Specific Refractive Index Increments of Polymer Systems at Four Wavelengths

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The accurate calibration of a Brice-Phoenix differential refractometer for use with laser light of 632.8 nm is described. Specific refractive index increments are reported at 435.8, 546.1, 589.3, and 632.8 nm for polystyrene in toluene, polydimethylsiloxane in chloroform, and aqueous solutions of bovine serum albumin, *D*-glyceraldehyde-3-phosphate dehydrogenase, amylopectin, and DNA.

To ascertain whether a low-angle laser scattering photometer under development (*16*) would give absolute molecular weights for biological macromolecules, it was necessary to obtain specific refractive index increments for light of wavelength 632.8 nm (He–Ne laser). Such data were unavailable for polymers although calibration data have been reported (*21*) for 589.3 and 632.8 nm for a series of electrolytic solutions. Anderson (*1*) also calibrated a commercial differential refractometer resembling that of Brice and Halwer (*5*) using the data obtained on salt solutions interferometrically by Kruis (*20*), and more recently Block (*4*) described a technique for using 632.8-nm radiation from a secondary source in differential refractometry.

None of these investigators determined specific refractive index increments of polymer systems. We report refractive index increments for a number of polymers determined with the commercially available Brice-Phoenix differential refractometer at four wavelengths from 632.8 to 435.8 nm. Calibration measurements were made at 24.2 ± 0.1°C by use of potassium chloride and sucrose, followed by studies of polystyrene, bovine serum albumin, *D*-glyceraldehyde phosphate dehydrogenase, polydimethylsiloxane, amylopectin, and deoxyribonucleic acid (DNA).

Experimental

Materials and equipment. Potassium chloride, potassium hydrogen phosphate, potassium phosphate monobasic, and sucrose were Baker-analyzed reagent quality. The potassium chloride was dried to constant weight under vacuo at 200°C before use (less than 0.1 wt % loss). All other inorganic salts were Mallinckrodt analytical reagent quality and were used without further purification. Chloroform and toluene were likewise Mallinckrodt analytical reagents. The chloroform was shaken with silica gel and further distilled through a column (*24*) before use. Silica gel of 6–12 mesh was from Matheson Coleman & Bell. Concentrated hydrochloric acid was Baker and Adamson reagent quality. Triple A Co. distilled water was used without further treatment. Tris [tris(hydroxymethyl)-aminomethane; Sigma 7-9 from Sigma Chemical Co.] was used for preparation of this buffer of pH 7.0 with HCl.

One polystyrene was NBS 706, a broad molecular-weight sample of weight average molecular weight 257,000 from the National Bureau of Standards. The other, of weight average molecular weight 1,800,000, was a special polystyrene standard (lot No. 149) of $\bar{M}_w/\bar{M}n$ less than 1.20, from the Pressure Chemical Co. There was no measurable loss of weight on drying under vacuum at 120°C.

A sample of polydimethylsiloxane (≂145c–03) was furnished by A. C. Ouano of IBM, San Jose. Bovine serum albumin (BSA), A grade, fraction V (cat. No. 12967, lot No. 100130), was from California Biochemical Co. specified as containing 0.05% ash and 4% moisture. Actual moisture content was determined by drying to constant weight under vacuum at 120° [National Formulary method (*23*) and was 4.6%.

D-Glyceraldehyde-3-phosphate dehydrogenase (GPDH) was obtained as an ammonium sulfate suspension of the crystalline enzyme from Boehringer Manheim Co., West Germany. Amylopectin from dent corn hydrolyzed 3 hr was furnished in an acetate buffer of pH 3.94 by Stig Erlander of Ambassador College, Pasadena.

One sample of the sodium salt of calf thymus deoxyribonucleic acid (DNA) (A grade, No. 2618, lot 900007) was obtained from the California Biochemical Co. (Specifications: N, 12.21%; P, 8.04%; $E_{260}^{1\%}$ = 192; and moisture, 14%). Another sample of calf thymus DNA was obtained from the Sigma Chemical Co., type VI, lab 558–1330. All the polymers except polystyrene and polydimethylsiloxane were stored at 4°C except when in use.

The differential refractometer used was the commercially available Brice-Phoenix instrument, 1000D Series, No. 940. Additional external light sources were a sodium vapor lamp (Gates and Co., SLA-5C) and a helium–neon laser (Spectra-Physics Model 133).

Absorbance measurements were made on either Beckman DB, Beckman DK2, or Cary Model 14 spectrophotometers. The pH was determined with a Beckman Model G pH meter. The temperature was determined with a thermometer calibrated against ice and $Na_2SO_4 \cdot 10H_2O$. Constant temperature (24.2 ± 0.1°C) in the differential refractometer was maintained by a Heto Temp-Adjust colorimeter cell (Denmark).

An acetate buffer solution of pH 4.9 and ionic strength 0.2, prepared from 72 ml of solution 0.2*M* in KCl and HCl and 1000 ml of solution 0.05*M* in sodium acetate and 0.15*M* in KCl, was used to prepare buffered BSA solutions. A phosphate buffer of pH 7.0 and ionic strength 0.14 prepared by dissolving 1.3382 grams of KH_2PO_4, 2.319 grams of K_2HPO_4, and 0.370 gram of the disodium salt of ethylenediaminetetraacetic acid (EDTA) to form a liter of solution 0.02*M* in total phosphate and 0.001*M* in EDTA, was used for preparation of GPDH solutions and their dialysis. Tris buffer of pH 7.0 and ionic strength 0.15, prepared by combining 1 liter of 0.1*M* Tris, 932 ml of 0.1*M* HCl, 11.69 grams of NaCl, and 68 ml of water, was used for preparation of DNA solutions and their subsequent dialysis. Some DNA samples were also studied in a BPES buffer of pH 6.8 and ionic strength 0.19 which was 0.006*M* in Na_2HPO_4, 0.002*M* in NaH_2PO_4, 0.001*M* in EDTA, and 0.179*M* in NaCl.

Stock solutions of calf thymus DNA (Cal Biochem) containing about 200 mg in 100 ml of the 0.05*M* Tris buffer were prepared at 4° and similarly DNA (Sigma) in BPES buffer. Both were dialyzed against their respective buffer solutions.

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Techniques. In determining scale deflections (Δd) in the refractometer, about 15 min were allowed after filling the cell for establishment of thermal equilibrium and adjustment of the eye to the darkened room. Precision was improved by adjusting the slit width to as narrow a width as could be seen, the width being less with the more intense laser light than with the other sources. Since there was good agreement between successive runs, in between which the position of the slit was readjusted, it appears there is no significant dependence of the value on the slit width.

When the He–Ne laser was used as an external source, a planocylindrical lens was placed at a distance of about 2 ft from the source and from a half-silvered mirror in the optical bench of the refractometer to expand the laser beam, which then flooded the slit. Use of a white card positioned in front of the half-silvered mirror, contrary to Block's experience (*4*), did not result in an adequate intensity of the laser beam through the refractometer. The micrometer microscope was refocused after each reading, the rotation knob turned back to the zero degree position, and readings were taken through both cell compartments until five or more readings of d_1 and d_2 were obtained agreeing within ±2 divisions in the sixth decimal place which is better than previously reported by others (*5*).

Even with the same solvent in both compartments, there was a constant difference (3 divisions in the case of water; less with other solvents) between the average values on interchanging the cell positions. Although inconsequential in the calibration runs, this was subtracted from all observed Δd values and was of greater importance in the case of dilute polymer solutions where the total difference in reading between solvent and solution was sometimes as small as 200 divisions. After measurements the cell contents were removed with a syringe, and the cells washed with five or more 2-ml portions of solvent until the equilibrium Δd value for the particular solvent was reached.

Values of K at 435.8, 546.1 and 589.3 nm could be readily calculated from the observed values of Δd and literature values (*2, 5, 20*) for the refractive index of the solutions at these wavelengths. To obtain the value of $K_{632.8}$, it was necessary first to determine the "magnification factor" for this wavelength, which is defined by the equation (5),

$$K_\lambda = (\cot i)/2\,M_0\,(a + b/2\,n_0 + t/n_w)$$

In this equation i, a, b, and t are determined by the geometry of the instrument and are constants independent of wavelength, whereas M_0 (the magnification of the instrument), n_0 (the refractive index of the solvent), and n_w (the refractive index of the glass) are dependent on wavelength. Consequently, the values of the calibration constant, K_λ, are inversely proportional to the magnification factor pertaining to the particular wavelength used. Hence, the constant for a desired wavelength can be calculated from that determined at a different wavelength if the magnification factors at the two wavelengths are known. Values of M were supplied by the manufacturer (*6*) as, respectively, 1.516, 1.512, and 1.509 at 589.3, 546.1 and 435.8 nm.

To determine $K_{632.8}$, the proportionality constant relating difference in scale displacement to difference in refractive index between solution and solvent at the wavelength of the He–Ne laser, the value of the magnification factor, $M_{632.8}$, had first to be determined. This was done utilizing the relation $M(\lambda_1) = \Delta d(\lambda_1)/\lambda d(\lambda_2) \times M(\lambda_2)$. To test the precision attainable, measurements were first made of Δd at 546.1 and 435.8 nm with the same slit width at each wavelength but four separate adjustments of the slit. The values of the ratio, $\Delta d_{546}/\Delta d_{435.8}$ obtained in these four runs were 1.0013, 1.0012, 1.0000, and 1.0021, averaging to 1.0012. This compares favorably with the value 1.0020 calculated from the ratio of $M_{546.1}/M_{435.8}$ using the manufacturer's values.

Measurements of the scale displacement ratios, $\Delta d_{632.8}/\Delta d_{435.8}$ and $\Delta d_{632.8}/\Delta d_{546.1}$, were then made similarly, and the results used to calculate the magnification factor at 632.8 nm from the given values at 435.8 and 546.1 nm. These were then used with the average values of $K_{435.8}$ and $K_{546.1}$, taken from Table II, to calculate the value of K at 632.8 nm from the relation $K(\lambda_2) = K(\lambda_1)\ M(\lambda_1)/M(\lambda_2)$. The results of this calibration are shown in Table I.

The cell constant, following Brice and Halwer (*5*), defined as $K_\lambda = \Delta n/\Delta d_{corr}$, was determined from measurements on four solutions of KCl in distilled water with the refractive index data of Kruis (*20*) and on two sucrose solutions using the data of Bates (*2*). As shown in Table II, both standards gave the same value for K.

Determination of dn/dc requires a measurement of the refractive index difference between solution and solvent, Δn, and of the concentration of the polymer. In the case of toluene solutions of polystyrene and chloroform solutions of polydimethylsiloxane, concentrations were calculated from the weight dissolved in 50 ml of solution, and a series of concentrations was prepared by quantitative dilution. The stock solution was kept at room temperature in a 50-ml glass-stoppered volumetric flask and lost only 6 mg out of an initial weight of 40 grams after four days' storage.

Measurements on buffered aqueous solutions of the other polymers were carried out at constant temperature and constant chemical potential of diffusible components (simple salts and solvent). This condition was met by dialyzing the buffered polymer solution against a buffer of fixed composition.

Dialysis was accomplished in a Crowe-Englander microdialyzer (*10*) with a measuring cylinder of about 5-cm diameter and a 500-ml capacity. Dialysis bags were made from cellophane tubing (Curtin Chemical Co. #077.032) of 1 cm-diameter and 25-cm length. The tubing was first boiled in 5% Na_2CO_3 solution about 10 min, boiled with distilled water, allowed to stand overnight in 0.1*M* HCl, rinsed with distilled water until there was no ultraviolet absorption or chloride present in the rinse, and stored in water until use. They were then rinsed with the appropriate buffer solution just prior to use.

Dialysis was carried out in a refrigerator near 0°C with magnetic stirring for 48 hr or more with two or more

Table I. Determination of Proportionality Constant Between Scale Displacement and Refractive Index at 632.8 nm

Displacement ratio		Magnification factor, M_{633}	Proportionality constant, K_{633}
$\Delta d_{633}/\Delta d_{436}$	Average	Calcd from M_{436}	Calcd from K_{436}
1.0045, 1.0056	1.0050	1.5165	1.0150
$\Delta d_{633}/\Delta d_{546}$		Calcd from M_{546}	Calcd from K_{546}
1.0045, 1.0031	1.0038	1.5177	1.0142
		Average	1.015

changes of dialysate in the outside chamber. That equilibrium was reached was shown by the fact that the end solution in the outside chamber had the same refractive index as the initial buffer solution as determined with the differential refractometer. After dialysis the assembly was allowed to stand at room temperature for an hour before opening the sack and transferring the contents to a 50-ml volumetric flask. Measurements of concentration and of change in refractive index of the dialyzed polymer with respect to solvent were made with the dialysate as a reference solution.

The concentrations of these solutions were determined from the ultraviolet absorption with a Beckman DB spectrophotometer except where otherwise stated. BSA was determined from the absorption at 280 nm, first demonstrating that the solutions obeyed Beer's Law and yielding a calculated value of 6.61 for the absorbance of a 1% solution in the acetate buffer of pH 4.9 in a 1-cm cell after appropriate allowance for the 4.6% moisture in the sample. This agrees well with the literature value of 6.60 (*8, 19*). GPDH concentration was likewise determined from the absorbance at 280 nm by using the molar absorptivity of Koshland and Conway (*17*).

DNA concentrations were determined from the absorbance at 260 nm by using both the Beckman DB spectrophotometer and the Cary Model 14, and an absorbance of a 1% solution in a 1-cm cell ($E_{1\%}{}^{1\,cm}$) of 1.92×10^2 as supplied by Cal Biochem. This value is almost exactly the average of values of 0.0181 and 0.0200 $cm^2/\mu g$ reported in the literature (*11, 12*). The ratio of the absorbance at 260 to that at 280 nm was also determined and found to be 1.93. The value of this ratio was used as a criterion that little or no denaturation of the DNA had occurred, native and denatured DNA giving values, respectively, of 1.90 and 1.41 (*22*).

The concentration of the amylopectin solution was determined by Stig Erlander in his laboratory with a Perkin-Elmer spectropolarimeter by use of $\alpha_D = 200°$.

Results and Discussion

The calibration data for determination of the proportionality constant, K_λ, for conversion of instrument scale displacements into refractive index differences between solvent and solution are given in Table II. Scale displacement on reversing solvent and solution positions (Δd) was determined for these solutions at 589.3 nm, leading directly to the value of $K_{589.3}$. Values of Δd were generally reproducible within ±2 divisions. Values of K at 546.1 and 435.8 nm were calculated from the values at 589.3 nm using the manufacturer's values for the magnification factor at these wavelengths, and at 632.8 nm from the magnification value at that wavelength determined as previously described.

Excellent agreement was obtained from the data on both sucrose and KCl solutions, with the exception of one KCl solution (0.04478 g/ml) where there must have been some systematic error since it gave values of K which were higher by about 0.5% than from any other solutions. The average value of K at each wavelength from Table II was then used to determine the specific refractive index increment for the polymer solutions from the observed values of Δd at each wavelength.

The results obtained for the specific refractive index increment of the various polymers are summarized in Table III. Actually, these are values of $\Delta n/\Delta c$, which are equal to dn/dc provided the solutions are sufficiently dilute that refractive index varies linearly with concentration. That such is the case is evident from the fact that the value of $\Delta n/\Delta c$, was independent of concentration within experimental error over the range investigated in the case of the two samples of polystyrene used and of the bovine serum albumin.

The greatest value of this work is the provision of accurate values of dn/dc at 632.8 nm which have not yet been reported in the literature. It may be useful, however, in assessing the reliability of these data to compare the present values of dn/dc at other wavelengths with those in the literature. The most comprehensive source of such data is the compilation made by Huglin (*14*), which should be consulted for all values not given a specific journal reference in the following discussion.

It is significant that our values for dn/dc for the two samples of polystyrene of different molecular weights and molecular-weight distributions should agree so closely at each of the four wavelengths. Previously reported values at 435.8 and 546.1 nm at (mostly) temperatures between 20 and 25°C range respectively from 0.111 to 0.118 and from 0.104 to 0.111 as compared to our values of 0.1155 and 0.1122. Although our values are slightly higher than many of those reported, the agreement is sufficiently good to confirm the reliability of the present work, and the accuracy of the new data at 632.8 nm.

Many authors have reported specific refractive index increments for bovine serum albumin, obtaining values in acetate buffer at 435.8 nm from 0.191 to 0.197 and at 546.1 nm of 0.184 to 0.187. The present values at these wavelengths are in excellent agreement, being correspondingly 0.191 and 0.186.

Values obtained in the present work for the specific refractive increment of glyceraldehyde-3-phosphate dehydrogenase in phosphate buffer at 435.8 and 546.1 nm are between 2 and 3% higher than the literature values (0.189 and 0.184, respectively). The difference here is likely attibutable to an uncertainty in the concentration owing to the use of different values for the specific absorbance by different investigators. Dandliker and Fox (*9*) used $E_{1\%}{}^{1\,cm}$ at 280 nm as 1000, whereas Jaenicke et al. (*15*) used a value of 1030. Koshland and Conway (*17*) calculated a value of $E_{1\%}{}^{1\,cm}$ of 1040, based on a molar absorptivity of 1.46×10^5 l./mol-cm from Dandliker and Fox and a molecular weight of 140,000. The present calculations of the enzyme concentration were based on a value of $E_{1\%}{}^{1\,cm}$ of 1040 at 280 nm.

Our values for dn/dc for amylopectin at 435.8 and 546.1 nm, respectively, 0.171 and 0.161, are significantly higher than the values of 0.156 and 0.152 reported by Erlander and coworkers (*14, 25*). There appears to be no obvious explanation for the discrepancy.

Values for the specific refractive index increment of DNA summarized by Huglin (*14*) vary widely, from 0.186 to 0.201 at 435.8 nm and from 0.174 to 0.185 at 546.1 nm. Some of the work, which shows the effect of changing ionic strength, solvent medium, and operating at constant chemical potential, is shown in Table IV. Particularly significant are the results of Cohen and Eisenberg (*7*) which show a considerable change in dn/dc of the DNA resulting from prior equilibrium dialysis against the solvent medium. However, in the present work (Table III), the difference between dialyzed and undialyzed samples was not found to be as large and was in the opposite direction.

The present value of dn/dc at 546.1 nm of 0.170 for calf thymus DNA in BPES buffer is in good agreement with the recent value of 0.166 ± 0.003 obtained by Krasna (*18*) by both interferometry and differential refractometry for DNA in the same buffer at concentrations from 0.05 to 1.6×10^{-3} g/ml, and of 0.172 reported by Harrington (*12*). Values obtained here at 435.8 nm fall comfortably within the range of values reported by previous investigators. There is only a slight dependence of the value of

Table II. Calibration Constants for Differential Refractometer at 24.2°C

Substance[a]	Concn, g/ml	$\Delta n \times 10^6$	Δd_{589}	$K_\lambda \times 10^6$ 589.3 nm	546.1 nm	435.8 nm	632.8 nm
KCl	0.01489	2004	1972	1.016	1.018	1.020	1.015
	0.02982	3960	3896	1.016	1.018	1.020	1.015
	0.05974	7766	7634	1.017	1.019	1.021	1.016
Sucrose	0.0200	2868	2824	1.016	1.018	1.020	1.015
	0.0400	5700	5612	1.016	1.018	1.020	1.015
Average				1.016	1.018	1.020	1.015

[a] Refractive index data for aqueous KCl solutions were from Kruis (**20**) and for sucrose from Bates (**2**), determined at 589.3 nm.

Table III. Specific Refractive Index Increments of Polymers at 24.2°C

Substance	Solvent	Concn, g/ml	dn/dc, ml/g 435.8 nm	564.1 nm	589.3 nm	632.8 nm
Polystyrene (NBS 706)	Toluene	0.00747	0.1154	0.1132	...	...
		0.01274	0.1149	0.1117	0.1108	0.1102
		0.01311	0.1149	0.1122	0.1117	0.1104
		0.01434	0.1151	0.1128	0.1123	0.1117
		0.01740	0.1142	0.1113	0.1107	0.1109
		Average	0.1149	0.1124	0.1114	0.1108
Polystyrene (Pressure Chem Co.)	Toluene	0.005914	...	0.1128	0.1123	0.1110
		0.00746	0.1160	0.1115	0.1114	0.1109
		0.01077	0.1160	0.1117	0.1102	0.1099
		0.01251	...	0.1120	0.1118	0.1112
		Average	0.1160	0.1120	0.1114	0.1108
Bovine serum albumin	Acetate buffer, pH 4.9	0.00597	0.190	0.186	...	...
		0.00719	0.191	0.188	0.184	0.184
		0.00729	0.192	0.186	0.185	0.185
		0.00741	0.190	0.185	0.182	0.182
		0.01028	0.191	0.185	0.183	0.184
		0.01032	0.193	0.186	...	0.183
		Average	0.191	0.186	0.184	0.184
Polydimethylsiloxane	Chloroform	0.001001	...	...	−0.059	−0.053
		0.01001	−0.064	−0.062	−0.059	−0.065
D-glyceraldehyde-3-phosphate dehydrogenease	Phosphate buffer, pH 7.0	0.00865	0.194	0.186	0.185	0.186
		0.00857	0.195	0.187	0.186	0.187
Amylopectin	Acetate buffer, pH 3.94	0.00347	0.171	0.161	0.159	0.160
DNA (Sigma Chemical Co.)	BPES buffer, pH 6.8	0.000865 (Dialyzed)	0.185	0.170	...	0.172
DNA (Cal Biochem Co.)	Tris buffer, pH 7.0	0.000860 (Undialyzed)	0.194	0.176	0.176	0.178
		0.000872 (Dialyzed)	0.197	0.178	0.178	0.183

Table IV. Specific Refractive Index Increments of DNA Solutions

Sample	Solvent	dn/dc, ml/g 435.8 nm	546.1 nm	Temp, °C	Ref
DNA	Various	0.186–0.201	0.174–0.185	...	*14*
Calf thymus DNA	Water	0.186	0.179	25	*7*
DNA, undialyzed	0.2M NaCl	0.182	0.176	25	*7*
DNA, dialyzed	0.2M NaCl	0.175	0.168	25	*7*
DNA, dialyzed	0.179M·NaCl, 0.006M $Na_2H\ PO_4$, 0.002M NaH_2PO_4, 0.001M EDTA pH 6.8	...	0.172	...	*12*
DNA, dialyzed	Same as above	...	0.166	...	*18*
DNA (Sigma Chemical Co.), dialyzed	BPES buffer, pH 6.8	0.185	0.170	24.2	This study
DNA (Cal Biochem), undialyzed	Tris buffer, pH 7.0	0.194	0.176	24.2	This study
dialyzed		0.197	0.178	24.2	...

dn/dc of DNA on wavelength except for the considerably higher value found at 435.8 nm than at the other wavelengths studied.

The value at 632.8 nm is slightly higher than that at 589.3 nm, opposite to the behavior found in solutions of polystyrene in toluene, where the value decreased monotonically with increasing wavelength. Likewise in the case of the other aqueous solutions (BSA, GPDH, amylopectin), the value of the specific refractive increment at 632.8 nm was either the same or slightly higher than that at 589.3 nm. This is contrary to the usual decrease in refractive index with increasing wavelength, described for pure substances empirically by the Cauchy equation (*3*). This effect, which was observed with all the aqueous solutions, may quite possibly be due to the 940.0- and 820.0-nm absorption bands of water (*13*) in the near infrared, which are not far removed from the longest wavelength at which refractive index measurements were made.

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Liquid-Liquid Distribution. Tributyl Phosphate Between Immiscible Solvents

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The extractability of tributyl phosphate from diluents by polar solvents, immiscible with the diluent, was measured at 25°. Distribution coefficients >1 resulted when using dipropylene glycol, methyl carbitol, and 1,2-propanediol with hexane as the diluent for the tributyl phosphate. 1,2-Propanediol extracted more tributyl phosphate from kerosine (Amsco 125–82), tri-*n*-butylamine, and petroleum ether than from hexane. The tributyl phosphate concentration had little effect on the distribution coefficients until enough was present to make the three components miscible.

Tributyl phosphate (TBP), in a hydrocarbon diluent, is an important and useful solvent for the separation and purification of electrolytes in nuclear energy technology. Because the purification or analysis of contaminated solvent mixtures may involve the separation of TBP from its diluent, this work was undertaken to demonstrate TBP's extractability from various diluents. The analysis of solutions was simplified by the use of ^{32}P-labeled TBP (*5*).

The hydrogen bonding ability of TBP (*10*) and related compounds (*13, 16*) suggested the possibility of removing the TBP by using polar solvents (*1*). Significant TBP extraction by polar compounds has been noted by several investigators (*1, 3, 4, 9, 11, 12, 14*). No data, however, other than ours in an institutional document (*1*), apparently have been published except those showing the small amount of TBP extracted by alkaline ethylene glycol (*3*) and by ethanolamine (*3, 4*). Therefore, this summary covering the distribution of P in a wide variety of immiscible solvent mixtures is presented.

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Experimental

Phosphorus-32-labeled tributyl phosphate (TBP-^{32}P) was prepared by using the ester interchange method (*5*). The distilled product (118°C at 1 torr) was treated with 1*M* NaOH and several water washes before being dried in vacuo (1 torr at room temperature for several hours). Its specific activity decreased from 5×10^3 d/m/mg TBP-^{32}P to 1.2×10^3 d/m/mg TBP-^{32}P during the course of the study.

The glycol solvents, purified by distillation, were vacuum dried at 1 torr for at least 2 hr at room temperature just prior to their use. The kerosine (Amsco 125-82), hexane, and petroleum ether were used as received since they were mixtures of hydrocarbons. Dibutyl ether was distilled before use; the remaining solvents were used as received.

Distribution measurements were made in 15-ml, glass-stoppered graduated centrifuge cones. Five-ml portions of each solvent were pipeted into the cone, and 0.050 ml of TBP-^{32}P was added. The cones were stoppered and tum-