A STUDY OF PARTICULATE CONTAMINATION IN 0.9% SODIUM CHLORIDE INJECTION IN POLYVINYL CHLORIDE PLASTIC CONTAINERS

BY

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INTRODUCTION

The dosage forms of drugs administered by the parenteral route are known as "Injections". Although these dosage forms are relatively new, their importance in therapy has increased rapidly. Injections require the highest purity standards because the parenteral route overcomes the protective barriers of the human body, i.e., the skin and the mucous membrane. It is important that injections be free, if possible, of particulate matter which has been defined [1] as "extraneous, mobile, undissolved substances, other than gas bubbles, unintentionally present in parenteral solutions". Particulate matter in parenteral solutions can produce psychological effects on the user since the visible particles would suggest that the solutions are of inferior quality. In addition, particulate matter can be actually harmful to the patient. It has been shown [3,5] that foreign particulate matter can produce capillary and arterial granulomas, and vascular bastosis in rabbits. In man, it has been shown that it may produce a chronic fibrous pneumonitis, pulmonary vascular bastosis, pulmonary microemboli and thrombi, in the cases in which intravenous fluids had been given before death. This suggests that the harmful effects may be related

to the presence of particulate matter in intravenous fluids.

There were several attempts to identify the particulate matter in parenteral solutions stored in glass containers [2,3,24,29]. Garvan and Gunner [2,3] used the dark-ground illumination method, the membrane filtration technique and the microscopic method to differentiate particulate matter in parenteral solutions. The particles were catagorized into: whole-rubber particles; chemical particles such as crystals; cellulose fibers; fungi; and miscellaneous objects such as starch granules. They found that most of the particles came from the rubber closures. The discovery that rubber closures were the source of particulate matter was reinforced by the work of Vessey and Kendall [8] and the work of Ernerot et al. [15]. It has also been shown that the particulate matter could originate from the break-down on the inner surfaces of the bottles [30].

Since glass containers for parenteral solutions have contributed to contamination which can be harmful to the patient, the polyvinyl chloride plastic containers have been developed to solve the contamination problems. These containers are collapsible, so the need for air tubes and other venting during infusion is eliminated, and, therefore, "air-born contamination" is reduced [20,36]. In general, a formulation

of a flexible polyvinyl chloride plastic contains apploximately 60% resin, 30% plasticizer, with the remainders which are stabilizers, antioxidants, and lubricants [31]. Davis et al. [12,13,32], using the membrane filtration technique and the microscopic method, reported that the solutions in plastic containers contained fewer particles than those in glass containers. Needham et al. [11], using the Coulter Counter technique, found that the plastic bags stored under quiescent conditions had a lower initial particle count than the glass bottles. By this same technique, it was reported in all cases that glass bottles produced fewer particles than did plastic bags under similar conditions [4,25].

It was found [14-18] that particle counts in glass bottles and plastic bags under non-agitated conditions were lower than the counts after shaking. Groves [16] has discussed that the increase in particle counts in glass bottles after shaking could be due to the air bubbles in the solution which would be counted as particles. After that, Ernerot [14] proved that the air bubbles had no effect on the results even if the particle counts were taken immediately after an intense shaking. He attributed the increase in particle counts in glass bottles to the break- down of particle agglomerates and the release of particles from the rubber closure and the

glass surface [14,15]. Blanchard et al. [17] reported that the increase in the number of particles in glass bottles after mild agitation was due to the particles that were dislodged from the inner surface of the container, and that the increase after vigorous agitation was due to the disintegration of large agglomerates into smaller sized particles.

In the case of polyvinyl chloride plastic containers, Whitlow et al. [4,25] demonstrated that the particulate matter in the solution could be generated by continuous shaking. They found that the increase in the particle counts was due to the plasticizer, di-2-ethylhexyl phthalate [DEHP], that leached from the plastic bags. Darby [18] attributed this increase in particle counts to the microspheres of liquid plasticizer which were generated after severe shaking. The study about the migration of ingredients from the container to the contents had been done previously by Autian et al. [22]. They studied the blood bags containing Anticoagulant Acid Citrate Dextrose [ACD] Solutions and demonstrated that microquantities of DEHP from the plastic bags had entered the ACD solutions. If the parenteral solution is given in large quantities to patients over periods of time, the microquantities of ingredients could produce subtle toxicity. The contaminants from the polyvinyl

chloride blood bags were also shown to be toxic to monolayers of L-cells [strain 929] and chick embryo cells. The toxicity of phthalate esters, including DEHP, has been reviewed [6,7]. These authors concluded that DEHP had an extremely low degree of acute toxicity to animals and man. Their research at the cellular level which determined the effects of phthalates on growth inhibition of mouse fibroblasts [L-cells] in tissue culture did indicate that the higher molecular weight ester had an extremely high intrinsic toxicity.

Polyvinyl chloride plastics containing DEHP have been used in industry and medicine for many years. Although the advantages of flexible polyvinyl chloride plastic bags were well-known, much less was known on the possible subtle toxic effects that low doses of phthalate esters might have on man. Furthermore, the information about contamination from PVC plastic bags has not been adequately investigated. Therefore, it is important that these PVC plastic bags be studied extensively.

The objectives of this study are:

[1] To determine the effect of agitation on the generation of particulate matter in PVC plastic bags and in glass bottles.

[2] To identify and determine the quantities of the

plasticizer entered 0.9% sodium chloride solution stored in PVC plastic bags.

[3] To determine the solubility of the plasticizer, DEHP, in 0.9% sodium chloride solution.

EXPERIMENTAL

A. Materials.

Sodium chloride crystals [A.R.grade, Mallinckrodt], isooctane [Spectro grade, Aldrich] and di-2-ethylhexyl phthalate [Aldrich] were all used as received. Carbon tetrachloride [A.R.grade, Mallinckrodt], n-hexane [Skelly-B] and water were distilled before use. The commercial Sodium Chloride Injection USP in 1000 ml viaflex containers [Travenol Laboratories] and in 1000 ml glass containers [Travenol Laboratories, and Baxter Laboratories] were examined. The empty 1000 ml viaflex containers [Travenol Laboratories] were used and filled with particle-free normal saline solution which was prepared by filtering the normal saline solution through a 0.45 um millipore membrane filter.

The polyvinyltoluene latex [Dow Diagnostics, lot 3M5C] of mean particle size 2.02 um dispersed in membrane-filtered normal saline solution. Dilutions of the latex were made with further amounts of the normal saline solution such that the Coulter Counter can count at a low coincidence factor [1% coincidence]. This latex dispersion was ultrasonified for 10 minutes to minimize the floccing of latex particles.

B. Instruments.

The Electrozone/Celloscope, model 111 LTH/ADC, fitted with a 24-um orifice was used to determine the number and sizes of particles present in the solutions. The instrument was connected with the Computerized Particle Data Processing MP 12--4K program PD/A-22. The Electrozone/Celloscope was calibrated using the latex dispersion as a standard calibration material. As a particle passes through the aperture, it changes the resistance between the electrodes and produces a voltage pulse having an amplitude proportional to the particle volume. The series of pulses is electronically scaled at the Electrozone/Celloscope and is converted to a logarithmic basis by the Particle Data System. Via programmed computer instructions, the particle data can be manipulated and printed out as desired.

All spectra were obtained on the Cary Recording Spectrophotometer, model 14. This instrument was fitted with a thermostatted cell compartment and a circulating water bath which maintained the compartment temperature to $\pm$ 0.1°C.

C. Methods.

[a] Determination of Particulate Matter

[1] Particle Counting

Particle counting was performed with the Electro-

zone/Celloscope connected with the Computerized Particle Data System and equipped with a 24-um orifice with 50 ul metering unit. The test solution was placed in a very clean beaker to minimize the possibility of contamination. During the experiments, the solution in the beaker was constantly stirred by a stirrer attached to the instrument to maintain a uniform suspension of particulate matter. the number of particles per ml exceeding the following diameters were determined: 1, 2, 3, 4, 5 um.

The commercial Sodium Chloride Injection USP in viaflex plastic bags [Travenol Laboratories] and in glass bottles [Travenol Laboratories and Baxter Laboratories] were turned upside-down carefully 20 times without air bubbles forming, to get an even distribution of sedimenting particles in the solution before counting. In the shaking trials, each bottle was shaken on a gyrotory shaker [Model G-10, New Brunswick Scientific Co., New Brunswick, N.J.] at a rate of 230 rpm for 20 minutes. Each viaflex plastic bag of the same batch was shaken at different periods of time: 10, 20, and 30 minutes. After each agitation, both bottles and plastic bags sat undisturbed at least 2 minutes before the particle counts were taken. This interval gives any air bubbles forming during agitation time to disappear.

After completion of this set of readings, each plastic bag was stored in an undisturbed condition for more than 48 hours; then the particle counts were taken again, without correction for the diminishing volume in the plastic bags.

The freshly-prepared, particle-free normal saline solution was tested for particulate matter before filling the empty viaflex containers. The particle counts were performed 10 minutes after filling the bags. In the shaking trials, the bags were shaken for 10 minutes on the gyrotory shaker right after the solutions were placed in these bags. The particle counts were taken after the bags sat undisturbed for 2 min.

[2] Microscopic Method

The apparatus was set up in the same way as that used in the second supplement, USP-NF [1]. The grid membrane filter [0.45 um pore diameter] was held in a verticle position and was washed both sides with particle-free isopropyl alcohol [filtered through 0.45 um membrane filter before using]. The membrane was allowed to dry and placed on the filter holder base. The filtering funnel was washed with particle-free normal saline solution and installed on the base. One group of the commercial Sodium Chloride Injection USP in viaflex containers [Travenol Laboratories] was shaken on the gyrotory shaker for 5 minutes and the other group for 20 minutes. A

sample of 200 ml from each bag was taken for particle counting. The rest of the solution was filtered through the prepared membrane filter and assembly by applying the vacuum. The inner wall of the funnel was rinsed with the particle-free normal saline solution. Then, the membrane filter was removed, placed in a plastic Petri-slide and allowed to dry with the cover of the Petri-slide. All this procedure was performed under a laminar flow hood equipped with ultra-HEPA [high-efficiency particulate absolute] filters. The slide was placed on the micrometer stage of the microscope. The particles on the membrane filter having effective linear dimensions equal to or greater than 2 um and equal to or greater than 5 um were counted under 100x magnification with the incident light at an angle of 10° to 20°.

[b] Isolation and Identification of the Plasticizer in Flexible Polyvinyl Chloride Container of Sodium Chloride Solution

[1] Thin Layer Chromatography

Thin layer chromatography was done to identify the plasticizer used in viaflex containers. The silica gel plate were prepared by the method of Lees and De Muria[19]. Ten ml of methanol was added to a viaflex container [Travenol Laboratories] to dissolve the plasticizer. This solution was

spotted on a silica gel plate; dibutyl phthalate solution and di-2-ethylhexyl phthalate solution were spotted on either side of this solution. Ethyl acetate - n-hexane [1:9] was used as the solvent system according to the method of Autian et al [22]. This silica gel plate was developed and then visualized with ultraviolet light.

[2] Extraction Method

The Sodium Chloride Injection USP in viaflex containers [Travenol Laboratories] was examined directly by measuring the absorbance on the Cary 14 Spectrophotometer, using particle-free normal saline solution as the reference solvent. The results showed that very small concentrations of the plasticizer had entered the solutions. For this reason, it was decided to extract sodium chloride solutions in plastic bags with a suitable organic solvent. The di-2-ethylhexyl phthalate [DEHP] exhibits absorption peaks at 224 and 275 nm but absorbs more at 224 nm. At first, the wavelength at 275 nm was chosen and carbon tetrachloride was used for extraction according to the method of Autian et al [22]. The extraction procedure was performed by using the whole volume of sodium chloride solution from 1000 ml viaflex containers. This solution was transferred into a separatory funnel and was extracted four times with - 100, 50, 50, 50 ml - of redistilled,

reagent grade, carbon tetrachloride. The combined carbon tetrachloride fraction was evaporated using the evaporator. The residue was dissolved with 10 ml isooctane and was assayed spectrophotometrically.

It was found later that the concentration of the plasticizer entering the sodium chloride solution in plastic bag was far below expected values and the wavelength at 275 nm was not appropriate in this study. Therefore, the measurements at the wavelength of 224 nm were made. Another problem arose from the absorption of carbon tetrachloride at the wavelengths lower than 265 nm, which is the cutoff wavelength of this solvent. Even a trace of carbon tetrachloride left after evaporation could have caused this absorption problem. To overcome this problem, it was decided to extract the sodium chloride solution and dissolve the residue with redistilled n-hexane using the same procedure as described above. N-hexane was selected because it possesses no absorption spectra at wavelengths longer than 210 nm.

[3] Spectral Studies

The residue dissolved in n-hexane from the extraction method was assayed spectrophotometrically on a Cary Model 14 using freshly-prepared normal saline solution extracted by the same procedure as the reference solvent.

The standard curve of DEHP in redistilled n-hexane was linear over the concentration range investigated. The molar absorptivities of DEHP at 224 and 275 nm determined from the slopes were 9.370×10^3 liter/mole-cm and 1.270×10^3 liter/mole-cm, respectively. Therefore, unknown concentrations of DEHP in viaflex containers could be obtained from the absorbance at 224 nm by inspection of the standard curve.

[c] Solubility Experiments

Stock solutions of DEHP were prepared by volumetric dilution of weighed quantities of DEHP with methanol. The desired concentrations of DEHP in normal saline solution were obtained by volumetric dilution of these stock solutions with normal saline solution. For the solubility studies, each 150 ml of different concentrations of DEHP in particle-free normal saline solution was placed in a 200 ml volumetric flask. The flasks were covered tightly with glass stoppers and placed in the constant temperature water bath at $25 \pm 0.1^{\circ}$ C. Then, they were subjected to continuous shaking for 2 days and stored undisturbed for 1 day. After this time, the excess amounts of DEHP on the surface of solutions were aspirated out. A 100 ml aliquot from each flask was withdrawn with a clean pipet and transferred to a separatory funnel. Each aliquot was extracted four times with - 50, 25, 25, 25

ml - of redistilled n-hexane using the same procedure as described earlier, and assayed spectrophotometrically at 224 nm.

The solubility of DEHP in normal saline solution could not be obtained from this procedure owing to the problem arising from the excess of DEHP remaining in the solution. Therefore, the Electrozone/ Celloscope- Computerized Particle Data System was applied to determine the solubility. Different concentrations of DEHP in particle-free normal saline solution were prepared as described above. After shaking and storing undisturbed, the flasks were turned upside-down carefully 20 times without air bubbles forming and the particle counts were taken in the same manner as described earlier. The number of particles per ml of DEHP solutions at different concentrations were plotted against the concentrations and the solubility of DEHP was obtained from this plot.

RESULTS

A. Determination of Particulate Matter

The observed particle size distributions and the total number of particles per ml of Sodium Chloride Injection USP in glass containers are given in Table 1. Since the main objective of this study was to examine the particulate matter generated in flexible plastic containers of normal saline solutions, only two groups of Sodium Chloride Injection USP in glass containers from two manufacturers were studied and compared. In each size range determined before shaking, there was little difference in the distribution of particles between the solutions from the two manufacturers, as shown in Figure 1. When studying the changes resulting from the shaking effect, only the solutions from one manufacturer [Baxter Laboratories] were used. It can be seen that shaking for 20 minutes causes the particle content to increase significantly and causes a little change in particle size distribution.

Table 2 shows the particle size distribution and the total number of particles per ml of Sodium Chloride Injection USP in flexible plastic containers [Travenol Laboratories]. The results given in Table 2 and Figure 2 illustrate

that shaking on the gyrotory shaker causes the particle content to increase very strongly as the shaking time increases. After shaking and taking the particle counts, the solutions are stored under undisturbed conditions for more than two days and the particle counts are taken again. The results show that the number of particles seems to decrease at all size ranges determined for the 10 minute shaken solution and seems to be about the same as that obtained before storage of the 20 minute shaken solution, as shown in figure 2. The particle size distribution does not change significantly from the shaking for different periods of time.

It will be seen from the results given in Table 3 that the total particle count of fresh, membrane-filtered normal saline solution is 1342 particles/ml. After this solution is put into the flexible plastic bag [viaflex container], the total particle count rises from 1342 to 4038 particles/ml. After the plastic bag is shaken for only ten minutes, the total particle count increases tremendously from 4038 particles/ml before shaking to 488,120 particles/ml after shaking. The particle size distribution is not much affected by the shaking, as shown in Figure 3.

The identification of the particles generated in flexible plastic bags of normal saline solution was performed

by microscopic method. The results given in Table 4 show that the total number of particles per ml increases significantly when the shaking time increases but the particle size distribution does not alter much from the shaking. These results confirm the results shown in Table 2 and 3.

After filtration of the shaken solution, the total particle count drops off very strongly. For the 5 minute shaken solution, the particle count is 1740 particles/ml as compared to 57,792 particles/ml before filtration. For the 20 minute shaken solution, the particle count decreases from 115,172 to 1860 particles/ml after filtration, which is comparable to the particle count of 1342 particles/ml of fresh, membrane-filtered normal saline solution [see Tables 3 and 4]. The particle size distribution of these solutions is shown in Figure 4. When the number of particles on the membrane filter is counted under 100 x magnification with the incident light at an angle of 10° to 20°, very few particles are found, as shown in Table 4. This result suggests that those particles can be the plasticizer.

B. Isolation and Identification of the Plasticizer

The thin layer chromatography result in Figure 5 shows that the plasticizer in viaflex containers is di-2-ethyl hexyl phthalate [DEHP]. It is reported that DEHP is the

plasticizer used in viaflex containers [20], which supports this observation.

An extraction and spectrophotometric assay procedure was developed in this study to determine the concentration of DEHP entering the sodium chloride solution in viaflex containers. The results are given in Table 5. It is obvious that the concentration of DEHP entering the solution increases as the storage time increases. Storage time is the time after the solution is put into the plastic bag. The concentration of DEHP found are in the range of 3.75×10^{-8}M [for freshly prepared normal saline solution stored in plastic bags for 1 hour] to 5.2×10^{-7}M [for commercial bags shaken for 20 minutes]. The commercial product [Sodium Chloride Injection USP in viaflex containers, Travenol Laboratories] shows higher concentration of the plasticizer than the solution prepared in this laboratory. It should be mentioned that the sodium chloride solution exhibits its own absorption at 224 nm. Therefore, freshly prepared normal saline solution undergoing the same procedure as that of the sample should be used as the reference solution. The effect of shaking sodium chloride solution prepared in this laboratory for 10 minutes and commercial bag for 20 minutes was studied. The results of shaking were compared to those

of storing in plastic bags under undisturbed conditions for the same periods of time, as shown in Table 5. It is seen that the concentration of DEHP entering the shaken solution is a little higher than that of the solution without shaking.

C. Solubility Determination by the Coulter Counter Technique

The solubility of DEHP in normal saline solution is determined, using the Coulter Counter technique. The number of particles obtained at different concentrations of DEHP in membrane-filtered normal saline solutions before and after equilibration at $25 \pm 0.1^{\circ}$ C is given in Table 6. It is obvious that when the saturated solution of DEHP in sodium chloride solution is attained, the number of particles increases markedly, as shown in Figure 6. Then, the solubility of DEHP in sodium chloride solution can be approximately determined from the plot of number of particles/ml versus concentration. Figure 6 shows that the solubility of DEHP in normal saline solution is in the range of 3×10^{-7}M to 6×10^{-7}M.

TABLE I

Number of particles and size distribution of Sodium Chloride Injection USP in glass containers [Travenol laboratories & Baxter laboratories], before and after shaking.

	Number of particles per ml					Total number of particles/ml
	>1um	>2um	>3um	>4um	>5um	
Before shaking[a]	1220±215	160±53	38±17	8±5	2±1.6	2230
Before shaking[b]	1624±29	168±17	39±11	10±5	3±0.5	3158
After shaking, 20 min.[b]	2960±6	395±55	106±25	25±6	7±3	5384

[a] Lot G97X2, Travenol labolatories; average of six determinations.

[b] Lot G161N9, Baxter laboratories; average of three determinations.

Fig. 1 Logarithmic plot of cumulative particle-size distribution in Sodium Chloride Injection USP in glass containers. (From Table 1)

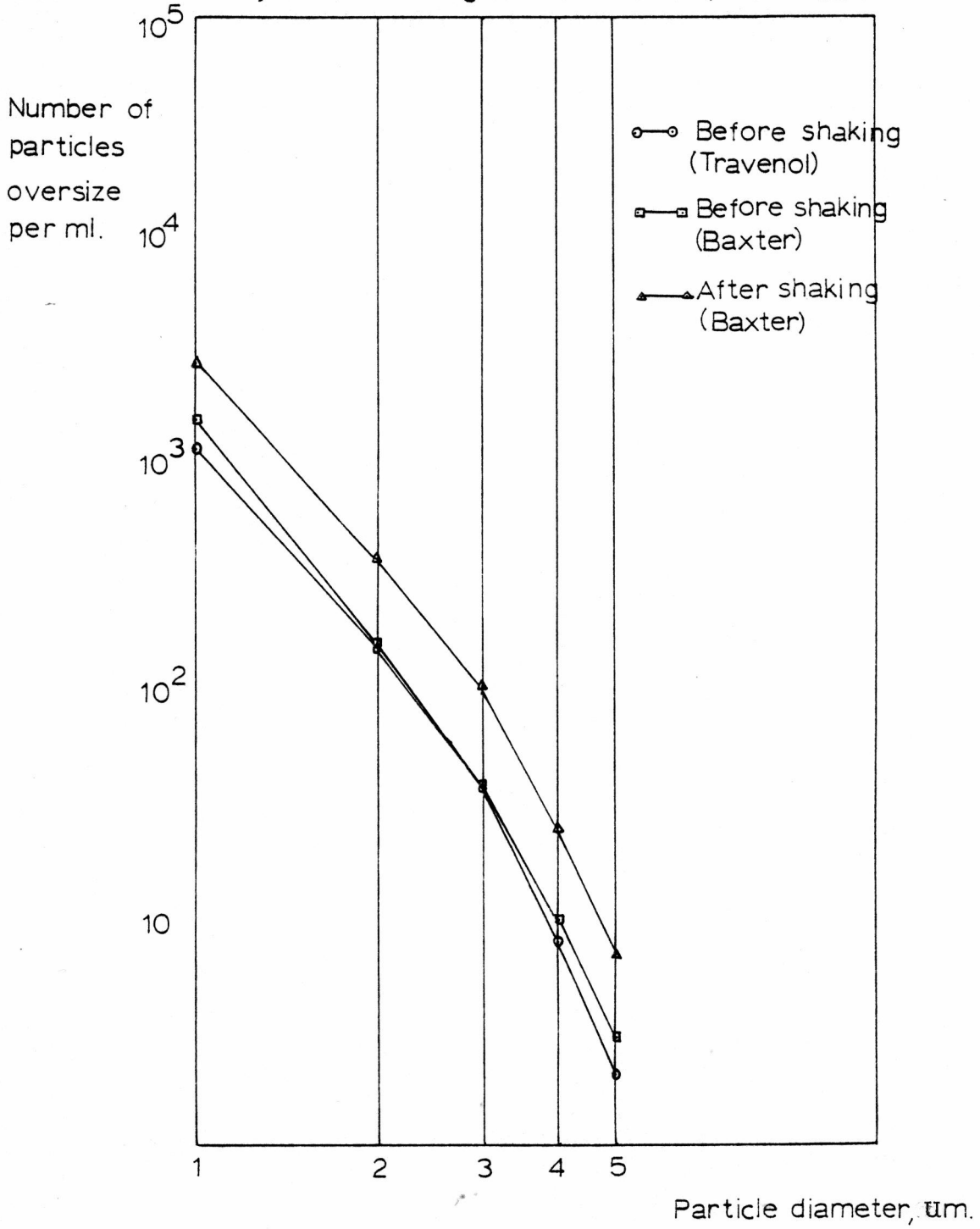


TABLE II

Number of particles and size distribution of Sodium Chloride Injection USP in plastic containers[a], before shaking, after shaking, and after storage for more than 2 days after shaking.

	Number of particles per ml					Total number of particles/ml
	> 1um	> 2um	> 3um	> 4um	> 5um	
Before shaking[b]	7986±594	1610±747	398±83	101±21	30±10	12304
After shaking, 10 min.[c]	52644±522	6415±398	1304±160	280±66	86±7	93232
After shaking, 10 min.&storage[d]	44552±646	5329±210	954±44	172±38	40±1·5	76320
After shaking, 20 min.[c]	122968±352	15014±122	3073±99	623±100	201±22	222768
After shaking, 20 min.&storage[c]	123252±1417	15054±500	2950±194	521±119	175±27	216916
After shaking, 30 min.[c]	366910±2174	45395±541	10087±354	2458±289	634±29	646652

[a] Lot CP290W7, viaflex container [Travenol laboratories]; [b] Average of 7 determinations. [c] Average of 5 determinations; [d] Average of 4 determinations.

Fig. 2 Logarithmic plot of cumulative particle-size distribution in Sodium Chloride Injection USP in viaflex containers (Travenol). From Table 2.

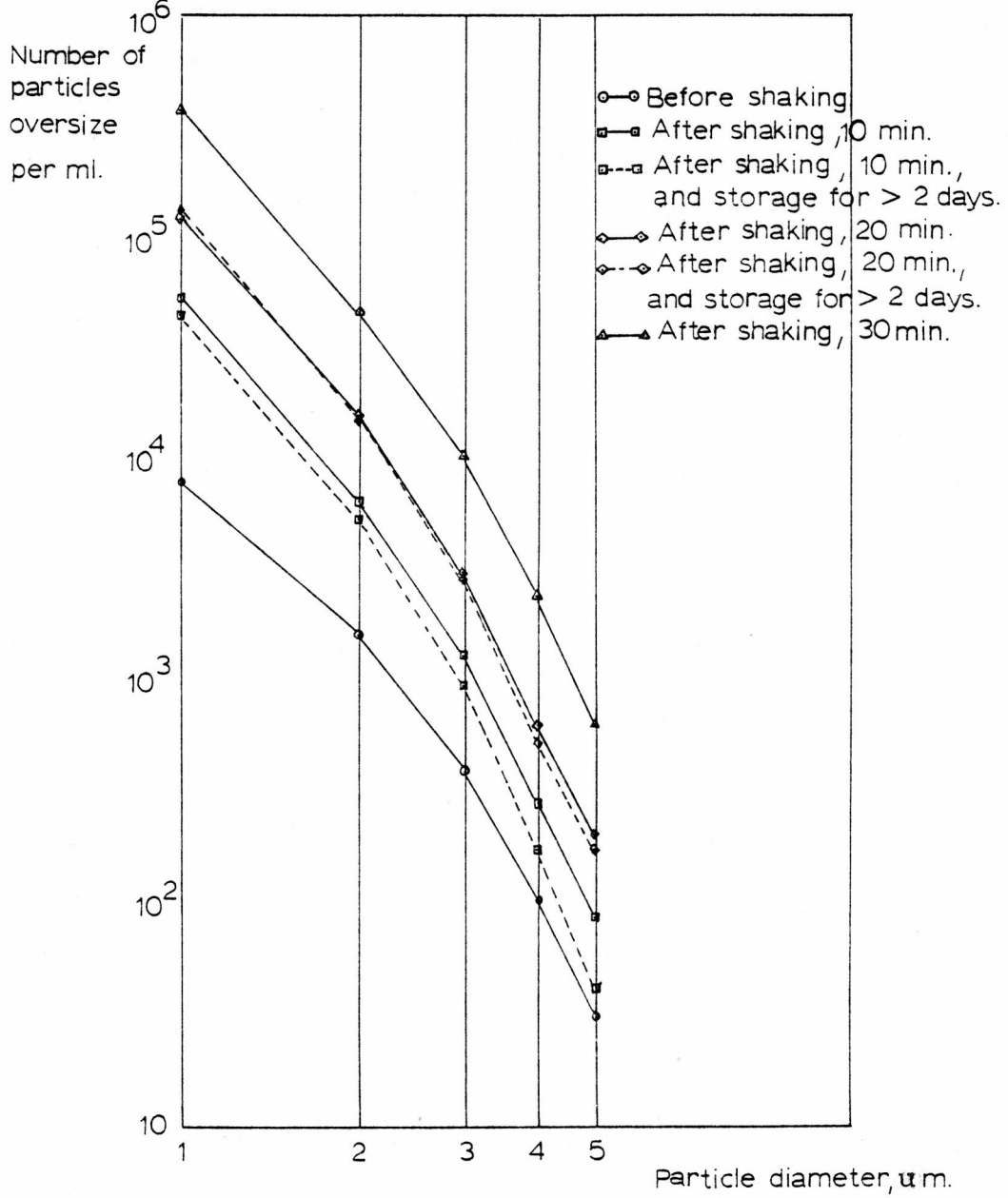


TABLE III

Number of particles and size distribution of fresh, particle-free normal saline solution and of this solution packaged in plastic bags, before and after shaking.[a]

	Number of particles per ml					Total number of particles/ml
	>1um	>2um	>3um	>4um	>5um	
Particle-free solution	702	91	32	12	5	1342
Solution in plastic bag, before shaking	2766	509	182	61	20	4038
Solution in plastic bag, after shaking, 10 min.	311909	47836	11227	2929	976	488120

[a] Lot NP37A7, viaflex containers [Travenol laboratories].

Fig.3 Logarithmic plot of cumulative particle-size distribution in normal saline solution in viaflex containers (Travenol). From Table 3

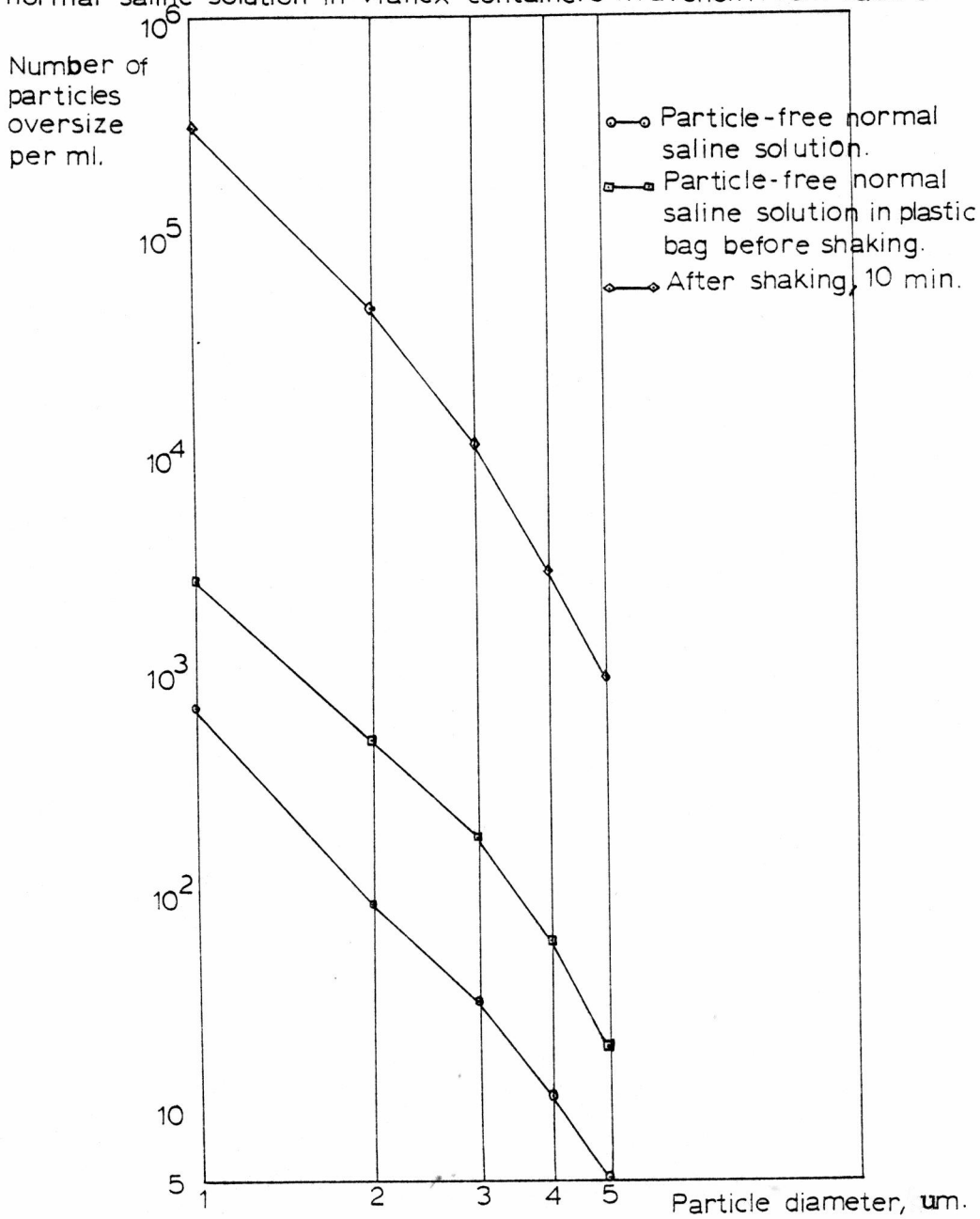


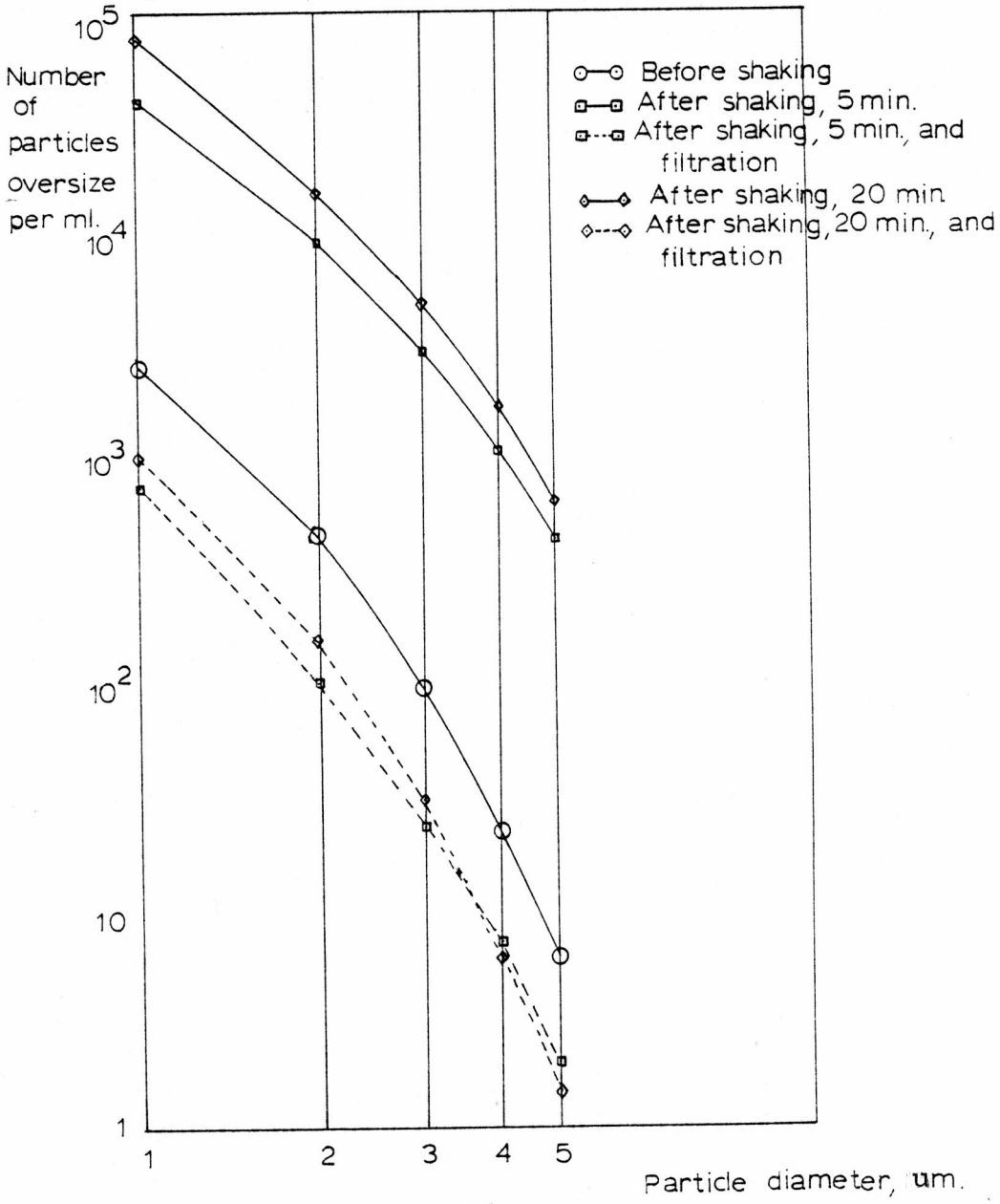
TABLE IV

Number of particles and size distribution of Sodium Chloride Injection USP in plastic containers[a], before&after shaking, and after filtration with a 0.45 um millipore filter. Column at far right gives values of number of particles on filter taken by microscopic method.

	Number of particles per ml					Total number	Microscopic counts	
	> 1um	> 2um	> 3um	> 4um	> 5um	of particles/ml	⩾2um	⩾5um
Before shaking[b]	2617±24	469±41	95±6	22±4	6±2	3754		
After shaking, 5 min.[b]	40397±100	9324±146	3082±34	1117±33	443±34	57792		
After shaking, 5min.&filtration	769	101	23	7	2	1740	-	9
After shaking, 20 min.[b]	76628±1038	15970±519	5067±230	1727±115	653±66	115172		
After shaking, 20min.& filtration	1041	156	30	6	2	1860	16	4

[a] Lot CP290W7, viaflex containers [Travenol laboratories]; [b] average of 3 determinations

Fig.4 Logarithmic plot of cumulative particle-size distribution in Sodium Chloride Injection USP in viaflex containers (Travenol). From Table 4



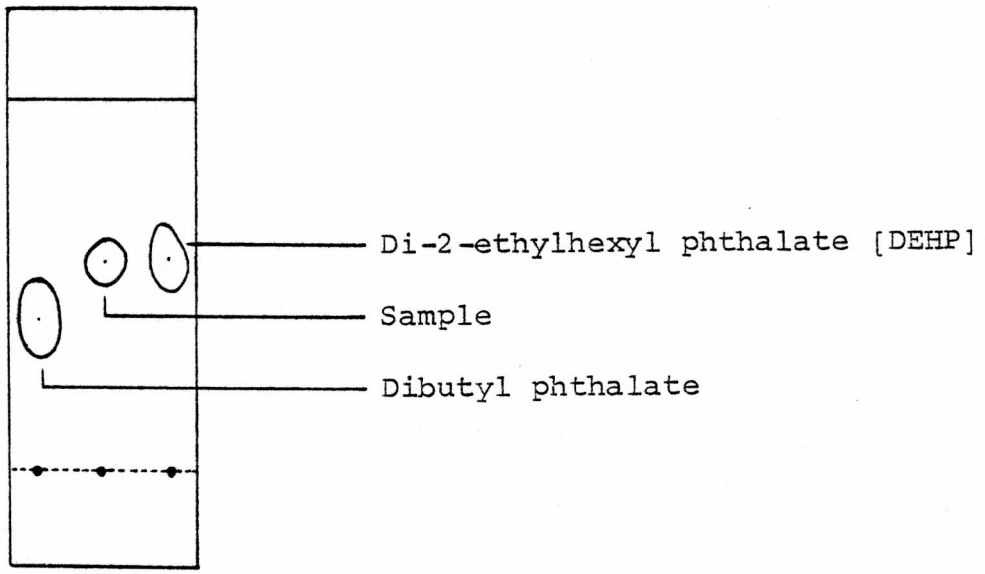


Figure 5. Thin-layer chromatography of sample solution from viaflex container compared with di-2-ethylhexyl phthalate and dibutyl phthalate.

TABLE V

Intensity of UV absorbance at 224 nm and corresponding concentrations of DEHP extracted from normal saline solution stored in viaflex containers[a] at different periods of time.

Storage times [hours]	Intensity[b] of UV absorbance at 224 nm	Concentration[c] detected
1	0.037	3.75×10^{-8}
2	0.048	4.93×10^{-8}
4	0.063	6.55×10^{-8}
8	0.064	6.6×10^{-8}
16	0.068	7.0×10^{-8}
32	0.100	1.05×10^{-7}
32 [shaking,10 min.]	0.115	1.2×10^{-7}
>1 mo.	0.146	1.52×10^{-7}
>1 mo.[shaking,10 min.]	0.217	2.26×10^{-7}
commercial bag[d]	0.168	1.74×10^{-7}
commercial bag[d] [shaking,20 min.]	0.486	5.2×10^{-7}

[a] Lot NP37A7, viaflex container [Travenol laboratories].
[b] Values using freshly prepared normal saline solution, undergoing the same procedure as that of the sample, as reference solution.
[c] Values after correction for dilution factor.
[d] Lot CP378X9, Sodium Chloride Injection USP in viaflex containers [Travenol laboratories].

TABLE VI

Number of particles determined at different concentrations of DEHP in fresh, particle-free normal saline solutions before and after equilibrating at 25 ± 0.1° C.

Concentrations [M]	Total number of particles per ml	
	Before equilibrating[a]	After equilibrating[b]
Blank	1023	1023
10^{-10}	1936	-
10^{-9}	2154	-
10^{-8}	1896	5062
10^{-7}	2134	7128
2×10^{-7}	-	7882
3×10^{-7}	12960	7727
6×10^{-7}	18333	15017
10^{-6}	20925	16416
10^{-5}	206921	1019362

a
The particle counts were taken right after the solutions were prepared.

b
The particle counts were taken after shaking in the constant temterature water bath at 25 ± 0.1° C for 2 days.

c
Fresh, particle-free normal saline solutions prepared by filtering solutions through a 0.45um millipore filter.

Figure 6. Plot of number of particles per ml versus concentrations of di-2-ethylhexyl phthalate [DEHP] in particle-free normal saline solution before and after equilibration at 25 $\pm$ 0.1° C. [Data shown in Table 6].

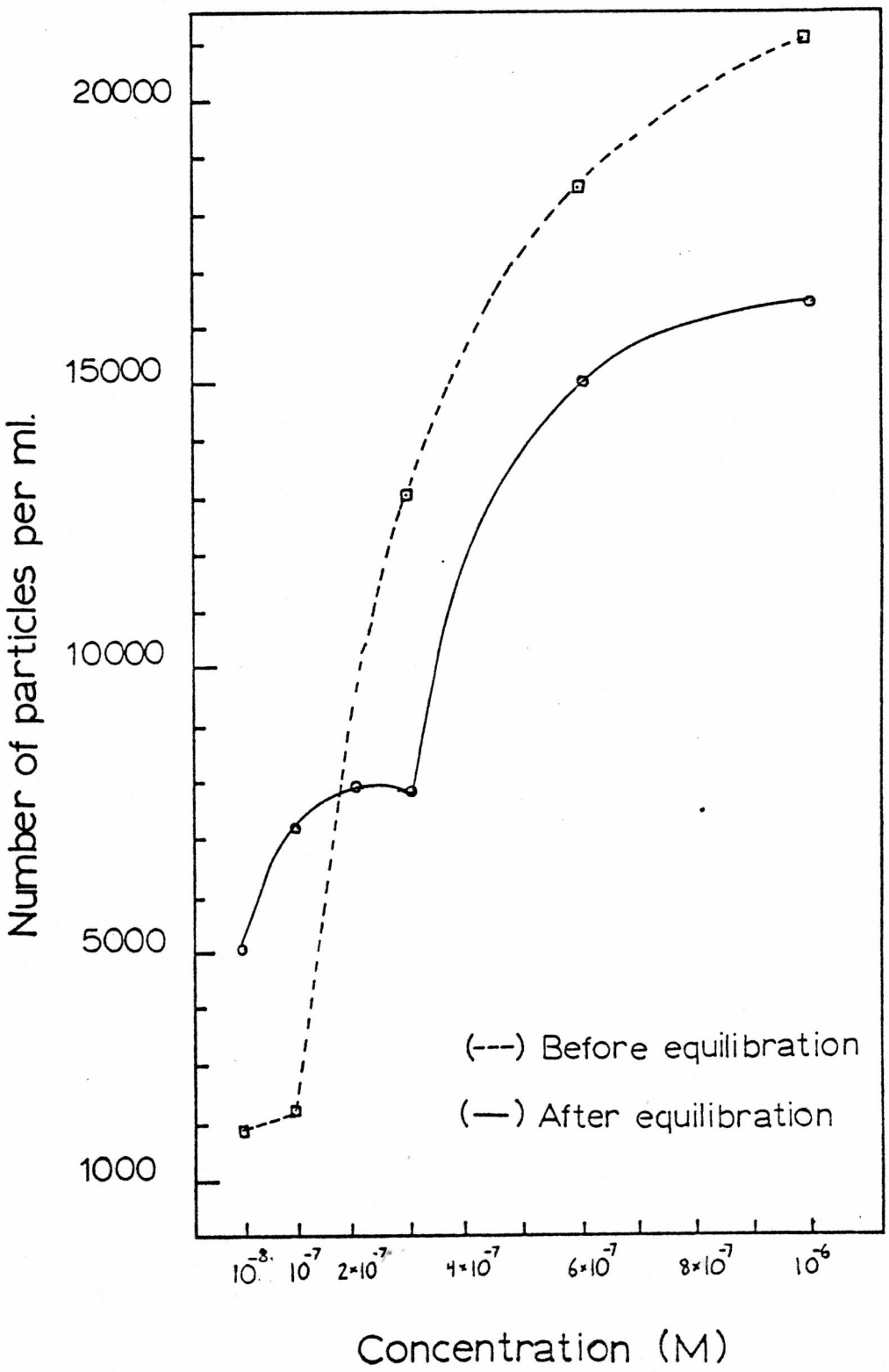

20000
15000
10000
5000
1000
Number of particles per ml.
(---) Before equilibration
(—) After equilibration
10⁻⁸
10⁻⁷
2×10⁻⁷
4×10⁻⁷
6×10⁻⁷
8×10⁻⁷
10⁻⁶
Concentration (M)

TABLE VII

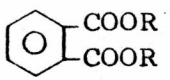
Comparison of number of particles in flexible plastic containers[a] and glass containers[b] before and after shaking.

Shaking time [min.]	Number of particles per ml	
	Glass bottles	Flexible plastic bags
0	3158	12304
20	5384	222768

[a] Lot CP290W7, viaflex containers [Travenol laboratories].

[b] Lot G161N9, [Baxter laboratories].

TABLE VIII

Physical and chemical properties of some phthalate esters[a] 

Compound	R	Molecular weight	Density	Bp, °C	Solubility in water, M
Dimethyl phthalate	$-CH_3$	194.19	1.1905	283.8	2.04×10^{-2} [b]
Diethyl phthalate	$-CH_2-CH_3$	222.24	1.1175	298	2.68×10^{-3} [b]
Dibutyl phthalate	$-[CH_2]_3-CH_3$	278.35	1.047	340	3.98×10^{-5} [b]
Di-2-ethylhexyl phthalate [DEHP]	$-CH_2-\underset{\displaystyle C_2H_5}{\underset{\vert}{CH}}-[CH_2]_3-CH_3$	390.56[c]	0.98[c]	384[c]	3×10^{-7} to 6×10^{-7} [d]

[a] From reference 28

[b] From reference 21

[c] From Aldrich catalog

[d] From the result in this laboratory; solubility in normal saline solution.

DISCUSSION

The BP standard states that the mean counts of particles per ml do not exceed 1000 equal to or greater than 2 um and 100 equal to or greater than 5 um; an instrument capable of counting the number of particles having equivalent sphere diameters equal to or greater than 2 um and equal to or greater than 5 um such as an electrical resistance counter or a light-scattering or a light-blocking device is required [23]. According to the BP specification, the Electrozone-Celloscope equipped with a 24-um orifice, which was capable of counting the number of particles having equivalent sphere diameters in the calibration range of 0.91 to 5.65 um, was used in this study to determine the number of particles exceeding the following diameters: 1, 2, 3, 4, and 5 um.

There is evidence [8, 11-13] that solutions packaged in glass bottles demonstrate greater particulate matter contamination than do solution stored in flexible plastic bags under quiescent conditions, which is in contrast to the results of Whitlow et al. [25]. Whitlow et al. demonstrate also that particulate matter can be generated in flexible plastic containers of normal saline solutions by continuous

shaking. Considering these phenomena, it was decided to determine and compare the number of particles in both glass and plastic containers under quiescent and agitated conditions. Tables 1-4 show that the number of particles increases at all size ranges determined after the solutions in both plastic and glass containers are subjected to mechanical shaking. This result agrees with the data reported by Whitlow et al. [25]. The results shown in Tables 1-4 also illustrate that the number of particles increases as the diameter of the particles decreases and the greatest number of particles is in the lowest range studied i.e., 1-2 um range. Comparision of the number of particles found in both plastic and glass containers before and after shaking is shown in Table 7. It is obvious that the total number of particles per ml found in plastic bags is significantly higher than the total number in the glass bottles in both agitated and nonagitated conditions, which agrees well with a previous report [25].

As noted by Garvan and Gunner [2,3], Bikhazi et al.[24], and Draftz and Graf [29], the particles in the solutions stored in glass containers are identified as rubber particles, cellulose fibers, carbon black, calcium carbonate crystals, glass, starch grains, fungi, etc. Garvan and Gunner consider that the rubber bungs are the main source of these particles.

In addition, the particles may arise from the other sources such as inadequate filtration, particles leached from the glass bottles themselves or contamination by the wash-water [8]. Figure 1 shows that the shaking effect causes a little change in the particle size distribution. This means that the slope of the log-log plot in Figure 1 does not alter much after shaking but the number of particles at all size ranges measured increases significantly. It has been suggested that there are two effects that may account for the increase in the number of particles occurring when the bottles are shaken [15]: the release of particles from the surface of the glass containers and rubber bungs, or the break-down of particle agglomerations. If the large agglomerates disintegrate into the smaller size particles, the slope of the log-log plot in Figure 1 must increase. Since there is no significant change in the slope after shaking the bottles for 20 minutes, the suitable explanation for this result is the dislodging of the particles from the surface of the glass containers and of rubber bungs whose size distribution is similar to those already present in the solution.

If we now examine the number of particles generated in flexible plastic containers after shaking for various periods

of time [see Tables 2 and 4], we find that the particle content increases very strongly as the shaking time increases but the size distribution seems to be about the same as before shaking, as shown in Figures 2 and 4. This suggests that these additional particles may originate from the particles that are dislodged from the inner surface of the plastic containers, which may have been improperly cleaned initially, or from the particles that may possibly leach from the plastic containers as the solution ages; but not from the disintegration of the large agglomerates. Table 2 demonstrates that after storage for more than two days, the number of particles tends to decrease at all size ranges. Probably, a certain degree of readsorption to the surface of the plastic container takes place.

In this study, the viaflex containers [Travenol Laboratories] were filled with membrane-filtered normal saline solution and subjected to shaking for ten minutes. The result [see Table 3] shows that the total number of particles found in the solution increases at all size ranges measured after the solution is placed in plastic bags, and that the number increases tremendously after shaking for only ten minutes. From this data, it is apparent that the origin of these particles is most likely particles that are

adsorbed on the inner surface of the plastic container which has been improperly cleaned. When subjected to the mild agitation, i.e., 20 inversions, some particles are dislodged from the surface, and after vigorous shaking, more particles are dislodged into the solution.

It has been suggested that the particulate matter contamination can be chemical reaction products which result from the reaction between the solution and the component parts of the containers [26], or can be leached ingredients from the plastic container into the solution [22,33]. In recent years, there have been attempts to identify the particles generated in the plastic bags [4,24]. Bikhazi et al. [24] identify those particles generated in the plastic bags as carbon black [1-3 um], a few particle contaminants, starch grains [15 um], and unidentified particles [1-5 um]. Needham and Luzzi [4] identify those particles as the plasticizer, DEHP. Considering these reports, it was decided to identify these particles by the microscopic method and determine the quantities of the plasticizer in the solution. The result from the microscopic method [see Table 4] illustrates that very few particles are found on the membrane filter. These particles are probably the particle contaminants from the filtration devices or from the environment. The number of

particles found in the filtrate after filtration is even less than the number in the solution before shaking. Theoretically, one would expect the number of particles counted on the membrane filter to be close to the number that disappeared from the filtrate. Two possible reasons may account for the disappearance of the particles: these particles which are in the range of 1-5 um are too small to be readily distinguished from the surface of the membrane filter unless they are black, highly colored or strongly birefringent [9,10]; these particles are likely to be adsorbed on the membrane filter if they are the plasticizer.

Therefore, the thin layer chromatography was employed to identify the plasticizer used in viaflex containers. Figure 5 shows that the plasticizer in viaflex containers was identified as DEHP which agrees with the previous report [20]. An extraction and spectrophotometric assay procedure was developed in this study to determine the concentration of the plasticizer entering the solution. From this procedure, the quantities of DEHP in the solution were found to be a function of the storage time after the solutions were placed in the plastic bags. It can be seen [Table 5] that the concentration of DEHP increases as the storage time increases and the concentration is higher when the solution is

subjected to shaking. The concentration found in the solution is in the range of 3.75×10^{-8}M to 2.26×10^{-7}M for the prepared normal saline solution stored in plastic bags between the periods of 1 hour to more than 1 month. In cases of the commercial products, the concentration detected for a bag shaken 20 minutes is 5.2×10^{-7}M which is higher than those solutions which are prepared in this laboratory. This suggests that some amount of the plasticizer is adsorbed at the inner surface of the plastic containers and dissolves in the solution when the solution is placed in the plastic bags. The quantities of the plasticizer leached into the solution increase as the contact time between the solution and the surface of the container increases. When the solution is shaken, the plasticizer is likely to be removed more from the surface into the solution. This effect may account for the increase in the quantities of the plasticizer entering the solution after shaking.

It has been suggested [18] that after the plastic bags are shaken, the particle content in the solution increases because of the microspheres of liquid plasticizer generated as pseudoparticles [not solids] in the solution. This is possible only if the quantities of the plasticizer in the solution exceed its solubility. The solubility of DEHP in

the normal saline solution was determined in this study. The result given in Table 6 shows that when the saturated solution of DEHP in normal saline solution is obtained, the number of particles increases significantly. The number of particles per ml is plotted against the corresponding concentration, as shown in Figure 6, and the solubility of DEHP is approximated from this plot to be in the range of 3×10^{-7} M to 6×10^{-7} M. When the physical and chemical properties of DEHP are compared to the other phthalate esters, as shown in Table 8, the solubility of DEHP obtained seems to be in a reasonable range. The concentration of DEHP entering the prepared solutions stored in plastic bags for 1 hour to more than 1 month, including the shaken bags, is in the range of 3.75×10^{-8}M to 2.26×10^{-7}M [see Table 5] which is lower than the solubility of DEHP. This result indicates that the particles generated in the plastic bags during this period of time, before or after shaking, may not be the plasticizer. In case of commercial bags shaken for 20 minutes, the concentration found is within the range of this solubility. Therefore, we try to calculate the volume of 115,172 particles/ml which is the total number of particles found after shaking for 20 minutes [see Table 4]. If these particles are microspheres of liquid plasticizer

DEHP, the calculated concentration of the plasticizer is approximately 2.42 x 10^{-7}M [assuming the average size of 1.17 um diameter which is obtained from the particle data analysis and using the density of 0.981]. The total quantities of the plasticizer in the solution must be the amount that dissolves in the solution plus the amount that exceeds the solubility which will occur in the form of the particles. The calculated quantities turn out to be in the range of 5.42 x 10^{-7}M to 8.42 x 10^{-7}M for 20 minute shaken commercial bags which is comparable to the concentration detected in these bags.

From these results, it can be seen that the increase in the number of particles in plastic containers after shaking is initially due to the particles that are dislodged from the inner surface of the plastic containers, which may not be the plasticizer. When the solution ages, for example, in the case of solution in a commercial bag, especially when it is subjected to shaking, the number of particles in the solution increases markedly and the quantities of the plasticizer also increase. There are two possible explanations for this increase in the particle content in the solution: [1] The quantities of the plasticizer migrated from the plastic bags into the solution increase which may finally

exceed the solubility. This excess of the liquid plasticizer will occur in the solution in the form of microspheres which are pseudoparticles [not solids].

[2] If the quantities of the plasticizer are less than the solubility, the plasticizer may appear as the particles by forming the micelles or the emulsified particles. These emulsified particles or micelles may exist if there are some surface active agents in the solution. Generally, soft flexible polyvinyl chloride is produced by the addition of liquid plasticizers to impart the flexibility; lubricants such as metallic stearates, stearic acid or mineral oil to function as processing aids; stabilizers such as calcium and zinc soaps, epoxidized soybean oil, and organic phosphite ester to decrease the problem of thermal instability, etc. [34,35]. It is possible that some of these additives possess the surface active property. Ross [36] was able to extract the surface active agents from both polyethylene and polyvinyl chloride articles used in hospitals, and identified these surface active substances as plasticizer and stabilizers. In the case of viaflex containers used in this study, the known additive is the plasticizer, DEHP. When the solution in the plastic bags ages, DEHP which is diester can be hydrolyzed. The product obtained in the form of phthalic

acid or monoester of phthalic acid may have surface activity, so that it may form micelles or the film around DEHP droplets which could be counted as the particles. In the presence of surfactants, the quantities of the plasticizer leached from the plastic bags into the contents may also increase. These phenomena could occur in the presence of other surface active substances leached from the plastic bags.

Therefore, the plasticizer can contribute to the increase in the particle content in the solution packaged in the polyvinyl chloride plastic bags after the solution ages and/or the solution is subjected to shaking.

CONCLUSION

This study has shown that by continuous shaking, the number of particles in both glass and polyvinyl chloride plastic containers of Sodium Chloride Injection USP increases at all size ranges determined but there is no significant change in the particle size distribution. It is obvious that solutions packaged in glass containers contain fewer particles than solutions stored in flexible plastic bags under similar conditions. After the bags are stored under undisturbed conditions for more than 48 hours, the number of particles tends to decrease. These particles generated in flexible plastic bags cannot be identified by the microscopic method.

The plasticizer used in viaflex containers is identified as DEHP by the thin layer chromatography. An extraction and spectrophotometric method is developed in this study to determine the concentration of the plasticizer entering the solution. The result indicates that the quantity of DEHP in a liter of normal saline solution increases as the time increases and/or as the solution is subjected to shaking. The quantities of the plasticizer are found to be in the range of 3.75×10^{-8}M [for freshly-prepared normal saline solution stored in plastic bags for 1 hour] to

5.2×10^{-7}M [for 20 minute shaken commercial bags]. The solubility of DEHP in normal saline solution determined by the Coulter Counter is found to be in the range of 3×10^{-7}M to 6×10^{-7}M. It may be seen that the concentration of the plasticizer entering the solution stored in plastic bags for less than 1 month is lower than the solubility. In the case of commercial bags, the concentration of DEHP detected is in the range of the solubility. Assuming that the particles detected in the solution are microspheres of liquid plasticizer, the concentration of these plasticizer particles is determined on the basis of a calculated volume of 115,172 microspheres/ml [using an average 1.17 um diameter and density of 0.981]. This calculated concentration is found to be 2.42×10^{-7}M and is combined with the saturated concentration of DEHP to give the total concentration calculated, which is comparable to the total concentration detected in commercial bags shaken for 20 minutes.

Thus, from this study, it may be concluded that the increase in the particle content in the solution may be initially due to the dislodged particles from the inner surface of plastic containers, which may have been improperly cleaned. With the aid of some surface active substances which may leach from the plastic container into the solution

or may be the product of chemical reactions, the plasticizer will be involved in the generation of the particles after the solution ages and/or the solution is agitated.

Further experimental designs should be planned to study the effect of surface active substances on the plasticizer, in view of the rate of migration of the plasticizer from the plastic containers and of the possible forming of micelles or emulsified plasticizer droplets in the solution. This effect should be examined in order to obtain the better explanation for the increase in particulate matter and to identify these particles in the plastic bags. In addition, it would be advantageous to determine the effect of lipoidal substances, for example, blood, serum or even fatty foods, on the extraction of plasticizers from the plastic containers.

REFERENCES

1. "Second Supplement to USP XIX - NF XIV," Mack Publishing Co., Easton, Pa., pp. 127-128, July 1975.

2. J.M. Garvan and B.W. Gunner, Med. J. Australia 50:2, 140 [1963].

3. J.M. Garvan and B.W. Gunner, Ibid, 51:2, 1 [1964].

4. T.E. Needham and L.A. Luzzi, N. Engl. J. Med. 289, 1256 [1973].

5. S.J. Turco and N.M. Davis, Hosp. Pharm., 8, 137-139 [1973].

6. J. Autian, Environmental Health Perspectives, June 1973, pp. 3-24.

7. R.F. Wallin, B. Klamer, R.W. Nicora, C.R. Thompson, Bull. Parenteral Drug Assoc., 28 , 278-286 [1974].

8. I. Vessey and C.E. Kendall, Analyst, 91 , 278 [1966].

9. M.J. Groves, Ibid, 94 , 995 [1969].

10. C.E. Kendall, Ann. N.Y. Acad. Sci., 158 , 642 [1969].

11. T.F. Stokes, E.D. Sumner, T.E. Needham, Am. J. Hosp. Pharm., 32 , 823 [1975].

12. N.M. Davis, S.J. Turco, E. Sivelly, Ibid, 27 , 825 [1970].

13. N.M. Davis, S.J. Turco, Ibid, 30 , 611-613 [1973].

14. L. Ernerot, Acta Pharm. Suec., 11 ,1-14 [1974].

15. L. Ernerot, L. Helmstein, and E. Sandell, Ibid, 7 , 501-507 [1970].

16. M.J. Groves, Proc. Soc. Anal. Chem., 8 , 271-275 [1971].

17. J. Blanchard, J.A. Schwartz and D.M. Byrne, J. Pharm. Sci., 66 , 935-937 [1977].

18. T.D. Darby and R.K. Ausman, N. Engl. J. Med., 290 , 579 [1974].

19. K.A. Connors, "A Textbook of Pharmaceutical Analysis", 2 nd Ed., Wiley-Interscience, NY , p.404 [1975].

20. Anon: Viaflex Containers, Med. Letter Drug Ther., 14 , 69 [1972].

21. N.C. Deno and H.E. Berkheimer, J. Chem. Eng. Data, 5 , 3 [1960].

22. W.L. Guess, J. Jacob and J. Autian, Drug Intelligence, 1 , 120-127 [1967].

23. "British Pharmacopoeia 1973," Her Majesty's Stationery Office, London, England, 1973, p. A123.

24. A.B. Bikhazi, J.A. Shiatis and A.F. Haddad, J. Pharm. Sci., 66 , 181-185 [1977].

25. R.J. Whitlow, T.E. Needham and L.A. Luzzi, Ibid, 63 , 1610-1613 [1974].

26. N.M. Davis and S.J. Turco, Am. J. Hosp. Pharm., 28 , 620-623 [1971].

27. J. Blanchard, C.M. Thompson and J.A. Schwartz, Ibid, 33 , 144-149 [1976].

28. "Handbook of Chemistry and Physics," 58th Ed., Chemical Rubber Publishing Co., Cleveland, p. C436 [1977-1978].

29. R.G. Draftz and Jean Graf, Bull. Parenteral Drug Assoc., 28 , 35-50 [1974].

30. S.M. Blaug and R.E. Sarabia, Ibid, 29 , 74-85 [1975].

31. N.J. Kartinos, Ibid, 22 , 33 [1968].

32. N.M. Davis, S. Turco and E. Sivelly, Ibid, 24 ,257 [1970].

33. J. Autian, Ibid, 22 , 276-287 [1968].

34. Jack Cooper, "Plastic Containers for Pharmaceutical-Testing and Control," World Health Organization, Geneva, pp. 5,8 [1974].

35. J. Haslam and H.A. Willis, "Identification and Analysis of Plastics," D. Van Nostrand Company, Inc., Princeton, New Jersey, p.46 [1965].

36. Alan J. Ross, J. Hosp. Pharm., [Dec.], 349 [1964].