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Title: Effects of pH, Temperature, Concentration, and Time on Particle Counts in Lipid-Containing TPN Admixtures

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Douglass J. Stennett

It has been standard practice in the United States to separate lipid emulsions from the other components of total parenteral nutrition due to the reported instability of admixed intravenous lipid emulsions. Some clinicians, however, have combined all TPN components into one container and administered these admixtures to patients without apparent difficulties. Infusion of all nutrients from one container has many advantages. In this study, standard and concentrated admixtures were aseptically prepared using generally accepted guidelines of the nutritional requirements for a 70 Kg patient. Treatments of standard and concentrated admixtures consisted of: 1) storing at 4°C without adjusting the pH; 2) increasing the pH to 6.6 and storing at 4°C; 3) increasing the pH to 6.6 and storing at room temperature. Samples were monitored for three weeks by means of Coulter Counter analysis, pH determinations, and visual observations.

The pH of the admixtures did not change over three weeks. Mean particle counts increased over time for each treatment group. Within treatments, concentrated admixtures had significantly greater particle counts than the corresponding standard admixtures. Within the standard and within the concentrated admixtures, the particle counts were significantly greater for group one than for group three. Particle counts in group two tended to lie between the values of group one and three. Visual signs of emulsion deterioration were greatest in those admixtures in which the pH was not adjusted and occurred earlier in concentrated admixtures.

Effects of pH, Temperature, Concentration, and Time on Particle Counts in Lipid-Containing TPN Admixtures

by

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INTRODUCTION

Intravenous fat emulsions have been firmly established as a source of calories and essential fatty acids in total parenteral nutrition (TPN). In the United States these emulsions have traditionally been administered either through a separate line or through a secondary set which enters the TPN administration set below an in-line filter (1). Lipid emulsions have been separated from nutrient and other intravenous solutions to avoid unstable admixtures (2-6). The development and use of stable lipid-containing TPN admixtures have several potential benefits; however, Intralipid[a] is the only lipid emulsion in the United States currently approved by the F.D.A. for admixing into TPN. The purpose of this study is to investigate the stability of complete TPN admixtures using Aminosyn[b] 7% as the amino acid source and Liposyn[b] as the lipid source.

Lawrence Trissel provides a basic overview of intravenous fat emulsion stability and compatibility and refers heavily to the studies of Black and Popovich (1). These authors found admixtures of Intralipid 10% fat emulsion to be stable for 72 hours when mixed with specific commercial amino acid solutions

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a. Cutter Laboratories, Inc., Berkeley, CA, 94710
b. Abbott Laboratories, North Chicago, IL, 60064

and stored at room temperature (3,6). The final pH of the admixtures approximated the pH of the amino acid solutions employed (FreAmine II[c], pH 6.7; Aminosyn 7%[d], pH 5.4; Travasol 8.5%[e], pH 6.0) rather than that of the lipid emulsion (pH 8.0). Admixtures of Intralipid with solutions of dextrose 10% or 25% U.S.P. resulted in a pH similar to that of the dextrose solution employed (pH 4.0, 4.25 respectively). However, after 48 hours a decrease in the pH was observed accompanied by coalescence of the emulsion. This was explained partially by an initial denaturation of the emulsion's lecithin surfactant at pH's below five with subsequent lowering of the pH by release of fatty acids from the internal phase into the aqueous phase. Amino acids are postulated to protect the emulsions from these deleterious effects of dextrose solutions by acting as a buffer system against pH changes (3,6).

Significant changes in the emulsion's integrity at room temperature were observed 48 hours after the addition of 50mEq of sodium chloride or potassium chloride to 500mls Intralipid. The relative ionic strength of the monovalent cation was reported to be proportional to its ability to cause coalescence. Sodium and potassium are believed to reduce the effective negative charge imparted by the surfactant which is responsible for maintaining oil globule separation (3).

Addition of 6.8mEq calcium chloride or magnesium chloride

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c. McGaw Laboratories, Irvine, CA, 92714
d. Abbott Laboratories, North Chicago, IL, 60064
e. Travenol, Deerfield, IL, 60015

to 500 mls of Intralipid caused immediate flocculation. Amino acids appeared to reduce this degradative effect of divalent cations. Dilution of the cations by addition of amino acid solutions only partially explained the protective action (3,6). Other authors recommend that lipid emulsions be added last when preparing lipid-containing TPN admixtures to reduce emulsion exposure to undiluted harmful solutes and to benefit from the protective action of amino acids (7,8). These solute-solute interactions, and the dilution of solutes which takes place when admixing TPN, demonstrate the importance of studying actual TPN admixtures as they are to be used clinically.

In search of an effective long-term regimen for hospital and home TPN patients, Solassol, Joyeux, Dubois and co-workers administered over 51,000 doses of complete (lipid-containing) TPN admixtures to 2,122 cancer patients (9). Patients received TPN preparations an average of 1.4 months with a range of 10 days to 18 months. Metabolic complications were minimal and were attributed to a poor match between admixture and the patient's nutritional requirement, not the admixture itself. Batches of complete TPN admixtures were tested for physicochemical stability, sterility, and apyrogenicity, and stored at 4°C for up to 5 weeks (9).

Jacobson et al. reported the stability of a specific complete nutrient admixture as well as demonstrated its clinical safety in a controlled short-term study (10). This mixture was stable for at least 24 hours at room temperature

and one week at 4°C when evaluated by microscopic examination and viscosity measurements.

Infusion of complete TPN admixtures from one container has several advantages. Fewer containers result in reduced TPN preparation and set-up time for pharmacy and nursing staff, fewer solution changes, fewer administration sets, single flow rates, and less manipulation of the feed line with its attending risks (11). Complete TPN admixtures have also been shown to increase compliance in home TPN patients (12-13). These advantages may become even more significant when considering that some clinicians believe certain patients, if not all, benefit from daily fat administration (14-15).

On the other hand, complete TPN admixtures have several disadvantages (11). In-line filters cannot be used due to the lipid component of the admixture. There may be considerable waste of fat emulsion as well as other TPN components if complete admixtures are prepared for unstable patients with fluctuating needs. Finally, limited brands, concentrations, and storage conditions have been tested (11). Caution is warranted when admixing lipid emulsions since the toxicity of an unstable emulsion may increase as the lipid particles coalesce to form larger particles (16-17). Atik et al. reported from animal studies that intravenous emulsions which caused adverse reactions contained lipid particles greater than or equal to the size of red blood cells (approximately six microns) (16).

Cutter Laboratories[f] currently is marketing in the United States 1.5 and 3.0 liter evacuated I.V. bags designed for admixing TPN with their brand of fat emulsion, Intralipid. Intralipid was found to be stable when added to commonly used TPN admixtures and stored three days at 5°C then two days at room temperature (7). It was, however, unstable after 24 hours at room temperature in TPN admixtures containing the amino acid solution Aminosyn 7%. Aminosyn 7%/Intralipid TPN admixtures were unstable unless refrigerated or adjusted to a pH of 6.3. Of the amino acid solutions tested, Aminosyn 7% had the lowest pH (5.1) and therefore produced the admixture with the lowest pH.

This study investigates the stability over time of several Aminosyn/Liposyn containing TPN admixtures. Three treatment groups of standard and three treatment groups of concentrated TPN admixtures were studied. The null hypothesis is that all treatment groups have identical mean particle counts at time zero, and the means do not change over time. Or:

Ho: $\mu1=\mu2=\mu3=\mu4=\mu5=\mu6$ Ha: $\mu1\neq\mu2\neq\mu3\neq\mu4\neq\mu5\neq\mu6$

at each time interval and among time intervals where u1, u2, u3, u4, u5 and u6 are the mean particle counts of the six different treatment groups.

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f. Cutter Laboratories, Inc., Berkeley, CA, 94710

METHODS AND MATERIALS

Admixtures

Standard admixture was designed to provide a 24 hour supply of TPN in 2500mls for a 70Kg patient (Table I). Concentrated admixture was designed to provide the 24 hour supply in 1500mls for a 70Kg fluid restricted patient (Table II). Vitamins and trace elements (Table III) were added to meet the American Medical Association/ Nutrition Advisory Group guidelines, and upper values for the range of 24 hour electrolyte requirements in metabolically uncomplicated patients were added to both admixtures (18-20). The proportion of carbohydrate solution to amino acid solution to lipid emulsion in the standard admixture was 1:1:0.5 (V:V:V). The proportion in the concentrated admixture was 1:1:1. Scaled-down versions of these admixtures (125mls standard, 150mls concentrated) were individually prepared in 250mls evacuated glass bottles[g] in a laminar flow hood[h] using aseptic technique. All solutions were injected into the bottles through 5µm filter needles[i] except the fat emulsion which was added last.

Standard and concentrated admixtures were each divided into three treatment groups (Table IV). Group one was admixed

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g. Abbott Laboratories, North Chicago, IL, 60064, lots 62367DM04, 64722DM01, 32620DE5
h. Abbott Clean Air Center, North Chicago, IL, 60064
i. Monoject, St. Louis, MO 63103, lot 213103

TABLE I

STANDARD ADMIXTURE

$D_{50}W$ USP[a]	1000mls
AMINOSYN 7%[b]	1000mls
LIPOSYN 10%[c]	500mls
TOTAL VOLUME	2500mls

2250 NONPROTEIN KCALS
70 GMS PROTEIN

AA:CHO:L (V:V:V) OF 1:1:0.5

a. 500mls partial fill, Abbott Laboratories, North Chicago, IL, 60064, lot 62463DM03
b. 500mls, Abbott Laboratories, North Chicago, IL, 60064, lot 61138DM01
c. 500mls, Abbott Laboratories, North Chicago, IL, 60064, lot 59132DE1

TABLE II

CONCENTRATED ADMIXTURE

$D_{70}W$ USP[a]	500mls
AMINOSYN 10%[b]	500mls
LIPOSYN 20%[c]	500mls
TOTAL VOLUME	1500mls

2190 NONPROTEIN KCALS
50 GMS PROTEIN

AA:CHO:L (V:V:V) OF 1:1:1

a. 500mls partial fill, Abbott Laboratories, North Chicago, IL, 60064, lot 57171DM03
b. 500mls, Abbott Laboratories, North Chicago, IL, 60064, lot 58310DM04
c. 500mls, Abbott Laboratories, North Chicago, IL, 60064, lot 60168DE4

TABLE III

TWENTY FOUR HOUR PARENTERAL REQUIREMENTS
OF ELECTROLYTES, VITAMINS, AND TRACE ELEMENTS

Na[a]	5mEq/100mls (125)[b](75)[c]
K[d]	2mEq/100mls + 20mEq/1000 NONPROTEIN KCALS (95)(74)
PO_4[e]	0.2mM/kg + 10mM/1000 NONPROTEIN KCALS (37)(36)
Cl[f]	2/3 Na REQUIREMENT (83)(50)
Mg[g]	0.1mEq/kg + 8mEq/1000 NONPROTEIN KCALS (25)(25)
Ca[h]	0.4mEq/kg (28)(28)
Ac[i]	AS NEEDED TO BALANCE
VITAMINS[j] AND TRACE ELEMENTS[k] ADDED TO MEET AMA-NAG GUIDELINES OF DAILY REQUIREMENTS	

a. NaCl, 30mls, 4mEq/ml, Invenex, Chagrin Falls, OH, 44022 lot 1878112SF
b. quantity in 2500mls standard admixture
c. quantity in 1500mls concentrated admixture
d. KCl, 10mls partial fill, 2mEq/ml, Abbott Laboratories, North Chicago, IL, 60064, lot 26492DK
e. Na_2HPO_4 & NaH_2PO_4, 15mls partial fill, 3mM/ml, Abbott Laboratories, North Chicago, IL, 60064, lot 35504DK & 86972DK
f. from sodium chloride and potassium chloride
g. $MgSO_4$, 2mls partial fill, 4mEq/ml, Invenex, Chagrin Falls, OH, 44022, lot 1598101RF
h. Calcium gluconate, 10mls partial fill, 0.47mEq/ml, Invenex, Chagrin Falls, OH, 44022, lot 410816NF
i. Potassium acetate, 20mls partial fill, 2mEq/ml, Abbott Laboratories, North Chicago, IL, lot 89648DK01
j. MVC Plus duo-vial, Ascot Pharmaceuticals, Inc., Skokie, IL, 60077, lot 83G243
k. Trace Metals Additive, Abbott Laboratories, North Chicago, IL, 60064, lot 58977DK

and immediately stored at 4°C[j]. Group two was adjusted to pH 6.6[k] with sodium bicarbonate[l] and immediately stored at 4°C. Group three was adjusted to pH 6.6 and stored at room temperature (22-35°C). Lipidless admixtures corresponding to the experimental groups were prepared by replacing lipid emulsion with an equal volume of extemporaneously compounded, double-filtered 0.9% sodium chloride electrolyte solution. These admixtures consisted of four groups: standard; standard, pH adjusted; concentrated; concentrated, pH adjusted. They were monitored only at time zero, thus storage temperature was not a variable.

It required 4.6mls of sodium bicarbonate solution (1mEq/ml) to raise the pH of each 125mls standard admixture and 125mls lipidless standard admixture from the initial pH of 5.5 to a final pH of 6.6. Nine mls were required to raise the pH of each 150mls concentrated admixture and 150mls lipidless concentrated admixture from the initial pH of 5.5 to a final pH of 6.6.

Each treatment group consisted of five bottles. Three were assayed at times 0, 6, 12, 24, 36, 48, 72 hours, one week, and three weeks post-admixing. The pH of the three bottles were measured at time zero, one week, and three weeks

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j. Rhodes Refrigeration, Inc., Portland, Or 97232
k. Corning model 12 pH meter, Corning Glassworks, Corning, NY, 14830
l. 1mEq/ml, 50mls, Abbott Laboratories, North Chicago, IL, 60064, lot 10489DK

post-admixing. A fourth bottle was created solely for pH determinations at every time interval. pH determinations required the use of larger needles which, after repeated puncture, could eventually undermine the integrity of a bottle's rubber stopper resulting in vacuum loss or contamination. A fifth bottle was left undisturbed for visual observation. The four lipidless groups each contained three sampling bottles.

Admixture Assay

A model TA II Coulter Counter[m] fitted with a 70µm aperture[n] was used to monitor particle counts in sample bottles and was calibrated weekly with monosized 3.4µm latex spheres[o]. Channels three through fourteen were used to count particles between 1.6 and 25.4 microns. Reagent grade sodium chloride[p] was accurately weighed[q] and dissolved in distilled water to make a 0.9% solution. The electrolyte was filtered consecutively through a 0.22µm[r] and 0.2µm[s] membrane filter and stored in clean glass containers. This electrolyte solution was used to calibrate the instrument and dilute the

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m. Coulter Electronics, Hileah, FL, 33010
n. Coulter Electronics, Hileah, FL, 33010, 53878/6102032
o. Coulter Electronics, Hileah, FL, 33010, lot 12005
p. American Scientific and Chemical, Portland, Or, 97217, lot SS270
q. Mettler H18, VWR Scientific, Seattle, WA 98124
r. Vented I.V. set with Millipore/Ivex HP 0.22 filter, Abbott/Shaw, North Chicago, IL, 60064, No. 1769, lot 25011DT01
s. Acro 50AS, Gelman Sciences, Inc., Ann Arbor, MI, 48106, lot 3019

TABLE IV

TREATMENT OF ADMIXTURES

TREATMENT GROUP	pH	STORAGE TEMPERATURE
ONE	5.5	4°C
TWO	6.6	4°C
THREE	6.6	(22-35°C)

TABLE V

SPLIT-PLOT ANOVA STATISTICAL DESIGN

source of variation	degrees of freedom
Concentration	1
Treatment	2
C x T	2
Error A	12
Total A	17
Time	7
Time x C	7
Time x T	14
Time x T x C	14
Error B	84
Total B	126
Grand total	143

admixture samples. All glassware used in the experiment was rinsed with distilled water and then the double-filtered electrolyte solution.

Admixture bottles were shaken immediately prior to sampling. A beaker of 200mls prepared electrolyte was flushed four times through the Coulter Counter for background particle counts. Sample (0.25ml standard, 0.1ml concentrated) was then drawn aseptically with an insulin syringe and added to the remaining 188mls of prepared electrolyte in the beaker to make either a 1:752 dilution of standard admixture or a 1:1880 dilution of concentrated admixture. Particles counts in 0.5ml of this dilution were determined four times. Sample particle counts at time zero served as controls for sample particle counts at later time intervals. Further dilutions were necessary for some samples at later time intervals in order to minimize coincidence error.

Statistics

For each sample at each time interval, the four background flushes were averaged and this mean subtracted from each of the four sample readings. Particle counts from the various dilutions were multiplied by the appropriate coefficients to make all data comparable with the data from 1:752 dilutions. A split-plot ANOVA (analysis of variance) for repeated measurements was performed on the adjusted data using the Statistical Package For the Social Sciences available on the

Cyber 170/720 at Oregon State University (21). The design was a two factorial whole-plot of concentration and treatment with a split-plot of time (Table V). In addition, the LSD test (least significant difference) was utilized to assess the significance of the difference between two mean particle counts from different treatments or concentrations (21).

Three-week data were omitted from statistical analysis for several reasons. ANOVA statistical analysis assumes equality of error variances for different treatments. The Welch statistic and Brown-Forsythe statistic which do not assume the equality of variance are limited to one-way ANOVA (21-22). Variance in this study tended to increase with increasing means. Three-week data had the largest means and thus error variance. Transformation of data (square root of data, log of data) sometimes can ameliorate the problem of unequal variance. In this study the results of split-plot ANOVA using transformed data (square root, log) were similar to the results of split-plot ANOVA using original data. For this reason all reported statistical analyses are based on original data.

RESULTS

The weekly calibration of the Coulter Counter demonstrated little or no channel drift. The coefficient of variation for particle counts within a sample's four runs through the Coulter Counter was small. The mean coefficient of variation for samples at time zero, one, and three weeks was 3.1% ± 2.4, 1.8% ± 2.0, and 2.2% ± 2.3, respectively.

The pH of the standard and concentrated admixtures did not change over the three weeks. No measurable depth of flocculation was present in any sample at any time; however, other visual signs of emulsion deterioration were manifest. In general, these signs were more obvious in admixtures whose pH had not been adjusted to 6.6 as well as in all admixtures at later time intervals. Clear rings along the meniscus of standard admixtures formed on the bottle surface 96 hours post admixing. Over time, these rings became thicker, yellow, oily in nature, and harder to dissipate upon shaking. Oil floating freely on the surface of standard admixtures was obvious by three weeks.

Rings started forming in concentrated admixtures 48 hours post-admixing. Free-floating oil was obvious by one week. Precipitated adhesions were seen 36 hours post-admixing in the concentrated admixtures with pH of 6.6. The precipitate was more abundant in those bottles stored at room temperature. No precipitate formed in the concentrated admixtures with pH of 5.5.

Mean particle counts generally increased over time for each treatment group (Tables VI and VII) (Figure 1). All variables in the split-plot ANOVA were statistically significantly different ($p < 0.001$). In addition, there was a significant three-way interaction between treatment, concentration, and time ($p < 0.001$). This rendered ANOVA impractical for individually assessing the effects of these variables on mean particle counts. The LSD test was utilized to assess the difference between two mean particle counts (Tables VIII and IX). A difference between two mean particle counts was significant if greater than: 13705, $p < 0.05$; 18169, $p < 0.01$; 19806, $p < 0.005$; 23398, $p < 0.001$.

By twelve hours, the mean particle counts in each of the three concentrated treatment groups were significantly greater ($p < 0.05$) than the corresponding means at time zero. Group one of the standard admixtures was significantly greater by 24 hours. Group two and three of the standard admixtures were not significantly greater until one week.

It was apparent that within treatment groups at most time intervals, concentrated admixtures had significantly greater particle counts than the corresponding standard admixtures (Table VIII). The extent to which this reflected a concentration effect as opposed to an effect of other variables or interactions is speculative. It was apparent that by 24 hours group one had significantly greater particle counts than group three within standard and concentrated admixtures (Table IX). Again, the extent to which this difference was due to

TABLE VI

MEAN PARTICLE COUNTS VS TIME, STANDARD ADMIXTURE

TIME (hrs)	GROUP 1[a](n=3) mean	sd	GROUP 2[b](n=3) mean	sd	GROUP 3[c](n=3) mean	sd
0	4321	1438	3860	856	7511	1551
6	7544	317	4837	1897	8250	2868
12	15206	1671	5983	1867	6924	2061
24	22779	2184	12163	1884	7452	2594
36	33132	11204	10562	2060	7509	2055
48	41957	16580	11105	2824	7854	1680
72	56964	18482	12923	2298	8050	1124
1wk	47663	15925	31359	18022	21500	6815
3wk			34119	30557	14322	5465

a. pH 5.5, refrigerated 4°C
b. pH 6.6, refrigerated 4°C
c. pH 6.6, room temperature

TABLE VII

MEAN PARTICLE COUNTS VS TIME, CONCENTRATED ADMIXTURE

TIME (hrs)	GROUP 1[a](n=3) mean	sd	GROUP 2[b](n=3) mean	sd	GROUP 3[c](n=3) mean	sd
0	16385	1676	22926	2711	15825	4290
6	39137	14164	35523	1562	23352	5855
12	53189	6487	51529	5902	30056	3526
24	56560	2543	65409	3604	23161	2515
36	68508	9839	77641	6595	29182	7118
48	91163	19080	85744	1388	29142	3142
72	102797	33323	101238	12142	29919	3633
1wk	133201	38105	94888	13255	27299	8930
3wk	247951	66745	102904	13063	87798	32768

a. pH 5.5, refrigerated 4°C
b. pH 6.6, refrigerated 4°C
c. pH 6.6, room temperature

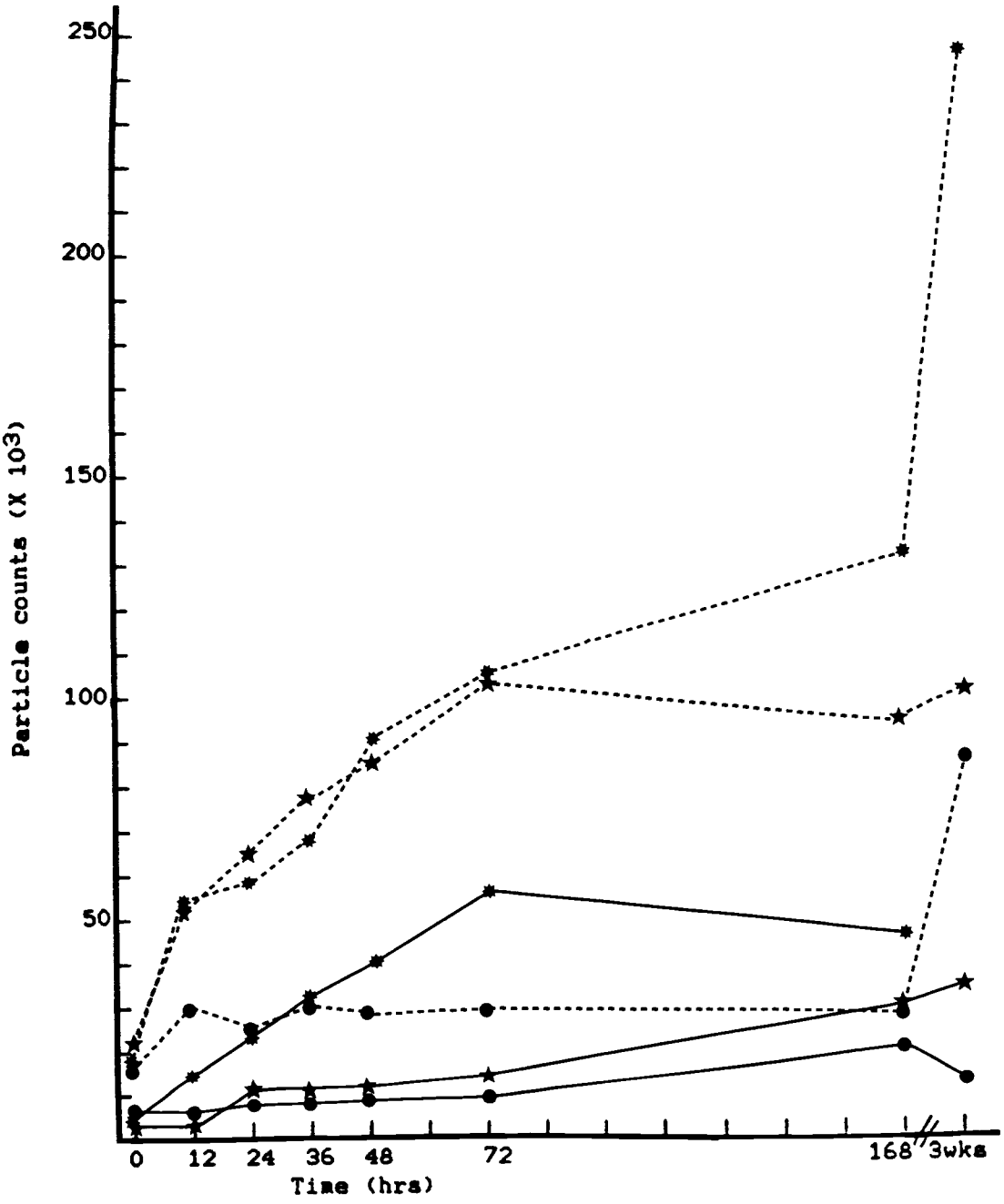

Figure 1. Mean particle counts within treatment groups vs time

TABLE VIII

STATISTICAL SIGNIFICANCE OF DIFFERENCE[a] BETWEEN STANDARD AND CONCENTRATED ADMIXTURES

	difference between standard and concentrated admixtures within:		
time (hrs)	group 1[b]	group 2[c]	group 3[d]
0	n[e]	***[g]	n
6	****[h]	****	*[f]
12	****	****	***
24	****	****	*
36	****	****	***
48	****	****	***
72	****	****	***
1 wk	****	****	n

a. LSD test for significance of difference between two means
b. pH 5.5, refrigerated 4°C
c. pH 6.6, refrigerated 4°C
d. pH 6.6, room temperature
e. no significant difference
f. $p < 0.05$
g. $p < 0.005$
h. $p < 0.001$

TABLE IX

STATISTICAL SIGNIFICANCE OF DIFFERENCES[a] BETWEEN TREATMENT GROUPS

STANDARD ADMIXTURE	time (hrs)							
difference between:	0	6	12	24	36	48	72	1 wk
group 1[b] and 2[c]	n[e]	n	n	n	***	****	****	*
group 2 and 3[d]	n	n	n	n	n	n	n	n
group 1 and 3	n	n	n	*	****	****	****	****
CONCENTRATED ADMIXTURE								
difference between:								
group 1 and 2	n	n	n	n	n	n	n	****
group 2 and 3	n	n	***[g]	****[h]	****	****	****	***
group 1 and 3	n	*[f]	***	****	****	****	****	***

a. LSD test for significance of difference between two means
b. pH 5.5, refrigerated 4°C
c. pH 6.6, refrigerated 4°C
d. pH 6.6, room temperature
e. no significant difference
f. $p < 0.05$
g. $p < 0.005$
h. $p < 0.001$

different treatments or an interaction of variables cannot be determined.

Although not consistently significant in both standard and concentrated admixtures, there was a trend for group two to have higher counts than group one. The only difference between these treatment groups was the storage temperature. Particle counts from the lipidless admixtures were indistinguishable from background particle counts. Supplemental assays of these lipidless admixtures (stored at room temperature) at one week demonstrated no change.

DISCUSSION

Channels three through fourteen of the Coulter Counter were monitored in order to avoid the extremely high particle counts at lower channels which would require greater dilutions of the admixtures, and to stay within the accurate range of the 70μm aperture. The instrument was calibrated so that the lower edge of channel seven monitored particles of four microns. Channel eight monitored the size range which included six microns. The mean particle size (internal phase) in Liposyn is approximately 0.4 microns. If an emulsion were to break, one would expect a change in particle counts as oil globules coalesced, grew in size, and passed through the monitored size range. Although Coulter Counters have been used to assess lipid emulsion particles, one must keep in mind that counts may be indicative of particles other than fat.

Calcium and magnesium can produce insoluble soaps in the presence of high molecular weight fatty acids (23). Liposyn contains predominately triglycerides; however, as the emulsion deteriorated small amounts of fatty acids may have leaked into the aqueous phase and combined with calcium and/or magnesium to form insoluble soaps. It is possible that the reported protection offered by amino acids against the deleterious effects of divalent cations may be due to soluble complexes formed between the cations and anionic amino acids. Calcium is known to bind with proteins and complex with anions such as citrate and phosphate (20).

The precipitate observed in the concentrated admixtures was most likely calcium phosphate. Calcium phosphate is more soluble at a pH below six and at temperatures below 75°F (20). Dibasic calcium phosphate ($CaHPO_4$)is very insoluble compared to monobasic calcium phosphate (30mg/dl and 1800mg/dl, respectively). Monobasic calcium phosphate ($Ca(H_2PO_4)_2$) is the predominant form found at low pH. As the pH increases, more dibasic phosphate is available to bind with calcium and precipitate. Increases in temperature increase the dissociation of calcium gluconate, thus, providing more calcium ion to bind and precipitate as dibasic calcium phosphate (24). In concordance with the above, no precipitate was observed in the admixtures with a pH of 5.5 whereas greatest precipitate was observed in those with a pH of 6.6 stored at room temperature. It may prove beneficial to divide a 24 hour supply of complete TPN into several containers and separate the calcium from the phosphate as is commonly done in traditional TPN.

The amount of precipitate observed in a bottle was not proportional to the bottle's particle count. Within the concentrated admixtures, the treatment group with the greatest precipitate had the the least particle counts. The treatment group without precipitate had the highest particle counts and the most obvious signs of emulsion deterioration. The opaque complete TPN admixtures may have posed a visibility problem for observing suspended and fine precipitation. The visible precipitation may have been out of the monitored size range.

It is possible that the high particle counts were derived from precipitated matter still in suspension and in the monitored size range. It is also possible that high particle counts reflected lipid emulsion coalescence since high particle counts were observed in conjunction with greater visual signs of emulsion instability.

Cutter Laboratories was able to produce stable complete TPN admixtures with Aminosyn 7% by either increasing the final pH to 6.3 or refrigerating the admixture at 5°C. The admixtures of Solassol and co-workers were refrigerated at 4°C and had a pH of 6.3. From these reports one may have anticipated that those admixtures with increased pH which were stored at 4°C would be most stable with least particle counts in both standard and concentrated preparations. Although not consistently statistically significant, the results suggest that admixtures with increased pH which were stored at room temperature had fewer particle counts than those with increased pH which were stored at 4°C. Further study may be needed to determine the optimum storage temperature of these admixtures. The results between these two treatment groups for concentrated admixtures was not as clear. Those bottles simply stored at 4°C had the greatest particle counts (at most time intervals) and the most obvious signs of emulsion deterioration. This suggests that pH may have a greater role in maintaining emulsion stability than refrigeration. Interestingly, particle counts at later time intervals decreased in some bottles. This may have represented particles leaving the size range monitored

and may partially account for the increased variance seen at later time intervals.

A supplemental study was performed with Liposyn 10%. 0.05ml Liposyn 10% was added to 188mls double-filtered electrolyte solution which had been previously assayed for background particle counts. This Liposyn dilution contained the same quantity of Liposyn 10% as that found in the 1:752 dilutions of standard admixture with double-filtered electrolyte. The mean particle counts in 0.5ml of this dilution was 635. The mean particle counts in 0.5ml of standard admixture dilutions at time zero were not statistically greater than 635; however, the large difference in particle counts suggests that the degradative process may begin immediately upon admixture of Liposyn with TPN components.

As seen in figure two, there was no obvious shift in particle counts from one channel into another over time. Particle counts increased proportionately in the various channels. Although figure two is derived from the data of one bottle (concentrated, stored at 4°C), it is representative of the type of curves seen in the other bottles. As total particles increased, so did the number of particles greater than six microns. At some point in time, one would expect the number of large particles to affect the safety of the admixtures. From particle counts alone, one may anticipate this time to occur earlier in concentrated admixtures and in admixtures with lower pH. The precipitation observed in

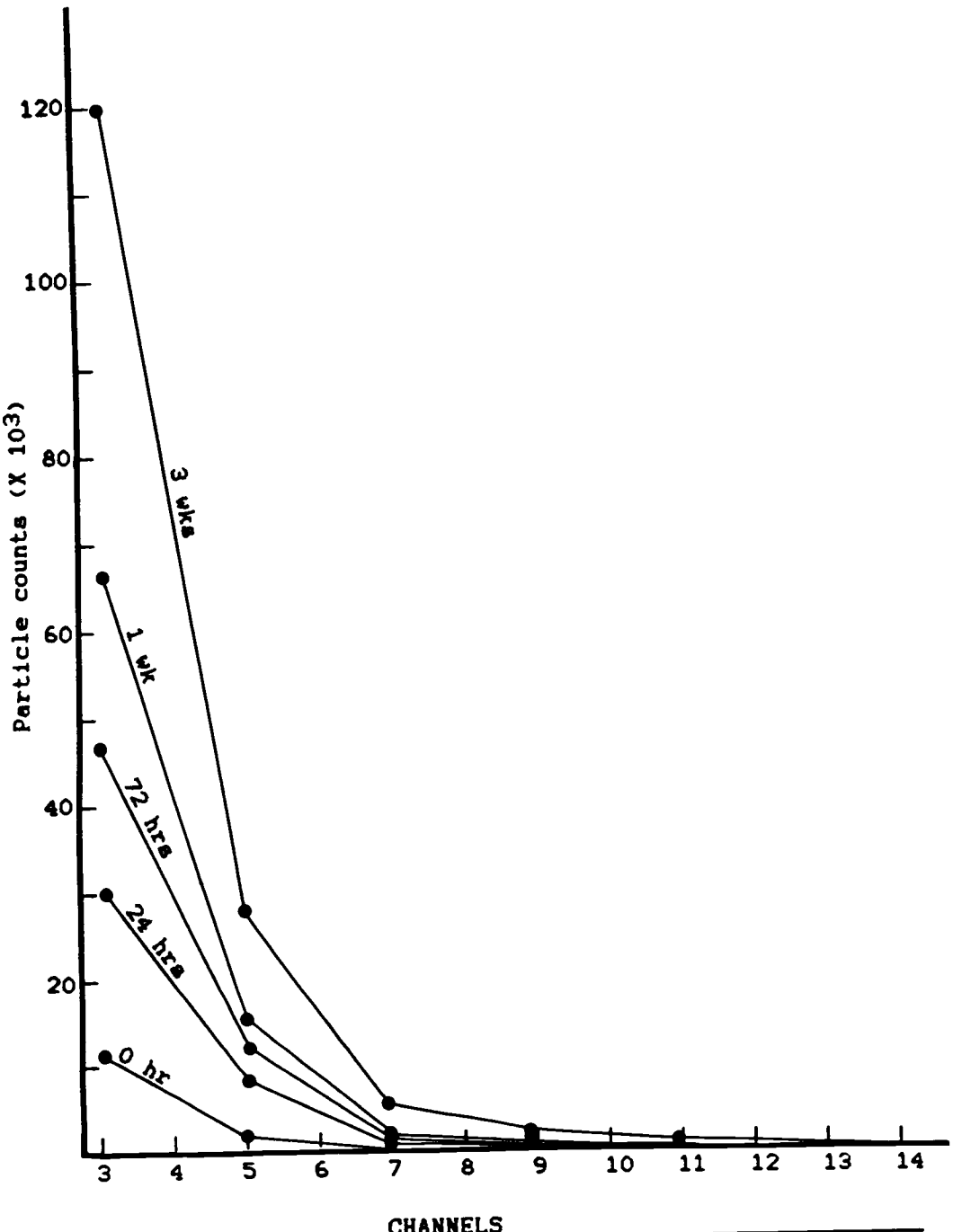

Figure 2. Channel particle counts within a bottle over time

concentrated admixtures and the decrease in particle counts in some emulsions with visual signs of deterioration emphasize that particle counts may not always be valid predictors of an emulsion's safety or stability. Likewise, the increase in particle counts prior to visual signs of instability emphasize the unreliability of visual observations for determining safety or stability.

It is suggested in the studies of Atik et al. that emulsions with few particles greater than four microns will cause the least acute adverse reactions, and that emulsions containing particles greater than six microns will almost invariably cause acute adverse reactions such as hypotension, and/or pulmonary hypertension, and acidosis (16). Interestingly, the internal diameter of a capillary ranges from four to eight microns (23). The particle size which may cause chronic adverse reactions upon repeated infusions may prove different from that which causes acute adverse reactions. A slight insult may be undetected initially and become significant with repeated infusions. Further study is needed using animal models.

SUMMARY

Within treatment groups, concentrated admixtures had significantly greater particle counts than the corresponding standard admixtures. Admixtures stored at room temperature with an adjusted pH of 6.6 had significantly less particle counts than those simply stored at 4°C. They also demonstrated slower onset and severity of visual signs of lipid emulsion instability. By one week, all admixtures had significantly greater mean particle counts than at time zero. For these reasons, it is necessary to reject the null hypothesis. A significant three-way interaction between time, concentration, and treatment prevented the individual assessment of a variable's ability to affect particle counts; however, it added to the evidence that lipid-containing TPN admixtures must be studied as they are to be prepared and stored clinically. No attempt was made to study the stability of non-lipid TPN components.

Emulsion deterioration appears to be a continual process which may begin immediately post-admixing. Admixtures stored at room temperature had the least particle counts; however, it is standard practice to refrigerate TPN admixtures in order to delay the growth of microorganisms. Increasing the pH of an admixture with sodium bicarbonate may be less economical than using an amino acid solution with a higher pH. The number of particles greater than six microns was proportional to the total number of particles. It is reasonable to assume that the

concentrated admixtures tested would be unsatisfactory for clinical use due to the early increase in particle counts and the precipitation. If, with further study, standard admixtures prove to be clinically safe at time zero, it is recommended that they are prepared immediately prior to administration.

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