alkaline medium. Further investigations for evaluating the distribution of different ions, specially of those which constitute the fission products, are receiving attention.

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The use of Teflon film in quantitative analysis by enclosed-strip paper electrophoresis

The separation and determination of the valence states of iron

The enclosed-strip method of paper electrophoresis has been used widely as a qualitative tool for the identification of unknown compounds, for the separation of mixtures and for the study of complexes, but it seems rarely to have been applied to quantitative work, probably for the following reason. The insulating films most commonly used for enclosure of the paper strips consist of polyethylene or Mylar [poly(ethylene terephthalate)] and when wet papers are removed from the apparatus after electrophoresis, traces of the impregnating electrolytes often adhere as droplets to the films, making quantitative recovery of the experimental compounds difficult. It is now shown that the method is readily adaptable to quantitative analysis if a film of Teflon (polytetrafluoroethylene) is used as the insulating envelope. The hydrophobic nature of Teflon allows the paper strips to retain aqueous electrolytes completely when they are parted from the film after electrophoresis. Mixtures of iron(II) and iron(III) were used to test the innovation and data are presented which show that, under the conditions to be described, the ionic species were recovered quantitatively from paper strips on which they were separated.

Experimental

Electrolyte. Sodium chloride solution (0.05 *M*).

Reagents. All reagents were of analytical grade or comparable purity. Aqueous solutions of 1,10-phenanthroline (0.25 % w/v), hydroquinone (1 %) and sodium citrate (25 %) were used for the colorimetric determination of iron[1]. Two test solutions were prepared in 0.1 *N* sulphuric acid, (A) containing ferrous ammonium sulphate (3.27×10^{-2} *M*) and ferric ammonium sulphate (2.34×10^{-3} *M*), and (B) containing the same salts in 4.1×10^{-3} *M* and 1.71×10^{-2} *M* concentrations, respectively.

Apparatus. Paper electrophoresis was conducted in the enclosed-strip apparatus described previously[2] except that a film (5×10^{-3} in. thick) of Teflon (from Ludowici and Son Ltd., Lane Cove, N.S.W.) replaced polyethylene film as the insulating envelope. Whatman No. 4 paper was used in strips 13.5 × 61 cm with 45 cm under pressure and cooled. Mains water at 24° was circulated through the coils of the cooling plate.

A Unicam SP600 spectrophotometer was used for measurements of absorbance.

Procedure. The paper strip was wetted with electrolyte and blotted between sheets of blotting paper ("Devon Valley 431 Mill", from Australian Paper Manufacturers Ltd.). A rubber roller was pressed firmly on the blotter during rolling to remove excess electrolyte from the paper strip which was then placed on the opened Teflon envelope covering the cooling plate of the apparatus. The test solution (100 μl) was applied immediately as a streak 10 cm long across the middle of the paper using a micropipette. 1 or 2 min was allowed for the complete absorption of the solution into the paper before covering it with a piece of Teflon film (14 × 45 cm). The envelope was closed, pressure applied to the assembly, and electrophoresis allowed to proceed at 22 V/cm for 15 min when less than 100 μg of iron(III) had been applied to the paper. Up to 25 min was allowed for increasing quantities of iron(III) to a limit of 200 μg.

The envelope was then opened and the Teflon cover peeled from the paper slowly enough to allow the paper to absorb the electrolyte completely from the surface of the film as it was removed. The paper itself was similarly withdrawn from the envelope surface upon which it rested. It was dried in an air-oven at 100° for 8 min and the separated iron(II) and iron(III) located as dark blue bands under a Hanovia "Chromatolite" UV lamp. Pieces (13.5 × 3 cm) containing the bands were cut from the pherogram and "paper blanks" of equal size were cut from the anode side of the starting-line. Each of the paper pieces was shredded into 4 mm strips and dropped into a 25 ml volumetric flask. (As a precaution against accidental contamination of papers with iron, new scissors and nickel-plated forceps were used to cut and transfer the paper strips[3].) Hydrochloric acid (10 ml, 0.1 *N*) was then added with gentle shaking to extract the iron. Hydroquinone solution (1 ml), 2 ml phenanthroline reagent and 0.75 ml sodium citrate buffer were added and the mixtures shaken occasionally during the following hour[1]. The mixtures were diluted to the mark with distilled water and a further addition of 0.25 ml water was made to compensate for the volume displaced by the paper in the flask. They were centrifuged at 2000 r.p.m. in covered tubes and the optical densities of the clear supernatants measured in the spectrophotometer at 508 mμ against paper blanks prepared similarly. The transmittance of the paper blanks measured against reagent blanks was about 97% (equivalent to 2 μg Fe). Determinations of the iron extracted from the papers were made by reference to a calibration curve prepared previously using standard solutions of ferrous ammonium sulphate.

Results and discussion

Electrophoresis of iron mixtures on papers impregnated with the sodium chloride electrolyte causes iron(II) to migrate freely as a compact cationic zone, possibly in the form of the complex ion, $FeOH^+$, resulting from hydrolysis of the Fe(II) (ref. 4). This separates cleanly from the iron(III) which remains fixed on the cellulose fibres at the point of application, probably as the insoluble, microcrystalline species, β-FeOOH, known to form and precipitate from solutions containing iron(III) and sodium chloride[5]. Table I, containing typical results for test solutions (A) and (B), illustrates the quantitative nature of the separations of the valence states and of the recoveries of iron from papers after compression between the surfaces of the Teflon envelope. Mixtures of iron(II) and iron(III) were selected to demonstrate the usefulness of Teflon film for quantitative applications because of the ease and accuracy with which iron can be determined. It appears, however, that the modification is of general application for quantitative work because it has also been applied, with success, to the separation and quantitative recovery of organic compounds[6]. Valuable features of the enclosed-strip method of paper electrophoresis thus retained for quantitative studies include the reproducibility with which the experiments may be conducted, and this stems from the ease with which effective control may be exercised over the experimental conditions. Positive control of the temperature of the working area of the paper strip is achieved by pressing the insulating envelope containing it firmly against the metal cooling plate[7]. Adequate cooling of the enclosed strip ensures, in turn, that water and other volatile components are not lost from the impregnating electrolyte which is then maintained at constant pH and concentration throughout the experiment. Enclosure of the paper strip in this way also ensures that the electrophoresis is conducted in the absence of air and the method therefore lends itself to investigations of compounds sensitive to oxygen and carbon dioxide[7]. The quantitative accuracy of the above results is probably due, in part, to the fact that iron(II) was protected from atmospheric oxygen during the separations.

TABLE I

QUANTITATIVE SEPARATION OF Fe^{2+} AND Fe^{3+}

Test solution (100 μl)	*Amt. applied* (μg)		*Amt. found* (μg)		*Error* (%)	
	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}
A	183	26	185	27	+1.1	+3.9
A	183	26	182	26	−0.5	0
B	23	191	22	194	−4.3	+1.6
B	23	191	24	196	+4.3	+2.6

When iron (III) is present in low concentrations in the applied test solutions it precipitates rapidly on the neutral papers as described above, but in higher concentrations, it begins to move cationically during the initial stages of electrophoresis. The acidity of the test solutions is probably partly responsible for this mobility[8], and it is not until the hydrogen ions migrate out of the band that the residual, slower-moving iron (III) is converted completely to the insoluble species. The effect is to broaden the bands of iron (III) and when quantities in excess of 150 μg are applied, it is often necessary to take larger areas of the pherograms for the determinations. The comparatively narrow, cationic bands of iron (II) are found approximately 4 cm from the starting line after electrophoresis for 15 min.

Regarding the preparation of the bands for spectrophotometry, the presence of Whatman No. 4 paper in solutions containing the colorimetric reagents does not interfere with the formation of the iron–phenanthroline complex, and the complex does not suffer preferential adsorption by the paper.

LINGREN *et al.*[3] recently reported a quantitative study of separations of mixtures of iron (II) and iron (III) by simultaneous paper chromatography and electrophoresis conducted in dilute sulphuric acid as the electrolyte. Under these conditions, iron (III) was mobile on the paper support. The results indicate that, as a method for the separation and estimation of the valencies of iron, the present one is the more rapid and accurate of the two, and this may be owing to the conversion of iron (III), under the present conditions, to an insoluble species which remains fixed at or near the origin allowing more rapid and complete separation of the cationically mobile iron (II).

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