

the question of whether there is a relationship between the concentration of freezing nuclei, meteor dust and the weather.

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Electrolytic Desalting with Ion-Exchange Membranes

SEPARATION of amino-acids and other substances by paper chromatography must often be preceded by the removal of inorganic salts. The common methods¹ require careful supervision, and under optimal conditions a loss of 10–20 per cent of many amino-acids may occur with 50 per cent loss of arginine by conversion to ornithine². A simplified form of desalter, employing ion-exchange membranes, as suggested by Astrup and Stage³, has been used in this Department for the routine preparation of plasma, urine and cerebrospinal fluid for paper chromatography. The apparatus consists of a small 'Perspex' container separated into three compartments by ion-exchange membranes (Fig. 1). The solution to be desalted in the centre compartment (3 ml.) is separated from the anode by an anion exchange membrane ('Permaplex' A.10) and from the cathode by a cation membrane ('Permaplex' C.10, both obtainable from Permutit, Ltd., London, W.4). The anode and cathode chambers are filled with 0.2 N sodium hydroxide and sulphuric acid respectively.

When a current is passed, the anion exchange membrane allows anions to move out of the centre compartment while preventing the ingress of cations,

Platinum electrode (negative) Air Platinum electrode (positive)

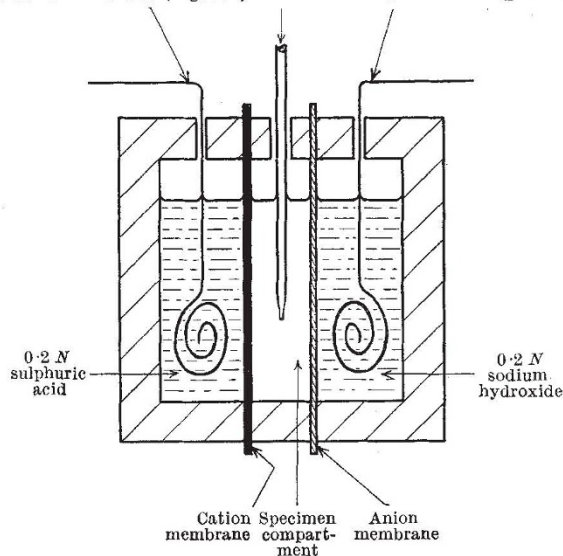


Fig. 1. Apparatus for electrolytic desalting with ion-exchange membranes

Table 1. RECOVERIES OF ELECTROLYTES AND AMINO-NITROGEN FROM VARIOUS SOLUTIONS AFTER DESALTING FOR 6 AND 10 MINUTES

| Solution | Initial pH | Concentrations | | | | | |
|---|------------|----------------------|--------|------------------------|--------|--------------------------------|---------|
| | | Sodium (m.eq./litre) | | Chloride (m.eq./litre) | | Amino nitrogen (mgm. per cent) | |
| | | 0 | 6 10 | 0 | 6 10 | 0 | 6 10 |
| | | (min.) | (min.) | (min.) | (min.) | (min.) | (min.) |
| A. Salts + Glycine Alanine Phenylalanine Valine | 7.2 | 126 | 55 37 | 100 | 52 29 | 5.6 | 5.3 4.8 |
| B. Salts + Glycine Alanine Glutamic acid Arginine | 7.3 | 124 | 56 22 | 103 | 49 31 | 6.0 | 5.8 4.6 |
| C. Salts + Glutamic acid Aspartic acid | 7.2 | 127 | 49 30 | 100 | 50 27 | 6.2 | 4.2 4.3 |
| | 4.4 | 125 | 53 28 | 100 | 45 30 | 6.2 | 4.9 4.4 |
| D. Salts + Arginine Lysine | 7.3 | 130 | 65 25 | 105 | 47 33 | 6.3 | 4.4 4.0 |
| | 8.8 | 130 | 60 20 | 105 | 53 30 | 6.3 | 5.3 5.0 |
| Serum ultrafiltrate | 7.1 | 139 | 40 19 | — | — | 10.9 | 9.0 7.0 |

and the cation membrane permits the passage of cations, but retains anions. The net result is a loss of electrolyte from the centre compartment with an increase in the salt concentration in the outer compartments. The current is supplied by a 6-V. accumulator, and a variable resistance (0–500 ohms) regulates the initial current to 50 m.amp.

Some loss of amino-acid is inevitable, and depends upon the dissociation of the various acids and also on the relative mobility of their ions. The rates of loss of the principal electrolytes of serum and of amino-acids were measured under standard conditions. A double-strength solution of plasma electrolytes was mixed with equal volumes of 0.01 M solutions of amino-acids, and desalted for 6 and 10 min.; the analytical results are shown in Table 1. The loss of neutral amino-acids was slight with an initial pH of 7.2 in the desalted solution. The greater losses of acidic and basic amino-acids could be reduced by bringing the initial pH nearer the isoelectric point of the amino-acids concerned. Desalting of serum ultrafiltrates and casein hydrolysate solutions gave similar results.

A steep fall in current occurred after 6 min., when the salt concentration fell to about 30 per cent of its initial value; this level permits satisfactory separation on paper of amino-acids in serum, cerebrospinal fluid and concentrated urine samples. A fall in the pH of the solution during desalting occurs which increases the loss of basic amino-acids. This can be largely overcome by the addition of 0.1 N potassium carbonate to adjust the initial pH of the solution to 8.0. For routine chromatography of biological material for diagnostic purposes, neutralization of the samples is all that is necessary, since 25 per cent differences in concentration are the least that can be detected on paper.

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