Multiple column chromatography of wheat gluten proteins

In studies on the chromatography of wheat gluten proteins it became necessary to perform chromatographic analyses on a relatively large number of protein samples. This paper describes apparatus that has been assembled for the simultaneous chromatography of up to six protein samples on columns of carboxymethyl-cellulose using salt gradient and step-wise salt and pH elution according to the procedure described by SIMMONDS AND WINZOR[1].

Columns (1.5 × 25 cm) were packed with carboxymethyl-cellulose (Whatman powder CM 70) and equilibrated with 0.005 *M* sodium acetate adjusted to pH 4.1 with acetic acid. The protein samples were applied to the columns in 0.005 *M* acetate buffer, pH 4.1, and prior to protein elution, the columns were equilibrated with 0.005 *M* sodium acetate containing 1 *M* dimethyl formamide (DMF) (also adjusted to pH 4.1 with acetic acid). The elution system is shown in Fig. 1. The gradient apparatus

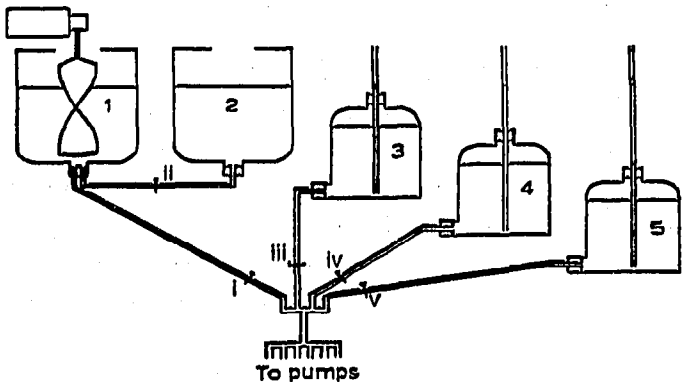

Fig. 1. Solvent-dispensing system for gradient and step-wise elution chromatography. i, ii, iii, iv and v are clamps on the delivery tubes of Solvents 1, 2, 3, 4 and 5 respectively.

consisted of two similar polyethylene bottles each with a hole drilled in the bottom. The two bottles, used in the inverted position, were joined by tubing passing through rubber bungs inserted in the necks of the two bottles. As solvent drained from the left hand bottle (originally containing Solvent 1, 0.005 *M* acetate–1 *M* DMF), Solvent 2 (0.2 *M* NaCl–0.005 *M* acetate–1 *M* DMF) entered from the right hand vessel and was mixed with the bulk of the solution by means of a perspex blade (5 × 15 cm twisted through 90°). A rotation speed of 80 rev./min ensured complete mixing without the formation of a vortex.

Following gradient elution, successive step-wise elutions with Solvents 3, 4 and 5 (0.5 *M* NaCl–acetate–DMF, 0.005 *M* phosphate–0.5 *M* NaCl–1 *M* DMF and 0.1 *N* NaOH, respectively) were produced by three bottles set up as shown in Fig. 1. The bottles were placed so that the lower opening of each Mariotte tube, passing through the stopper of the bottle, was below the opening of the delivery tube of the previous bottle. (Although the delivery tubes are shown entering by an opening at the bottom of each bottle, entry via the neck of the bottle is equally satisfactory.) Fine tubing is recommended for Mariotte and delivery tubes, but for the filling procedure to be effective the volume of the delivery tube must exceed the volume of the Mariotte tube.

With all clamps closed the bottles were filled with the required volumes of the respective solvents and the stoppers carrying the Mariotte tubes were inserted. After filling the delivery tubes with the respective solvents, the clamps were opened in the order v, iv, iii, i, allowing time for the solvent heights to come to equilibrium. As the system was drained, virtually all the solvent came initially from the two gradient bottles. When the liquid level dropped below the lower opening of the Mariotte tube of bottle 3, virtually pure Solvent 3 drained from the system, and so on through the succession of solvents. As the liquid levels fell in the Mariotte tubes, a small amount of solvent drained from each of the delivery tubes. By filling the tubes in the prescribed manner, this latter solvent had the same composition as the main solvent being drawn off at any one time. Contamination of later solvents by earlier solvents draining from the delivery tubes cannot be avoided, but can be minimised by using small diameter tubing. Large fluctuations in temperature should be avoided in the Mariotte bottle system as the resulting change in volume of the airspace displaces solvent from the bottle.

The above system is also useful for the automatic regeneration of the columns with step-wise solvent changes in the order, distilled water, 0.05 *M* acetate buffer, 0.005 *M* acetate buffer. The solvent dispensing apparatus is similar to that described by ANDERSON *et al.*[2]. However, the use of Mariotte tubes in standard reagent bottles saves costly construction of the special containers recommended by these authors, allows the bottles to be used only partly filled and also permits closer spacing of the bottles with respect to height.

Of a number of methods tested for the collection of fractions, the only method found to be satisfactory was the combination of a metering pump with a timer-operated fraction collector (Fig. 2). A four-channel peristaltic pump, supplied by Sigmamotor Inc., Middleport, New York (Model T8), was coupled to a variable speed changer (Zero-max Co., Minneapolis, Model 142 X). The pumping unit was modified so as to take an additional two channels, and a screw adjustment was fitted to the speed change lever to facilitate regulation of flow rate. The modified unit was capable of delivering up to 15 ml/min to each of six channels, with accuracy at 1 ml/min of $\pm$ 0.01 ml/min, using Tygon tubing of 1/16 in. internal diameter. For fraction collection, two LKB RadiRac Automatic Fraction Collectors (LKB-Produkter AB, Stockholm) were used in conjunction with a pair of times (E. Dold u. Söhne, Furtwangen, Baden, Germany, Models ZR1U 712 f, 40 sec and ZR1U 701 f, 60 min). The advantage of the common solvent system for multiple column chromatography has been pointed out previously by MARR AND GILBO[3], but the fraction collection system described above avoids the tedious measuring of individual fractions performed by these workers.

The efficiency of the solvent-dispensing system was tested by substituting increasing concentrations of the dye auramine for Solvents 1–5. The system was drained at the rate of 1 ml/min by each of the six channels and 10-min fractions were collected from one of the solvent streams. The absorbancy at 430 mμ of the fractions (Fig. 3) indicated that a linear gradient was produced, that the change-overs between step-wise elutions were sharp and that the emergent concentrations of each of Solvents 3, 4, and 5 were unchanged from each of the original concentrations.

The chromatographic profiles obtained by the simultaneous chromatography of the gluten proteins of six wheat varieties are shown in Fig. 4. The gluten proteins were extracted from the crushed wheat samples using 0.05 *M* acetic acid, after preliminary

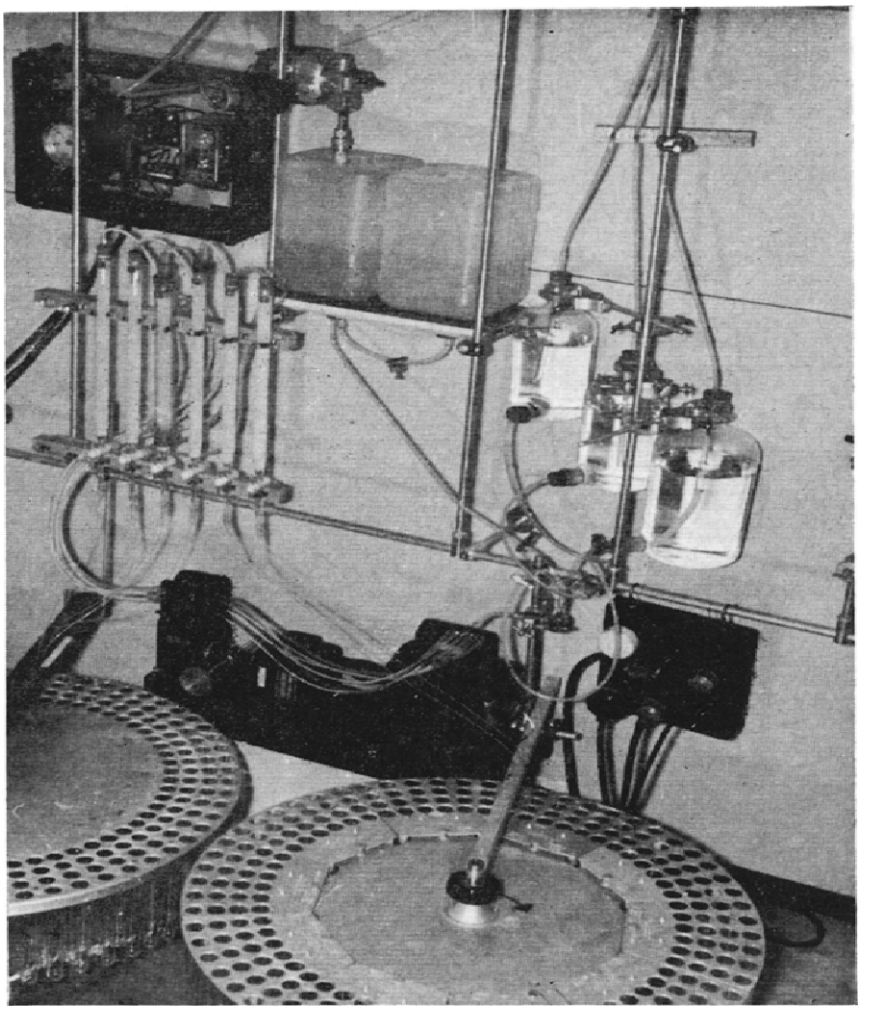

Fig. 2. Apparatus for the simultaneous chromatography of six protein samples.

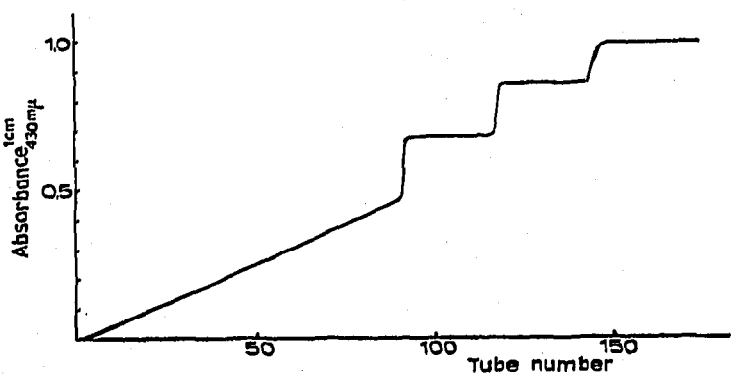

Fig. 3. Absorbance at 430 mμ of fractions delivered from the solvent-dispensing system substituting increasing concentrations of the dye auramine for Solvents 1–5. The original absorbance values of Solvents 1–5 were 0.00, 0.48, 0.68, 0.86 and 1.02 respectively.

extraction with 0.01 *M* sodium pyrophosphate, pH 7.0, as previously described[4]. Included in the six extracts chromatographed is an extract of the wheat variety Gabo, which is routinely used as a standard. The appearance of the profile that is characteristic of this variety provides a check on the correct functioning of the procedure. The Gabo profile further serves as a standard for estimating the distribution of protein between the chromatographic peaks. The eluted fractions corresponding to each peak were pooled in the standard manner for the Gabo extract and the fractions obtained

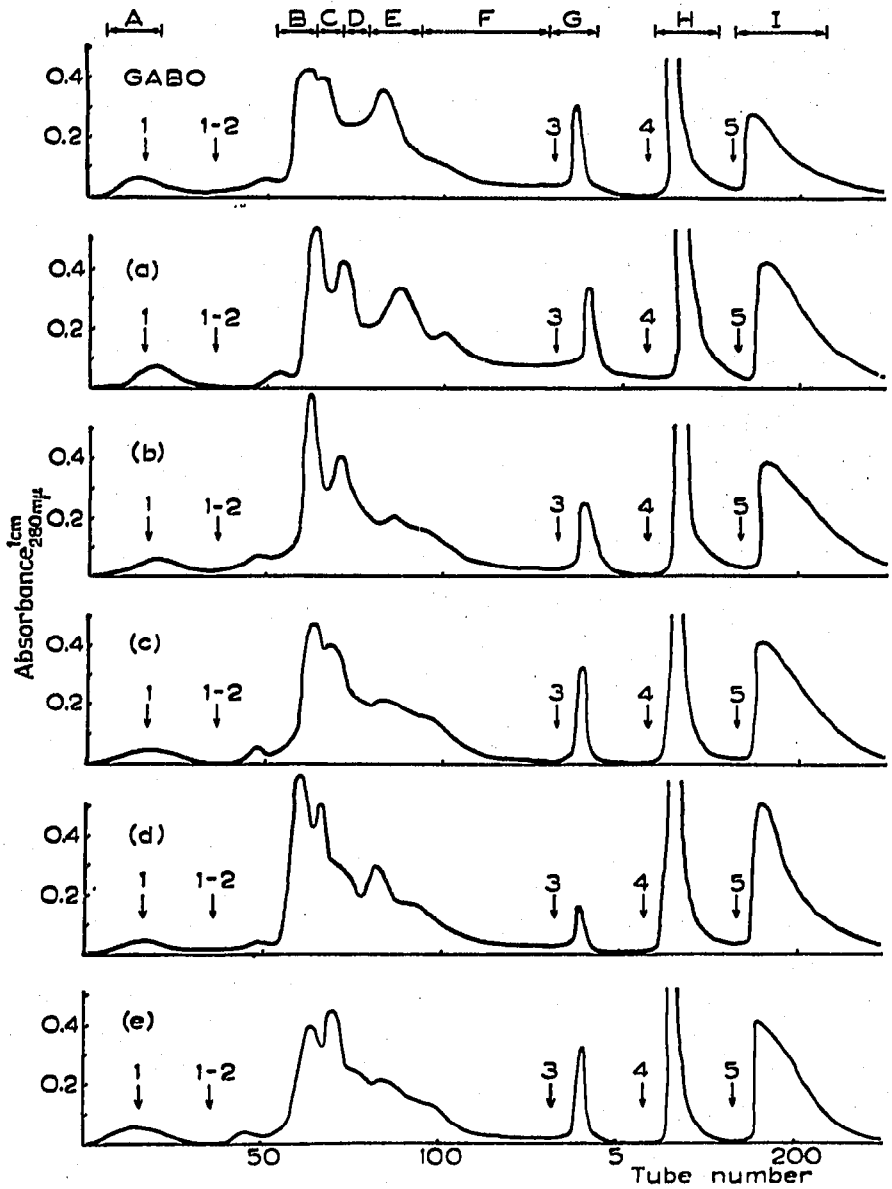

Fig. 4. Chromatographic profiles resulting from the simultaneous chromatography of the gluten extracts of six wheat varieties using the apparatus described in this paper. Column loadings (mg N) were as follows: Gabo, 65.0; (a) 61.4; (b) 58.3; (c) 52.4; (d) 67.8; (e) 55.3. The manner of pooling the tubes that correspond to each of the peaks A to I is indicated at the top of the figure. Changes of solvents are indicated as follows: (1) 0.005 *M* acetate containing 1 *M* DMF (pH 4.1); (1)–(2) gradient from 0 to 0.2 *M* NaCl in acetate–DMF; (3) 0.5 *M* NaCl in acetate–DMF; (4) 0.005 *M* trisodium phosphate–1 *M* DMF–0.5 *M* NaCl, pH 12; (5) 0.1 *N* NaOH. Flow rate, 1 ml/min for each column. Fraction size, 10 ml.

from the other five extracts were pooled in an identical manner preparatory to colorimetric protein determination[5]. Pooling tubes according to a standard profile thus allows comparison of protein distribution between corresponding peaks from one set of chromatographic analyses to another.

The apparatus described provides a system whereby up to six protein samples can be eluted simultaneously from chromatographic columns under identical conditions by the automatic introduction of a succession of solvents. The routine inclusion of a standard protein sample provides a check on the reproducibility of the analysis, thus allowing comparison between sets of chromatographic analyses.

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Some remarks on the paper "Centrifugally Accelerated Chromatography of Steroids"

In a recent paper by MATTHEWS AND CERVANTES[1] on centrifugal chromatographic separation of steroids, velocities of 200–250 r.p.m. were used and it was found that the steroids did not move from the line of application. These authors thought that "this is probably because at high velocities the solvent travels too fast for partitioning to occur". However, the present authors consider that the unsatisfactory results of separation were due to an unsuitable arrangement of the centrifugal chromatography, for the following reasons.

(1) The velocity of 200–250 r.p.m. is not sufficiently high to cause a substantial acceleration of the mobile phase flow. According to our own experience the run will take about 30 min instead of 40, which is the necessary time for developing a standard circular chromatogram.

(2) The velocity necessary to establish the partition equilibrium is much higher than the velocity of the solvent flow. Even a velocity of the mobile phase which amounts to *ca.* 1,700 r.p.m. will not interfere with the partition.

No reason can be seen why these compounds (hydrocortisone, cortisone and 11-desoxy-17-hydroxycorticosterone) should not be separated by centrifugal chromatography under high velocities and actual separations are shown in Fig. 1. These were performed at 300, 600, 900 and 1,200 r.p.m. Paper preparation and sample application were the same as described by MATTHEWS AND CERVANTES, but Whatman paper No. 3