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A Desalting Apparatus for Amino Acids

TAGE ASTRUP, AGNETE STAGE and ERIK OLSEN

Biological Institute, Carlsberg Foundation, Copenhagen, Denmark

The desalting apparatus described by Consden, Gordon and Martin[1] has probably been modified in several laboratories; the apparatus described here has been developed during the last two years, and was demonstrated recently[2]. The original apparatus of Consden, Gordon and Martin may be used with only minor alterations for treating small volumes of amino acid mixtures. We have tried, however, to design an apparatus which could be used for small amounts of fluid, and at the same time had a high desalting capacity without overheating the amino acid solution. It should further be able to remove other salts than chlorides and to prevent any back-diffusion of dissolved chlorine. The apparatus in its final form appears in Fig. 1. The essential modifications introduced concern the construction of the cathode vessel and the anode holder.

Sulphuric acid (0.1 *M*) is passed continuously through the anode compartment during the electrolysis in order to remove the heat evolved by passage of the current. The acid may be cooled first by passing through a glass coil placed in an icebath, but this is not always necessary. The anode compartment is constructed in a manner which allows the sulphuric acid to flow immediately over the dialysis membrane (a piece of Visking casing, size 36/32, which is renewed at least once a day), before it enters an inner tube containing the anode. The anode is a coil of platinum wire reaching to about 1 cm from the end of the inner glass tube. In this manner the flowing H_2SO_4 will carry away all the ions passing through the dialysis membrane into the anode compartment. It will at the same time remove all chlorine produced at the electrode, thus avoiding any back-diffusion of dissolved uncharged chlorine molecules into the compartment containing the amino acid mixture. It is possible to remove ions, such as phosphate and acetate, from the solutions and thus avoid the time consuming conversion into salts of sulphuric acid used by Consden *et al*[1]. The sulphuric acid on its flow from the anode passes a small thermo-

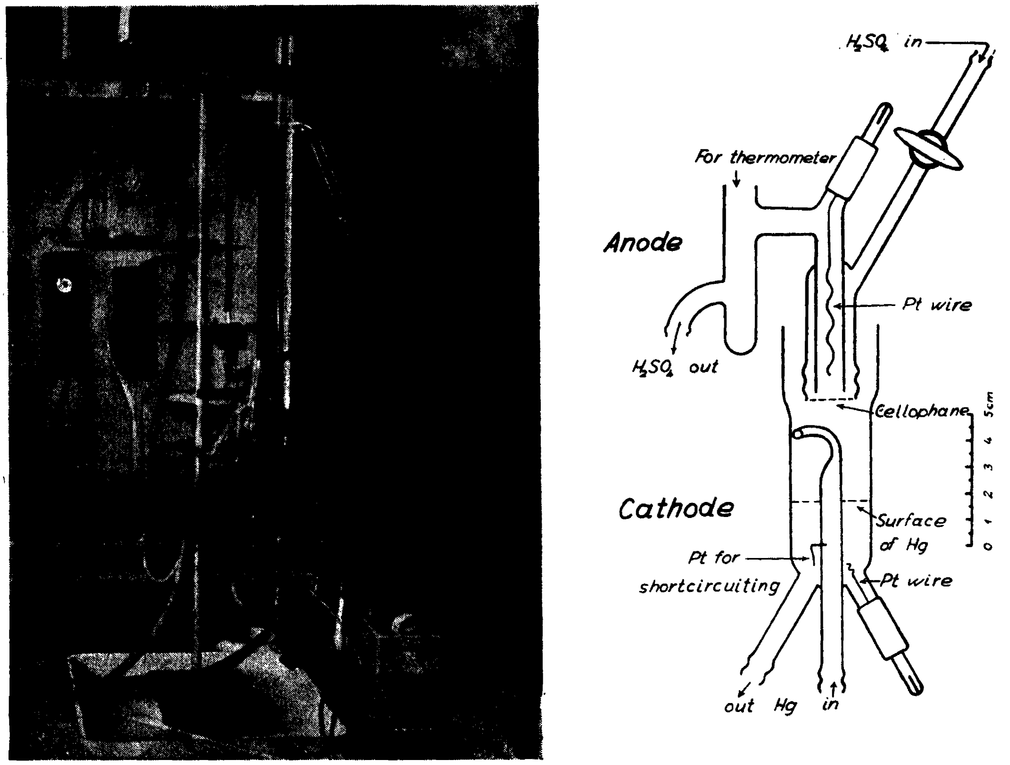

Fig. 1. Construction of electrode compartments of the desalting apparatus and the assembled apparatus with ammeter and buzzer to the right.

meter pocket for controlling its temperature. From there the acid runs to the sink.

The mercury is circulated through the cathode vessel by means of the water lifting pump introduced for this purpose by Consden *et al*[1]. It is, however, not passed directly into the cathode vessel but is introduced through an inner glass tube, which protrudes up into the amino acid solution. The tip of the glass tube is narrowed and bent so that mercury leaves it tangentially to the wall of the vessel and appears as a jet introduced into the solution. In this way the amino acid solution undergoing desalting is given a rotational movement, which helps to mix it, and at the same time is efficiently cooled by the cold, washed mercury. The stream of mercury falls down on the mercury surface and thus continuously furnishes a fresh electrode surface to the solution. In this manner the ions taken up by the Hg during electrolysis are rapidly removed. In order to form an electrical connection between the Hg in the bottom of the cathode vessel and the Hg introduced into the inner tube a platinum wire is inserted through the walls of the latter. In this manner the Hg system is short-circuited and the appearance of sparks during or at the beginning of the desalting process is avoided. The capacity of the cathode vessel depends upon the amount of Hg present. When the vessel is filled with amino acid solution to about 3 cm above the tip of the inner tube, it contains 10—20 ml solution. By increasing the amount of the mercury in the bottom smaller volumes may be handled. From the cathode vessel the mercury passes in the usual manner to the water lifting pump, from which it flows to a wide vertical glass tube where it settles after being washed by the water from the pump, and finally into the cathode vessel again. The mercury can be removed from the system by means of a side tube at the lowest point of the connection between the vertical settling tube and the inner tube of the cathode vessel. This system contains a much smaller amount of Hg than the apparatus described by Consden *et al.* and may need renewal more often. The water appearing from the settling tube passes through a sedimenting flask in the usual manner in order to prevent Hg escaping from the settling tube into the sink. In order to avoid breckage of the tip of the inner tube and the platinum wires the fluid is emptied and the vessel washed out by means of a pipette provided with a short rubber tubing (5 cm) on its tip.

Solutions of 0.9 per cent NaCl (10 ml) are desalted in 15—20 minutes starting with a current of about 0.7 amps. and ending with about 0.1 amps. Such a desalted solution gives practically no reaction with silver nitrate. Because the time used for carrying out the desalting is only a fraction of that needed in the original apparatus it is convenient to have a buzzer to indicate the endpoint. It can be regulated to indicate this endpoint at any strength of current wanted. The use of an automatic current breaker is not recommended, as the back-diffusion will set in as soon as the electrical potential is removed.

TESTING THE APPARATUS

The desalting capacity of the apparatus was tested with solutions of NaCl, Na-acetate and Na_2HPO_4. Table 1 presents the results of such an experiment.

It is seen that even solutions containing high concentrations of phosphate or acetate may be desalted with reasonable speed in the apparatus here described. This is of importance when the solutions, as is often the case, have been prepared with other salts than chlorides. Paper chromatograms prepared after desalting amino acid mixtures in 1 *M* phosphate or acetate are however usually slightly inferior to those prepared from solutions containing 1 *M* NaCl.

Table 1. Desalting of 5 ml 1 M salt solution diluted to about 10 ml with H_2O.

Salt	Time mins.	Amps.		Resistance (arbitrary in ohms)		H_2SO_4 solution used
		Start	Stop	Start	Stop	liters
NaCl	55	1.6	0.3	30	900	8
Na-acetate	120	1.2	0.3	35	800	15
Na_2HPO_4	120	1.5	0.3	35	1000	15

No measurable loss in amino acid content was observed when a solution was washed with the circulating mercury alone for 2 hours (measured as mg N per ml solution by mikro-Kjeldahl).

The amount of uncharged compounds able to pass through the membrane during a desalting period was determined by measuring the amount of amino acid removed from the solution, when the mercury and the sulphuric acid were circulated in the usual manner but with no potential applied to the electrodes. Table 2 shows such an experiment.

Table 2. 10 ml glycine solution (530 mg in 100 ml 0.9 per cent NaCl). The solution was removed from the vessel, diluted with wash water to an appropriate vol. and an aliquot removed for analysis. No electrical potential applied.

Time	mg N/ml	Diffusion loss	H_2SO_4 used in liters
0	0.980	—	—
$^1/_4$ h	0.920	0.060 mg = 6.1 %	3
$^1/_2$ h	0.855	0.125 mg = 12.8 %	6.5
1 h	0.805	0.175 mg = 17.8 %	9.4
2 h	0.675	0.305 mg = 31.1 %	23.0

The desalting procedure was tried out on selected neutral, acid and basic amino acids. The acids were dissolved in 0.9 per cent NaCl in an amount corresponding to about 0.5 mg N per ml. 10 ml was desalted in about 20 minutes.

The total solution was removed from the desalting apparatus and diluted to 25 ml. 5 ml was used for N determination (Kjeldahl). A control was analyzed similarly. Table 3 presents such an experiment. Aminoethylphosphoric ester was prepared according to Outhouse [3]. The other acids were commercial samples (Hoffmann-La Roche).

Table 3. Loss of nitrogen during desalting.

Amino acid	mg N/2 ml		Loss in %
	before	after	
Glycine	0.90	0.90	0
Aspartic acid	0.64	0.64	0
Arginine	1.00	0.93	7
Taurine	0.96	0.89	7
Aminoethyl phosphoric ester	0.91	0.91	0

Arginine was included in these investigations at the suggestion of Dr Stanford Moore, because he had observed a conversion of arginine into ornithine during desalting in the original apparatus of Consden *et al.* (Stein and Stanford Moore [4]).

Paper partition chromatograms using phenol in NH_3-atmosphere were run on the concentrated desalted solutions and compared with controls. All the acids mentioned, except arginine, showed normal chromatograms. While the recovery of most acids is almost quantitative, it is supposed that the loss of taurine is caused by diffusion of this strong acid against the sulphuric acid in the anode compartment. Arginine behaved otherwise. Here a spot for ornithine was always found, so that the total loss of arginine amounted to more than the observed loss of 7 % in nitrogen. Another basic amino acid, lysine, was tried in other experiments (as dihydrochloride). Here only an insignificant loss was observed.

In a number of experiments the conversion of arginine to ornithine during the desalting was followed by means of the colorimetric method of Chinard [5], which method was kindly made available to us by Dr Moore. To the salt solution used by Dr Moore, containing in 200 ml H_2O: Na_2SO_4 (200 mg), NaCl

(900 mg), K_2HPO_4 (275 mg), $CaCl_2$ (20 mg) and $MgSO_4$ (50 mg), was added 20 mg arginine. 5 ml was diluted to about 10 ml with H_2O and desalted in 15 mins, starting with 0.6 amp. and ending with 0.1 amp. The conversion of arginine into ornithine amounted to 40—50 %. When instead of a solution of different salts only NaCl was used the desalting took about 11 mins and the loss of arginine amounted to 20—25 %. These losses, although large, are considerably lower than those observed by Stein and Moore for the original Consden apparatus. Several experiments have been performed in order to reduce the loss, but so far without much success. The reactions occurring have been found more complicated than expected and the results will be described in a subsequent communication.

SUMMARY

A modified desalting apparatus for amino acids is described *. Some of its advantages are: Small volumes may be used. High desalting capacity without heating the solution. No back-diffusion of chlorine. Anions other than chloride may be removed, *e.g.* phosphates and acetates. Small Hg-surface with reduction of secondary processes (arginine).

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* Messrs. *Buch & Holm, Copenhagen,* deliver an apparatus according to this description.