A PERSPEX* ELECTROLYTIC DESALTER

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INTRODUCTION

The interference caused by electrolytes in paper sheet partition chromatograms creates a major difficulty in the satisfactory separation of amino acids from biological sources. At present four main techniques are available for the solution of this problem. In the first the amino acids are extracted with acetone containing hydrochloric acid[1] or ammonia[2] prior to being placed on paper. In the second a buffer is added to the developing solvent to stabilise the ionic equilibria in the paper[3]. In the third the salts are removed by ion-exchange resins[4–8]; and in the fourth the salts are removed by various electrolytic techniques[9–11].

In the course of work using a densitometric method for the determination of urinary amino acids on paper chromatograms[12–14], it was found that none of the techniques cited above fulfilled both of the following requirements:

(1) That there should be a quantitatively reliable recovery of taurine, glutamine, β-aminoisobutyric acid, glycine and alanine (the five amino acids in which I was mainly interested);

(2) That the method should be rapid (this, because of the large number of samples to be estimated).

It was decided, therefore, to examine the possibility of developing an electrolytic desalter, using the same principle as that of CONSDEN, GORDON AND MARTIN[9], which would satisfy the above requirements, and, together with these, would satisfy two further requirements, these being:

(3) That the electrolysis chamber should be designed for maximum efficiency with 1 ml of fluid (this volume is usually ample for experiments involving the paper chromatography of urinary amino acids); and

(4) That the design of the instrument should provide for a maximum degree of cooling.

DESIGN OF THE DESALTER

Fig. 1 shows the design of the desalter. The entire structure is made of perspex (1/8 in. thick). Both of the electrodes are platinum. All angle joints must be care-

* Methyl methacrylate polymer manufactured by I.C.I., England.

fully made and reinforced so as to guard well against the possibility of leakage. This is most likely to occur in the mercury circulation system as the result of wear caused by the high rate of flow of mercury and water.

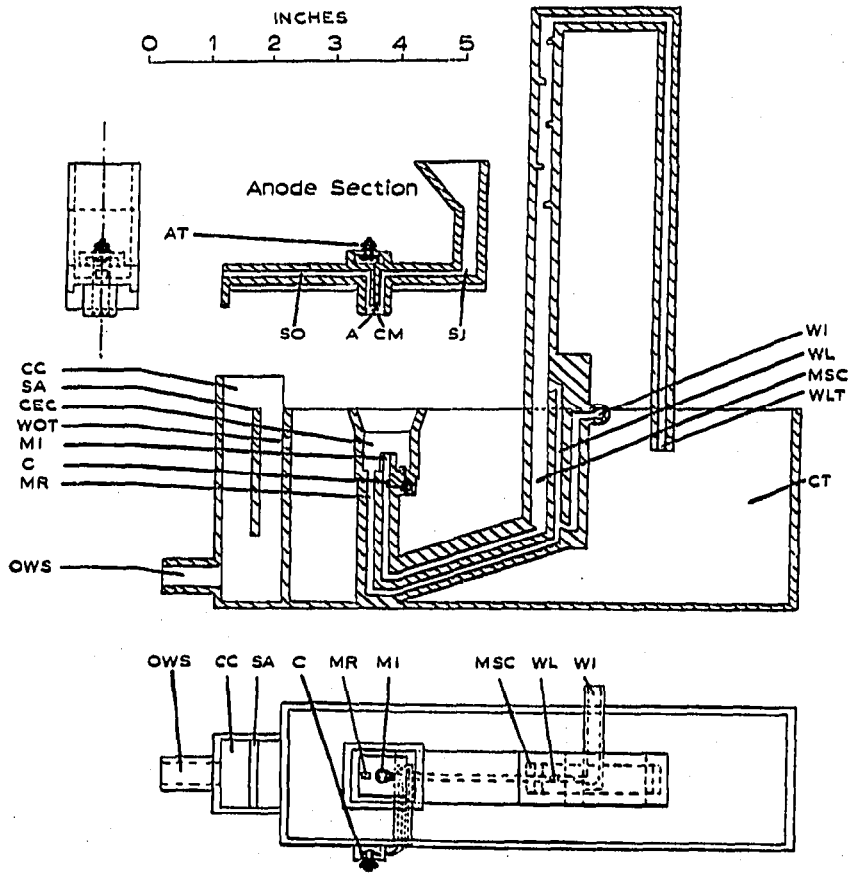

Fig. 1. Perspex electrolytic desalter. A = anode; At = anode terminal; C = cathode and cathode terminal; CC = channel for combined outflow of sulphuric acid from the anode compartment and water from the cooling tank; CEC = cathode electrolysis chamber; CM = cellophane dialysis membrane; CT = cooling tank; MI = mercury inlet for electrolysis chamber; MR = mercury return channel from electrolysis chamber to water lift; MSC = mercury settling channel; OWS = outlet for water and sulphuric acid; SA = support for anode section; SI = sulphuric acid inlet to anode compartment; SO = sulphuric acid outlet from anode compartment; WI = water inlet to water lift pump; WL = water lift pump channel; WLT = water lift pump outflow to cooling tank; WOT = water overflow from cooling tank.

The instrument is powered by a rectified supply from the mains using a Westalite Rectifier Set (Style No. 253, 3 A D.C.). The current supply was controlled by passing the rectified current through a fixed 100 Ω resistance and then through a variable 2000 Ω resistance. Once the variable resistance has been set so that the current through the electrolysing fluid is at 0.9 A for a 2.5 % solution of sodium chloride, no further adjustment is necessary. The E.M.F. applied under these conditions was found to be 300 V. (Measured by an Avometer placed across the terminals with the desalter out.)

The shape of the electrolysis chamber and its size are such that it will contain the bulk of any foam formed. The use of an anti-foam such as the Silicone Anti-foam M430 made by the General Electric Co. of America in the manner described

under "Experimental Procedure" will remove all difficulties connected with foaming.

Nylon suture (size 3N) is used to bind the cellophane membrane to the bottom of the anode compartment because it is relatively inert and non-absorbing.

A thin dialysis cellophane membrane is used, because thick membranes seriously cut down the efficiency of the desalter.

The sulphuric acid which is passed through the anode compartment has a concentration of 1 % (v/v). It is supplied directly from a 5-l reservoir.

EXPERIMENTAL CONSIDERATIONS

Requirement (3) decided the size of the electrolysis chamber. The electrolysing fluid depth was made as small as possible, and the electrodes were brought as close together as was practicable. The limiting factor here was the size of the voltage to be applied to the electrolysing fluid, since too high a voltage resulted in sparking between the electrodes, and rupture of the cellophane membrane. It was found that a fluid depth of about 3 mm was feasible if the voltage applied to the electrolysing fluid was adjusted so that a starting current of 0.9 A was passed through 1 ml of a solution containing 2.5 % sodium chloride. Under these conditions no sparking occurred between the electrodes, even though the surface of the mercury cathode was no more than about 5 mm from the anode.

Solutions containing 2.5 % of sodium chloride were desalted to a negative reaction with ammoniacal silver nitrate in 12 min. Most urines were desalted to a faint reaction with ammoniacal silver nitrate within 5 min. The sodium chloride solutions gave pH values which were never less than 5 after desalting, and the urines gave pH values which were never less than 4. The increased acidity in the case of the urines was due mainly to the presence of organic acids.

The whole apparatus was placed in a water tank in order to provide maximum cooling, the tank being fed from the water lift pump. This had the added advantage that any mercury being carried away from the water lift pump was trapped in the tank. In general, overheating never became a problem with this apparatus because of the very short desalting period and the relatively low electrical resistance of the apparatus.

Variation in the pressure of the water supplied to the water lift pump seriously affected the mercury level in the electrolysis chamber of one previous design of desalter used by the author. In the case of the present design, variation in the pressure of the mains supply caused insignificant variations in the level of the mercury in the cathode electrolysis chamber. Once the water rate was adjusted by means of the water tap at the commencement of desalting, little further adjustment was required. The water column above the water lift pump was equipped with baffles so as to cut down the rate of loss of mercury to the cooling tank.

The anode compartment was designed in such a manner that the sulphuric acid was channelled rapidly between the anode and the cellophane membrane. The rate of flow of the sulphuric acid in this region was extremely fast.

EXPERIMENTAL PROCEDURE

The cellophane membrane is bound onto the bottom of the anode compartment with the nylon suture. The membrane should then be tested for leaks by carrying out the electrolysis procedure with distilled water. If there are no leaks, the current will rapidly drop to 0.15 A. This also serves the purpose of cleaning the mercury. If the membrane is leaking, the current will rise rapidly to 0.9 A and the membrane should then be renewed.

The height of the mercury in the cathode electrolysis chamber should be such that the surface of the mercury just covers the top of the mercury inlet nozzle. The mercury circulation rate should be adjusted by means of the water tap so that only a small amount of mercury comes over into the cooling tank.

Having ensured that the membrane is not leaking, the inside of the cathode electrolysis chamber and the outside of the anode compartment are greased with the silicone anti-foam. The electrolysis chamber and the cellophane membrane are washed three times with the fluid to be desalted, and 1 ml of the fluid is then run into the cathode electrolysis chamber for desalting.

The anode section is next lowered into position on top of the cathode electrolysis chamber, care being taken to ensure that the cellophane membrane makes good contact with the fluid to be desalted. The sulphuric acid is turned on and its flow rate is adjusted to about ten litres per hour. At this stage everything is ready for the desalting to commence, and the current is turned on.

In the case of sodium chloride solutions, it is possible to let the desalting continue until the current has dropped to 0.15 A. In the case of urine, however, the desalting is stopped when the current reaches 0.65 A, or as soon as very severe bubbling begins at the cathode. This bubbling is presumed to be due to a highly increased rate of hydrogen ion discharge at the cathode following the discharge of the major part of the sodium and potassium ions. The current rarely drops below 0.65 A with urine owing to the presence of a relatively large quantity of organic acids, which are not discharged or are discharged very slowly.

On switching off the current, the flow of sulphuric acid to the anode compartment is stopped, and the anode section lifted off the cathode electrolysis chamber. The required volume of desalted fluid is then withdrawn with a pipette.

A volume of desalted fluid usually containing 15 μg of creatinine is then placed on paper for the chromatography of urinary amino acids[14].

RESULTS AND DISCUSSION

The change in concentration of the amino acids during desalting has been used as a basis for measuring the extent to which the desalter can be used in quantitative determinations, since fluid losses due to splashing during the electrolysis cannot be accounted for. Losses by this means could amount to 0.05 ml which represents 5 % of the total fluid present.

TABLE I

EFFECT OF VARYING THE STARTING CURRENT AND THE CONCENTRATION OF THE ANODE SULPHURIC ACID ON THE CONCENTRATION OF AMINO ACIDS

Starting concentration of salt in each case = 2.5 % NaCl. Starting concentration of amino acids in each case = 1 μ mole/ml. The final concentration is shown as a percentage of the original concentration.

Starting current (A)	*Concn. of anode H_2SO_4 (% v/v)*	*No. of samples*	*Change in concentration (Mean values with standard errors in brackets)*				
			Glycine	*Taurine*	*β-Amino-isobutyric acid*	*Alanine*	*Glutamine*
0.6	1.0	3	83.3	80.7	87.0	88.3	81.7
0.6	2.0	3	80.0	78.0	97.0	80.3	69.3
0.6	0.1	2	72.5	57.5	81.5	65.5	61.0
0.9	1.0	5	104.5 (1.5)	99.8 (1.5)	98.2 (2.3)	101.8 (1.5)	89.9 (2.3)

Table I shows the effect of varying the starting current and the concentration of the anode sulphuric acid on the concentration of the amino acids in the desalted sample. It can be seen that losses of nearly 20 % are incurred with a starting current of 0.6 A and an anode sulphuric acid concentration of 1.0 %. Raising the concentration of the anode sulphuric acid brought about little change in the concentration of the β-aminoisobutyric acid, but resulted in an increased loss of glutamine. This was probably due to an increased degree of hydrolysis at the cellophane membrane brought about by the increased concentration of acid. Lowering the anode sulphuric acid concentration to 0.1 % further increased the losses of all the amino acids. It was decided therefore that the hydrogen ion concentration at the membrane was probably not sufficient to pass on positive charges to all the amino acids approaching the membrane, and that this allowed a certain proportion of the amino acids to go through. This difficulty was solved by raising the electrical potential applied to the electrolysing solution. By this means it was possible to increase the hydrogen ion concentration in the cellophane membrane without increasing the rate of diffusion of sulphuric acid. As can be seen from Table I, raising the starting current to 0.9 A, resulted in little change in the concentration of four of the amino acids, but there was a 10.1 % decrease in the concentration of glutamine.

Table II shows what occurred when the same conditions of electrolysis were applied to the desalting of ten different samples of urine. It can be seen from the mean values obtained that relatively insignificant changes in the expected increase in concentration occurred when known amounts of the amino acids had been added to the urine prior to desalting. Glycine was not included in this experiment because urinary hippuric acid was hydrolysed during electrolytic desalting[15], resulting in an increase in the concentration of free glycine. There was no significant loss of glutamine in this experiment. This was probably due to the fact that the average concentration of salt in the urines examined was very much less than that of the salt solutions considered in Table I.

TABLE II

CHANGE IN CONCENTRATION OF AMINO ACIDS DURING ELECTROLYTIC DESALTING AFTER ADDITION TO TEN DIFFERENT SAMPLES OF URINE

The amount added to each sample of urine gave an increased concentration of 1 μmole/ml for each of the amino acids. The concentration after desalting is shown as a percentage of the expected increase in concentration. Starting current = 0.9 A. Concentration of anode sulphuric acid = 1 % v/v.

Sample No.	*Change in concentration*			
	Taurine	*β-Amino-isobutyric acid*	*Alanine*	*Glutamine*
1	89.4	85.1	91.8	89.6
2	113.8	86.3	96.0	91.8
3	103.0	121.2	99.8	97.9
4	107.0	98.1	98.9	99.3
5	98.4	101.2	97.8	88.2
6	98.7	90.0	97.3	107.0
7	107.8	102.9	102.3	97.9
8	93.3	93.5	92.4	103.9
9	106.6	106.7	101.1	102.4
10	92.5	105.1	95.3	97.3
Mean	101.0	99.0	97.3	97.5
S.E.	2.5	3.4	1.1	1.9

Table III shows a comparison of estimations of alanine in ten different samples of urine using both ion-exchange desalting (method of CLARKSON AND KENCH[7]) and electrolytic desalting. It can be seen from the figures presented at the bottom of the Table that the mean difference of 0.05 mmoles/g creatinine is not significant.

There is every indication, therefore, that the desalter can be used quite exten-

TABLE III

COMPARISON OF ESTIMATIONS OF ALANINE IN TEN DIFFERENT SAMPLES OF URINE USING (1) THE ION-EXCHANGE DESALTER AND (2) THE PERSPEX ELECTROLYTIC DESALTER

Mean difference ($\bar{x}$) = + 0.015. Standard deviation of mean difference (s) = 0.036. Standard error of mean difference ($s_{\bar{x}}$) = 0.011.

Sample No.	*Amounts of alanine (mmoles/g creatinine)*	
	Ion-exchange desalter	*Electrolytic desalter*
1	0.22	0.19
2	0.27	0.29
3	0.15	0.16
4	0.53	0.49
5	0.40	0.36
6	0.23	0.18
7	0.42	0.43
8	0.31	0.35
9	0.34	0.34
10	0.54	0.47
Mean	0.341	0.326

sively for the desalting of fluids prior to the paper chromatography of amino acids. Associated with this are the important advantages of the compactness of the instrument, its ease in handling, and the rapidity of the method. The amount of time saved is very great, for up to ten urine samples were desalted in an hour, as against two days using a bank of ten ion-exchange columns.

With respect to the desalting of urine, the main disadvantage of electrolytic desalting is the resultant hydrolysis of hippuric acid. It is not considered advisable, therefore, to use electrolytic desalting prior to the determination of free glycine. The instrument has not yet been tested for arginine destruction. There is every likelihood, due to the very short electrolysis time, that the destruction of arginine will be minimised.

ACKNOWLEDGEMENTS

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SUMMARY

A perspex electrolytic desalter has been designed on the basis of the principle originally used by CONSDEN, GORDON AND MARTIN[9]. The instrument has been specifically designed for the desalting of 1-ml samples of fluids in preparation for partition chromatography on paper. An investigation was carried out of the changes in concentration of taurine, glutamine, β-aminoisobutyric acid, glycine and alanine which occurred under different conditions of electrolysis in salt solutions and in urine. The optimum conditions found for the E.M.F. applied to the electrolysing fluid and the concentration of the sulphuric acid passing through the anode compartment of the desalter were 300 V and 1 % (v/v) respectively. Under these conditions no significant decrease took place in the concentration of taurine, β-aminoisobutyric acid and alanine during desalting in either salt solutions or urine. In the case of glutamine there was a mean decrease of 10.1 % (± 2.3 %) when solutions containing 2.5 g% of sodium chloride were desalted, but no significant decrease in concentration when urine was desalted. The concentration of glycine was unaffected by desalting in sodium chloride solutions, and was not examined in the case of urine due to the hydrolysis of urinary hippuric acid during electrolytic desalting[15].

A 2.5 g% solution of sodium chloride was desalted in 12 min. Most urines were desalted in under 5 min.

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