Behaviour of phenylphosphinic and phenylphosphonic acids in paper electrophoresis

Many workers[1] have given their attention to the chemical forms of dialkylphosphonates as well as to their parent acids.

Recently studies on the hydrogen exchange reaction of phenylphosphonous acid[2,3], and on the precipitation of diphenylphosphine oxide with silver nitrate[4] have been reported. It is known that electrophoresis is useful in the measurement of stability constants of chelate compounds[5,6] and the detection of functional groups in a molecule[7].

In this paper, the electrophoretic behaviour of phenylphosphonous and phenylphosphonic acids is reported and the chemical forms of the acids briefly discussed.

Experimental

The apparatus[5,7] used is illustrated in Fig. 1.

Background buffer solutions of different pH for electrophoresis were prepared

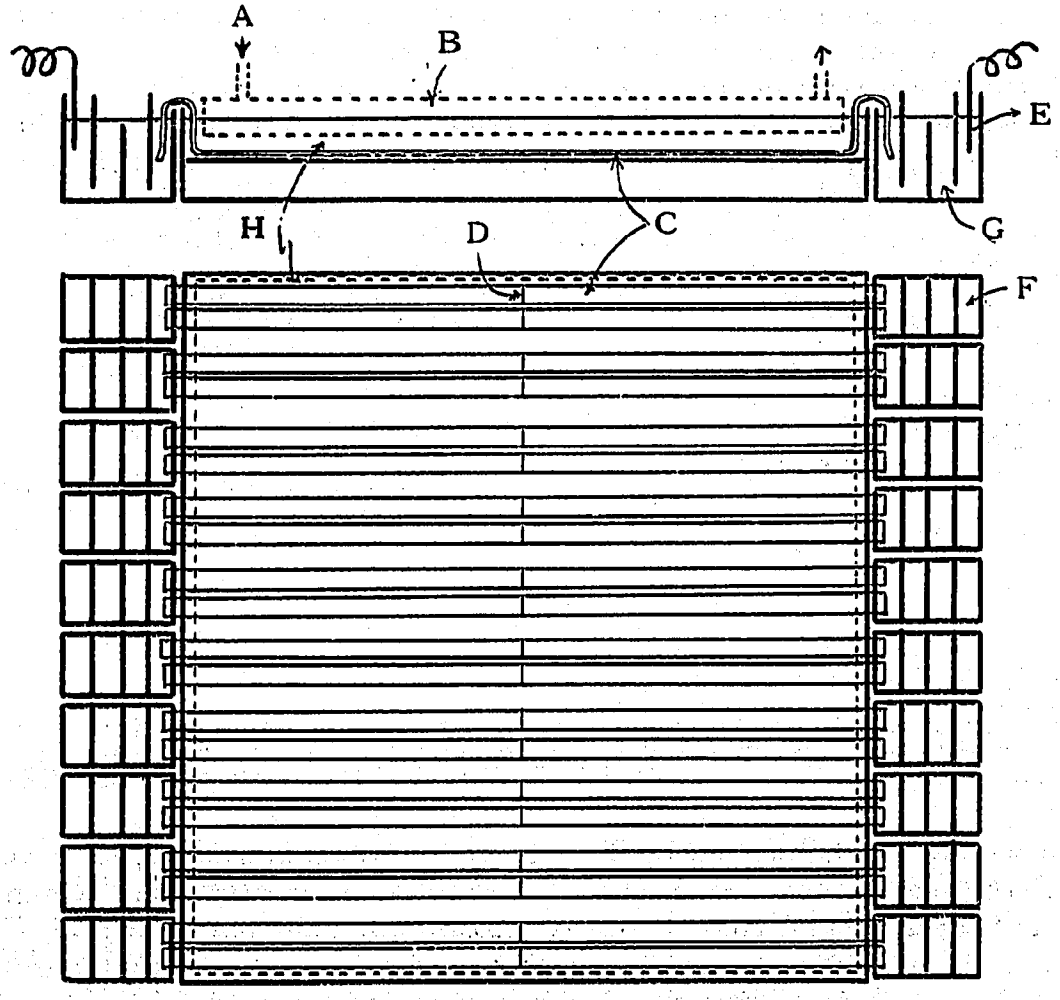


Fig. 1. The migration apparatus with multi-compartment cell. A = Water input; B = flowing-water jacket; C = paper; D = starting line of migrant; E = electrode (Pt); G = background electrolyte; F = unit cell; H = carbon tetrachloride.

by mixing 0.1 *N* hydrogen chloride, 0.1 *M* ammonium acetate and 0.1 *M* aqueous ammonia (ionic strength $\mu = 0.1$ over a range of pH = 1.0–11). A strip (Toyo-roshi No. 50, 2 × 40 cm) was uniformly dipped in a given buffer solution and the excess of the solution removed, after which an aliquot (5 μl) of the solution (0.1 mole/l) containing phenylphosphonous or phenylphosphonic acid was spotted at the starting position on the strip. The migration was carried out at potential gradient of 500 V/34 cm for 30 min, keeping the temperature of the migration box at 20°. After drying under an infrared lamp, the developed strip was subjected to a thermal neutron flux of 5×10^{12} n/cm^2 sec for 30 min. After cooling for a week, the radioactivity on the strip was localized by autoradiography.

Results and discussion

As the electrophoretic mobility of a compound in a filter paper depends upon many factors such as pH value, ionic strength, temperature, nature of filter paper and molecular shape of migrating substance, a relative mobility was used to compensate these factors as far as possible. Although tetraethylammonium cation[8] or picrate[9] are often used as standard substances, the use of hypophosphorous acid is more suitable in the case of phosphorus compounds because it has only one dissociation constant ($pK = 1.1$, and is fully ionized over the pH range 2.5–11), its mobility is larger than that of usual phosphorus compounds, and it can easily be detected by activation analysis together with other phosphorus compounds. Relative mobilities of the samples to that of $H_2PO_2^-$ over the range of pH=1.0–11 are shown in Fig. 2.

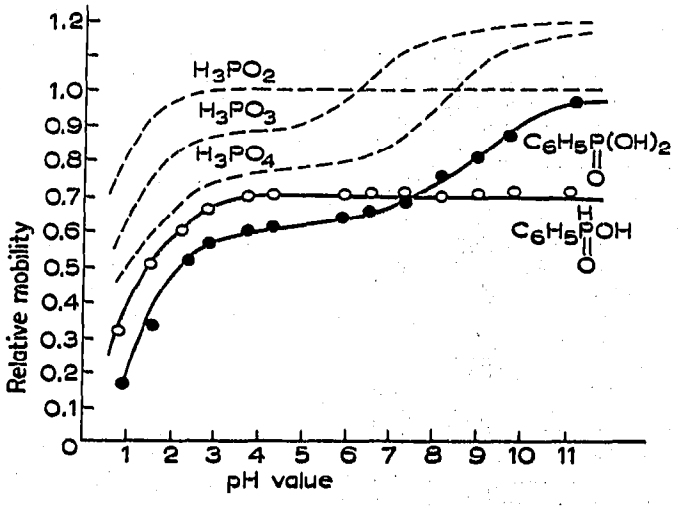
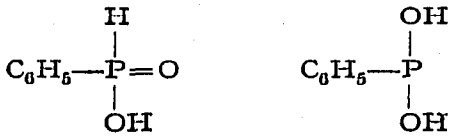


Fig. 2. Relative mobilities of phenylphosphinic and phenylphosphonic acids at a range of pH= 1–11 of background solution.

The dotted lines show the behaviour of three inorganic phosphorus oxyacids as references. The curve of phenylphosphonous acid had only one inflection point which agreed with titration[10] results while that of phenylphosphonic acid had two points. As the pH value of an inflection point corresponds to a dissociation constant[9] and the number of the inflection points with the number of hydrogen atoms which can dissociate in a molecule, it was concluded that phosphonous acid has only one dissociatable hydrogen while phosphonic acid has two.

We can assign the so-called phenylphosphonous acid two chemical forms, *viz.* A and B.



(A) (B)

If the phosphonous acid is present in form B, the electrophoretic curve of the phosphonous acid must show two inflection points, considering that the rate of the hydrogen exchange reaction of hydrogen directly bonded to oxygen atom was much higher than that bonded to the phosphorus atom[3] and that two inflection points were found in the curve for phosphonic acid. The actual results that the curve had one point is in agreement with the form A, which must be named phenylphosphinic acid.

Research Reactor Institute Kyoto University, Kumatori-cho, Sennan-gun, Osaka (Japan)

Y. KISO
M. KOBAYASHI
Y. KITAOKA
K. KAWAMOTO
J. TAKADA

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Received October 8th, 1967

J. Chromatog., 33 (1968) 561–563

A starch gel electrophoretic demonstration of the effect of pH on the aggregation of arginine-rich histones

The arginine-rich histone f3 comprises about 20 % of the histones of calf thymus and can be prepared by the extraction of deoxyribonucleoprotein with ethanol–1.25 *N* HCl (80:20, v/v) and subsequent precipitation by dialysis against ethanol[1]. The molar ratio of lysine to arginine is about 0.65 when the fraction has been purified by reprecipitation[2], and alanine is the main N-terminal amino acid (40–50 μmoles/g of protein), amounting to over 95 % of all such groups found.

The analytical evidence suggests that this is substantially a single protein but