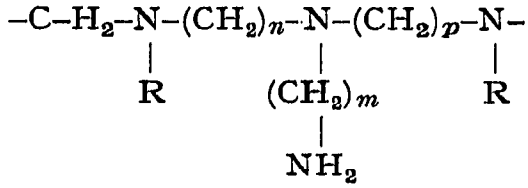
CHROM. 4003

Hydrolytic decomposition products of some carrier ampholytes used in electrofocusing

Electrofocusing is a relatively new technique for the separation of protein mixtures at their isoelectric points along a pH gradient by means of high voltage[1]. The aqueous solution in the electrofocusing tube usually contains (a) a series of low molecular weight amino-carboxylic acids which, under the influence of the electric current, arrange themselves in order of their isoelectric points to form a pH gradient (the carrier ampholyte) and (b) a density gradient produced by sucrose in varying concentrations to avoid diffusion of the protein bonds by thermal agitation. It has been reported previously that artefacts may arise from protein impurities present in the sucrose[2] and in this investigation an examination has been made of spurious amino acid analyses which could arise from contamination of the separated proteins with the carrier ampholyte.

A frequently used carrier ampholyte system consists of a mixture of a large number of synthetic aliphatic amino-carboxylic acids of molecular weight 300–600 and of general formula[3]



where R = H or $-(CH_2)_x-COOH$ and m, n, p and $x < 5$.

Such material is manufactured by LKB Ltd. under the name of "Ampholine".

In view of the fact that complex formation may occur between the carrier ampholytes and accompanying protein fractions during electrofocusing, methods have been described for disrupting such complexes with mild hydrogen-bond breaking reagents such as molar sodium chloride solution[4] as opposed to simple dialysis. Since little is known about the nature of possible protein–ampholyte complexes, it is possible that such mild methods may not break them down prior to hydrolysis of the separated protein and its analysis for amino acid content. Although these synthetic ampholytes contain no peptide linkages and their structures do not suggest that they would yield amino acids on hydrolysis, nevertheless it has been reported that amino acids of undisclosed structure are, in fact, formed on hydrolysis[5]. It was decided, therefore, to subject a range of commercial carrier ampholytes to the normal protein hydrolytic procedure and analyse the products on an ion-exchange column to determine the nature of the artefacts which may arise if a protein is contaminated by such material.

Materials and methods

"Ampholine" solutions from LKB Ltd. of pH ranges 3–5, 4–6, 5–8 and 7–10 (equivalent to 40 mg solid) were hydrolysed with 5.6 *N* hydrochloric acid (5 ml) in a sealed tube under nitrogen for 17 h at 120°. The hydrolysate was evaporated to

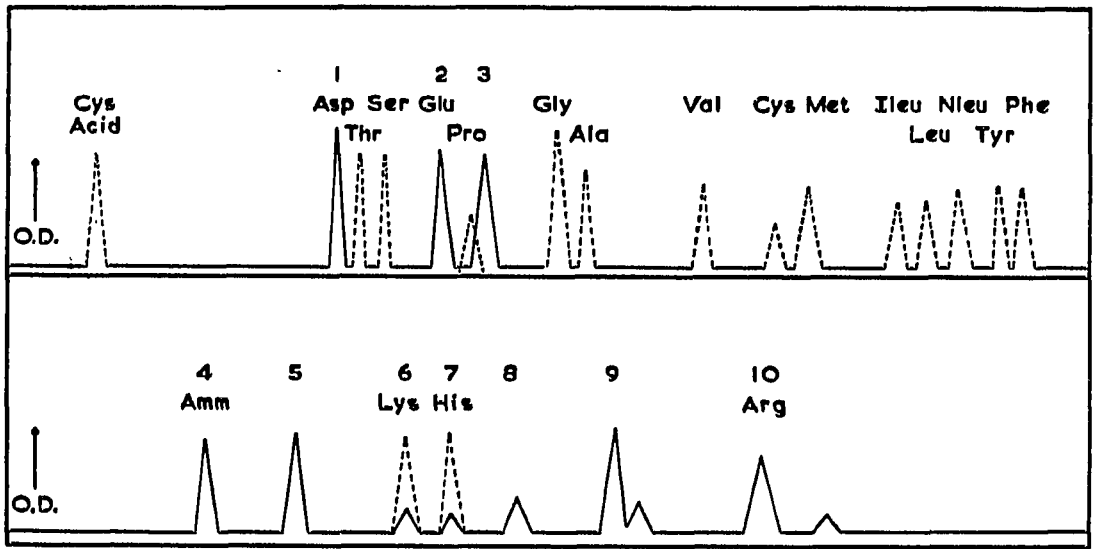


Fig. 1. Ninhydrin-positive substances absorbing at 570 mμ produced by hydrolysates of LKB "Ampholines". "Ampholine" peaks: continuous lines and numbered. Amino acid peaks: discontinuous lines and lettered.

dryness in vacuum over phosphorus pentoxide and sodium hydroxide. The residue was dissolved in water (5 ml) and aliquots (1.0 ml or 0.01 ml) subjected to automatic amino acid analysis using a Technicon single column (6 mm I.D.) machine and standard Technicon procedure for 24-h chromatograms. Colour development was by the ninhydrin–hydrazine sulphate method.

The reference standard amino acid solution used was supplied by Calbiochem, Inc.

Results and conclusions

The major peaks given by the hydrolysates of the four LKB synthetic carrier ampholytes have been combined in the chromatogram shown in Fig. 1, which includes also the peaks of the amino acids normally found in proteins. All these peaks had a 1:4 red:green absorption ratio which makes them indistinguishable from amino acids. Numerous minor peaks produced by the "Ampholines" have been omitted. The distribution of these peaks among the different "Ampholines" is given in Table I.

TABLE I

POSITION OF PEAKS PRODUCED BY DIFFERENT "AMPHOLINES"

pH range of "Ampholine"	*Peaks present*		*Normal amino acids affected*[a]
	Major	*Medium*	
3–5	1, 2, 3	4,	Asp, Glu, (Amm)
4–6	1, 2, 3	4, 5	Asp, Glu, (Amm)
5–8	5, 8, 9	4, 10	(Amm), (His), (Lys), (Arg)
7–10	9, 10	4	Arg, (Amm), (His), (Lys)

[a] The amino acids in parentheses are affected only slightly.

From this work two broad conclusions may be drawn.

(a) Even with much larger concentrations of "Ampholine" carrier ampholyte present in a protein than would be expected after dialysis, the values of the majority of amino acids are unchanged. The exceptions are glutamic and aspartic acids, arginine, lysine and histidine. The latter two are the least affected.

(b) The appearance of peaks which do not correspond to known amino acids or which correspond to uncommon acids may be indicative of the presence of "Ampholine". Thus if an acid "Ampholine" has been used, even the slightest deviation from the straight base line in the region of citrulline (peak 3), when that amino acid is known to be absent, indicates an inaccuracy in the aspartic and glutamic acid values. Similarly, in the basic range, peak 9 approximately midway between histidine and arginine, is characteristic of the presence of "Ampholine" and indicates an inaccurate arginine value, the values for lysine and histidine being also possibly in error.

Postgraduate School of Textile Technology,
The University,
Bradford 7 (Great Britain)

C. EARLAND
D. B. RAMSDEN

1 H. SVENSSON, *Acta Chem. Scand.*, 15 (1961) 325.
2 C. EARLAND AND D. B. RAMSDEN, *J. Chromatog.*, 35 (1968) 575.
3 *Acta Ampholinae No. 5*, published by LKB, Stockholm, 1968.
4 R. QUAST AND O. VESTERBERG, *Acta Chem. Scand.*, 22 (1968) 1499.
5 LKB LTD., private communication.

Received February 10th, 1969