

out exception, the carboxylic acid polymers (acrylic and maleic acids), demonstrated the most potential as prolonged-action coatings based on solubility in simulated gastric and intestinal fluids. Solubility of a maleic acid polymer varied in aqueous media at a range of pH levels with the degree of esterification of the polymer and with the ester chain length. Butyl half esters of the maleic acid polymer demonstrated good potential solubility properties for a prolonged-action coating. The intact films studied were impermeable to drug in solution. Polymer-drug binding was significant with only one of the three final polymers studied. Poly(methyl vinyl ether)/maleic anhydride *n*-butyl half ester demonstrated the best *in vitro* potential as a sustained-

action coating. The polymer showed no toxic effects in a preliminary short-term feeding study.

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One-Phase Solvent Systems for Paper Chromatography

Mixtures of *n*-Butanol, Acetic Acid, and Water

By T. J. BETTS

Whenever possible, one-phase solvent systems, made up of the minimum number of constituents, should be used for paper chromatography. Two-phase systems should be replaced by their equivalent one-phase mixtures giving stability, accuracy, and simplicity. A "phase diagram" of the type shown is required for all commonly used three-component paper chromatographic solvent mixtures to facilitate the design of one-phase solvents for particular problems. Mixtures of *n*-butanol, glacial acetic acid, and water were studied with this concept in mind.

SOLVENT MIXTURES used for paper chromatography may consist of one or two phases. The two-phase mixtures have been traditionally used for paper chromatography, and may themselves be divided into *direct phase* mixtures (1) in which the phase flowing over the paper is an organic substance such as *n*-butanol or phenol which has been saturated with a more polar liquid such as water, and *reversed phase* mixtures (2) in which the mobile phase is the more polar of the two. *Zaffaroni type* paper chromatography is an example of the direct phase method in which the paper is impregnated with a relatively nonvolatile stationary phase such as formamide, the mobile phase being a less polar liquid such as benzene (3). The paper may also be impregnated for reversed-phase chromatography, being made hydrophobic with a grease.

With the Zaffaroni method, the results obtained obviously depend greatly on partition effects

between the two phases. The term "partition" has been almost automatically associated with paper chromatography since the original paper of Consden *et al.* in 1944 (4), but some workers consider that in some cases other phenomena are involved such as adsorption (5,6) or ion exchange (7,8). Considering, for example, that tannins can be resolved with pure water as solvent (9), or hydroxyanthraquinones with pure toluene (10), the mechanism of partition is unlikely to be an explanation for all separations obtained by paper chromatography, even allowing for the formation of cellulose-water complexes (11).

The preoccupation with partition, however, has resulted in the use of many two-phase solvent systems; the conception apparently was that this would insure that partition takes place. The use of a two-phase system means that the composition of the mobile phase is not known, so that it cannot be varied in a controlled manner. In 1960, Smith (12) advocated the use of "monophasic" solvents in place of two-phase mixtures because "emulsions sometimes formed (during their preparation) and... a drop in

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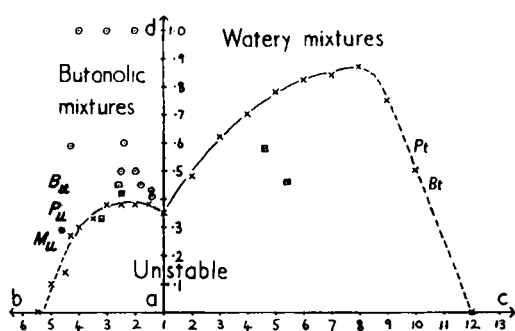


Fig. 1.—Quantities of glacial acetic acid required to just form one phase with various mixtures of *n*-butanol and water. Key: *a*-*b*, volume of *n*-butanol mixed with unit volume of water; *a*-*c*, volume of water mixed with unit volume of *n*-butanol; *a*-*d*, volume of glacial acetic acid required to form one phase of the above mixtures, in terms of the unit volume, *n*-butanol or water (whichever least). Temperatures of mixtures, 19–23°. Points marked *x* were calculated from experimental results. Points marked *o* represent one-phase mixtures in the literature. Points marked *□* were obtained from analyses, and represent the composition of the upper (*P_u*) and lower (*P_l*) phases of the Partridge mixture, the upper (*B_u*) and lower (*B_l*) phases of the Baker mixture, and the upper phase of the Munier mixture (*M_u*).

temperature during the overnight run sometimes led to the separation of water on the paper and resulted in unsatisfactory separations." This paper offers further support to his plea for simplicity. Although one-phase systems have been used since 1948 (13), the literature abounds with two-phase systems, those consisting of *n*-butanol, glacial acetic acid, and water being probably the most frequently used of all. Examination of the literature revealed 15 apparently different two-phase mixtures of this type, and 14 one-phase mixtures. The composition of some of the one-phase mixtures is shown in Fig. 1. This multiplicity led to the choice of mixtures of butanol, acetic acid, and water for study in relation to the concept of one-phase solvent systems.

EXPERIMENTAL

First, the minimum quantity of glacial acetic acid required to form one phase of various mixtures at approximately 21° of *n*-butanol and water was determined. From these results, the values used in plotting Fig. 1 were calculated. (Similarly shaped curves are obtained if ethanol or acetone is used instead of acetic acid.) Figure 1 indicates whether a particular mixture will form one phase. It might be anticipated that unstable mixtures which break down into two phases would form layers each of just stable composition. This hypothesis was tested by the analysis of the phases of typical two-phase mixtures. These were analyzed for their content of acetic acid and water, the butanol present being calculated. The acetic acid content

of a phase was determined by titration of 5 or 10-ml. portions with normal aqueous alkali to phenolphthalein. The water content was determined by titration of 0.05 to 0.25-ml. portions (measured with a micrometer syringe) with Karl Fischer reagent.

Analysis of Partridge's Solvent Mixture

This well-known solvent mixture was first used by Partridge in 1948 to examine sugars (14) and has been used for many other substances since then. It is prepared by shaking together 40 ml. of *n*-butanol, 10 ml. of glacial acetic acid, and 50 ml. of water. The upper phase (of approximately 51 ml.) which separates on standing is usually used as the mobile phase. Analytical results:

Upper Phase.—63.5% butanol, 10.7% acetic acid, 25.8% water are contained. [Smith (12) analyzed this upper phase as butanol-acid-water = 62:12:26 at 20°.]

Lower Phase.—16.1% butanol, 9.4% acetic acid, 74.5% water are contained. Converting these analyses to proportions corresponding to Fig. 1 gives:

Upper Phase.—2.5 butanol:0.42 acetic acid:1 water.

Lower Phase.—1 butanol:0.58 acetic acid:4.6 water.

From Fig. 1 it is apparent that the upper phase should be a stable mixture as it contains acetic acid in slight excess of the amount required to form one phase from the proportions of butanol to water present. Surprisingly, the lower phase appears to be an unstable mixture containing insufficient acetic acid for stability.

Analysis of Baker's Solvent Mixture

This solvent mixture was first used by Baker *et al.* (15) to examine amines and has since been used for hydroxyanthraquinone aglycones (16) and glycosides (17). If it is prepared by shaking together 50 ml. of *n*-butanol, 10 ml. of glacial acetic acid, and 40 ml. of water, an upper phase of approximately 72 ml. separates on standing and is the mobile phase. Analysis gives:

Upper Phase.—63.8% butanol, 11.2% acetic acid, 25.0% water, or a ratio of 2.6 butanol:0.45 acetic acid:1 water. This stable mixture is very similar to the upper phase of the Partridge mixture, although it is prepared from different proportions of butanol and water. From the results for the upper phase, the following was calculated:

Lower Phase.—1 butanol:0.46 acetic acid:5.4 water. Like the lower phase of the Partridge mixture, a mixture of this composition should be unstable.

Analysis of Munier's Solvent Mixture

This represents solvent mixtures even more rich in butanol. It was used by Munier and Macheboeuf (18) to examine alkaloids, but is imprecisely defined. *n*-Butanol-glacial acetic acid (100:10) saturated with water may be prepared with the minimum quantity of water to give two phases, or with—for example—a quantity of water equal to that of the organic solvent mixture. For this work, 50 ml. of *n*-butanol with 5 ml. of glacial acetic acid required 17 ml. of water to form two phases. An upper phase of approximately 69 ml. separated on standing. Analysis results:

Upper Phase.—70.7% butanol, 7.3% acetic acid, 22.0% water, or a ratio of 3.2 butanol:0.33

TABLE I.—AVERAGE R_f VALUES ($\times 100$) OBTAINED WITH VARIOUS MIXTURES OF *n*-BUTANOL AND WATER MADE INTO ONE PHASE^a

Solvent Mixture	Morphine	Thebaine	Quercetin ^b	Quercitrin	Aloe-emodin	Barbaloin	Leucine	Rhamnose
<i>n</i> -Butanol-glacial acetic acid-water								
(1) 1.5:0.4:1	59	78	70	79	90	80	66	45
(2) 2.25:0.4:1	53	74	68	77	89	76	61	36
(3) 2.5:0.4:1 ^c	46	71	69	78	89	74	61	33
(4) 2.8:0.4:1	43	70	73	79	90	75	59	31
(5) 2.8:0.8:1	54	75	65	72	87	73	67	38
(6) 2.8:1.6:1	64	80	56	66	84	71	72	45
(7) 3.0:0.4:1	42	70	72	78	90	73	57	32
(8) 4.5:0.2:1	33	60	st ^d	st ^d	87	71	41	27
(9) 5.5:nil:1	st ^d	st ^d	st ^d	st ^d	85	67	28	22
<i>n</i> -Butanol-ethanol-water								
(10) 3.0:0.6:1	45	63	67	75	86	72	47	35
<i>n</i> -Butanol-acetone-water								
(11) 3.0:0.6:1	46	65	81	80	91	76	42	32

^a These results were obtained at 16–20°, on Whatman No. 1 paper (unwashed), using the ascending technique overnight. The spots of morphine and thebaine were revealed by spraying with Dragendorff's reagent (Munier's modification). The rhamnose spots were revealed by aniline phthalate solution and the leucine spots by ninhydrin solution, with the subsequent application of hot air. Only solvent mixtures 5 and 6 contain acetic acid in excess of the amount required to form one phase. The other mixtures contain practically the minimum of acetic acid, or ethanol, or acetone to form one phase. With *n*-butanol-water (3:1), 0.6 part of ethanol or acetone are required to form one phase, but only 0.4 part acetic acid. ^b R_f values of quercetin are difficult to estimate, as the spots are prone to tailing. ^c This one-phase mixture represents the upper phase of the Partridge mixture. Using the two-phase Partridge mixture (with both phases present in the tank) the following R_f values were obtained: 45, 71, 68, 75, 89, 76, 61, 32 (substances in the same order as in Table). ^d Streaks obtained instead of spots.

acetic acid:1 water. Figure 1 suggests that this is hardly a stable mixture, and if kept apart from the lower phase, it does deposit globules.

Paper Chromatography with One-Phase Solvent Mixtures

The changes in R_f values of a selection of naturally occurring compounds (representative of those normally subjected to paper chromatography in these laboratories) in response to change in composition of solvent mixtures of *n*-butanol, glacial acetic acid, and water was studied. This could be done readily with one-phase mixtures. Results are given in Table I.

DISCUSSION

In addition to the points against the use of two-phase solvent mixtures for paper chromatography made by Smith (see above), the following emerge from the above results. The preparation of two-phase mixtures may result in the use of a mobile phase readily inclined to become unstable. The formation of phase apparently unstable on separation may be due to the separated phase providing a vapor phase for its new container, or just due to partial evaporation or slight temperature change. The visible breakdown of separated phases may be just due to the formation of butyl acetate. But whatever the cause, this potential instability, noticed under the conditions in which chromatography is performed, is something to be avoided. Also, change in the formula of a two-phase three-component mixture may cause no (or very little) change in the composition of the mobile phase. In addition, the preparation of the solvent is always easier and quicker than even nonemulsifying two-phase mixtures, possible variation in the composition of the mobile phase of a two-phase mixture due to temperature differences when it is prepared

is prevented, and wastage of nonmobile phase is eliminated. The vapor of a one-phase mixture can fill the atmosphere of the chromatographic tank as well as the nonmobile phase of a two-phase mixture if it is placed in the tank for a suitable period before running the chromatogram.

Figure 1 represents the type of curve likely to be obtained with most three-component mixtures where two components are relatively immiscible, and the third is miscible with both the others. Such curves should be available for all commonly used three-component paper chromatographic solvents to facilitate selection and modification of solvent mixtures. Figure 1 reveals that the upper phase of the Partridge solvent mixture contains *n*-butanol and water in proportions representing one of their least miscible combinations, so that a relatively large amount of acetic acid is needed to form a stable mixture. In fact, this upper phase contains a slight excess of acetic acid for this purpose. These observations may be of value in designing other paper chromatographic solvent mixtures, a procedure which is rarely recorded by workers.

Table I indicates that the upper phase of the Partridge mixture can be replaced with complete success by a one-phase mixture of *n*-butanol-glacial acetic acid-water (63:11:26), or roughly (25:4:10), or still more approximately but satisfactorily (5:1:2). The lower phases of the Partridge and Baker mixtures present a problem not resolved here. The analysis results indicate that they have a composition which is thermodynamically impossible in that it is unstable, yet the lower phase of the Partridge mixture, separated from the upper phase, does not break down on chilling to minus 5° (unlike the upper phase). Yet it is impossible to prepare a one-phase mixture of the composition indicated from the analysis by mixing the con-

stituents in any manner. The tendency to form actually or theoretically unstable phases is a point against the use of two-phase mixtures, for they might break down during chromatography. Another point against their use is that superficially dissimilar two-phase systems (Partridge and Baker) use a mobile phase of practically identical composition. A change in formula may be meaningless.

The function of the acetic acid in mixtures with butanol and water has been implied to be the regulation of the amount of water that will dissolve in the butanolic phase and vice versa. But substances with basic or acidic character may be affected by the acetic acid. An acidic response could be the suppression of ionization so that ion exchange does not take place with the paper. A basic response could be the formation of the acetate, this salt being the substance actually chromatographed. From Table I (mixtures 7, 10, 11), as acetic acid can be replaced by a suitable quantity of ethanol or acetone without greatly affecting the R_f values of aloë-emodin, barbaloin, morphine, quercitrin, and rhamnose, these substances would appear to be unaffected by the acid. However, there are signs of some response by morphine and rhamnose and also leucine when the acetic acid is reduced or eliminated, as is possible with butanol-rich mixtures. In some cases this may be due to the high butanol content of the solvent mixture. But whatever the cause of these responses, the correct amount of ethanol or acetone may be substituted for acetic acid in some cases, thus avoiding the problem of esterification which otherwise occurs. Table I should be helpful in designing solvents for particular problems.

The results in Table I agree with the observation of Harasawa and Danjo in 1957 (19) that "generally effects of variation of composition (of solvent mixtures) are relatively small." With the mixtures examined, increase in the proportion of butanol to the other constituents tends to decrease R_f values. But increasing the proportion of acetic acid at the same time tends to offset this effect in some cases.

The ideal one-phase paper chromatographic solvent is a pure liquid such as water or toluene. Two-component one-phase mixtures may cover the many cases where this ideal is unrealizable. Only if these fail should three-component mixtures be used. It is an admission of unsuccessful application to invoke the use of mixtures with four or more components. The proportions of solvent mixtures should also be expressed as simply as possible. It is unlikely that the mixture of butanol-acetic acid-water "40:12:29" (20) will produce

any result that 3:1:2 will not do. Chromatographers should remember that there is no virtue in complexity for its own sake.

SUMMARY

1. A "phase diagram" in modified form was obtained for mixtures of *n*-butanol and water, made into one phase by the addition of the minimum quantity of glacial acetic acid when necessary.
2. The phases of some well-known two-phase mixtures of *n*-butanol, glacial acetic acid, and water used as paper chromatographic solvents were separated and analyzed, and found to be apparently unstable in some cases, by reference to the "phase diagram."
3. Some naturally occurring compounds were paper chromatographed with various one-phase mixtures of *n*-butanol, glacial acetic acid, and water designed with reference to the "phase diagram," and changes in R_f values produced by changes in the solvent mixture studied.
4. The use of one-phase mixtures as solvents for paper chromatography whenever possible is recommended and comments are made on their design.

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