Effect of Phthalate Plasticizer on Blood Compatibility of Polyvinyl Chloride

S. W. KIM ˣ, R. V. PETERSEN, and E. S. LEE

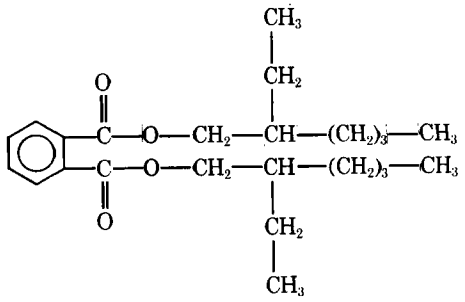
Abstract □ The amount of a phthalate plasticizer on the surface of a sheet of polyvinyl chloride used in the fabrication of blood storage bags was quantified using attenuated total reflectance spectroscopy, water contact angle measurement, and weight loss due to methanol extraction. Water wettability increased as the amount of surface phthalate extracted by methanol increased, which indicates that the accumulation of phthalate on the surface increases hydrophobicity. The extraction of phthalate by methanol consists of two steps: (*a*) methanol first dissolves surface phthalate, and (*b*) phthalate in the bulk then diffuses through the surface. The adsorption of plasma proteins was investigated to determine the initial events as blood contacts the surface. The composition of adsorbed proteins on the methanol-cleansed surface differs from that on the uncleansed polyvinyl chloride surface. Albumin adsorption onto phthalate-contaminated surfaces is less than on cleansed surfaces while adsorption of γ-globulin and fibrinogen is greater on phthalate-contaminated surfaces. Protein adsorption can be related to surface thrombus formation. Increases in platelet numbers appeared on phthalate-contaminated surfaces as compared with methanol-treated surfaces. A phthalate may enhance platelet adhesion and aggregation when it exists on a polymer surface.

Keyphrases □ Phthalate plasticizer—effect of polyvinyl chloride on blood compatibility, quantified by attenuated total reflectance spectroscopy □ Polyvinyl chloride—blood compatibility, effect of phthalate plasticizer, quantified by attenuated total reflectance spectroscopy □ Attenuated total reflectance spectroscopy—measurement of effect of phthalate plasticizer on blood compatibility of polyvinyl chloride

Polyvinyl chloride plasticized with a phthalate ester, principally bis(2-ethylhexyl) phthalate (I), has been utilized in the fabrication of various medical devices such as blood storage bags, transfusion assemblies, and extracorporeal circulation devices used in cardiopulmonary bypass and renal hemodialysis units. It was recognized (1–3) that small quantities of additives, primarily I, are leached out of polyvinyl chloride by blood or various injectable solutions. Several investigators (4–12) quantitated the rate of extraction of I from blood storage bags (4–11), medical grade polyvinyl chloride tubing (4–6, 12), hemodialysis units (7), and cardiopulmonary bypass units (7) under various conditions and by various blood components. Of particular interest was the fact that hemodialysis units gave a zero-time concentration of 7.75 mg/100 ml and cardiopulmonary bypass tubing gave a zero-time concentration of 5.05 mg/100 ml in circulating blood (7).

In 1970, I was identified in tissues of human patients who had been transfused with blood stored in polyvinyl chloride blood storage bags (13). Similar observations subsequently were made in this laboratory and by other researchers (14–17) in studies on animals and humans directly exposed to polyvinyl chloride or to blood stored in polyvinyl chloride blood bags.

Although the effects of I on platelets and other formed elements of blood have been studied, little is known regarding the effects of I or other phthalates on the thrombogenicity of polymer surfaces. The studies reported here evaluate the alteration of polyvinyl chloride surface characteristics with the accumulation of I. It is postulated that exposed I on a polyvinyl chloride surface increases hydrophobicity, resulting in greater adsorption of γ-globulin and fibrinogen onto the surface, an important factor for platelet adhesion and aggregation (22).



I

EXPERIMENTAL

Several experiments characterized the surface nature of polyvinyl chloride contaminated with I and the same material after extraction of I from the surface to determine the possible adverse effects of I upon contacting blood. The plastic used was a sheet of polyvinyl chloride supplied by a major manufacturer of blood bags used extensively in clinics and hospitals for the collection and storage of blood or blood fractions.

Since different batches vary considerably in composition and physical characteristics, polyvinyl chloride from a single batch was used in all studies. The age of the sheet ranged from about 6 to 8 months during the experiments. Similar formulations are employed in the manufacture of tubing used in blood administration assemblies and extracorporeal circulation devices.

Phthalate Extraction and Surface Characterization of Polyvinyl Chloride—Polyvinyl chloride samples (2 × 2.5 cm) were stored in 20 ml of methanol for varying periods. The quantity of I extracted was determined after drying at 50° for 15 min. Water contact angles were determined[1]. Attenuated total reflectance spectroscopy[2] was used to calculate the amount of surface phthalate.

The standard IR bands used were the carbonyl stretching vibration at 1720 cm^{-1} for phthalate ester and the methylene bending vibration at 1430 cm^{-1} for polyvinyl chloride. The plateau time was determined from the contact angle measurements and the weight of extracted phthalate. Surface phthalate was considered to be removed when the contact angle reached a plateau. The standard IR bands ratio, methanol extraction time, and decreasing weight of the sample provided data for the calibration curve for surface phthalate amounts.

Adsorption of Plasma Proteins on Polyvinyl Chloride Surfaces—The plasma protein solutions used consisted of albumin (human crystallized 4×), γ-globulin (bovine fraction II), and fi-

[1] Zisman goniometer, Rame-Hart, Inc.

[2] Perkin-Elmer model 521 grating IR spectrophotometer with Wilks Scientific Corp. attenuated total reflectance attachment.

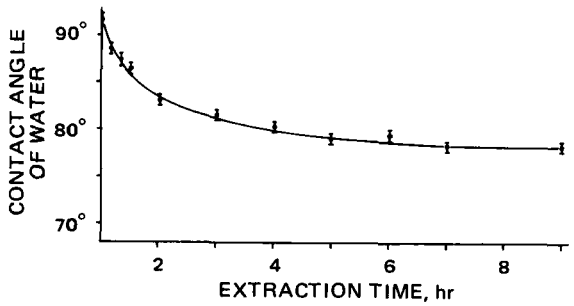


Figure 1—*Water contact angle as a function of methanol extraction time.*

brinogen (bovine fraction I) at physiological concentration ratios. In each experiment, one of the three proteins was radiolabeled with iodine-125. The polymer samples were clamped in cells designed for static adsorption. The samples were exposed for varying periods, following which they were rinsed with pH 7.4 isotonic phosphate buffer saline at a flow rate of 10 ml/sec for 1 min to remove excess solution (23).

The adsorbed amount of labeled protein was determined as a function of time by direct measurement of radioactivity on a section of polyvinyl chloride, using a scintillation counter[3]. The experiment was performed on phthalate-contaminated and methanol-extracted polyvinyl chloride surfaces. The latter was extracted with methanol overnight.

***Ex Vivo* Platelet Adhesion**—Phthalate-contaminated and methanol-extracted polyvinyl chloride surfaces were exposed to unheparinized animal blood. Sheep were cannulated, and the blood was allowed to flow through a specially designed polycarbonate cell wherein polyvinyl chloride was exposed to the flowing blood for 3 min by a technique described previously (22). The samples were rinsed with saline, and the platelets were fixed with glutaraldehyde and stained with Wright's staining solution. Twenty fields of each sample were used to determine the average number of platelets. A microscope[4] equipped with incident and transmitted light was used to visualize the platelets.

RESULTS AND DISCUSSION

Bulk phthalate constantly diffuses to the surface of polyvinyl chloride due to the concentration gradient. As shown in Fig. 1, the water contact angle decreases (wettability increases) as methanol extraction time increases until a plateau is reached, which requires about 6 hr at ambient temperature. The hydrophobic nature of the contaminated surface (water contact angle of 91.6°) decreases by dissolution of the surface phthalate until the plateau (water contact angle of 78.2°) is reached. The plateau contact angle can be considered the water contact angle on a pure polyvinyl chloride surface.

The amount of extracted phthalate starts with 0.00162 g/cm^2 at 10 min and increases asymptotically until the plateau quantity of

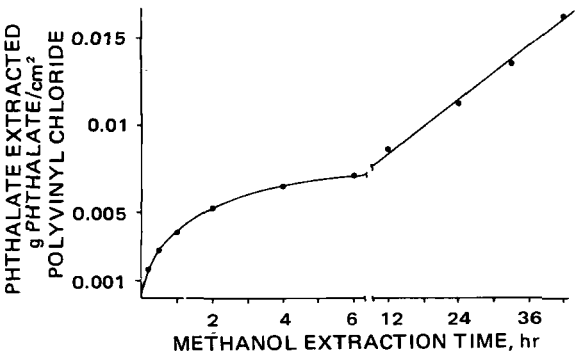


Figure 2—*Bis(2-ethylhexyl) phthalate (I) extraction as a function of methanol extraction time. The initial phase is a dissolution process followed by diffusion of bulk I through the surface.*

[3] Two-channel Nuclear Chicago model 725 liquid scintillation spectrometer.

[4] Nikon Apophot.

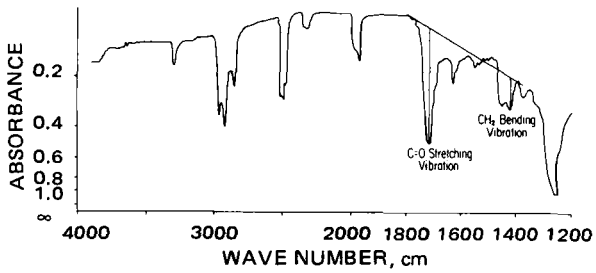


Figure 3—*Typical attenuated total reflectance tracing. The carbonyl stretching vibration for I is at 1720 cm^{-1}. The methylene bending vibration for polyvinyl chloride is at 1430 cm^{-1}.*

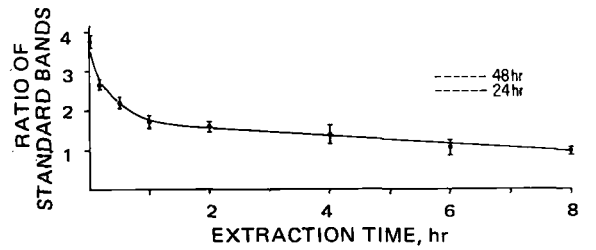


Figure 4—*I–polyvinyl chloride standard attenuated total reflectance bands ratio as a function of methanol extraction time. The dashes indicate surface I after 24 and 48 hr of air exposure following 6 hr of methanol extraction.*

0.00710 g/cm^2 is reached at 6 hr (Fig. 2). After 6 hr, the extracted amounts of phthalate increase linearly. The extraction and contact angle data indicate that surface phthalate is removed by a dissolution process. After dissolution of surface phthalate, bulk phthalate begins diffusion through the surface according to a linear curve fitting Fick's law of diffusion. These observations are valuable in obtaining the amount of surface phthalate and extraction time necessary for complete removal. There should be an overlapping step between dissolution and diffusion. The overlap would be expected to be indicated by a smooth curve at or near 6 hr rather than a sharp discontinuity.

A typical attenuated total reflectance tracing of polyvinyl chloride with surface phthalate is shown in Fig. 3. The characteristic band for I is at 1720 cm^{-1} for the carbonyl stretching vibration. A methylene bending vibration at 1430 cm^{-1} is characteristic of polyvinyl chloride. The peak for I includes the phthalate existing to a depth of approximately 1 μm. The ratio of the standard band of I to the polyvinyl chloride standard band *versus* methanol extraction time is plotted in Fig. 4. If a surface extraction time of 6 hr is assumed, a unique calibration curve is obtained to quantify the amount of phthalate existing on the surface. The calibration plot shown in Fig. 5 is a combination of Figs. 1, 2, and 4. Therefore, the quantity of surface phthalate can be determined using Fig. 5.

The kinetic curves for competitive protein adsorption are shown in Figs. 6–8. Albumin adsorption is greater on the methanol-cleansed surface. γ-Globulin and fibrinogen adsorptions are higher on the untreated polyvinyl chloride surfaces. It was noticed in ear-

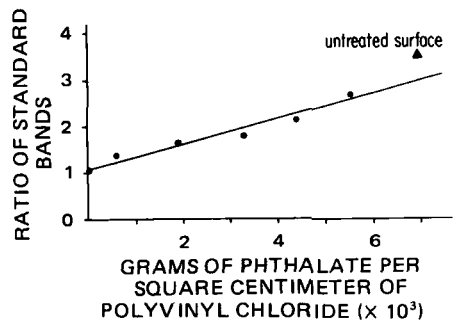


Figure 5—*Surface phthalate quantity measured as a function of the ratio of I–polyvinyl chloride standard attenuated total reflectance bands. Key: ▲, surface phthalate quantity on uncleansed polyvinyl chloride surface.*

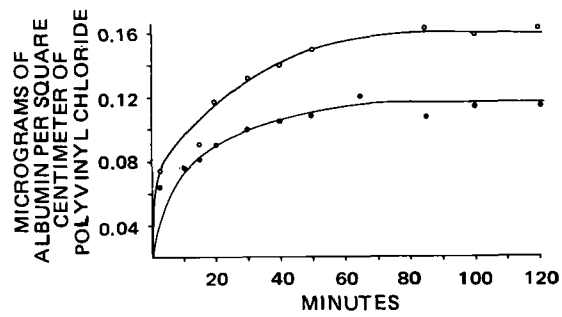


Figure 6—*Competitive adsorption of albumin from protein mixture. Key:* O, *methanol-extracted polyvinyl chloride surfaces; and* ●, *uncleansed polyvinyl chloride surfaces.*

lier studies (23, 24) that adsorbed protein amounts, plateau times, and adsorption rates depend upon the polymer surface. The governing factors might be the hydrophobic interaction between adsorbate and adsorbent, water structuring at the interface, hydrogen bonding, and the configurational entropy of proteins at the adsorbed sites.

In Fig. 6, it can be seen that the less hydrophobic extracted surface contains less phthalate, which increases albumin adsorption. However, the phthalate-containing surface shows an increase in adsorption of γ-globulin and fibrinogen when compared to the extracted surface (Figs. 7 and 8). When calculated on a weight percent basis after 2 min of adsorption, the phthalate-contaminated surface contained 6% albumin, 54% γ-globulin, and 40% fibrinogen; the extracted surface contained 19% albumin, 46% γ-globulin, and 35% fibrinogen. Following this initial determination, the weight percentages tended toward equalization over the 2 hr of observation. From these data, it can be seen that the initial events favor albumin adsorption onto the extracted surface; however, even pure polyvinyl chloride surfaces are not ideal for blood compatibility.

Another remarkable observation concerns the recontamination of the surface after methanol extraction. Polyvinyl chloride surfaces will recover the phthalate from the bulk polymer. After 1 day of exposure to air, 60% of the original quantity of surface I was restored *via* the diffusion process. After 2 days of exposure, 80% of the original quantity of I had diffused to the surface (Fig. 4).

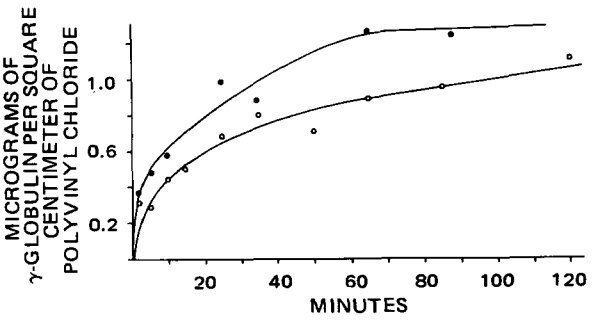


Figure 7—*Competitive adsorption of γ-globulin from protein mixture. Key:* O, *methanol-extracted polyvinyl chloride surfaces; and* ●, *uncleansed polyvinyl chloride surfaces.*

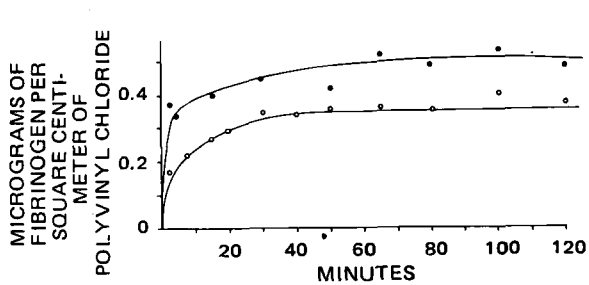


Figure 8—*Competitive adsorption of fibrinogen from protein mixture. Key:* O, *methanol-extracted polyvinyl chloride surfaces; and* ●, *uncleansed polyvinyl chloride surfaces.*

Two fibrinogen adsorption patterns were obtained, one for the original methanol-cleansed surface and the other following 2 days of air exposure after 6 hr of methanol extraction. In the latter, the fibrinogen adsorption pattern was similar to that on unextracted surfaces.

The number of adhered platelets differed for contaminated and extracted surfaces. According to the *ex vivo* study, the platelet counts were 3.1 ± 0.6/20,000 and 13.1 ± 2.8/20,000 μm^2 on methanol-extracted surfaces and untreated surfaces, respectively. Aggregated clumps of platelets also were observed on the untreated surfaces.

Although many extensive studies have been conducted on platelet adhesion and aggregation as related to hemostasis in the physiological environment (25, 26), few workers have focused on the foreign surface and certain chemical agents for aggregation. Since platelet adhesion to a surface and aggregation is the first observable event occurring in blood upon contact with a foreign surface, platelets play a major role in the *in vivo* initiation of thrombus formation on foreign surfaces.

Adenosine diphosphate, thrombin, collagen, and polylysine are platelet-aggregating agents (27). Adsorbed γ-globulin on a surface has been known to induce platelet aggregation on a surface (28). Fibrinogen has been shown to be an important factor for platelet adhesion to glass (29–31). However, it has not been fully explored whether or not plasticizers such as phthalate esters play a role as platelet-aggregating agents in the blood. When blood contacts a foreign surface, plasma proteins adsorb onto the surface until an equilibrium plateau is reached, and this proteinated surface determines the platelet adhesion. It was postulated (32) that glycosyl transferase on a platelet interacts, together with a nucleoside diphosphate monosaccharide and a divalent cation, with a glycoprotein acceptor adsorbed onto the foreign surface. The stability of the complex determines the strength and duration of the adhesion.

It has been shown that adsorbed γ-globulin and fibrinogen can be acceptors for enzyme complex formation while albumin is not. According to previous studies in this laboratory on competitive protein adsorption, the more hydrophobic polymers[5] adsorb fibrinogen and γ-globulin much faster than less hydrophobic polymers like polyurethane and dimethicone rubber (24). *Ex vivo* experiments have shown that about 10 times more platelets adhere to the more hydrophobic surfaces than to the polyurethane surfaces (22).

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High-Pressure Liquid Chromatographic Determination of the 15-Epimer of Dinoprost in Bulk Drug

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Abstract □ The *p*-nitrophenacyl esters of dinoprost and its 15-epimer are well resolved using high-pressure liquid chromatography. Quantitation was achieved using the internal standard technique. The specially synthesized diphenylurea ester of cholic acid was found to be a model internal standard. Graphs of peak height ratios of the prostaglandin to the internal standard were linear with respect to the amount of prostaglandin injected, with the lower detection limit of the 15-epimer being about 0.5%. Data are presented that demonstrate the usefulness of this analytical technique in determining the concentration of the 15-epimer present during studies on the kinetics of decomposition of dinoprost.

Keyphrases □ Dinoprost—high-pressure liquid chromatographic analysis of 15-epimer in bulk drug □ High-pressure liquid chromatography—analysis, dinoprost and its 15-epimer □ Prostaglandins—dinoprost and its 15-epimer, analysis, high-pressure liquid chromatography

Development of stability-indicating assays is necessary for evaluating the feasibility of pharmaceutical dosage forms before clinical testing programs are initiated. Prostaglandins, for example, are being developed in many different drug delivery systems (1), *e.g.*, solutions, suppositories, and tablets, where the spectrum of decomposition products depends upon the particular prostaglandin and dosage form. To obtain accurate rate constants for the decomposition process, more than one analytical technique may be needed to distinguish between various degradation products.

A case in point is the separation and quantitation of the 15-epimer of dinoprost in the presence of dinoprost. It was reported previously that dilute solutions of dinoprost are unstable in acidic media (2), with a major route of degradation being epimerization of the C-15 hydroxyl group[1]. To date, the 15-epimer has not been resolved from dinoprost by GLC (3), but it can be separated by TLC in a reported solvent system (4).

Recently, however, radioisotopically labeled methyl esters were used to determine the 15-epimer content in a prostaglandin closely related to dinoprost (3), while the 15-epimer content in bulk dinoprost was determined using a refractive index detector on a high-pressure liquid chromatograph (5). High-pressure liquid chromatography (HPLC) was used to detect and quantitate various prostaglandins (6).

Utilizing the discovery that prostaglandins can be rapidly converted to *p*-nitrophenacyl esters for HPLC (7), a procedure is presented that quantitatively separates the 15-epimer from naturally occurring dinoprost. The procedure involves the reaction of the carboxylic acid group of the prostaglandin with *p*-nitrophenacyl bromide to yield the UV-absorbing *p*-nitrophenacyl ester. The derivatives are well separated and are quantitated using the internal standard technique. This analytical procedure is not only useful in calculating the amount of 15-epimer in bulk dinoprost but also can be utilized to determine the concentration of the 15-epimer in solution during studies on the kinetics of decomposition of dinoprost.

[1] T. J. Roseman, unpublished data.