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Capillary sodium dodecyl sulfate gel electrophoresis of proteins I. Reproducibility and stability

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Abstract

This paper investigates the use of a non-cross-linked polymer gel as a separation matrix for sodium dodecyl sulfate (SDS) capillary gel electrophoresis of proteins. The method employs a polyacrylamide coated capillary filled with a polyethylene oxide–gel buffer solution. A standard seven-protein mixture was chosen for the evaluation of coating stability and reproducibility. It was found that the coating is stable for more than 400 runs with a 1 *M* HCl wash between each run. The hydrophilic nature of polyethylene oxide also allows high resolution and high efficiency for all protein peaks. The linear plot of log M_r vs. mobility demonstrates a pure sieving mechanism of polyethylene oxide matrices. The molecular masses of twenty-nine standard proteins determined by SDS capillary gel electrophoresis are in good agreement with those obtained from the SDS polyacrylamide gel electrophoresis (PAGE) slab gel method. The capability to replace the gel after each run allows improved run-to-run and batch-to-batch reproducibility. The relative standard deviation (R.S.D.) in migration time of 19 injections of the seven-protein standard mixture, with the 190 injections of crude fetal calf serum in between, falls in the range of 0.351 to 0.453%. The application of this technique for the separation of proteins in chicken egg white and bovine milk is demonstrated.

1. Introduction

Sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis (SDS-PAGE) has been a well established analytical method for the separation and characterization of proteins [1,2]. The technique has proved useful for the estimation of protein molecular mass, purity assessment, and the structure of subunits. Although the method is widely used for these applications, it can be time consuming and difficult to quantitate and automate. Capillary gel electrophoresis (CGE) [3–6], a recently developed technique, offers an alternative approach to gel electrophoresis with the advantages of fast separation, quantitative analysis, on column detection, and automation.

Both cross-linked and non-cross-linked polyacrylamide gels have been used for the SDS-PAGE separation of proteins in slab gels [7,8]. These matrices can also be used in CGE. Hjerten was the first to show SDS-PAGE separation of membrane proteins by a polyacrylamide gel filled capillary [9]. Cohen and Karger provided a detailed description of SDS protein and peptide separations by cross-linked and non-cross-linked polyacrylamide gels [3]. Tsuji also reported the

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separation and quantitation of recombinant proteins by a similar method [10]. These fixed polyacrylamide gel capillaries, however, have several limitations. One problem is poor detection at low UV wavelengths due to the strong absorption of the polyacrylamide polymer. Higher wavelengths can be used, but with a lower detection limit. A second limitation is that the gel in the capillary can be easily contaminated or damaged by sample matrix if it is not replaceable, limiting the number of useful runs.

Recently, Ganzler *et al.* reported SDS separation of proteins using a UV transparent, replaceable, non-cross-linked polymer as the sieving matrix [11]. Guttman *et al.* also reported the separation of proteins in the molecular mass range of 29–205 kDa using a replaceable linear polymer matrix [12,13]. The use of these polymer networks as a sieving matrix offers several advantages compared to fixed gel SDS-PAGE capillary electrophoresis. First, the replaceable gel offers longer life time of the gel capillary compared to SDS-PAGE capillary electrophoresis. Secondly, the polymers employed have no absorption in the low UV range, and hence provide the possibility to detect proteins at wavelengths which allow higher sensitivity than the standard 280 nm wavelength. Thirdly, the capability of replacing gel after each run prevents sample carry over from previous runs, allowing better quantitation.

This paper evaluates the use of polyethylene oxide and polyethylene glycol mixtures as the sieving matrix for protein separations by SDS capillary electrophoresis in the molecular mass range of 14–205 kDa. A hydrophilic coating was applied on the capillary wall to reduce non-specific adsorption on the capillary surface and to minimize electroosmotic flow. The polymer network has low viscosity and is UV transparent at 214 nm. Protein molecular mass determinations by this method are similar to the results obtained from SDS-PAGE slab gels. A standard seven-protein mixture was used to evaluate the performance of the capillary. Results are presented regarding capillary stability, reproducibility and protein molecular mass determinations. Finally, separations of proteins in chicken egg white and bovine milk are presented to demonstrate the general applicability of the method.

2. Experiment

2.1. Instrumentation

Capillary gel electrophoresis was performed on a P/ACE 2200 (Beckman Instruments, Fullerton, CA, USA) with cathode at the injection end and anode at the detector end. The eCAP SDS 14-200 kit with coated capillary (100 μm I.D., 27 cm total length, 20 cm to detector) and replaceable polymer–buffer (Beckman Instruments) were used for all SDS capillary electrophoresis studies. The temperature of gel filled capillary was controlled to $+/-0.1$°C by a liquid cooling cartridge system of the P/ACE instrument. The analysis was monitored at 214 nm and the data was analyzed by System Gold software (Beckman Instruments) with IBM personal computer.

2.2. Materials

The replaceable polymer, a mixture of polyethylene glycol and polyethylene oxide was obtained from the eCAP SDS 14-200 kit (Beckman Instruments). The molecular mass standard mixture, containing α-lactalbumin (bovine milk), carbonic anhydrase (bovine erythrocytes), ovalbumin (chicken egg white), albumin (bovine serum), phosphorylase *b* (rabbit muscle), β-galactosidase (*Escherichia coli*), and myosin (rabbit muscle), is also supplied with the kit. All other protein standards and 2-mercaptoethanol were purchased from Sigma (St Louis, MO, USA). Chicken egg and bovine milk were purchased from local grocery store.

2.3. Protein sample preparation

Protein standard mixture

One vial of protein standard mixture (3.4 mg/vial) was dissolved in 750 μl of sample buffer and 750 μl of doubly deionized (DDI) water. A volume of 200 μl of protein solution was transferred to a polypropylene vial. A volume of 5 μl

of 2-mercaptoethanol and 10 μl of 1% orange G were added to the vial and mixed for 10 s. The final mixture was boiled for 5 min and then cooled in an ice bath. The sample was stored in a freezer prior to the injection.

Proteins of interest

A volume of 2 ml of chicken egg white or bovine milk was diluted with DDI water in 1:1 ratio and filtered through a 0.4-μm filter. A volume of 100 μl of filtrate was transferred to a polypropylene vial and 85 μl of sample buffer, 5 μl of 2-mercaptoethanol and 10 μl of 1% orange G were added to the vial. The final mixture was boiled for 5 min and cooled in an ice bath before CGE analysis.

2.4. HPCE methods

CGE was performed under the following conditions. Prior to the run, the capillary was rinsed with 1 *M* HCl solution for 1 min followed by eCAP SDS 14-200 gel/buffer for 3 mins. The samples were injected by pressure [0.5 p.s.i. (3447.38 Pa) for 30 s] into the capillary. The separation was carried out at 300 V/cm (8.1 kV for 27 cm capillary) and 20°C. Since the protein SDS complexes were negatively charged, reversed polarity was used for all separations.

3. Results and discussion

3.1. Linearity study

In SDS capillary electrophoresis, the protein molecule is fully denatured by boiling in excess amount of SDS and thiol reducing agent such as 2-mercaptoethanol. After denaturation, the protein S–S bond was reduced and the three dimensional structure is extended to a rod shape. The SDS in the sample buffer binds to the proteins, regardless of protein size or shape, in a 1:1.4 ratio. The charge per unit mass of the protein–SDS complex can be assumed constant since all the charge on protein was masked by SDS. As the mobility of each complex in electrophoresis is practically identical, assuming no other interaction between proteins and the polymer network, the separation is based only on the sieving mechanism. Fig. 1 demonstrates the separation of the seven-standard protein mixture (M_r 14 200–205 000 daltons) by SDS capillary electrophoresis in less than 15 min. The separation was found to be similar to those on the cross-linked and non-cross-linked polyacrylamide gel. Fig. 2 shows the linear plot of logarithm of molecular weight and mobility in capillary gel electrophoresis. The mobility was found to be proportional to the logarithm of molecular mass of proteins. The high degree of linearity of the plot ($r = 0.99$) suggests a pure sieving mechanism of the polymer matrix. This fact was also supported by a Ferguson analysis study [14]. The calibration curve obtained in this way can be used for protein molecular mass estimation of unknown samples. Table 1 compares the molecular mass estimation of 32 standard proteins by SDS capillary and slab gel electrophoresis. From the results, both methods are in good agreement for most proteins.

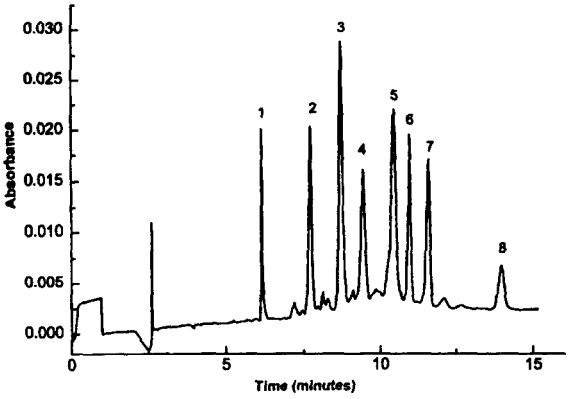

Fig. 1. Separation of seven SDS–protein complexes by the eCAP SDS 14-200 kit. Conditions: see Experimental section. Solutes: 1 = orange G, reference marker; 2 = α-lactalbumin; 3 = carbonic anhydrase; 4 = ovalbumin; 5 = bovine serum albumin; 6 = phosphorylase *b*; 7 = β-galactosidase; 8 = myosin.

3.2. Stability study

Most fixed, gel filled capillaries have a limited life time due to bubble formation or sample contamination inside the capillary. Since the

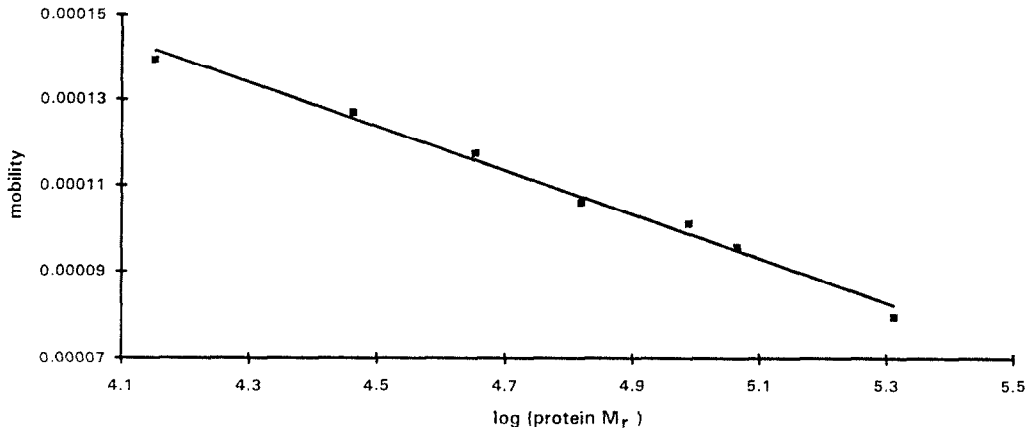

Fig. 2. Linearity plot of logarithm of protein molecular mass vs. mobility.

Table 1
Protein molecular mass estimation by slab gel SDS-PAGE and SDS capillary electrophoresis

Proteins	CGE	Slab gel SDS-PAGE
α-Lactalbumin	14 251	14 200
Lysozyme	14 313	14 300
Myoglobin	17 231	18 800
β-Lactoglobulin	18 464	18 000
Monoamine oxidase	65 121	60 000
Alcohol dehydrogenase	41 932	41 000
Glyceral phosphate dehydrogenase	36 135	35 700
Enolase	50 333	42 000
α-Amylase	58 866	50 000
Carbonic anhydrase	29 960	29 000
Ovalbumin	43 264	45 000
Bovine serum albumin	66 004	66 000
Fumarase	44 302	49 000
Luciferase	42 854	42 000
Hexokinase	51 786	51 000
Catalase	56 614	60 000
Soybean tripsin inhibitor	21 267	20 100
Cytochrome oxidase	191 925	200 000
Urease	92 420	83 000
Phosphorylase *b*	98 970	97 400
Puruvate carboxylase	134 902	130 000
L-Amino acid oxidase	70 019	70 000
β-Galactosidase	126 000	116 000
L-Lactic dehydrogenase	32 683	36 500
Asparagenase	40 460	37 000
Triosephosphate isomerse	27 306	26 600
α-Macroglobulin	188 853	180 000
Transkatolase	74 468	70 000
Heptoglobin	84 233	85 000

matrix can not be replaced, a new capillary is required when performance deteriorates. In this paper, a low viscosity polymer solution and a hydrophilic polymer coated capillary were used for SDS–protein separations. Since the polymer solution can be replaced after each run, a fresh matrix is used for every analysis. The hydrophilic polymer coating also prevents sample interaction with the capillary surface. If the capillary coating is not stable, a charge from the capillary wall or coating may be exposed, which can lead to band broadening due to sample adsorption or the generation of electroosmotic flow. Fig. 3 illustrates the separations of the seven-protein standards after the 1st, 200th and 400th injections, respectively. After 400 runs, good peak shape and constant migration time are still observed. These results demonstrate that the capillary can be used for more than 400 injections without any noticeable degradation. The capability of replacing gel after each run combined with the stable coating used in this study significantly increases overall capillary stability.

3.3. Reproducibility study

A critical consideration of capillary gel electrophoresis is the reproducibility of sample migration time. Several factors, such as temperature control, coating stability, and protein adsorption affect this performance. Using the P/ACE capillary electrophoresis system, the temperature of the gel filled capillary was controlled to +/− 0.1°C by the liquid cooling system. The neutral coating also reduces any interaction between proteins and capillary surface. In our experiments, we tested the reproducibility of the capillary with protein standard mixture, and with the injection of fetal calf serum sample between runs. The crude fetal calf serum contains large amounts of protein including albumins and immunoglobulins. The serum sample was diluted with DDI water (1:1 ratio) and pretreated with the standard denaturation procedure. Table 2 shows the R.S.D., range from 0.351 to 0.453%, of uncorrelated migration times for the first and last few runs of standard proteins. The highly reproducible results may be attributed to the

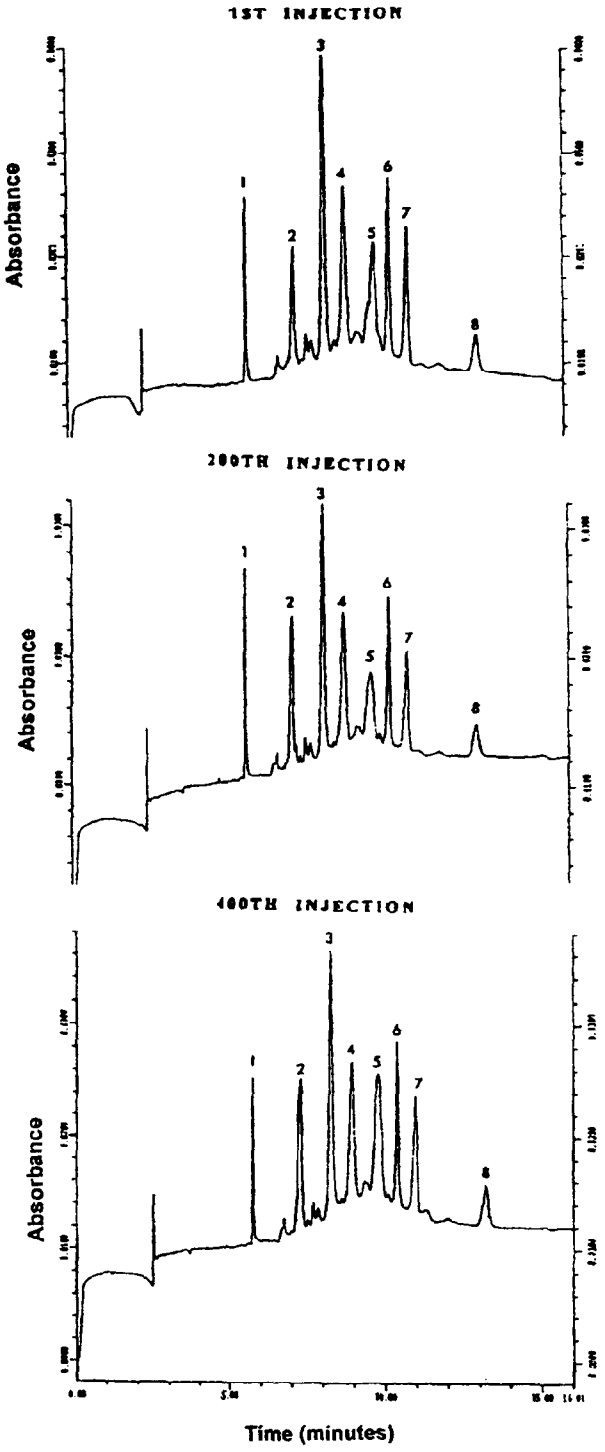

Fig. 3. Stability study of the eCAP SDS 14-200 kit. Conditions: see Experimental section. From top to bottom, 1st injection, 200th injection, and 400th injection, respectively.

Table 2
SDS 14-200 reproducibility study

Run[a]	OG	α-LAC	CA	OVA	BSA	PHOS	β-GAL	MYO
1st	5.94	7.50	8.44	9.15	10.12	10.60	11.19	13.40
2nd	5.94	7.50	8.43	9.14	10.12	10.59	11.18	13.40
3rd	5.93	7.49	8.42	9.13	10.10	10.58	11.17	13.38
4th	5.90	7.44	8.38	9.07	10.05	10.53	11.12	13.32
5th	5.90	7.45	8.39	9.08	10.04	10.53	11.12	13.31
6th	5.93	7.47	8.42	9.11	10.07	10.55	11.14	13.34
7th	5.94	7.50	8.45	9.16	10.14	10.61	11.21	13.43
8th	5.89	7.43	8.38	9.08	10.04	10.52	11.11	13.31
9th	5.88	7.42	8.36	9.06	10.01	10.50	11.09	13.29
191st	5.93	7.45	8.44	9.08	10.08	10.58	11.20	13.45
194th	5.90	7.44	8.42	9.07	10.06	10.57	11.18	13.43
195th	5.90	7.44	8.40	9.06	10.04	10.56	11.15	13.41
199th	5.88	7.45	8.40	9.08	10.05	10.56	11.16	13.42
200th	5.87	7.43	8.39	9.06	10.03	10.55	11.15	13.41
Av.	5.90	7.46	8.41	9.09	10.06	10.56	11.15	13.39
S.D.	0.024	0.030	0.030	0.041	0.042	0.038	0.043	0.061
R.S.D. (%)	0.400	0.398	0.351	0.448	0.413	0.358	0.388	0.453

[a] Injections 1–9: protein standard; Injections 10–190: fetal calf serum; Injections 191–200: protein standard.

good temperature control, stable coating, HCl wash and replaceable gel.

Another important issue of capillary gel electrophoresis is the batch-to-batch reproducibility of gel capillaries. For replaceable polymer solutions, the chain length of polymer and viscosity of solution can be significantly increased. Tables 3 and 4 show the reproducibility study of three different batches of gel buffers and capillaries. R.S.D. values range from 0.59 to 1.12%.

3.4. Applications

Fig. 4 shows the separation of commercial low fat milk by SDS capillary electrophoresis. Proteins can be identified by their molecular mass estimated from the migration of the standard proteins. High speed separation and good resolution were obtained from the analysis. Fig. 5 illustrates the SDS-protein separation of chicken egg white. Proteins such as lysozyme, ovalbumin, conalbumin are baseline separated in less than 15 min. In comparison to native protein separation with uncoated silica capillary, the SDS capillary electrophoresis offers much higher peak efficiency and resolution [15].

4. Conclusions

Fast separations of SDS–protein complexes have been demonstrated on a UV transparent polymer network matrix. The straight-line plot of log protein molecular mass vs. mobility demonstrates the pure sieving mechanism during the separation. The column can be used for more than 400 sample injections without loss in performance when employing a HCl wash in between runs. Migration time reproducibility in different runs and batches are in general less than 1% R.S.D. The method provides fast analysis of protein mixtures on the basis of the difference in protein molecular masses. Real samples without sample pre-treatment may be directly analyzed by this method. As a final note, evaluation of proteins higher than 205 000 daltons subjected to further investigation.

Table 3
SDS 14-200 gel buffer lot-to-lot reproducibility

Buffer	OG	α-LAC	CA	OVA	BSA	PHOS	β-GAL	MYO
Lot No. 1								
Inj. 1	6.45	8.08	9.11	9.83	10.89	11.42	12.07	14.48
Inj. 2	6.43	8.05	9.08	9.80	10.87	11.39	12.03	14.42
Inj. 3	6.43	8.06	9.08	9.80	10.88	11.40	12.03	14.45
Inj. 4	6.42	8.05	9.08	9.80	10.87	11.40	12.03	14.44
Inj. 5	6.42	8.04	9.07	9.80	10.86	11.39	12.02	14.42
Lot No. 2								
Inj. 1	6.32	8.25	9.22	9.98	11.92	11.56	12.17	14.46
Inj. 2	6.30	8.28	9.18	9.96	11.00	11.54	12.13	14.44
Inj. 3	6.29	8.26	9.19	9.96	11.02	11.54	12.14	14.45
Inj. 4	6.29	8.25	9.20	9.96	11.02	11.55	12.16	14.47
Inj. 5	6.30	8.25	9.21	9.96	11.00	11.56	12.17	14.47
Lot No. 3								
Inj. 1	6.43	8.24	9.28	9.98	11.03	11.57	12.20	14.55
Inj. 2	6.42	8.22	9.26	9.96	11.00	11.54	12.16	14.51
Inj. 3	6.42	8.22	9.25	9.96	10.99	11.53	12.14	14.50
Inj. 4	6.41	8.21	9.25	9.94	10.98	11.52	12.14	14.48
Inj. 5	6.41	8.21	9.24	9.94	10.97	11.52	12.14	14.49
R.S.D. (%)	0.96	1.12	0.82	0.77	0.59	0.62	0.51	0.24

Table 4
SDS 14-200 capillary lot-to-lot reproducibility

Buffer	OG	α-LAC	CA	OVA	BSA	PHOS	β-GAL	MYO
Lot No. 1								
Inj. 1	6.13	7.68	8.75	9.41	10.27	10.91	11.53	13.83
Inj. 2	6.13	7.67	8.72	9.40	10.28	10.91	11.52	13.82
Inj. 3	6.12	7.68	8.70	9.39	10.28	10.89	11.50	13.79
Inj. 4	6.12	7.68	8.70	9.38	10.28	10.87	11.47	13.76
Lot No. 2								
Inj. 1	6.23	7.82	8.87	9.54	10.57	11.09	11.73	14.06
Inj. 2	6.22	7.84	8.85	9.52	10.54	11.06	11.69	14.00
Inj. 3	6.22	7.84	8.84	9.51	10.53	11.05	11.67	13.99
Inj. 4	6.21	7.83	8.83	9.50	10.51	11.04	11.66	13.98
Lot No. 3								
Inj. 1	6.12	7.71	8.68	9.37	10.35	10.87	11.46	13.73
Inj. 2	6.09	7.69	8.65	9.36	10.35	10.88	11.48	13.81
Inj. 3	6.09	7.70	8.66	9.37	10.36	10.90	11.51	13.86
Inj. 4	6.10	7.71	8.68	9.38	10.37	10.92	11.53	13.88
R.S.D. (%)	0.89	0.92	0.93	0.73	1.10	0.77	0.83	0.77

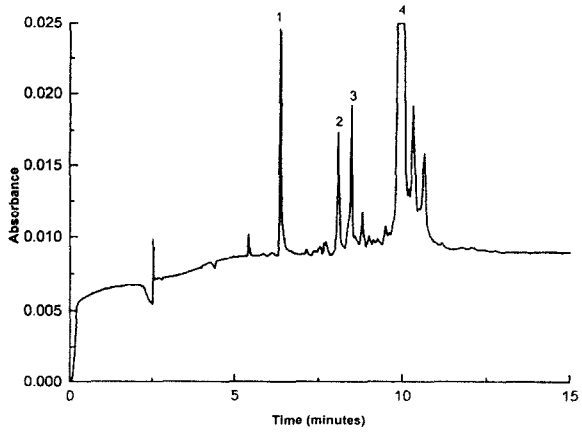

Fig. 4. SDS–protein separation of bovine milk. Conditions: see Experimental section. Solutes: 1 = orange G, reference marker; 2 = α-lactalbumin (M_r 14 200); 3 = β-lactoglobulin (M_r 18 000); 4 = casein.

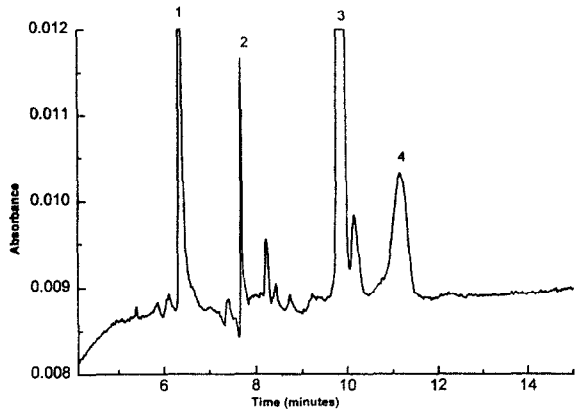

Fig. 5. SDS–protein separation of egg white. Conditions: see Experimental section. Solutes: 1 = orange G, reference marker; 2 = lysozyme (M_r 14 300); 3 = ovalbumin (M_r 45 000); 4 = conalbumin (M_r 77 000).

References

[1] K. Weber and M. Osborn, *J. Biol. Chem.*, 244 (1969) 4406.
[2] D.B. de Wald, L.D. Adams and J.D. Pearson, *Anal. Biochem.*, 154 (1986) 502.
[3] A.S. Cohen and B.L. Karger, *J. Chromatogr.*, 397 (1987) 409.
[4] A. Widhalm, C. Schwer, D. Blass and E. Kenndler, *J. Chromatogr.*, 546 (1991) 446.
[5] A. Guttman, A.S. Cohen, D.H. Heiger and B.L. Karger, *Anal. Chem.*, 62 (1990) 137.
[6] D. Wu and F.E. Regnieer, *J. Chromatogr.*, 608 (1992) 349.
[7] T.S. Work, *J. Mol. Biol.*, 10 (1964) 544.
[8] A.H. Gordon and L.N. Louis, *Anal. Biochem.*, 21 (1967) 190.
[9] S. Hjerten, in H. Hirai (Editor), *Electrophoresis' 83*, Walter de Gruyter, New York, 1984, pp. 71–79.
[10] K. Tsuji, *J. Chromatogr.*, 550 (1991) 823.
[11] K. Ganzler, K.S. Grene, A.S. Cohen, B.L. Karger, A. Guttman and N. Cooke, *Anal. Chem.*, 64 (1992) 2665.
[12] A. Guttman, J. Nolan and N. Cooke, *J. Chromatogr.*, 632 (1993) 171.
[13] A. Guttman, J. Horvath and N. Cooke, *Anal. Chem.*, 65 (1993) 199.
[14] A. Guttman, P. Shieh, J. Lindal and N. Cooke, *J. Chromatogr. A*, 676 (1994) 227.
[15] J.D. Mcculloch, *J. Liq. Chromatogr.*, 16 (1993), 2025.