to this effect, although it seems likely in view of Table II that increase in size of the polymer ion with increase in charge is the principal factor involved.

V. Summary

1. An investigation of the system polyacrylic acid–sodium hydroxide, with water as solvent, has been made by transference experiments using radioactive sodium tracer to determine the total flow of sodium. Measurements were made over the range 0 to 100% neutralization at two stoichiometric acid concentrations, namely, 0.0151 and 0.0378 *N*, and at 61.7% neutralization for an acid concentration of 0.1189 *N*.

2. A considerable fraction of the sodium ions are associated with polymer over most of the range; about one fourth of them at 25% neutralization, about two thirds of them at 100% neutralization.

3. The fraction of sodium ions associated increases monotonically with increase in neutralization at constant acid concentration. However, at constant neutralization, this quantity appears to decrease slightly with increase in acid concentration over the six-fold range investigated.

4. Over the range 25 to 100% neutralization the fraction of the current carried by polyacrylate ion is surprisingly high and roughly constant (0.4 to 0.5).

5. With increase in neutralization, the charge and size of the polymer ion increase and the equivalent conductance of the polymer ion goes through a maximum in the neighborhood of 60% neutralization.

6. With increase in acid concentration over the range 0.015 to 0.12 *N*, the charge and number of sodium ions on the polymer ion remain practically constant, but the polymer ion mobility decreases appreciably.

7. The effect of finite ion exchange on transference experiments is briefly considered.

URBANA, ILLINOIS RECEIVED SEPTEMBER 2, 1949

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Separation of Organic Acids[1]

BY C. S. MARVEL AND R. D. RANDS, JR.

In connection with the problem of the structure of diene polymers we have encountered the need for a rapid and efficient method for the separation of organic acids which have some degree of water solubility. Such a method has now been developed using partition chromatography. The apparatus is simple and the procedure has been standardized so that it may be used in qualitative examinations of acid mixtures by reference to a table of threshold volumes determined with known acids. Quantitative data are obtained at the same time for acids that are separated well and the standard procedure can be easily modified or extended to allow better separation of overlapping bands. Only 5–80 mg. of an acid mixture is required.

Several methods have been used for the separation of mixtures of organic acids. They include fractional distillation of methyl esters,[2] fractional crystallization,[3] and fractional extraction.[4] Separation by distillation of esters is limited to those acids having sufficiently volatile esters. Fractional crystallization usually fails with compounds of similar solubility properties. Extraction technics have been more successful in recent years but still are not always easy to apply to complex mixtures. Partition chromatography[5] has recently been used with considerable success to separate amino acids,[6] fatty acids,[7] and some polybasic acids.[8]

In the present investigation we have tried to develop a system which would separate as many of the water-soluble acids as possible. The system reported here is similar to those described by Isherwood[8a] and by Claborn and Patterson[8b] except we have systematically increased the polarity of the developing solvent. This scheme makes it possible to separate mixtures containing from two to seven water-soluble acids and also of separating these acids from less-polar acids or other less-polar compounds. As little as 0.5 mg. of an acid can be detected in an 80-mg. mixture under proper conditions. With a larger column than the one described here, several grams of acid mixtures have been separated into their component parts.

Water adsorbed on silicic acid acts as the immobile phase. The developing liquid or eluant is made progressively more polar in order to develop the more water-soluble acids. This is

(1) This investigation was carried out under the sponsorship of the Office of Rubber Reserve, Reconstruction Finance Corporation, in connection with the Government Synthetic Rubber Program.

(2) (a) Desparmet, French Patent 663,425, Feb. 17, 1928; *C. A.*, **24**, 628 (1930); (b) Rabjohn, Bryan, Inskeep, Johnson, and Lawson, THIS JOURNAL, **69**, 314 (1947).

(3) Lehman and Schroter, U. S. Patent 2,323,061, June 29, 1944.

(4) (a) Cassidy, THIS JOURNAL, **63**, 2735 (1941); (b) Ney, Crouch, Rannefeld and Lochte, *ibid.*, **65**, 770 (1943); (c) Craig, *J. Biol. Chem.*, **155**, 519 (1944); (d) Martin and Synge, *Biochem. J.*, **35**, 91 (1941); (e) Sato, Barry and Craig, *J. Biol. Chem.*, **170**, 501 (1947).

(5) Martin and Synge, *Biochem. J.*, **35**, 1358 (1941).

(6) (a) Consden, Gordon and Martin, *ibid.*, **38**, 224 (1944); (b) Stein and Moore, *J. Biol. Chem.*, **176**, 337 (1948).

(7) (a) Ramsey and Patterson, *J. Assoc. Official Agr. Chem.*, **28**, 644 (1945); (b) Ramsey and Patterson, *ibid.*, **31**, 139 (1948); (c) Ramsey and Patterson, *ibid.*, **31**, 441 (1948); (d) Elsden, *Biochem. J.*, **40**, 252 (1946).

(8) (a) Isherwood, *ibid.*, **40**, 688 (1946); (b) Claborn and Patterson, *J. Assoc. Official Agr. Chem.*, **31**, 134 (1948).

accomplished by adding increasing amounts of *n*-butyl alcohol to chloroform. The effluent is collected in measured fractions which are titrated. A graph of milliequivalents of acid per unit fraction *vs.* volume of effluent (chromatograph) shows clearly the efficiency of separation as well as the peak effluent volume of each acid. The peak effluent volume is defined here as that volume of effluent collected while a given compound moves from the top of the column to the bottom and is measured at the point at which the greatest concentration of the compound is eluted. It is apparent that such a factor is dependent upon the dimensions of the column, solvents used, and rate of change of the polarity of the eluant. When these variables are held constant, each compound has a characteristic peak effluent volume. A knowledge of these constants can be used in the tentative identification of the acid components of an unknown mixture. Positive identification can be obtained by combining succeeding fractions containing the same acid, evaporating off the solvent, and using the usual methods on the isolated compounds. It is often desirable to run the separation on a larger scale in order to have more material for identification purposes.

Table I lists the peak effluent volumes of thirty-six acids run on the partition chromatography system described here. All of the acids were added to the column as known mixtures containing from three to seven components. The identity of each separate peak in the chromatographs obtained from the titration data was determined by correlating the amount (milliequivalents) of each acid eluted with the amount added to the mixture. The mixtures were made up with different amounts of each acid so this would be possible. Figure 1 and Table II give an example of this for a mixture of five acids. The recovery of each acid is essentially quantitative.

TABLE I

PEAK EFFLUENT VOLUMES OF SOME ORGANIC ACIDS ON THE STANDARD COLUMN

Acid	Peak effluent volume, ml.	Acid	Peak effluent volume, ml.
m-Hydroxybenzoic	15[b]	Cyanoacetic[a]	275
Benzoic[a]	30	Formic[a]	285
Salicylic[a]	46	Glutaric[a]	295
n-Butyric[a]	55	Citraconic	315
Crotonic[a]	70	Itaconic	315
Mandelic[a]	105	Lactic	340
Sebacic[a]	125	Succinic	365
Propionic[a]	130	Maleic	395
Suberic[a]	135	Malonic	455
Trichloroacetic[a]	145	Aconitic	465
p-Hydroxybenzoic	146	Gallic	494
Chloroacetic[a]	155	β-Carboxyadipic	520
Adipic	173	Glycolic	577
Acetic[a]	185	Tricarballylic	618
Phthalic	185	Malic	775
Trimesic	230	Citric	925
Adipic[a]	235	Tartaric	1015
Glutaric	255	Glyceric	1070 (?)
Fumaric	265	Oxalic	1000 (?)

[a] Added to the column in chloroform solution. The others were added in alcohol–chloroform solution. [b] This peak effluent volume is low because the acid is washed through the column without development when using the standard procedure. If it is added to the column in less alcohol, it will be developed normally but it will come out at the same value as *p*-hydroxybenzoic acid.

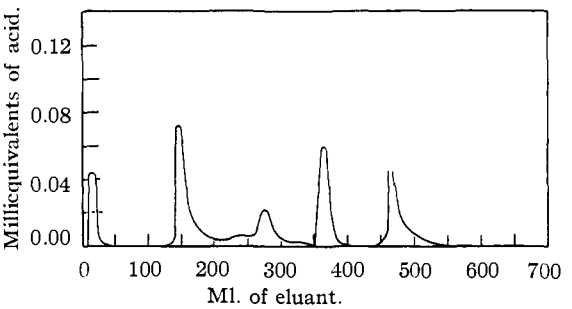

Fig. 1.—Threshold volumes of *m*-hydroxybenzoic acid (15 ml.); trichloroacetic acid (145 ml.); cyanoacetic acid (275 ml.); succinic acid (365 ml.); aconitic acid (465 ml.).

There must be at least a 20-ml. difference in the peak effluent volumes of two successive acids in order to be able to establish the existence of separate peaks. The exact difference necessary depends on the amount of the acids present and the sharpness with which they are eluted.

TABLE II

DETERMINATION OF PEAK EFFLUENT VOLUMES OF KNOWN ACIDS

Acid	Peak effluent volume, ml.	Amount added, meq.	Amount found, meq.	Recovery, %
m-Hydroxybenzoic	15	0.0493	0.0497	100.9
Trichloroacetic	145	.1702	.2494	102.6
Cyanoacetic	275	.0727		
Succinic	365	.1154	.1147	99.3
Aconitic	465	.1201	.1209	100.7

The manner in which the mixture is added to the column is very critical for those acids eluted in the first part of the run. If the mixture can be dissolved in chloroform, this is done but many of the water-soluble acids are insoluble in chloroform and a small amount of *n*-butyl alcohol must be used. In mixtures containing acids of both high and low water solubility some of the less soluble acids may be washed through the column without fractionation when alcohol is used as the solvent. This can usually be prevented, when the peak effluent volumes of known acids are to be determined, by first adding the water-soluble acids dissolved in *n*-butyl alcohol and chloroform, followed by a small volume of pure chloroform and then by addition of the others in chloroform solution. In this way the alcohol has been displaced down the column before the water-insoluble acids have been added. The extra chloroform does

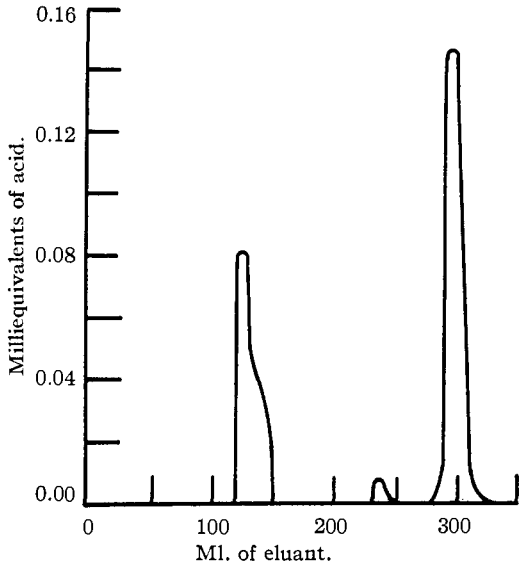

Fig. 2.—Separation of dibasic acids by standard procedure: sebacic–suberic acid mixture (125–150 ml.); adipic acid (235–250 ml.); glutaric acid (295–305 ml.).

not have any effect on the peak effluent volumes of acids developed later in the run.

When unknown mixtures are to be determined, any chloroform-soluble material is first extracted and run separately or added just after the non-chloroform-soluble acids have been added. Some acids which cannot be dissolved in chloroform and are highly alcohol-soluble and only slightly water-soluble, may be washed through the column without development or may be partly washed through and partly developed, thus giving two peaks. When 7 mg. of *m*-hydroxybenzoic acid was run with the standard procedure, it was eluted at a peak effluent volume of 15 ml., but when 18 mg. was dissolved in 0.2 ml. of *n*-butanol (instead of the standard 0.4 ml. of butanol), its peak effluent volume was 157 ml. *p*-Hydroxybenzoic acid which was run simultaneously came through at the higher value in both instances. Hence, these isomers are readily separated by the standard procedure.

The reproducibility of peak effluent volumes is good when a standard procedure is adhered to. Usually the same values are obtained in duplicate runs or in runs in which different components are present. Sometimes a difference of 10 ml., or more for those acids with higher peak effluent volumes, may be noted. The amount of acid present does not ordinarily have much effect on these values as long as all of the acid is in solution when added to the column.

One factor which may make a decided difference in the rate at which the acids are eluted is the silicic acid used. Different batches of silicic acid usually give different peak effluent volumes even when all other conditions are kept constant. It is necessary, therefore, on a new lot of silicic acid, to make a preliminary run with known acids if threshold volumes are to be used as evidence for the presence of these acids in an unknown mixture. In the work reported here, only one lot of silicic acid has been included. The peak effluent volumes given in Table I can be used to show the relative positions of the acids listed.

When two acid peaks overlap and it is necessary to have a better separation for a quantitative determination or for isolation and identification, the standard procedure can usually be modified to accomplish this. Figure 2 shows how sebacic and suberic acids are developed as a single band when 5% *n*-butanol in chloroform is added to pure chloroform. If these acids are developed first with 300 ml. of 1% *n*-butanol and then with 3% *n*-butanol, a complete separation is obtained as shown in Fig. 3.

Essentially, the same procedure can be utilized in large-scale separations of from 2 to 10 g. of acid mixture. Simple proportionality factors can be used to calculate the materials needed and the threshold values found. Here, 100- or 200-ml. fractions are collected from which 5- or 10-ml. aliquots are taken for titration. The remainder is set aside in numbered flasks until the titration data have been plotted. Consideration of the chromatograph will indicate which fractions should be combined and evaporated to obtain as pure acid samples as possible. Preliminary runs on a small scale to determine a good separation procedure will usually save time and material.

It will be noted in Fig. 1 that the acid peaks do not have a symmetrical shape but have more extended trailing edges. Isherwood[8a] ascribes this to partial adsorption of the acids and shows that essentially symmetrical peaks can be obtained by preliminary treatment of the adsorbent (silica gel) with concentrated hydrochloric acid and by the use of dilute sulfuric acid on the column to suppress ionization. Figure 4 shows the

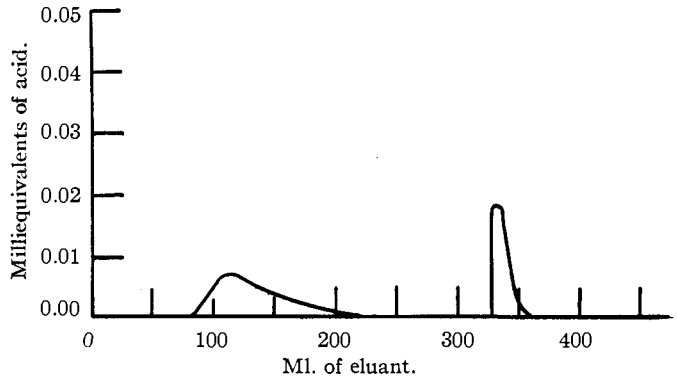

Fig. 3.—Separation of sebacic and suberic acid by modified procedure: sebacic acid (100–200 ml.); suberic acid (325–350 ml.).

effect of using 0.3 *N* sulfuric acid on our column. Trichloroacetic acid is entirely eluted in three 10-ml. fractions, whereas with water alone on the column ten or twelve fractions are required (see Fig. 1). The crotonic acid peak is improved but still tends to trail off. The use of dilute acid in the procedure described here would necessitate a blank run because increasingly more acid would be brought through as the alcohol concentration is increased.

Experimental

Apparatus.—The chromatographic tube is made from 1.8 cm. inside diameter Pyrex tubing 48 cm. long, constricted at the bottom and sealed to a stopcock. At the constriction a plug of cotton and a disk of filter paper act as support for the column.

Pressure is applied from a compressed air line or a tank of nitrogen with a suitable pressure regulating valve, or the following apparatus may be used: A rubber pressure bulb is connected through a three-way stopcock to the top of the column and to an air reservoir (2-l. bottle) provided with a mercury manometer for measuring pressure. After the system has been pumped up to the desired pressure, the stopcock is turned to connect only the reservoir and the tube thus preventing leakage back through the valve in the bulb.

Preparation of the Chromatographic Column.—In a mortar 20 g. of silicic acid[9] and 12 ml. of distilled water[10] is thoroughly mixed. The fine slurry prepared by stirring in 80 ml. of chloroform (technical) into the mixture is added to the tube which is then tapped to insure uniformity of the slurry. A pressure of 20–60 cm. is applied until the top of the column becomes firm but without drying out or cracking. If the latter occurs before the acids are added, the gel must be removed and reslurried. The amount of chloroform collected at the bottom of the column subtracted from the amount used in slurrying is the holdup volume. After some further settling, the column height is about 18.5 cm. and the holdup volume is 25–30 ml.

Standard Separation Procedure.—From 10–80 mg. of the acid mixture is completely dissolved in 1 or 2 ml. of chloroform. If all of the acids will not dissolve, the chloroform-soluble material is extracted and the remaining acids are dissolved in 0.4 ml. of *n*-butanol and diluted with 0.6 ml. of chloroform. The solution is carefully added to the top of the column by means of a pipet and forced into the column under a little pressure. To the original container is added 0.1 ml. of *n*-butanol and then 0.9 ml. of chloroform is introduced. A second rinse of 2.0 ml. of chloroform is also used.

The chloroform-soluble acids may be run separately or they may be dissolved in 1 or 2 ml. of chloroform and added to the column after the above solutions. Two 1.0 ml. chloroform rinses are used. The receiver at the bottom of the column is changed and the collection of 10 ml. fractions is begun. The developing liquid used is increased in polarity as follows:

1	100 ml.	chloroform[11]	
2	100 ml.	5% *n*-butanol[12]	95% chloroform V/V
3	100 ml.	10%	90%
4	100 ml.	15%	85%
5	100 ml.	20%	80%
6	100 ml.	25%	75%
7	100 ml.	30%	70%
8	100 ml.	40%	60%
9	100 ml.	50%	50%
10	100 ml.	70%	30%
11		100%	0%

Each solution is saturated with water by shaking with excess water and separating the excess. The wet solution (after the first 100 ml.) is added to the column before the last 10 ml. of the preceding solution has run in. This mixing allows a more gradual change in polarity of the solvent. A pressure of 60 cm. forces the eluant through the column at a rate of about 2–3 ml. per minute until high concentrations of alcohol decrease this rate considerably.

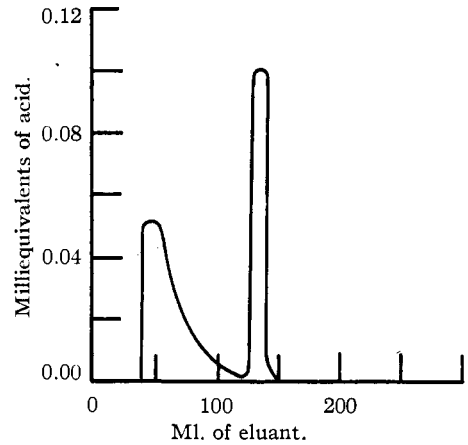

Fig. 4.—Separation of crotonic acid and trichloroacetic acid on silicic acid column acidified with 0.3 *N* H_2SO_4: crotonic acid (50–100 ml.); trichloroacetic acid (125–150 ml.).

The effluent is collected in 10-ml. fractions and titrated. It is convenient to calibrate the top of the tube at 10-ml. intervals and to collect the eluant in the containers used for titration. One drop of 0.1% phenol red indicator solution[13] and one drop of 2% Dreft solution are added and the solution is titrated with standard 0.02 *N* aqueous sodium hydroxide. Because there are two phases present, it is necessary to provide vigorous stirring. This can be done with a magnetic stirrer using an erlenmeyer flask or with the usual mechanical stirrer using a beaker. The former is preferred because there is less possibility of loss from spattering.

A small blank correction may have to be applied to the titration of the first 700–800 ml. of eluant. When high concentrations of butanol are used, slightly higher blank corrections are usually necessary. High blank values are caused by acid impurities in the solvents, particularly in the *n*-butanol.

Sample Separation of a Known Mixture.—Sebacic acid (8.9 mg.), suberic acid (6.8 mg.), adipic acid (1.0 mg.), and glutaric acid (14.7 mg.) were weighed out in a 10-ml. erlenmeyer flask and 2 ml. of chloroform was added. Heat and agitation were used to dissolve the mixture. A 1-ml. pipet was employed to transfer the solution to the top of a column prepared as described above. The tip of the pipet was placed about 5 or 6 mm. above the top of the silicic acid and the solution was added dropwise so that the smooth surface of the column was not disturbed. About 15 cm. pressure was used to force the solution into the column. The flask and pipet were washed with two 1-ml. portions of chloroform which were added and forced into the column separately. The container at the bottom of the column was replaced by a 50-ml. erlenmeyer flask. Fifty milliliters of chloroform saturated with distilled water was carefully poured into the tube with the aid of a

(9) Mallinckrodt's Analytical Reagent, 100-mesh; specify for chromatographic use.

(10) The amount of water for best results varies from one batch of silicic acid to another but can be determined easily by trial. Usually a little less than the maximum amount that can be mixed with the silicic acid and still give a smooth slurry with chloroform is satisfactory.

(11) Technical chloroform is satisfactory.

(12) Distilled over potassium carbonate.

(13) Lange, "Handbook of Chemistry," Handbook Publishers, Inc., Sandusky, Ohio, sixth edition, 1946, p. 1101.

stirring rod held against the side. The rubber stopper containing the air pressure line was plugged firmly into place and 60 cm. pressure was applied. The top of the tube had been calibrated at 10-ml. intervals starting from the top of the column and as the level of the liquid passed each mark, a new flask was put under the column, care being taken not to miss a drop in the transfer. When 10 ml. was left above the column, another 50 ml. of chloroform saturated with water was added and the pressure reapplied. In this manner 100 ml. of chloroform, 100 ml. of 5% *n*-butanol–95% chloroform V/V, 100 ml. of 10% *n*-butanol, and 100 ml. of 15% *n*-butanol were added.

To the 10-ml. fractions collected, one drop of phenol red indicator and one drop of Dreft solution were added and the solutions were titrated with 0.0203 *N* aqueous sodium hydroxide. The milliequivalents of acid in each fraction was calculated using a blank value of 0.01 ml. of base. A graph of milliliters of effluent *vs.* milliequivalents of acid was plotted and the points connected by a smooth curve (Fig. 2).

Determination of Peak Effluent Volumes.—The peak effluent volumes given in Table I were determined using the following known mixtures:

(1) Adipic (0.0845),[a] glutaric (0.0954), succinic (0.1154), malonic (0.1295), oxalic (0.1911).
(2) Mandelic[b] (0.0449), lactic (0.0978), glycolic (0.1008), malic (0.0929), citric (0.1422), tartaric (0.1640), glyceric (0.0355).
(3) β-Carboxyadipic (0.1557), tricarballylic (0.1092).
(4) *n*-Butyric[b] (0.0788), propionic[b] (0.1217), acetic[b] (0.1732), formic[b] (0.2590).
(5) Benzoic[b] (0.0594), phthalic (0.1156), trimesic (0.1440), gallic (0.0744), citric (0.1255).
(6) Chloroacetic (0.1197), trichloroacetic (0.0765), cyanoacetic (0.1028), itaconic (0.1451), citraconic (0.1014), aconitic (0.1201).
(7) Sebacic[b] (0.0835), suberic[b] (0.0731), adipic[b] (0.0120), glutaric[b] (0.2061).
(8) *m*-Hydroxybenzoic (0.0493), crotonic[b] (0.0712), *p*-hydroxybenzoic[c] (0.0652), fumaric (0.0677)[c], maleic (0.1121).
(9) Salicylic[b] (0.0478), chloroacetic[b] (0.0777), trimesic (0.1012), itaconic (0.1272).
(10) Benzoic[b] (0.1035), trichloroacetic[b] (0.1702), cyanoacetic[b] (0.0727).

[a] Milliequivalents, calculated from the weight and measured neutral equivalent. [b] Added to the column in chloroform solution after the others were added in alcohol–chloroform solution. [c] The peak effluent volumes of these acids were verified in other experiments not reported here.

Summary

A procedure for the separation of water-soluble organic acids using partition chromatography is described. This method is suitable for qualitative and quantitative determination of acids in mixtures.

The "peak effluent volumes" of thirty-six acids as determined by a standard procedure are listed. Mixtures containing acids of such closely related structures as *o*-, *m*- and *p*-hydroxybenzoic, lactic and glycolic, malic and tartaric, aconitic and tricarballylic, or adipic, glutaric, succinic, malonic and oxalic are easily separated. Sebacic and suberic or other acid mixtures that are not separated by the standard procedure may be separated by modification of it.

URBANA, ILLINOIS RECEIVED DECEMBER 16, 1949

[COMMUNICATION NO. 1274 FROM THE KODAK RESEARCH LABORATORIES]

The Structure of Ester-Lactone Polymers.[1] I. Ester-Lactones of the Maleic Anhydride–Vinyl Acetate Interpolymer

BY L. M. MINSK, G. P. WAUGH AND W. O. KENYON

Though maleic anhydride does not homopolymerize, it forms interpolymers with a variety of unsaturates.[2] The interpolymer with vinyl acetate consists of equimolar ratios of the two monomers in alternate array.[3] Upon treatment with alcohol and an acid catalyst, this interpolymer undergoes deacetylation, esterification and lactonization.[4] Resins of ester-lactone structures result from similar chemical treatment of the interpolymers of vinyl acetate with fumaric or maleic esters.

The structure assigned in the original disclosure of the ester-lactone resin[4] was based on certain chemical transformations and analyses of the product,[5] which involved determinations of the acetyl, free carboxyl and total saponification values. To confirm the compositions of these derived polymers, it was necessary to refine old and to devise additional analytical methods, particularly for those ester-lactones containing alkyl groups higher than ethyl.

The structures given may be assigned to the intermediate interpolymer and the derived ester-lactones.

Both γ- and δ-lactones are possible. The analytical methods here described do not discriminate between the two forms, since the analytical fragments are similar in both.

The anhydride interpolymer possesses acetyl and anhydride groups, and a small amount of free carboxyl, not indicated in the structural formula, which arises from slight hydrolysis. All three components should be determined in order that the summation of the analyses yield a complete description of the resin structure. The acetyl groups can be calculated to vinyl acetate, the anhydride groups to maleic anhydride, and the free carboxyl groups to maleic acid.

(1) Presented before the High Polymer Forum of the American Chemical Society at the Atlantic City, N. J., meeting, September, 1949.

(2) Voss and Dickhauser, German Patent 540,101, November 26, 1931, to I. G. Farbenindustrie A.-G.

(3) Tong and Kenyon, THIS JOURNAL, **71**, 1925 (1949).

(4) McNally and Van Dyke, U. S. Patent 2,306,071, December 12, 1942, to Eastman Kodak Company.

(5) Unpublished research of the late Dr. F. P. Pingert, formerly of these Laboratories.