[Chem. Pharm. Bull. 25(7)1791—1796(1977)] UDC 615.451.011.3 : 615.014.24

Particles of Di(2-ethylhexyl) Phthalate in Intravenous Infusion Fluids Migrating from Polyvinyl Chloride Bags

MASAYOSHI HORIOKA, TOSINOBU AOYAMA, and HIRONOBU KARASAWA

Hospital Pharmacy, Faculty of Medicine, Kyushu University[1)]

(Received February 25, 1977)

Mechanical measurement of particles in the infusion fluid contained in a polyvinyl chloride (PVC) bag revealed the presence of a large number of particles and principle of these particles was examined. It was thereby confirmed that the majority of particles in the solution contained in a PVC bag are liquid particles of di(2-ethylhexyl) phthalate (DEHP) used as a plasticizer for PVC, and these particles adhere to the inner wall of the bag or float in the solution. When the PVC bag is shaken before sampling the fluid, the liquid particles of DEHP are liberated and dispersed as microparticles which are counted as particles by HIAC automatic counter, but not observed on membrane filter under a microscope. Detecting of such a liquid particle, a considerable difference between the results from a mechanical method and that from a microscopic observation was pointed out.

Keywords——di(2-ethylhexyl) phthalate, (DEHP); liquid particle; polyvinyl chloride intravenous bags; plasticizers; adverse effects of particulate matter in *i.v.* solutions; *i.v.* infusion fluid; normal saline, lactated Ringer's; gas-liquid chromatography; HIAC Automatic Particle Counter; microscopic observation of DEHP particles; particulate matter

With the progress in modern medicine, a large quantity of intravenous (*i.v.*) infusion fluids is used for treatment, and the effect of such an infusion on patients, especially the hazardous effect of foreign particulate matter present in infusion fluids, has become a great problem. As the hazardous effects of foreign particulate matter in *i.v.* infusion fluids, plumonary embolism, granuloma, renal infarct, narrowing of visual field, and phlebitis are known to occur.[2)]

There are many methods for detecting these foreign particulate matter and many of them are much more advanced than a macroscopical light testing procedure. For example, the method stipulated in the British Pharmacopeia[3)] calls for measurement with a counting apparatus, and the U.S. Pharmacopeia[4)] stipulates counting under a microscope after filtration through a membrane filter.

Examination of particulate matter in commercial parenteral solutions using a counting apparatus has shown that there are a large number of particles in the solutions placed in a

1) Location: *Maidashi 3-1-1, Higashi-ku, Fukuoka, 812, Japan.*
2) *a*) E.J. Brüning, *Virchows Archi. Pathol. Anat. Physiol.*, **327**, 460 (1955); *b*) S. Sarrut and C. Nezelof, *Presse Med.*, **68**, 375 (1960); *c*) J.L. Chason, J.W. Landers, and R.E. Swanson, *Neurology*, **13**, 558 (1963); *d*) J.M. Garvan and B.W. Gunner, *Med. J. Australia*, **50** (2), 140 (1963); **51** (1), 1 (1964); *e*) F.J.C. Roe, R.L. Carter, M.S. Walters, and J.S. Harington, *Int. J. Cancer*, **2**, 628 (1967); *f*) *Can. J. Hosp. Pharm.*, **24**, 226 (1971); *g*) M.J. Groves, *Pharm. J.*, **210**, 185 (1973); *h*) S. Turco and N.M. Davis, *Hospital Pharmacy*, **8**, 137 (1973); *i*) P.B. Ryan, R.P. Rapp, P.P. DeLuca, W.O. Griffen, Jr., J.D. Clark, and D. Cloys, *Bull. Parenter. Drug Assoc.*, **27**, 1 (1973); *j*) J. Collin, D.E.F. Tweedle, C.W. Venables, F.L. Constable, and I.D.A. Johnston, *Br. Med. J.*, **4**, 456 (1973); *k*) T. Aoyama, *Gekkan Yakuji*, **16**, 1847 (1974); *l*) R.G. Swift, Jr., D.M. Searcy, and A.S. Pickard, *Drug Intell. Clin. Pharm.*, **9**, 76 (1975); *m*) P.P. DeLuca, R.P. Rapp, B. Bivins, H.E. McKean, and W.O. Griffen, *Am. J. Hosp. Pharm.*, **32**, 1001 (1975); *n*) E.E. William, F.B. Larry, and V.S. Joseph, *Am. J. Hosp. Pharm.*, **33**, 1160 (1976).
3) "British Pharmacopeia," Her Majesty's Stationary Office, London, 1973.
4) "United States Pharmacopeia," 19th Ed., 1st Supp., United States Pharmacopeial Convention, Inc., Washington, D.C., 1975.

polyvinyl chloride (PVC) bag.[5] Whitlow, *et al.*[6] reported that the number of particles in an infusion fluid contained in a PVC bag increased to about 70 fold when the bag was shaken vigorously, but they did not make any mention of its reason.

About foreign particulate matter in the *i.v.* infusion fluid in a PVC bag, it was found in this work that there was a vast difference in the counts between the use of HIAC automatic particle counter and the USP method. We were able to clarify the principle of foreign particles by mechanical measurement in the present series of works.

Experimental

Materials——Normal saline solution (500 ml) and lactated Ringer's injection (500 ml) in a polypropylene container (Otsuka), in a glass container (Fuso, Kyosei), and in a PVC bag (Terumo) were used for the test. Di(2-ethylhexyl) phthalate (Tokyo Kasei Co., special grade) (DEHP) was used in this experiment, which is commonly used as a plasticizer for the manufacture of PVC.

Measurement and Observation of Foreign Particles——Foreign particle measurements were made with HIAC automatic particle counter, Model PC-305 SSTA (5 channels), automatic sampler, Model BS-1331 (10—120 ml), and a sensor, Model D-5-150 (for particle size of 5—150 μm). This whole assembly will be referred as HIAC hereafter. Polyvinyl chloride tube was employed for collecting samples of the fluid after being washed thoroughly with ultraclean distilled water. Shimadzu Model GC-2C gas chromatograph was used for the identification and determination of particulate matter, and Olympus FH microscope for examination of foreign particles.

Measurement of Particle Number——HIAC channel was set for 5 ranges of 5—10, 10—25, 25—50, 50—100, and 100—150 μm, and the number of particles in 10 ml of sample solution was counted (counting was made six times). Collection and preparation of sample solutions were made with the tube and in a laminer flow unit, Hitachi Clean Bench, Model PCV-841 AN.

Identification and Determination of DEHP——After extraction of about 200 ml of the sample solution with 50 ml (the first time) and 30 ml (the second time) of dichloromethane, the solvent was evaporated from the combined extract, and the residue was then dissolved in 0.2 ml of acetone containing dinonyl phthalate (0.6 μg/μl solution). Two microliters of the acetone solution were injected into the chromatograph. Gas-liquid chromatography (GLC) was carried out on a gas chromatograph equipped with a flame-ionization detector. The stainless steel column (0.5 m$\times$3 mm i.d.), packed with 3% OV-17-coated Shimalite W (80—100 mesh), were maintained at 250° with the injection port and detector at 250°. Nitrogen, carrier gas, was maintained at 100 ml/min, with hydrogen and air at 30 ml/min and 1.58 kg/cm^2, respectively.

Changes in the Number of Particles by Shaking——The number of particles was counted with a fluid collected from the PVC bag allowed to stand for some time and after shaken vigorously. The fluid (200 ml) in PVC bag was passed through a membrane filter of 0.45 μm pore size before and after shaking, and particles on the filter were examined and counted under a microscope ($\times$60).

Number of Particles and Solvent Effect——Acetone filtered through Fluoropore (Sumitomo Electric Ind., Osaka) with 0.45 μm pore size was added stepwise to the infusion fluid in a PVC bag and changes in the number of particles were measured by HIAC.

Effect of Shaking on Concentration of DEHP——The solution was collected from the PVC bag before and after shaking and each was submitted to identification and determination of DEHP by GLC. Conditions for extraction and determination were the same as described above.

Particles in Infusion Fluids in PVC Bag——The fluid in a PVC bag was stained with Methylene blue and a drop of this fluid was examined under a microscope.

Comparison of Particles in Fluid in PVC Bag and in Infusion Fluid added DEHP——DEHP was added to the normal saline solution in a polypropylene container to make a concentration of 1 ppm, and the container was shaked vigorously. The number of particles counted by HIAC and microscopic observation of stained fluid were compared with those found in the fluid from a PVC bag.

Concentration of DEHP and Number of Particles——DEHP was added to the normal saline solution in a polypropylene container to make a concentration of 0.1—0.6 ppm, and the number of particles was counted by HIAC. This result was compared with the relation between the amount of DEHP in the fluid in a PVC bag and the number of particles. In this case, the number of total particles in the range of 5—150 μm was counted.

At the same time, the concentration of DEHP was calculated from the number of particles, assuming that the particles counted were spheres of DEHP, and the value was compared with the quantity of DEHP added.

5) M.J. Groves, *J. Pharm. Pharmacol.*, **18**, 161 (1966).
6) R.J. Whitlow, T.E. Needham, and L.A. Luzzi, *J. Pharm. Sci.*, 63, 1610 (1974).

Results and Discussion

Number of Particles in Infusion Fluid

Table I shows the result of measurement of particles in commercial intravenous infusions. It will be seen from this table that the number of particles is larger in the solution in a PVC bag compared with other products in polypropylene or glass containers.

Table I. Particle Counts in Commercial Infusion Fluid

Infusion	Brand	Particle distribution (μm)				
		5—10	10—25	25—50	50—100	100—150
Normal saline solution	Otsuka	12	2	0	0	0
	Fuso	125	6	0	0	0
	Terumo	16293	1304	3	0	0
Lactated Ringer's injection	Otsuka	11	2	0	0	0
	Fuso	172	13	0	0	0
	Terumo	2752	191	0	0	0
	Kyosei	2190	12	1	0	0

Otsuka: in polypropylene container (500 ml), Fuso and Kyosei: in glass container (500 ml), Terumo: in polyvinyl chloride container (500 ml).

Changes in the Number of Particles before and after Shaking

The number of particles in a solution sampled from a PVC bag in a static state and after vigorous shaking is shown in Table II. As was reported by Whitlow, *et al.*,[6] there was about 100 fold increase in the number of particles, but in this study there were some cases where the increase was not very large, for these samples had high particle counts before shaking. It is assumed that the marked increase in the number is due to substances which migrated from the container and were adhering to the inner wall, and these substances have been freed by the vigorous shaking and dispersed in the solution.

Table II. Change of Particle Counts in Infusion Fluid in PVC Bag before and after Shaking

		Particle distribution (μm)				
		5—10	10—25	25—50	50—100	100—150
A	Before shaking	97	6	0	0	0
	After shaking	11224	159	0	0	0
	Ratio	116	27	—	—	—
B	Before shaking	201	10	0	0	0
	After shaking	16157	66	0	0	0
	Ratio	80	7	—	—	—
C	Before shaking	2934	19	0	0	0
	After shaking	5542	28	1	1	0
	Ratio	2	2	—	—	—

Normal saline solution, in 10 ml counts; Ratio=particle counts after shaking/counts before shaking.

The sample solutions before and after shaking were each passed through a membrane filter of 0.45 μm pore size and the filter was examined under a microscope (Table III). If the substances were the usual kind of foreign particulate matter (*e.g.*, fiber, talc, starch, *etc.*), they should be collected on the filter, for they were solid particles, and there should be an increase in the number. Result of measurement did not reveal a significant increase. Consequently, the increased number of particles measured by HIAC must be liquid state particles which cannot be observed as particles on the membrane filter under a microscope.

Table III. Change of Particle Counts collected on Membrane Filter

	I	II	III
Before shaking	45	115	462
After shaking	58	84	705

White grid filter, 0.45 μm pore size.
Particles were counted under a microscope.

Number of Particles and Solvent Effect

In order to take changes in the number of particles in the infusion fluid in a PVC bag by addition of acetone, residual number of particles was measured. As shown in Fig. 1, addition of acetone resulted in sudden decrease in the number, and this was considered to be due to dissolution of such particles in acetone.

Figure 1 shows changes in the number of particles by the addition of acetone to the infusion fluid added DEHP, and a similar decrease was observed.

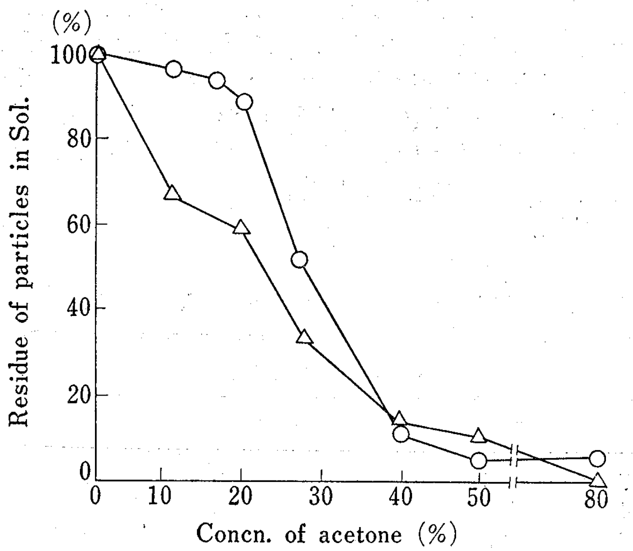

Fig. 1. Decrease of Particle Counts in Infusion Fluid by Increasing Solvent Concentration

—○—, DEHP+normal saline;
—△—, fluid from PVC bag.

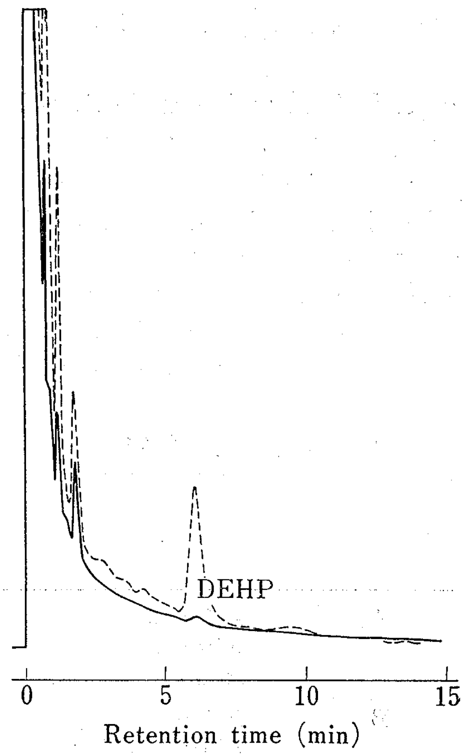

Fig. 2. Gas Chromatogram of DEHP extracted from Infusion Fluid in PVC Bag before and after Vigorous Shaking

———, before shaking;
---------, after shaking.

Number of Particles before and after Shaking and Result of GLC

Since it seemed that the particles in the solution in a PVC bag were liquid particles, the fluids from a PVC bag, before and after shaking, were each extracted with dichloromethane and the extract was submitted to GLC (Fig. 2). There was an increase in the peak height in samples obtained after shaking. The peak, had a retention time of 6 min, agreed with that of DEHP. Determination of DEHP gave values listed in Table IV, which indicated that DEHP increased 3—21 fold in the fluid sampled after shaking.

These results suggest that DEHP migrated from the PVC bag during and after sterilization or during storage of the infusion fluid, adheres on the inner wall or floats in the fluids, and shaking of the bag frees DEHP which disperses and floats in the solution.

Table IV. Concentration of DEHP in Infusion Fluid in PVC Bag

Sample	DEHP (ppm)			
	I	II	III	IV
Before shaking	0.060	0.046	0.032	0.025
After shaking	0.292	0.148	0.142	0.523
Ratio	4.87	3.22	4.44	20.92

DEHP was analysed by GLC.

Examination of Particles in Infusion Fluid in PVC Bag

Photomicrograph of particles in the infusion fluid in a PVC is shown in Fig. 3, left, indicating the presence of spherical particles of around 5 μm in diameter, same as in the case of the fluid added DEHP, shown on the right in Fig. 3.

Considering the results of solvent effect on the number of particles described above concurrently, presence of DEHP in the solution as oily, spherical microparticles has thus been proved. This agrees with the report of Needham and Luzzi[7] that DEHP in the fluid in PVC bag is present as colloidal particles.

Concentration of DEHP and the Number of Particles

Relation between the quantity of DEHP added and the number of particles in the infusion in PVC bag is shown in Fig. 4. It will be seen that there is a linear relation between the concen-

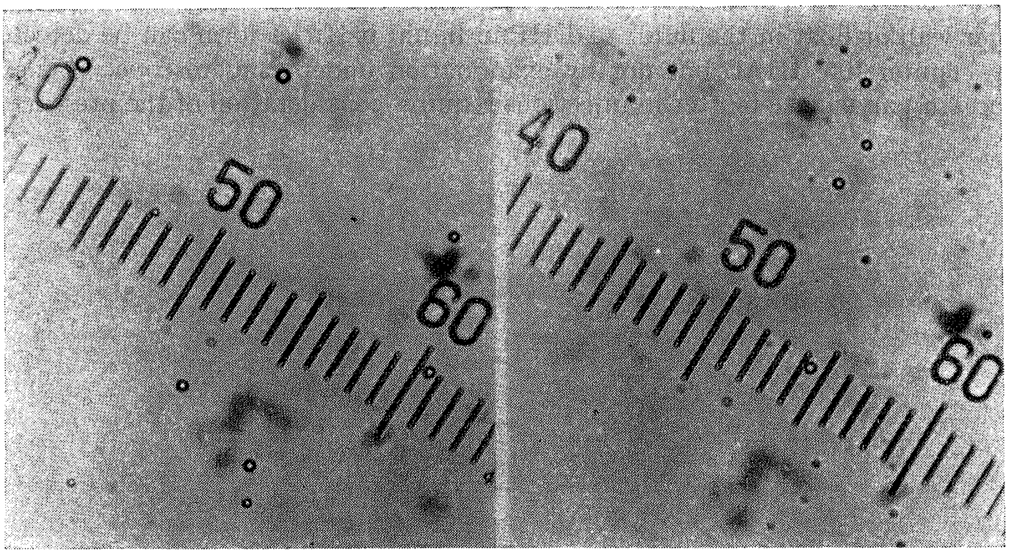

Fig. 3. Photomicrographs of Floating Particles of DEHP in Infusion contained in PVC Bag and Floating Particles of DEHP added to Saline Solution in Polypropylene Containers

The solution was stained with Methylene blue and observed microscopically. The left photograph shows particles in solution in PVC bag and the right shows ones in polypropylene container (normal saline+DEHP). In both solutions, the liquid particles of DEHP were about 5—10 μm in diameter.

7) T.E. Needham, Jr. and L.A. Luzzi, *N. Engl. J. Med.*, **289**, 1256 (1973).

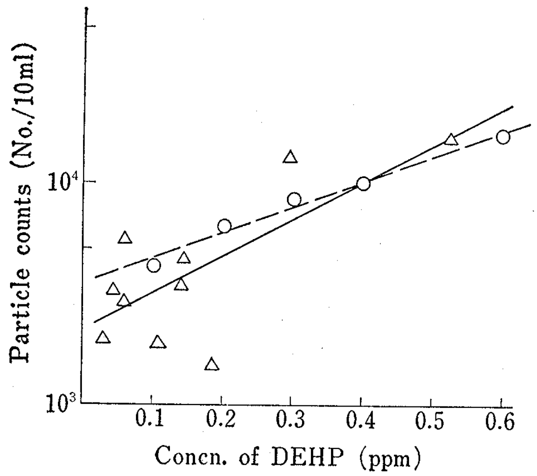

Fig. 4. Comparison of Added and Analysed DEHP

—△—, DEHP found, log $Y=3.34+1.68\,X$ ($r=0.732$, $p<0.05$); —○—, DEHP added, log $Y=3.55+1.15\,X$ ($r=0.989$, $p<0.01$).

Table V. Comparison of Concentration of DEHP Added and Calculated DEHP (ppm)

Added	Calculated	Ratio
0.1	0.09	0.90
0.2	0.11	0.55
0.3	0.16	0.53
0.4	0.35	0.88
0.6	0.59	0.98

Particles from range 2—50 μm were used. Particles from range 5—50 μm were counted by HIAC and ones from range 2—5 μm were computed from the former particle counts.

tration of DEHP and the logarithm of the number of particles in both cases, and there is no difference between the two straight lines. Comparison of the amount of DEHP added and the amount calculated showed a high correlation (Table V).

These experimental results confirmed that the majority of particles mechanically counted in the infusion fluid in PVC bag is DEHP.

Conclusion

The present experimental results indicate that the principle of particles in the infusion fluid in PVC bag is the plasticizer, DEHP, and that it forms liquid particles which adhere to the inner wall or float in the fluid. DEHP in liquid particles form can be detected by an automatic counter like HIAC but not by examination under a microscope. Consequently, test for foreign particulate matter in injections requires consideration of the presence of liquid particles.