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The use of peak height as the sole index of concentration in amino acid chromatography*,**

The determination of the area under a curve is one of the more common operations in experimental work. In the ion-exchange chromatography of amino acids, area computation is the procedure usually employed to convert the graphical output to concentration. In repetitive analyses, the degree of precision is of major concern, since the area obtained is subsequently converted by means of a factor into concentration or some similar unit. In most of these analytical procedures, standards are submitted to analysis at the same time as the sample under investigation, in order to have a current, meaningful conversion factor. Hence, the precision of the determination of the area is quite important. Many differing procedures are employed, and each procedure has its advantages.

In 1967, MONDINO[1] reported that peak height can be used successfully in the determination of amino acids eluted from ion-exchange columns where the ninhydrin reaction effluent is plotted by means of a linear response recorder, and the net height is estimated by means of a ruler. In 1968, MEFFERD *et al.*[2] reported equal precision for seven methods applied to 184 curves obtained by gas chromatography.

This communication reports the results obtained in using peak height as the sole parameter for similar amino acid chromatographs where the curves are plotted as optical density. The precision obtained with peak height estimated manually is of the same order of magnitude as that obtained using a Simpson's rule integration executed by an IBM 360 computer.

Method

The analyses were performed on an automatic column system employing a gradient based on the procedure of PIEZ AND MORRIS[3].

The area is the output from a computer program that performs the integration based on punched tape digital information. This has been our method in the past. Table I lists the results from two sets of standards. The area values are from the computer output mentioned above.

The peak height is the net peak height expressed in optical density units as read from the printed chart. The base line values which are subtracted from the total optical density values are estimated on the basis of the base line before and after the peak. In the case of peaks incompletely resolved, the base line is estimated on the basis of the base line value after the trace returns to a steady base value.

In both cases, the number obtained is normalized to the value for norleucine, the internal standard. The relative areas or relative peak heights for a number of analyses is listed in Table I.

In this laboratory, we group the data on the basis of a ninhydrin batch of 19 l. Buffers are made up in quantities sufficient to prevent any need for change during the consumption of a given batch of ninhydrin reagent. Color yield factors are newly determined for each batch of ninhydrin. The fact that we group our data on this basis

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probably explains the precision of the peak height values. The actual configuration of the peak will depend to a large degree on the actual buffer composition during elution, since temperature, elution rate, resin particle size, etc., do not change from run to run. A sequential buffer system should show a greater degree of precision than the gradient system used here.

Our experiences with the area computation approach has been that grouping or unitizing our operation results in greater precision. It follows, then, that such a procedure should result in improved precision in determinations based on peak height.

TABLE I

RELATIVE PEAK HEIGHTS AND AREAS OF SEVERAL AMINO ACIDS

Amino acid	*Standards A*[a] *(0.1 μM)*				*Standards B*[b] *(0.1 μM)*			
	Peak Height		*Area*		*Peak Height*		*Area*	
	Mean	*C.V.*	*Mean*	*C.V.*	*Mean*	*C.V.*	*Mean*	*C.V.*
Asp	0.85	0.03	0.82	0.02	0.98	0.05	0.79	0.05
Thr	0.92	0.02	0.90	0.01	1.10	0.03	0.94	0.04
Ser	0.99	0.03	0.97	0.02	1.12	0.02	1.04	0.04
Glu	0.82	0.05	0.94	0.01	0.78	0.04	0.83	0.05
Pro	0.19	0.04	0.20	0.04	0.19	0.04	0.20	0.05
Gly	1.29	0.03	1.20	0.02	1.30	0.07	1.38	0.06
Ala	0.85	0.05	0.85	0.01	0.77	0.04	0.88	0.04
Val	0.77	0.03	0.77	0.01	0.71	0.05	0.79	0.04
Cys	0.77	0.05	0.57	0.04	0.72	0.03	0.57	0.08
Met	1.09	0.02	1.11	0.01	1.04	0.03	1.14	0.08
Ile	0.83	0.02	0.77	0.02	0.83	0.02	0.79	0.04
Leu	1.10	0.01	1.12	0.01	1.10	0.02	1.11	0.02
Tyr	0.92	0.01	1.07	0.02	0.94	0.02	1.04	0.03
Phe	0.90	0.02	1.07	0.01	0.92	0.04	1.09	0.05
Lys	1.25	0.01	1.01	0.02	1.30	0.04	1.05	0.07
His	1.30	0.02	1.20	0.01	1.34	0.04	1.22	0.06
Arg	1.02	0.01	1.00	0.01	1.06	0.03	1.03	0.05

[a] Standards A analyses were performed with *tert.*-butyl alcohol in the pH 2.75 buffer[4]; number of analyses = 5.

[b] The values for glutamic and aspartic acids were corrected for progressive loss[5] since methyl alcohol was used; number of analyses = 5.

Results and discussion

Table I lists the means and coefficients of variation (C.V.) for a series of analyses performed on a multiple column system. Selection by column has not been performed here, since our experimental design is an attempt to randomize. The absolute values are different, but the precision is of the same order of magnitude. This table proves the precision at a column loading of 0.1 μmole.

These results are of significance since they indicate that the complexity of data treatment can be much reduced. A manual procedure becomes economically feasible, particularly with the desk-type programmable calculators. For greater amount of data, the computer program can be simplified from the extreme of a Simpson's rule approach to a search for a change in sign of the slope.

The two methods appear equally precise for all practical purposes, and we now

employ the peak height procedure both for peptides and proteins. The use of peak height provides a simpler approach to the determination of concentration of components of such chromatographic effluents without loss of precision.

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Colorimetric detection of peptides with *tert.*-butyl hypochlorite and potassium iodide

The detection of peptides on paper chromatograms by exposure to chlorine gas to form the labile N-chlorinated derivatives of peptide bonds has been proposed by RYDON AND SMITH[1]. Recently, MAZUR, ELLIS AND CAMMERATA have used a solution of *tert.*-butyl hypochlorite in cyclohexane instead of chlorine gas[2]. The application of starch–iodide or *o*-tolidine–iodide to chlorinated peptides on a paper chromatogram reveals the peptides as blue black spots[1–5]. This is due to the liberation of iodine from the potassium iodide and the reaction of the starch or *o*-tolidine with iodine. The spectrophotometric measurement of the triiodide ion is known as a sensitive method for the determination of iodine[6].

In this paper, the chlorination of peptides with *tert.*-butyl hypochlorite, followed by the colorimetric measurement of the triiodide ion which is formed from the potassium iodide solution, is adopted for the monitoring of peptides eluted from a chromatographic column with a volatile buffer system.

Materials

Egg white lysozyme (6 × recrystallized) and *tert.*-butyl hypochlorite were standard commercial products (Seikagaku Kogyo Co., Tokyo). All chemicals were reagent grade and used without further purification. A tryptic digest of carboxymethylated lysozyme was prepared according to the method of CANFIELD AND ANFINSEN[7]. The peptides were a gift from Dr. N. IZUMIYA.