perimental percentage composition and the calculated values varies from 0.4 to 2.2%. This agreement is satisfactory for routine control purposes.

As the amine solutions used in the regeneration study contained some phenol, a test was made to determine the effect of small amounts of phenol on the end point by adding 0.5 gram of phenol to 25 ml. of mixture 3. The titration curve for this case, mixture 4 in Table I, is shown as curve *B* in Figure 3. By comparing curves *A* and *B*, it is evident that the effect of a small amount of phenol on the accuracy of the titration is rather small.

To determine whether or not other acidic materials, such as carboxylic acids, generally present in coal-tar distillates, would interfere with this determination, a carbonated amine solution actually used in a tar acid extraction experiment was titrated to determine the amine and the carbon dioxide contents. Curve *C* of Figure 3 shows the titration curve. Examination of this curve reveals no interference. This method was therefore adopted in the study of the regeneration of the carbonated amine solution used in the phenol extraction process.

In general, while the presence of acids weaker than carbonic acid, such as phenols, will not interfere with determination of the end points of titration, the presence of sulfur dioxide, carbonyl sulfide, and acids such as hydrogen sulfide, acetic acid, etc., as usually encountered in commercial synthesis gas purification processes using ethanolamine as absorbent, is expected to cause trouble. However, the procedure as outlined was developed specifically for a tar acid–amine–carbon dioxide system in which these constituents are not generally present. Therefore, judgment must be exercised in using this procedure for analyzing samples containing appreciable amounts of interfering materials.

Table I. Results of Titration

	Calculated Values, %		Titration Values, %		Difference, %	
	Free amine	Amine carbonate	Free amine	Amine carbonate	Free amine	Amine carbonate
Stock solution 1	100.0	0	100.0	0	0	0
Stock solution 2	29.8	70.2[a]	30.2	69.8	+0.4	−0.4
Mixture 1[b]	47.5	52.5[c]	46.9	53.1	−0.6	+0.6
Mixture 2[d]	65.0	35.0[c]	65.8	34.2	+0.8	−0.8
Mixture 3[e]	82.6	17.4[c]	83.3	16.7	+0.7	−0.7
Mixture 4[f]	82.6	17.4[c]	84.8	15.2	+2.2	−2.2
Actual sample	...	...	23.5	76.5	...	...

[a] Based on carbon dioxide as determined by evolution method.
[b] 75 vol. % stock 2 + 25 vol. % stock 1.
[c] Based on compositions of stock solutions.
[d] 50 vol. % stock 2 + 50 vol. % stock 1.
[e] 25 vol. % stock 2 + 75 vol. % stock 1.
[f] 25 vol. % stock 2 + 75 vol. % stock 1 + 0.5 gram phenol.

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Estimation of Serum Phospholipide and Total Phosphorus Using Chloric Acid

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▶ Chloric acid is a most suitable reagent for the digestion of serum and serum extracts in the colorimetric estimation of phosphorus. A sensitive and uncomplicated method for phosphorus is obtained in combining chloric acid digestion with the heteropoly blue color formed by hydrazine sulfate reduction of the molybdiphosphate complex.

CHLORIC acid has been used successfully for serum protein destruction in the estimation of protein-bound iodine (*5*). This reagent has the property of destroying the organic components of serum rapidly and requires very little attention during the process.

The phosphorus content following chloric acid digestion of serum was determined using the heteropoly blue color reaction of Boltz and Mellon (*2*), which employs hydrazine sulfate as a reducing agent. The color produced is not only more stable than that produced using 1-amino-2-naphthol-4-sulfonic acid (*3*) in the reduction of the phosphomolybdic acid complex but also far more sensitive.

REAGENTS

CHLORIC ACID. Weigh 500 grams of potassium chlorate into a 3-liter beaker, add 900 ml. of distilled water, and heat on a hot plate until solution is effected. Remove from the hot plate and add slowly with constant stirring 375 ml. of 72% perchloric acid which precipitates potassium as the perchlorate. Cool and place the covered beaker in a refrigerator for 12 to 24 hours. Filter on a Büchner funnel with suction through a Whatman 41H paper. The filtrate is approximately 28% chloric acid.

STANDARD PHOSPHORUS STOCK SOLUTION. Dissolve exactly 0.351 gram of pure, dry monopotassium phosphate in distilled water and transfer quantitatively to a 1-liter volumetric flask. Add 10 ml. of 10*N* sulfuric acid, dilute

to the mark with distilled water, and mix. This solution contains 0.08 mg. of phosphorus per ml.

WORKING STANDARD PHOSPHORUS SOLUTION. Quantitatively transfer 50 ml. of the standard stock solution to a 200-ml. volumetric flask and dilute to the mark. Each milliliter now contains 0.02 mg. of phosphorus.

MOLYBDATE SOLUTION. Dissolve 2.5 grams of sodium molybdate in 100 ml. of 10*N* sulfuric acid.

PROCEDURE FOR PHOSPHOLIPIDE PHOSPHORUS

Extraction. Pipet 1 ml. of serum dropwise into a 25-ml. volumetric flask containing approximately 18 ml. of alcohol-ether (*1*) mixture (3 parts of 95% reagent alcohol and 1 part of reagent grade ether). Place the extract in a 70° C. water bath and permit the alcohol-ether mixture to boil for approximately 30 seconds. Remove the flask, cool to room temperature, and dilute to the mark with alcohol-ether mixture. Filter rapidly through a fluted No. 1 Whatman paper into a 50-ml. graduate. Cover the funnel with a watch glass to prevent evaporation of the solvent.

Table I. Comparison of Methods for Phospholipide Phosphorus in Blood Serum

(Mg. of phosphorus per ml.)

Chloric Acid Method, at 830 mμ	Chloric Acid Method, at 655 mμ	Modified Youngburg Method, at 655 Mμ
8.8	...	9.0
9.1	...	9.0
9.6	...	9.5
9.1	...	9.0
8.9	...	9.1
8.1	...	8.2
9.0	...	8.0
8.4	...	8.2
8.7	...	8.9
9.0	...	9.0
10.5	...	10.6
10.3	10.1	10.1
8.6	8.4	8.5
9.6	9.6	9.7
10.8	10.1	10.3
9.3	9.2	9.4
9.8	9.6	9.9
8.3	8.2	8.3
8.5	8.3	8.2
8.9	...	8.9

Digestion. Transfer 5 ml. of the filtrate (0.20 ml. of serum) to a 180-ml. electrolytic beaker and evaporate to dryness on an electric hot plate at low temperature. Add 5 ml. of chloric acid and 2 drops of a 10% sodium chloride solution. Add a boiling stone and cover the beaker with a borosilicate watch glass. Digest the sample in a fume hood on a hot plate at medium temperature with the watch glass in place until white fumes of perchloric acid appear in the beaker. Remove the watch glass and continue the digestion to incipient dryness. Remove the beaker from the hot plate and allow it to cool. Add approximately 10 ml. of distilled water from a wash bottle, using the stream to wash the walls of the beaker. Warm the beaker to redissolve the perchlorate crystals.

Color Development. When the solution has cooled, neutralize it by adding 2*N* sodium hydroxide dropwise, using phenolphthalein (1% solution in 95% alcohol) as the indicator. Add 5 ml. of sodium molybdate solution. Use water washings to transfer the sample to a 50-ml. volumetric flask. Add 2 ml. of 0.15% hydrazine sulfate solution. Dilute the sample to 50 ml. with distilled water, then stopper the flask at once. Heat the stoppered flask in boiling water for 15 minutes to develop the color, then cool in cold water. Determine the absorbance of the sample with a Beckman Model DU spectrophotometer at 655 or 830 mμ after setting the reagent blank at zero. The color is stable for at least 12 hours.

Table II. Total Phosphorus Determination of Pooled Serum

(Mg. of phosphorus per ml.)

Chloric Acid Method, at 830 Mμ	Modified Youngburg Method, at 655 Mμ	Difference
12.8	12.6	0.2
13.6	13.5	0.1
14.2	14.1	0.1
14.3	14.4	−0.1
15.8	15.8	0.0
14.0	14.1	−0.1
13.7	13.8	−0.1
16.5	16.4	0.1
12.9	12.5	0.4
10.4	10.4	0.0
11.6	11.2	0.4
13.8	14.0	−0.2

Standard Curve. Prepare a reagent blank and three standards by measuring 0.0, 0.5, 1.0, and 2.0 ml. (1.0 ml. ≈ 0.02 mg. of phosphorus) of the working standard into a series of four 180-ml. electrolytic beakers. Add 2 drops of 10% sodium chloride solution to the contents of each beaker. Digest the blank and the standards, develop their colors, and determine the absorbances as described above.

PROCEDURE FOR TOTAL PHOSPHORUS

To 0.1 ml. of whole serum in a 180-ml. electrolytic beaker, add 7 ml. of chloric acid and proceed with digestion and color development as for phospholipide phosphorus.

EXPERIMENTAL

Table I compares the chloric acid method with the modified Youngburg method (*4*) using the Fiske-Subbarow (*3*) color development for phospholipide phosphorus in serum. At both 830 and 655 mμ the chloric acid procedure agrees well with the Youngburg method. Table II shows a comparison of the two methods for total phosphorus in serum.

By following the described procedure, as little as 5 γ of phosphorus may be determined quantitatively at 830 mμ. The method may be scaled down for much smaller amounts. Phospholipide determinations may be made on 0.1 ml. of serum by extracting in 25 ml. of alcohol-ether mixture. Anywhere from 1 to 10 ml. (≈0.4 to 4 γ of phosphorus) of this extract may be used for digestion. For these small amounts the digestion mixture was reduced to 3.0 ml. of chloric acid and 1 drop of 10% sodium chloride. The sample was neutralized before color development with 1*N* sodium hydroxide. The other reagents were reduced to 1.0 ml. of sodium molybdate and 0.5 ml. of hydrazine sulfate. The final volume was made up to 10.0 ml. In all instances the color developed obeys Beer's law. Blanks are uniform and reproducible, an advantage not always obtained with aminonaphtholsulfonic acid. The absorbancy index of the color developed is 300 at 655 mμ and 837 at 830 mμ, while that of 1-amino-2-naphthol-4-sulfonic acid is 166 at 655 mμ and 138 at 830 mμ.

DISCUSSION

Over a 2-year period, chloric acid has been a most suitable agent for the digestion step in the estimation of serum phospholipide and total phosphorus. The chief advantages are that individual attention during the digestion procedure is unnecessary and the number of samples that may be determined at one time is limited only by the facilities for processing them. As many as 15 samples in duplicate are routinely handled in the authors' laboratory at a time. Chloric acid reagent begins to boil just above 100° C. and reaches a maximum temperature of approximately 200° C., where only salts and perchloric acid remain. Boiling is gentle, minimizing possible loss by spray or froth.

Heating to develop color by hydrazine sulfate is an added step, but the greatly increased sensitivity and stability of the heteropoly blue produced more than compensate for this. Hydrazine sulfate reagent is stable for months at room temperature, which is a distinct advantage over 1-amino-2-naphthol-4-sulfonic acid. The latter reagent may not be used to reduce samples which have been digested with chloric acid, because it causes erratic color production.

The addition of 1 or 2 drops of 10% sodium chloride solution to the standards before digestion with chloric acid enhances reproducibility. While the role of sodium chloride is not precisely known, it seems reasonable that some pyrophosphate may be formed in its absence. Naturally occurring sodium chloride should have a similar protective influence.

Volumetric flasks must be stoppered immediately after adding hydrazine sulfate, because exposure to air leads to erroneous results.

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The Rouy Method for Photoelectric Polarimetry

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▶A set of adapters, each employing two polaroid plates, can convert the photoelectric colorimeter to a highly sensitive polarimeter. The theory and experimental results for this method are given. When the phase angle between the polarizer and analyzer for the two adapters is the same but opposite in sign, the sensitivity will increase as the phase angle approaches 90°. Reproducible rotations of ±0.001° have been attained.

RECENT publications (*1, 2, 5*) have indicated several advantages of photoelectric polarimetry over visual polarimetry. All experimental work published in photoelectric polarimetry making use of polaroids has been based on the use of a single set of polaroids with their optical axes crossed usually at an angle $\theta = 45°$. Keston and Laspalluto (*3*) reported a method in which polaroids were employed at angles larger than $\theta = 45°$. Rouy (*4*) has pointed out that two sets of polaroids, each set having the same fixed angle but opposite in sign and having the phase angle greater than 45°, considerably enhance the sensitivity of the photoelectric polarimeter. Because an increased sensitivity as high as 10^2 over previous instruments of this type has been observed, the theory, apparatus, and experimental data for this method are considered.

THEORY OF METHOD

Measurements are taken with a set of adapters, constructed to hold the cuvette containing the optically active solution. One adapter has the axes of its front and rear polaroid plates crossed at $+\theta$, the other at $-\theta$. This is illustrated in the vector diagram Figure 1. When a sample having an optical activity, α, is placed between the polarizer and analyzer, the polarized amplitudes, $\overline{OP}_1$ and $\overline{OP}_2$, will have corresponding emerging amplitude projections:

$$\overline{OA_1} = \overline{OP_1'} \cos(\theta + \alpha) \quad (1)$$

$$\overline{OA_2} = \overline{OP_2'} \cos(-\theta + \alpha) \quad (2)$$

The corresponding light energies which are proportional to the square of the vector projections on the common axis, $\overline{OA}$, are given by Equations 3 and 4.

$$E_1 = \overline{OP_1'^2} \cos^2(\theta + \alpha) \quad (3)$$

$$E_2 = \overline{OP_2'^2} \cos^2(-\theta + \alpha) \quad (4)$$

Sources of extinction such as light absorption or scattering will alter Equations 3 and 4 by the same factor. One method of preventing this factor from affecting polarimetric determinations, is making use of a polarimetric scale such as R, where

$$R = \frac{E_2 - E_1}{E_2 + E_1} \quad (5)$$

Substitution of Equations 3 and 4 in Equation 5 leads to

$$R = \frac{\sin 2\theta \sin 2\alpha}{1 + \cos 2\theta \cos 2\alpha} \quad (6)$$

Equation 6 may be derived as follows: substitution of Equations 3 and 4 in Equation 5 and making use of the relationship that

$$\overline{OP_1'} = \overline{OP_2'}$$

yields

$$R = \frac{\cos^2(-\theta + \alpha) - \cos^2(\theta + \alpha)}{\cos^2(-\theta + \alpha) + \cos^2(\theta + \alpha)} \quad (5a)$$

Because

$$\cos^2(\theta + \alpha) = {}^1/_2[1 + \cos 2(\theta + \alpha)]$$

substitution in Equation 5a results in

$$R = \frac{1 + \cos 2(-\theta + \alpha) - 1 - \cos 2(\theta + \alpha)}{1 + \cos 2(-\theta + \alpha) + 1 + \cos 2(\theta + \alpha)} \quad (5b)$$

The identities,

$$\cos 2(\theta + \alpha) = \cos 2\theta \cos 2\alpha - \sin 2\theta \sin 2\alpha$$

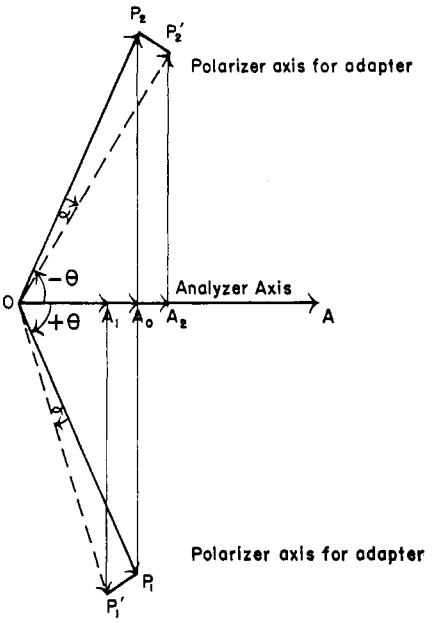

Figure 1. Effect of placing sample with optical activity, α, between polarizer and analyzer

$$\cos 2(-\theta + \alpha) = \cos 2\theta \cos 2\alpha + \sin 2\theta \sin 2\alpha$$

may be used in Equation 5b. Upon simplification, Equation 6 is obtained.

The latter equation may be put into the form of a series which may be used for experimental purposes, by using

$$\sin 2\theta = \frac{2 \tan \theta}{1 + \tan^2 \theta}$$

$$\cos 2\theta = \frac{1 - \tan^2 \theta}{1 + \tan^2 \theta}$$

and similar expressions for sin 2 α and cos 2 α. Substitution of these in Equation 6 yields

$$R = 2 \tan \theta \tan \alpha \, [1 + \tan^2 \theta \tan^2 \alpha]^{-1} \quad (6a)$$

The latter equation may be expanded in a series