# NOMOGRAM FOR CALCULATING PERCENTAGE IONIZATION OF ACIDS AND BASES

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## SUMMARY

A nomogram is described for the rapid calculation of the percentage ionization of acids and bases at various pH values. Predicted electrophoretic mobilities, based on charge:molecular weight ratios, can be calculated at the rate of more than 120 individual determinations per hour. Phosphoenolpyruvate, orthophosphate and pyrophosphate and seven organic acids have been subjected to electrophoresis at various pH values on unwashed and on formic acid-washed papers. Deviations of experimental mobilities from predicted mobilities are discussed.

## INTRODUCTION

Accurate prediction of the electrophoretic mobilities of organic compounds has been the subject of numerous reports and many of these serve to illustrate the variety of factors which cause retardation of compounds during electrophoresis in supporting media such as cellulose. In particular some recent studies[1-4] have contributed greatly to the detailed understanding of these factors and to the accurate prediction of mobilities for members of several groups of organic and inorganic compounds.

A high order of precision in the prediction of mobilities is not necessary, however, for most practical purposes of electrophoretic separation. Generally, all that is required is a rapid method for estimating the pH at which maximum separation is likely to occur and the present nomogram has been prepared expressly to fill this need.

## PREPARATION OF THE NOMOGRAM

An equation and alternative tabulated data for calculating the percentage ionization of a base of known $pK_a$ value at any given pH has been published by ALBERT[5] (eqn. 1).

$$\text{Percentage of base ionized} = \frac{100}{1 + \text{antilog}\,(pH - pK_a)} \tag{1}$$
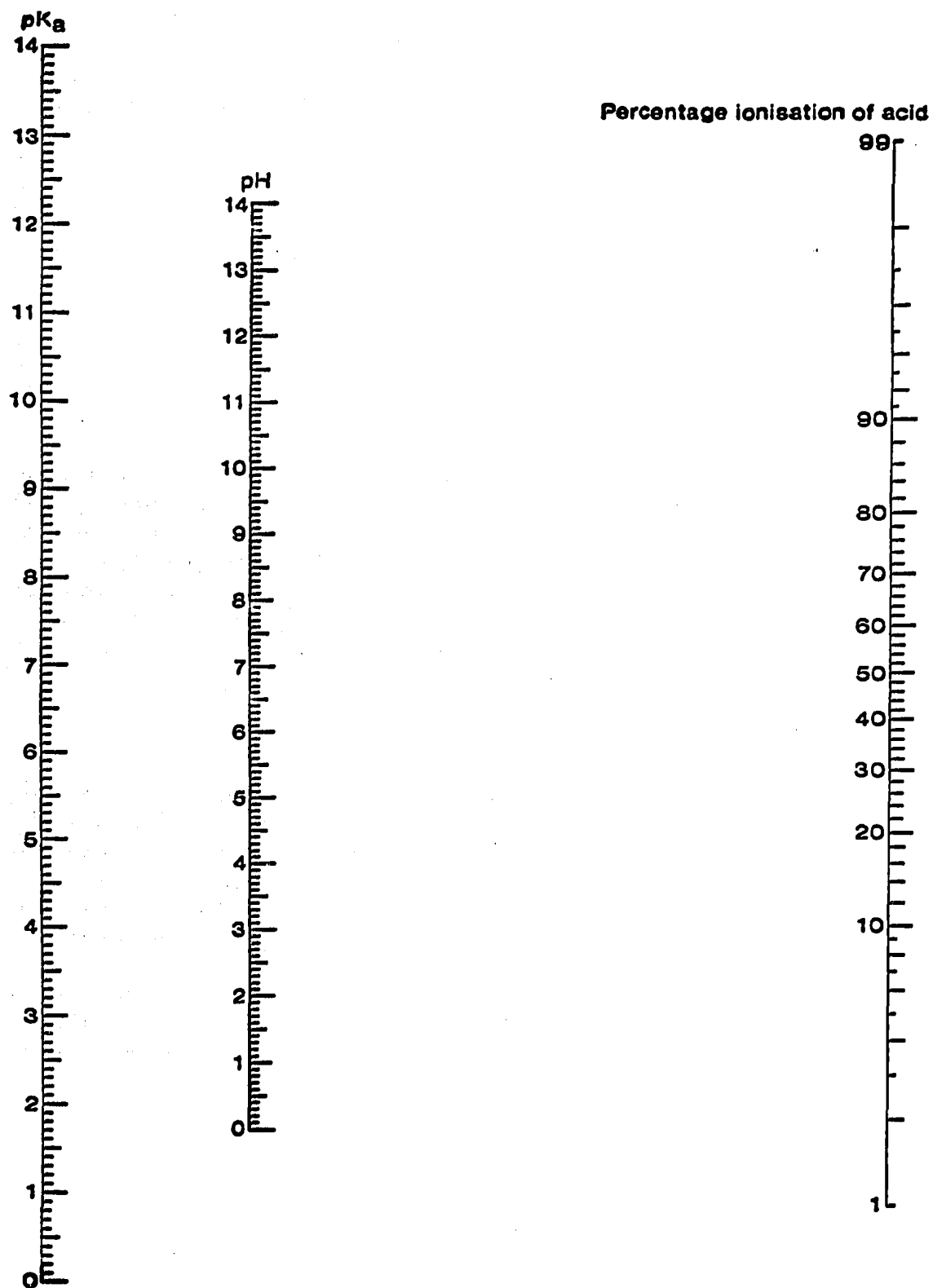
Fig. 1. Nomogram relating $pK_a$ values of acids to percentage ionization at various pH values.

This can be expressed in the related form (eqn. 2) for the ionization of an acid.

$$\text{Percentage of acid ionized} = \frac{100}{1 + \text{antilog (p}K_a - \text{pH)}} \tag{2}$$

In order to simplify and consequently speed up calculations of degree of ionization, the nomogram (Fig. 1) was devised, eqn. (2) being used for the construction of the percentage ionization scale. The nomogram is thus directly applicable to estimations for organic acids and weak to moderately strong inorganic acids. Corresponding values for bases are given by subtracting from 100 the percentage ionizations derived from the nomogram. The three scales of the nomogram are so placed that lines joining identical numerical values of p$K_a$ and pH intersect at the 50% ionization point.

For compounds with more than one ionizing group, the degree of ionization of each group at a particular pH is read separately and the values are added together to indicate the total charge[6] or in the case of a zwitterion, the net charge.

## EXPERIMENTAL

All chemicals used were of the highest available purity, and generally were of analytical grade. PEP* was synthesized in this laboratory[7] as the monocyclohexyl-ammonium salt.

Electrophoresis was performed at approximately 20° in the apparatus described by FRAHN AND MILLS[8] using a potential gradient of approximately 20 V per cm. Whatman No. 4 paper was used throughout, sometimes after pretreatment by washing with 1.5 $M$ formic acid followed by de-ionized water. After electrophoresis, phosphates were detected with ferric chloride–sulphosalicylic acid[9] and organic acids with chromium trioxide–permanganate–sulphuric acid[10].

Caffeine was applied to papers as the marker for electroendosmotic flow and the $p$-nitrobenzenesulphonate ion was used as the standard of rate. The mobilities of the experimental organic acids, calculated relative to the latter, are expressed as $M_N$ values[10]. The mobilities of PEP and PPi are expressed relative to Pi ($M_{Pi}$ values) but are uncorrected for electroendosmotic flow.

The required p$K_a$ values were taken from the literature for DL-malic acid[11], the other organic acids[12], Pi and PPi[4], and PEP[13,14].

## RESULTS AND DISCUSSION

The rapidity with which mobilities can be predicted from the nomogram was demonstrated by the calculation of the charge:molecular weight ratios of Pi, PPi and PEP for every whole and half pH value in the range of 2 to 12. The 66 readings from the nomogram and associated calculations were accomplished in less than 30 min.

The ratio charge:molecular weight has been used to predict mobilities and for simplicity and speed, other relevant factors such as size and shape of the ion[2,15] and its degree of hydration[2] have been ignored. Nevertheless, the experimental electrophoretic mobilities of Pi, PPi and PEP (Table I) agree in most cases with the order of their charge:molecular weight ratios (Fig. 2). Two exceptions occur at about pH 9.5 where PEP runs ahead of rather than behind the two phosphate ions, and in the pH region 2.5 to 3.5 where PEP runs faster than Pi. The p$K_a$ value of 1.4 for PEP used

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* Abbreviation: PEP = phosphoenolpyruvic acid.

TABLE I

ELECTROPHORETIC MIGRATIONS OF PHOSPHATES AT VARIOUS pH VALUES

Electrolytes used were 1.5 $M$ formic acid (pH 2.5), 0.1 $M$ ammonium formate (pH 3.5–4.0), 0.1 $M$ ammonium acetate (pH 6.0–6.5), and 0.1 $M$ sodium carbonate (pH 9.8–10.0). $Na_4P_2O_7$, $KH_2PO_4$ and PEP applied to the paper as 0.1 $M$ aqueous solutions (1 $\mu$l). Electrophoresis for 60–100 min at approx. 20 V per cm.

| pH | Pretreatment of paper | $M_{Pi}$ (uncorrected) | |
|---|---|---|---|
| | | PPi | PEP |
| 2.5 | W[a] | 1.74 | 1.28 |
| 3.5 | None | 1.29 | 1.15 |
| 3.6 | W | — | 1.22 |
| 4.0 | W | 1.31 | 1.20 |
| 6.0 | W | 1.18 | 1.27 |
| 6.0 | None | 1.19 | 1.28 |
| 6.25 | W | 1.08 | 1.22 |
| 6.5 | None | 1.19 | 1.28 |
| 9.8 | W | 0.97 | 1.08 |
| 10.0 | None | 0.90[b] | — |

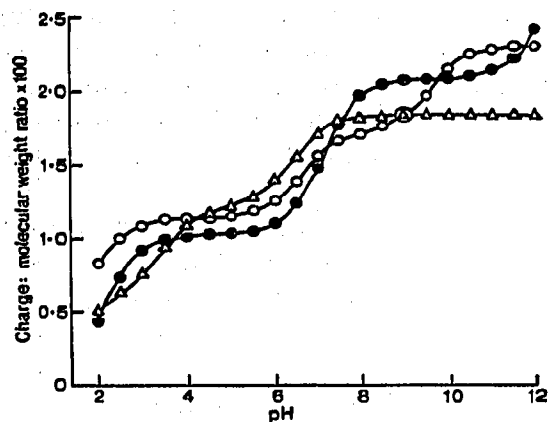[a] Formic acid-washed.
[b] Markedly elongated spot.



Fig. 2. Charge:molecular weight ratios of phosphates at various pH values. O—O, inorganic pyrophosphate ($pK_a$ values 1.0, 2.05, 6.6, 9.6); ●—●, inorganic orthophosphate ($pK_a$ values 2.1, 7.2, 12.3); △—△, PEP ($pK_a$ values 1.4, 3.4, 6.35).

in the calculations was measured at 35° (see ref. 14) and is probably not applicable to the lower temperature (approx. 20°) at which electrophoresis was conducted. However, any error should significantly affect the calculation of the charge on the PEP molecule only at or below pH 2.5.

The use of formic acid-washed papers prevents tailing of PPi at alkaline pH, but appears not to affect the mobilities of these three compounds at acidic pH values.

The calculations and experimental results for Pi, PPi and PEP illustrate the value of the nomogram for the rapid determination of conditions by which compounds may be separated in a desired order. For example, [32]PPi can be separated from a large excess of [32]Pi by electrophoresis at pH 2.5 and its radioactivity can be measured without contamination from [32]Pi which has a lower mobility. At pH 9.5 to 10 the mobilities are reversed and [32]Pi can be separated from a large excess of [32]PPi, although

TABLE II

ELECTROPHORETIC MOBILITIES OF ORGANIC ACIDS AT TWO pH VALUES

The electrolyte used was either 0.1 $M$ ammonium formate (pH 3.6) or 0.1 $M$ ammonium acetate (pH 6.6). Acids applied to the paper as 1 $\mu$l of an 0.1 $M$ solution (fumaric and citric as sodium salts, lactic as lithium salt). Temperature of cooling water 20–21°. Time 1 h at approx. 20 V per cm.

| Acid | $pK_a$ values | pH 3.6 | | | | | pH 6.6 | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | Charge: molecular weight ratio × 100 (A) | Unwashed paper $M_N$ (B) | B/A | Washed paper $M_N$ (C) | C/A | Charge: molecular weight ratio × 100 (D) | Unwashed paper $M_N$ (E) | E/D | Washed paper $M_N$ (F) | F/D |
| Oxalic | 1.27, 4.27 | 1.31 | —a | — | 1.42 | 1.08 | 2.27 | 1.81 | 0.80 | 2.01 | 0.89 |
| Maleic | 1.95, 6.25 | 0.86 | 1.19 | 1.38 | 1.24 | 1.44 | 1.47 | 1.55 | 1.05 | 1.57 | 1.07 |
| Fumaric | 3.02, 4.38 | 0.80 | 0.92 | 1.15 | 0.90 | 1.13 | 1.75 | 1.73 | 0.99 | 1.73 | 0.99 |
| meso-Tartaric | 3.22, 4.82 | 0.51 | 0.68 | 1.33 | 0.66 | 1.29 | 1.34 | 1.45 | 1.08 | 1.55 | 1.16 |
| Citric | 3.13, 4.76, 6.40 | 0.42 | 0.67 | 1.59 | 0.68 | 1.62 | 1.37 | 1.05 | 0.77 | 1.51 | 1.10 |
| DL-Malic | 3.40, 5.26 | 0.48 | —b | — | 0.60 | 1.25 | 1.48 | 1.53 | 1.03 | 1.63 | 1.10 |
| Lactic | 3.86 | 0.39 | 0.48 | 1.23 | 0.43 | 1.10 | 1.12 | 1.13 | 1.01 | 1.13 | 1.01 |

a Streaked from the point of application.
b Not located.

a reliable degree of separation is achieved only by the use of unwashed paper which causes retardation of PPi. Similarly, if [32]PEP is to be measured in the presence of an excess of either [32]Pi or [32]PPi, or both, electrophoresis in the pH range 6 to 6.5 would provide the required separation.

The results of electrophoresis of seven organic acids at two selected pH values are given in Table II. Comparisons between the actual and calculated orders of mobilities were made by dividing $M_N$ values by the appropriate charge:molecular weight ratios. Correlations are generally good but some anomalies are again evident. Perhaps the retardation on unwashed papers of citric acid at pH 6.6 and of oxalic acid at both pH values can be ascribed to chelation effects by residual metal ions in the paper, but the absence of retardation effects with citric acid at pH 3.6 on unwashed papers is more difficult to explain. Citric acid does, however, have a much lower net charge at pH 3.6 than at 6.6, and probably would chelate less effectively at the lower pH. The migrations of maleic and citric acids at pH 3.6 are much larger than expected by comparison with the other acids. However, all the acids vary considerably in their net charge between pH 3.6 and 6.6, and therefore the degree of hydration of the partially ionized acid anions would also be expected to vary over this pH range. Consequently the effective weight of any of these ions may be considerably in excess of its molecular weight.

These investigations illustrate the fact that prediction of electrophoretic mobilities may be complicated by factors which are not easily evaluated. Even with the more elaborate procedures suggested by JOKL[1] and others[2,4] an occasional anomaly occurs. The present nomogram is intended only to provide a rapid guide to conditions likely to achieve a desired separation, given only the dissociation constants and molecular weights of the components.

## ACKNOWLEDGEMENTS

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