromium, manganese and copper. ** Water-soluble multiple vitamins.

higher weight percentage of oil globules with a size larger than 5 μm as a result of coalescence of the submicron oil globules.

Results and discussion

The visual evaluation of the admixtures prepared in this study showed no sign of yellow oily streaks, rings, or patch formations at the admixture-container interface. Table 3 presents the HIAC results for all the admixtures evaluated in this study. The HIAC data were subsequently analysed statistically using the analysis of variance procedure in SAS (Statistical Analysis System). The results indicate the significant main effect of dextrose concentration ($P<0·01$), storage conditions ($P<0·01$), and the pumping method ($P=0·03$) on the HIAC data of the admixtures. The effect of interaction between dextrose concentration and storage condition was shown to be significant ($P=0·06$), while the remaining effects of interactions between these variables were concluded to be insignificant ($P>0·3$).

In statistical terminology, the lack of additivity of variable effects is known as interaction (Anderson & McLean 1974). When the effect of interaction between two variables is significant, the magnitude of the effect of one variable is always dependent on the level of the interacting variable. Since the interaction effect between the dextrose concentration and storage conditions is shown to be significant, it is important to evaluate the influence of these two variables on 3-in-1 admixtures in a specific combination. Graphic presentation is the most convenient way to interpret the effects of interaction between variables. A lack of interaction between two variables always results in two parallel lines when the means of the combined effects are plotted. Fig. 1 displays the two plots for the mean HIAC data resulting from the four different combinations of dextrose concentration and storage conditions. The nonparallel nature of the two plots is a good indication of a significant interaction between these two variables. For admixtures with a high dextrose concentration (30%), the weight percentage for

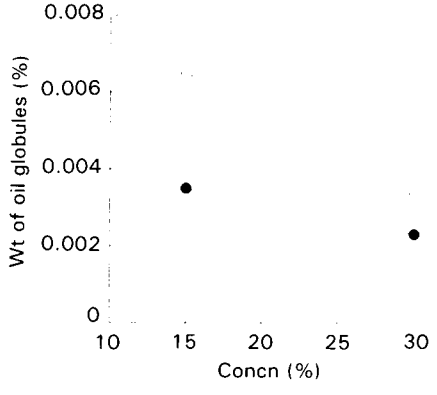

FIG. 1. The combined effect of dextrose concentration and storage conditions on the HIAC data for 3-in-1 admixtures. ○ Stored two days at 5 C and one day at room temperature, ● stored three days at 5 C.

Table 3. The weight percentage of oil globules larger than 5 μm (HIAC) for 3-in-1 admixtures.

	Variables							
	D1				D2			
	M1		M2		M1		M2	
	S1	S2	S1	S2	S1	S2	S1	S2
R1	0·008	0·004	0·006	0·004	0·004	0·003	0·002	0·001
R2	0·006	0·003	0·006	0·002	0·005	0·003	0·003	0·002

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

VALIA DILOVA, VALENTINA SHISHKOVA, *Department of Galenical, Chemical Pharmaceutical Research Institute, 1156 Sofia, 3a Kliment Ohridsky, Bulgaria*

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.

Correspondence: V. Dilova, Department of Galenical, Chemical Pharmaceutical Research Institute, 1156 Sofia, Bulgaria.

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).