Sodium Dodecyl Sulfate Capillary Electrophoresis of Wheat Proteins. 1. Uncoated Capillaries†

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Four different polymer/buffer systems (a commercial polymer from Bio-Rad, dextran, poly(ethylene oxide) (PEO), and non-crosslinked poly(acrylamide)) were evaluated for use in sodium dodecyl sulfate capillary electrophoresis (SDS−CE) separations of wheat proteins. These polymers were chosen on the basis of published reports of their use in uncoated or dynamically coated capillaries. Each polymer was optimized (where possible) by manipulating the polymer concentration and buffer concentration, and through the use of organic modifiers such as methanol and ethylene glycol. The addition of ethylene glycol to the separation buffer was found to improve the resolution of the separations, despite dilution of the sieving polymers. When PEO was used as the sieving polymer, however, no improvement was seen when ethylene glycol was added. Despite producing similar separations of molecular mass markers, the polymers did not all produce similar wheat protein separations. The commercial reagent and dextran produced similar separations, while the poly(acrylamide) produced faster separations than either. The poly(acrylamide) displayed much lower resolution in the 40−60 kDa range than the other polymers, though this polymer was able to separate the high molecular mass glutenin subunits (HMM-GS) without the use of added organic solvent. PEO produced much different wheat protein separations than the other polymers, despite similar separations of the molecular weight markers. This may have been due to interaction between the wheat proteins and PEO. Each polymer system also predicted different molecular masses of the various wheat protein fractions separated, with the PEO and poly(acrylamide) grossly overestimating the masses for all protein classes. This could have been due to protein−polymer interactions. Further work was done with the Bio-Rad buffer modified by the addition of ethylene glycol. Several different wheat protein fractions as well as proteins extracted from several different cultivars were separated with this buffer and compared. SDS−CE separations were also compared to SDS−poly(acrylamide) gel electrophoresis (PAGE) and several differences in the migration pattern of HMM-GS were noted.

Keywords: *Capillary electrophoresis; proteins; polymers; glutenins; wheat*

INTRODUCTION

Since its introduction into the cereal chemistry field by Bietz and Wall (1972), sodium dodecyl sulfate−poly(acrylamide) gel electrophoresis (SDS−PAGE) has been a widely used, if not the most widely used, method to separate cereal proteins. Although many modifications and improvements in SDS−PAGE protocols for separating wheat proteins have been reported in recent years (Graybosch and Morris, 1990; Khelifi and Branlard, 1991; Zhen and Mares, 1992; Kasarda et al., 1998), SDS−PAGE has its drawbacks including the use of toxic reagents, the requirement for post-separation detection (staining), and a lack of direct, easy quantification (although this has been addressed with wheat proteins in recent papers (Hou and Ng, 1995 and references therein). SDS−PAGE has the advantage of low equipment costs and high throughput capabilities.

In addition to traditional slab gel electrophoresis, high performance capillary electrophoresis (HPCE) has recently been applied to the separation of cereal proteins (for a recent review, see Bean et al., 1998a). HPCE offers the ability to perform rapid, high-resolution electrophoretic separations of proteins analogous to PAGE, but with the automation and digital output of data that modern HPLC instruments provide. Although both HPLC and HPCE currently lack the throughput capability of traditional slab gels, this limitation may soon be overcome with HPCE through the use of multicapillary arrays (e.g., Huang et al., 1992), chip-based separations (Effenhauser et al., 1997), and novel buffers which allow extremely high-voltage separations (Hjerten et al., 1995; Righetti et al., 1997).

Although a number of papers have utilized one particular mode of HPCE, referred to as free zone capillary electrophoresis (FZCE), to separate cereal proteins and considerable development has been done with FZCE (Bean et al. 1998a), comparatively little work has focused on development of methods for sodium dodecyl sulfate capillary electrophoresis (SDS−CE) separations, analogous to SDS−PAGE, of cereal

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10.1021/jf990413n CCC: $18.00
Published on Web 09/24/1999

proteins. To take full advantage of the benefits of HPCE (automation, unattended operation, and digital output which is easily quantified), SDS−CE methods need to be developed for rapid, rugged separations analogous to SDS−PAGE for wheat and other cereal proteins. Earlier work with a commercial sieving buffer, Prosort (Applied Biosystems, Foster City, CA), composed of a proprietary commercial non-crosslinked poly(acrylamide) (PAA) (Werner et al., 1993), showed that rapid, high-resolution SDS−CE separations were feasible (Werner et al., 1994; Weegels et al., 1995; Sutton and Bietz, 1997); however, to the best of our knowledge, this reagent is no longer available (W. Werner, personal communication). Other SDS−CE separation systems have been employed to separate wheat (Scholz et al., 1997; Pollard et al., 1998) and maize proteins (Parris et al., 1997) although a systematic evaluation of the various polymers available has not been completed.

Originally, SDS−CE separations of proteins were carried out in gels polymerized inside capillaries (Wu and Regnier, 1992). Because of problems with polymerization of the gels and their breakdown during use, fluid "entangled polymers" have been used to replace the rigid gels (Ganzler et al., 1992). These polymers can be forced into the capillary after each separation, providing high resolution and repeatability (Ganzler et al., 1992). Several types of polymers have been proposed for these types of separations and several reviews have covered this topic (Kenndler and Poppe, 1994; Heller, 1995; Guttman, 1996; Chrambach, 1996; Takagi, 1997). This paper compares four types of polymers for separating various fractions of wheat proteins according to molecular mass. The polymers selected for this study were chosen primarily on the basis of their ability to be used in uncoated capillaries (a similar project comparing polymers that must be used in coated capillaries is underway). The four polymers selected were a commercial polymer from Bio-Rad ("CE SDS protein run buffer", Bio-Rad, Hercules, CA) specifically formulated for use in uncoated capillaries; dextran; poly(ethylene oxide) (PEO); and non-crosslinked PAA.

MATERIALS AND METHODS

Reagents. Bio-Rad CE SDS protein run buffer and wide range molecular mass standards were obtained from Bio-Rad (Hercules, CA). PEO (M_r = 100 000) was from Polysciences (Warrington, PA). *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) (ultrapure) was obtained from Gibco-BRL (Grand Island, NY). Dextran ($M_r = 2 \times 10^6$) and all remaining chemicals were obtained from Sigma (St. Louis, MO) and were of the highest possible purity.

Capillary Electrophoresis. Beckman PACE 5510 or PACE 2100 instruments were used for all separations. Either 50 or 75 μm i.d. uncoated fused silica capillaries (Polymicro Technologies, Phoenix, AZ) 27 cm in length (20 cm to detector) were used for separations. The larger diameter 75 μm i.d. capillaries were used when the Bio-Rad SDS reagent was used due to its high viscosity. Samples were separated at 25 °C and 8 kV unless stated otherwise in the text. All samples were injected by pressure at 0.5 psi. Injection times varied depending on the polymer and sample being used. UV detection at 214 nm was used for all separations with all polymer types. Individual high molecular mass glutenin subunits (HMM-GS) were identified in SDS−CE separations by comparing glutenin electrophoregrams from several cultivars with known HMM-GS composition and special genetic lines with limited numbers of HMM-GS.

Capillary Rinsing Protocols. A variety of capillary rinsing protocols were used depending on the polymer being studied at the time. The Bio-Rad buffer was used according to the manufacturer's directions. Briefly, capillaries were rinsed for 5 min each with 1 M NaOH and 1 M HCl before being used for the day. Before each separation, capillaries were rinsed with the polymer solution for 5 min. After each separation, capillaries were flushed with 1 M NaOH for 2 min, followed with 1 M HCl for 1 min.

When the dextran buffer was used, capillaries were first flushed with Bio-Rad SDS run buffer for 5 min followed by the dextran containing buffer for 5 min. After each separation capillaries were rinsed with 1 M NaOH and 1 M HCl as described above.

For PEO and non-crosslinked PAA containing buffers, capillaries were rinsed with 1 M NaOH for 5 min and 1 M HCL for 5 min, and then were rinsed with separation buffer for 30 min at the beginning of each day. Flat, stable baselines could only be achieved by following this rinsing protocol. Prior to each analysis, capillaries were rinsed with separation buffer for 5 min. No post separation rinses were made.

Sample Preparation. Total protein extracts were prepared by extracting 250 mg of flour with 1 mL of 1% SDS and 5% β-mercaptoethanol (β-ME) for 30 min with continual vortexing using a Vortexgenie2 equipped with a 30 place vial holder (Scientific Instruments, Bohemia, NY). Gliadins were extracted from flour by mixing 250 mg flour with 1 mL of 1% SDS and vortexing as described above for 5 min. Note that these extracts would also contain albumin and globulin proteins as well as soluble polymeric glutenins and other polymeric proteins.

The insoluble glutenins (polymeric proteins), were extracted from flour (250 mg) with 1 mL of 1% SDS and 5% β-ME after removal of "soluble" proteins with three 50% 1-propanol extractions as described in Bean et al. (1998b).

HMM-GS were prepared by a combination of extraction with 50% 1-propanol + 1% dithiothreitol (DTT) and acetone precipitation as described in Bean and Lookhart (1998). Precipitated HMM-GS were redissolved in 400 μL of 1% SDS and 5% β-ME by stirring at 95 °C for 10 min. Molecular mass standards were prepared by mixing 10 μL of sample with 20 μL of 1% SDS and 5% β-ME. The HMM-GS peaks in the SDS−CE separations were identified by comparing results to the data of Werner et al. (1994) and Sutton and Bietz (1997) and by comparing the HMM-GS patterns of approximately 20 different wheats with various combinations of HMM-GS.

For SDS−PAGE analysis, insoluble polymeric proteins (mainly glutenins) were isolated with three 50% 1-propanol extracts (Bean et al., 1998b) and then extracted with a 247 mM Tris-HCl, buffer pH 8.5 containing 5% β-ME, 2% SDS, 10% sucrose, and tracking dye for color. All flour extracts were heated at 95 °C for 10 min prior to analysis.

Formation of PAA. Non-crosslinked PAA was formed with slight modifications (maintaining constant temperature and purging the solution with nitrogen during polymerization) to the method of Bode (1977) which utilized the amount of catalyst to control the size of the polymer formed during polymerization (a similar method was also used to create short-chain PAA polymers for the separation of DNA [Ruiz-Martinez et al., 1993]).

"Long-chain" polymer (Bode, 1977) was formed by adding 15 g of acrylamide into 190 mL of water and dissolving completely. Once dissolved, the solution was vacuum degassed and 40 mg of ammonium persulfate (dissolved in 10 mL of water) and 300 μL of TEMED were added. The solution was then heated in a water bath at 40 °C, slowly rotated, and allowed to polymerize for 70 min with nitrogen gas slowly bubbled through the solution. After polymerization, the polymer was precipitated by the addition of 200 mL of methanol (Thomas and Wang, 1985). The precipitated polymer was recovered by centrifugation, redissolved in 200 mL of water and precipitated once again with 200 mL of methanol. The precipitate was recovered and dried under vacuum overnight at room temperature.

"Medium-chain" polymer (Bode, 1977) was formed with the same procedure except the amount of catalyst was changed to 190 mg ammonium persulfate (in 10 mL of water) and 1.5

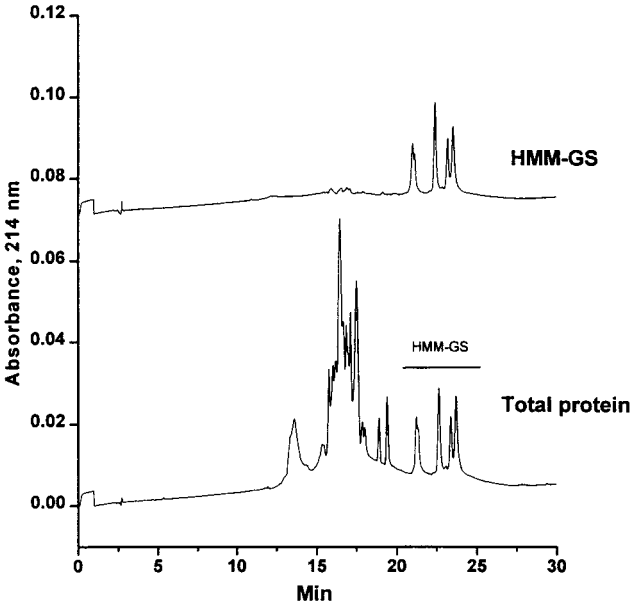

Figure 1. Separation of HMM-GS and total proteins from the cultivar Karl separated with CE SDS protein run buffer. Separations were carried out in an uncoated capillary 75 μm i.d. × 27 cm long (20 cm L_D) at 25 °C and 8 kV. Samples were pressure injected (0.5 psi) for 15 s.

mL of tetramethylethylenediamine (TEMED). When these polymers were dissolved, gentle stirring was used at all steps in order to limit the amount of polymer breakage due to shear (Goetzinger et al., 1998). At no time was the polymer ground into a fine powder before use.

SDS−PAGE. SDS−PAGE was performed as described in Bean and Lookhart (1998). SDS−PAGE gels were stained with Gelcode Blue stain (Pierce) according to manufacturer's directions. Gels were photographed with a digital camera (Eastman Kodak Company, Rochester, NY) and lane profiles were generated using Kodak 1D software. Profiles were generated solely to allow easy comparison of the SDS−PAGE separations and were not intended to be used in a quantitative fashion.

RESULTS

Separations with Bio-Rad Reagent. To initially evaluate the Bio-Rad reagent, total proteins and isolated HMM-GS from the wheat cultivar Karl were separated (Figure 1). Separations were completed in ca. 30 min and with good resolution. HMM-GS of cultivar Karl were well-separated from other proteins in the total protein extract (Figure 1). However, all five HMM-GS of Karl were not clearly resolved from each other (Figure 1).

Addition of Organic Modifiers. Reportedly, previous SDS−CE separations of wheat proteins indicated that methanol had to be added to the separation buffer to achieve optimum resolution of HMM-GS (Werner et al., 1994; Sutton and Bietz, 1997). Therefore, methanol was added to the Bio-Rad reagent and HMM-GS were well separated with this mixture. Upon addition of 5% methanol, the resolution of the HMM-GS was improved and by increasing the amount of methanol to 10%, baseline resolution of all five HMM-GS was seen (data not shown). The addition of methanol, however, caused a white precipitate to form in the Bio-Rad reagent, which was difficult to redissolve. Thus an additional organic modifier, ethylene glycol, was tested. When ethylene glycol was added to the Bio-Rad reagent at 5% concentration, resolution of the HMM-GS was improved (Figure 2). Increasing the concentration to 15% led to

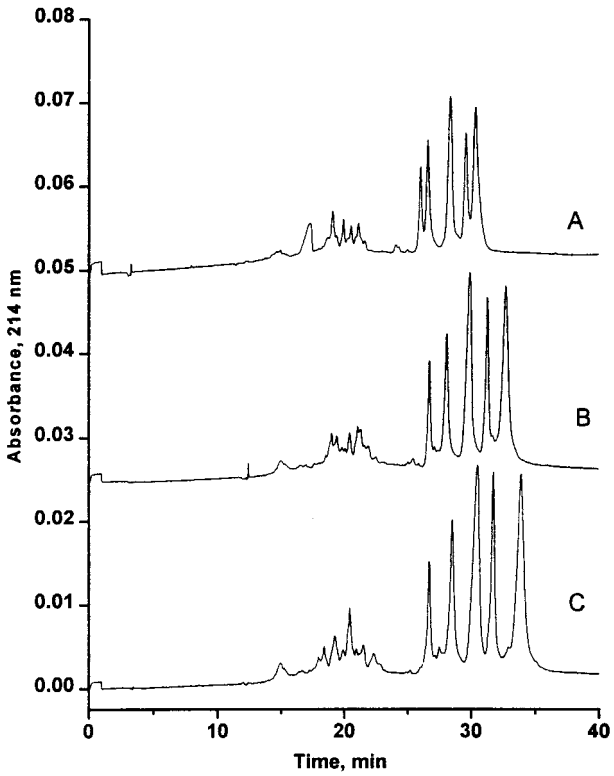

Figure 2. Effect of addition of ethylene glycol to Bio-Rad SDS run buffer on the resolution of HMM-GS. (A) Bio-Rad buffer + 5% ethylene glycol, (B) Bio-Rad buffer + 10% ethylene glycol, (C) Bio-Rad buffer + 15% ethylene glycol. Separation conditions are the same as in Figure 1.

even better separations and it was judged to be the optimum concentration (Figure 2). No precipitation in the Bio-Rad reagent was seen when ethylene glycol was added.

Optimization with Bio-Rad Buffer. Separation conditions for the Bio-Rad buffer modified by the addition of 15% ethylene glycol were optimized by varying the voltage (4−16 kV) and temperature (20−45 °C). Optimum conditions were judged to be 8 kV and 30 °C (data not shown).

Separation of Protein Fractions. Several different protein fractions from the cultivar Karl, along with standard molecular mass markers, were separated and compared using the Bio-Rad reagent/15% ethylene glycol mixture (Figure 3). Good separations of all the protein classes were obtained with this buffer, including the standard proteins. Total protein extracts showed a number of well-resolved peaks, although there was some overlap between the HMM-GS and the other lower molecular mass proteins, which was not seen when the ethylene glycol was not present (compare to Figure 1). It was apparent from the electrophoregrams of the gliadin and glutenin separations that this overlap was due to some late migrating gliadins overlapping with the lower molecular mass HMM-GS (Figure 3). The glutenin separation showed good resolution of the HMM-GS and resolved the low molecular mass glutenin subunits (LMM-GS) into several well-resolved peaks. Likewise, gliadins were resolved into several peaks, though resolution was substantially lower than typically seen with free zone separations of these proteins (Bean et al., 1998a). Note that both the gliadins and LMM-GS showed better resolution in their respective fractions than in the total protein extract. This is

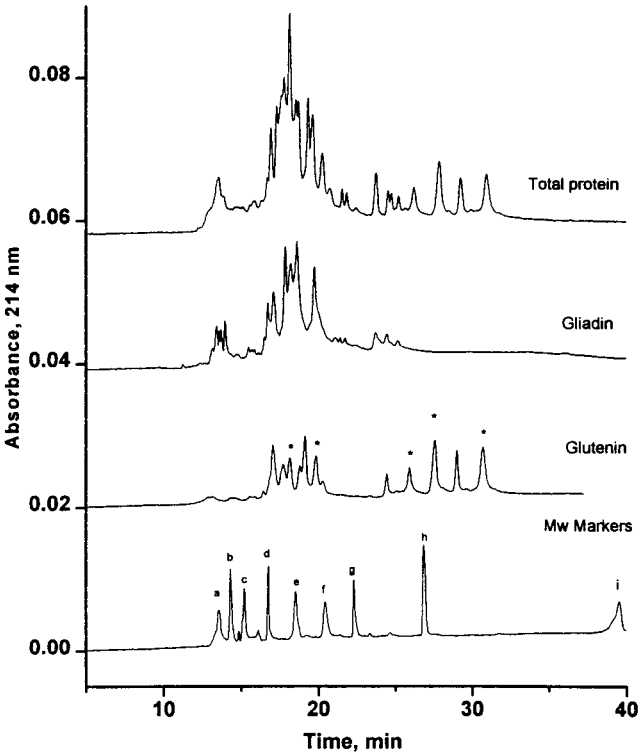

Figure 3. Separation of wheat protein fractions in Bio-Rad CE SDS run buffer + 15% ethylene glycol. Separation conditions are the same as in Figure 1, except temperature was 30 °C. Molecular mass markers were as follows: (a) aprotinin (6.5 kDa), (b) lysosyme (14.4 kDa), (c) trypsin inhibitor (21.5 kDa), (d) carbonic anhydrase (31 kDa), (e) ovalbumin (45 kDa), (f) serum albumin (66.2 kDa), (g) phosphorylase b (97.4 kDa), (h) β-galactosidase (116.25 kDa), and (i) myosin (200 kDa). Asterisks on the glutenin separation denote peaks used to determine repeatability data.

Table 1. Predicted Approximate Molecular Mass (kDa) of Wheat Protein Fractions

	protein class		
polymer	HMM-GS	LMM-GS	gliadins[a]
Bio-Rad	94−142	32−67	32−66
Dextran	109−160	43−81	50−83
PEO	57−351	18−203	57−219
PAA	139−216	53−110	73−150
SDS−PAGE	92−149[b]	31−51[c]	35−63[d]
cDNA	65−90[b]	−	−

[a] Numbers for gliadins are for the major gliadin peaks only and do not include the peaks corresponding to albumin and globulins or late migrating ω-gliadins. [b] Data taken from Werner, 1995. [c] Data taken from Payne and Corfield, 1979. [d] Data taken from Lookhart and Albers, 1988. Numbers represent only the major gliadins, and do not include ω-gliadins with unusually high values.

because both LMM-GS and gliadins are present in the total protein extract and have similar mass ranges and thus interfere with each other. This does not occur in the isolated protein fractions.

The molecular mass marker proteins were all well resolved. Correlation between the molecular mass versus migration time for the molecular mass standards was high (r^2 = 0.98). The molecular mass ranges predicted for the various wheat protein fractions this buffer system was used are shown in Table 1.

Separations in Dextran. Several factors were manipulated to optimize wheat protein separations when using dextran as a sieving agent. Buffer (Tris-Borate, pH 8.5 + 0.1% SDS) concentration (100−800 mM),

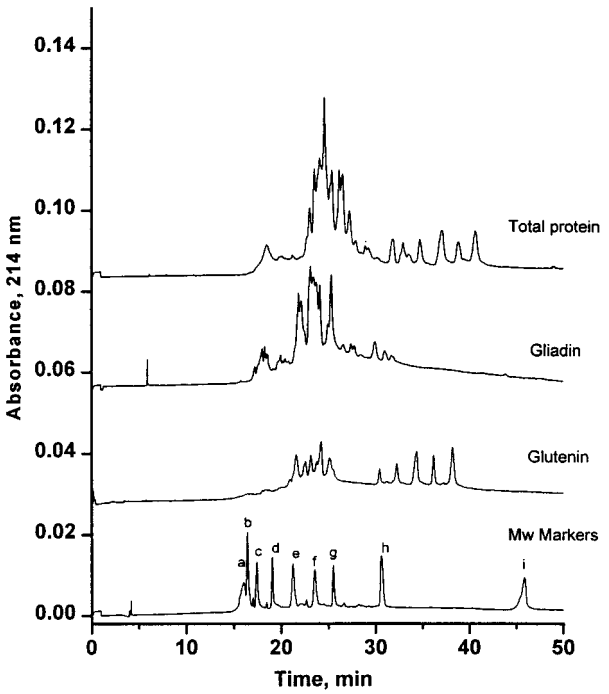

Figure 4. Separation of wheat protein fractions in 10% dextran in 600 mM Tris-Borate buffer, pH 8.5 + 0.1% SDS mixed 9:1 with ethylene glycol (final concentration of ethylene glycol 10%). Separation conditions were 25 °C and 8 kV using a 75 μm i.d. × 27 cm long (20 cm L_D) uncoated capillary. Samples were pressure injected (0.5 psi) for 15 s. Molecular mass markers are the same as in Figure 3.

dextran concentration (10−20%), and the addition of ethylene glycol (5−15%) were all tested. Optimum conditions were found to be 10% dextran in either 400 or 600 mM buffer containing 10% ethylene glycol. Separation of several wheat protein fractions in the optimized dextran system, along with molecular mass markers, are shown in Figure 4. Separations in the optimized buffer were nearly identical to that of the Bio-Rad buffer for all protein classes. Baseline resolution of all five HMM-GS of the cultivar Karl were obtained and good resolution of LMM-GS and gliadins was also seen. Molecular mass standards were well resolved and the correlation between molecular mass and migration time was good (r^2 = 0.98). The migration times of the wheat proteins relative to the standard proteins were slightly different than for the Bio-Rad reagent. This is evident in the predicted molecular mass ranges for the wheat protein fractions when the dextran system was used (Table 1).

Separations in Poly(ethylene oxide). Again several parameters were optimized, including buffer concentration, PEO concentration, and the addition of ethylene glycol. Optimum conditions were found to require 400 mM buffer (Tris-2-(cyclohexamino)ethanesulfonic acid (CHES)), pH 8.5 + 0.1% SDS) containing 4% PEO. Increasing the PEO concentration beyond 4% resulted in decreased resolution and no improvements were noted when the buffer concentration was increased beyond 400 mM. Interestingly, the addition of ethylene glycol to PEO containing buffers caused decreased resolution of the HMM-GS (data not shown).

Separation of several protein fractions in the optimized PEO buffer is shown in Figure 5. Substantial differences between the separations shown in Figure 5 and those obtained with the Bio-Rad reagent (Figure 3) or dextran (Figure 4) were found. The total protein

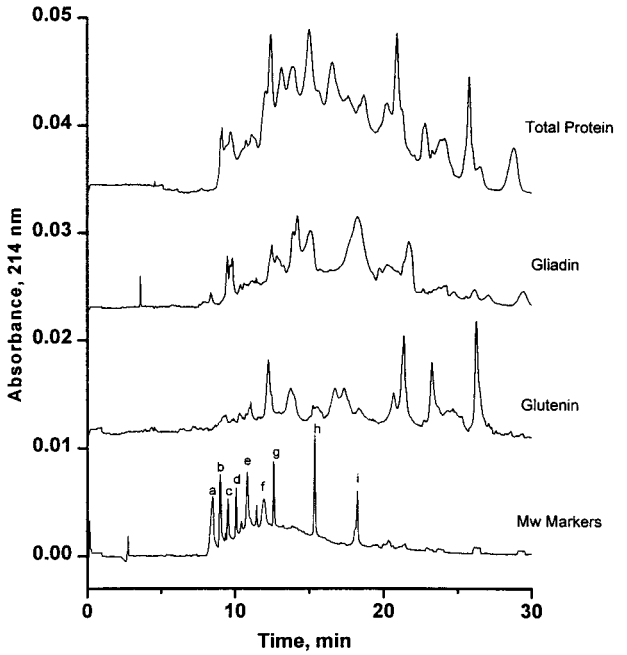

Figure 5. Separation of wheat protein fractions in 4% PEO in 400 mM Tris-CHES buffer, pH 8.5 + 0.1% SDS. Separation conditions were 25 °C and 8 kV using a 50 μm i.d. × 27 cm long (20 cm L_D) uncoated capillary. Samples were pressure injected (0.5 psi) for 10 s. Molecular mass markers are the same as in Figure 3.

extract did not show good separation between the HMM-GS and the other proteins when PEO was used. Both gliadins and glutenins were separated into several peaks, although patterns were distinctly different from the previous polymers tested. However, the molecular mass standards were well resolved and showed good correlation between migration time and molecular mass (r^2 = 0.98). The high end of the molecular masses predicted for the various wheat protein classes were grossly overestimated in the PEO buffer system (Table 1), possibly suggesting wheat protein−PEO interactions.

Separations in PAA Polymers. A neutral buffer was chosen for use with the PAA polymers to reduce hydrolysis of the polymer over time (Werner et al., 1994). A Tris-HEPES buffer was selected as the buffer species due to its low conductivity and good buffering capacity at neutral pH (Reijenga et al., 1996).

Initial separations were carried out using 3% "medium"-chain PAA in 100, 200, and 400 mM buffer. As with the other polymers, this matrix produced acceptable separations of the wheat protein fractions as well as the standard proteins (data not shown). However, better separations were achieved when 2% solutions of "long" chain PAA were used (Figure 6). Because of the higher viscosity of the long-chain polymer, a 2% solution was the highest concentration that was practical to use in 50 μm i.d. capillaries. Optimum separation of HMM-GS with this polymer was found with 200 mM buffer and 2% PAA.

Separation of protein fractions from the cultivar Karl along with molecular mass markers is shown in Figure 6. Good separations of the HMM-GS and LMM-GS were found in the glutenin extract. All five HMM-GS were well resolved without the need to add ethylene glycol to the separation buffer. Gliadins however, were poorly resolved, as was the low molecular mass range in the total protein extract. This range was also poorly separated in the molecular mass markers, with several of

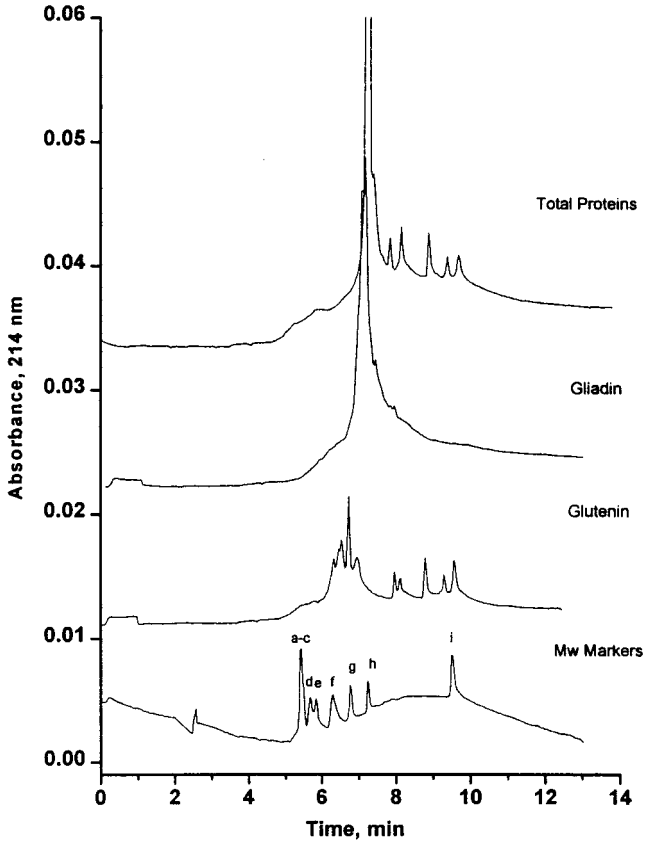

Figure 6. Separation of wheat protein fractions in 2% non-crosslinked PAA in 200 mM Tris-HEPES buffer, pH 7.0 + 0.1% SDS. The capillary was an uncoated 50 μm i.d. × 27 cm (20 cm L_D). Temperature was maintained at 25 °C and voltage at 8 kV. Samples were injected by pressure for 30 s. Molecular mass markers are the same as in Figure 3.

the smaller proteins comigrating (Figure 6). Correlations between migration time and molecular mass of the standard proteins was similar to the other polymers tested (r^2 = 0.98) The predicted molecular masses for the various wheat protein fractions were higher than those predicted in either the modified Bio-Rad reagent or dextran, but lower than those found with PEO (Table 1).

Repeatability. The Bio-Rad/ethylene glycol combination was selected for further evaluation and testing. Separation-to-separation repeatability of migration times and peak areas were measured. The migration times of selected peaks from a glutenin extract of the cultivar Karl varied by <0.60% RSD over a 10 run period (Table 2). These same peaks varied by <2.18% RSD in peak area (Table 2).

Day-to-day variability was assessed by analyzing a glutenin extract of Karl on 5 separate days over a 7 day period. Migration time variation was similar to the run-to-run variation (<0.5%) except for the first peak, which showed much higher variation. Peak areas showed higher variation day-to-day than run-to-run. Note that the separation-to-separation repeatability tests were performed several months apart from the day-to-day tests, and as such utilized a different capillary, sample, and buffer lot.

Gliadin and Glutenin Separations. Finally, glutenins and gliadins of several different wheat cultivars were separated and compared. Separations of gliadins isolated from five different wheat cultivars are shown in Figure 7. The electrophoregrams for each cultivar

Table 2. Run-to-Run Repeatability of Migration Times and Peak Areas[a]

	migration time peak					corrected peak area[b] peak				
run	1	2	3	4	5	1	2	3	4	5
1	18.1	20.8	25.5	28.6	31.7	7284	4816	1393	4307	4088
2	18.1	20.9	25.5	28.6	31.7	7573	5023	1458	4511	4259
3	18.2	21.0	25.6	28.7	31.8	7210	4739	1385	4293	4116
4	18.1	20.9	25.5	28.6	31.8	7311	4779	1418	4361	4179
5	18.1	20.8	25.4	28.6	31.6	7293	4764	1393	4352	4154
6	18.1	20.9	25.5	28.6	31.7	7407	4769	1417	4447	4194
7	18.1	20.8	25.4	28.5	31.6	7298	4725	1394	4380	4137
8	18.1	20.8	25.4	28.6	31.6	7358	4809	1400	4379	4155
9	18.0	20.7	25.3	28.4	31.5	7294	4663	1392	4383	4141
10	17.8	20.6	25.2	28.3	31.4	7228	4651	1365	4402	4109
av	18.1	20.8	25.4	28.6	31.6	7326	4774	1402	4382	4153
SD	0.11	0.11	0.11	0.11	0.11	104	104	25	63	49
RSD (%)	0.59	0.55	0.46	0.41	0.36	1.41	2.17	1.78	1.45	1.18

[a] Migration times and peaks areas measured from 10 consecutive separations of glutenins isolated from the cultivar Karl. Peaks were selected to span the entire time range of the separation. [b] Corrected peak area = peak area/migration time of peak (Altria, 1993).

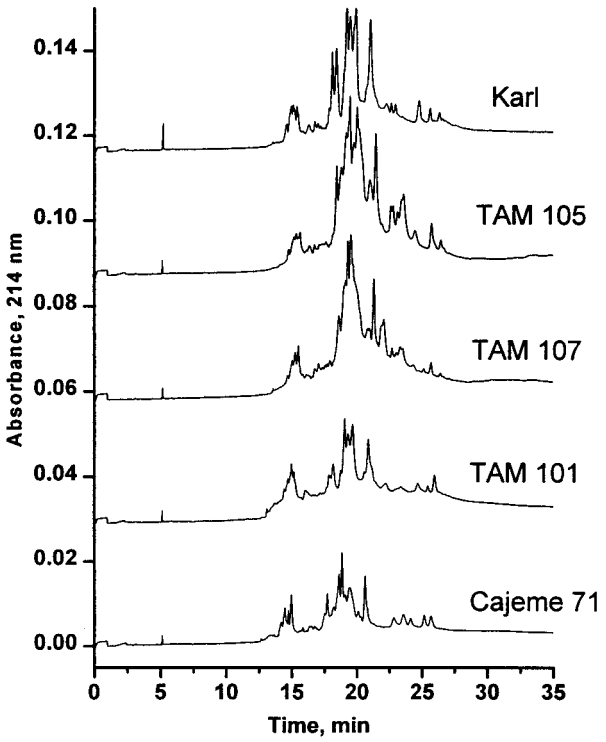

Figure 7. Separation of gliadins from five different wheat cultivars. Samples were separated with Bio-Rad CE SDS run buffer modified by addition of 15% ethylene glycol. All separation conditions are the same as in Figure 3.

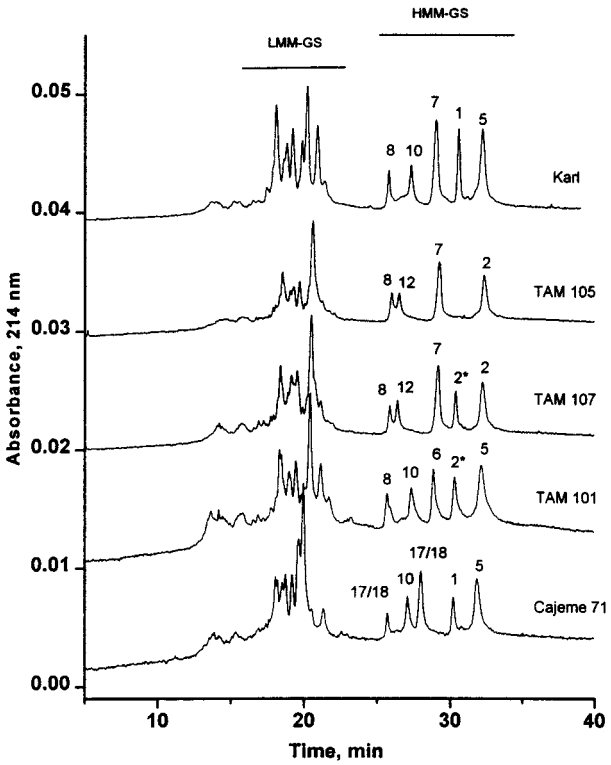

Figure 8. Separation of glutenins of different wheat cultivars in Bio-Rad CE SDS protein buffer modified with 15% ethylene glycol. Numbers above peaks identify individual HMM-GS and are numbered according to accepted nomenclature (Payne and Lawrence, 1983). All separation conditions are the same as in Figure 3.

were unique, showing that this method could be used to discriminate cultivars. The peaks migrating between 12 and 16 min were not present if the flours were first extracted to remove the albumins and globulins (data not shown); thus these peaks probably belong to the albumin/globulin class of proteins. Note that some unreduced glutenins would be present in these extracts, with molecular masses ranging into the 100 000s (Larroque et al., 1997). However, no peaks that should have corresponded to these proteins were noticed in the time frame of these separations.

Glutenins from the same cultivars shown in Figure 7 were separated to compare the resolution of various allelic HMM-GS (Figure 8). For comparison to the SDS–CE separations, lane profiles from SDS–PAGE separations of glutenins from these cultivars were generated and are shown in Figure 9. The HMM-GS of all the cultivars were well resolved in the Bio-Rad/ethylene glycol buffer, with many pairs showing higher resolution by the SDS–CE separation than by SDS–PAGE (compare Figure 8 to Figure 9). For example, 2 and 2* are difficult to separate by SDS–PAGE, though some improvements in separating them have been reported (Zhen and Mares, 1992; Huang and Khan, 1998); however, 2 and 2* were well separated by the SDS–CE method employed here. Also 2* and 5 were easily separated by SDS–CE, but are sometimes difficult to separate from one another by SDS–PAGE. The pair of HMM-GS 17 and 18 found in Cajeme 71 are typically not well resolved on the gels, but were well separated by SDS–CE (though it could not be determined which peak was 17 and which was 18) and display much

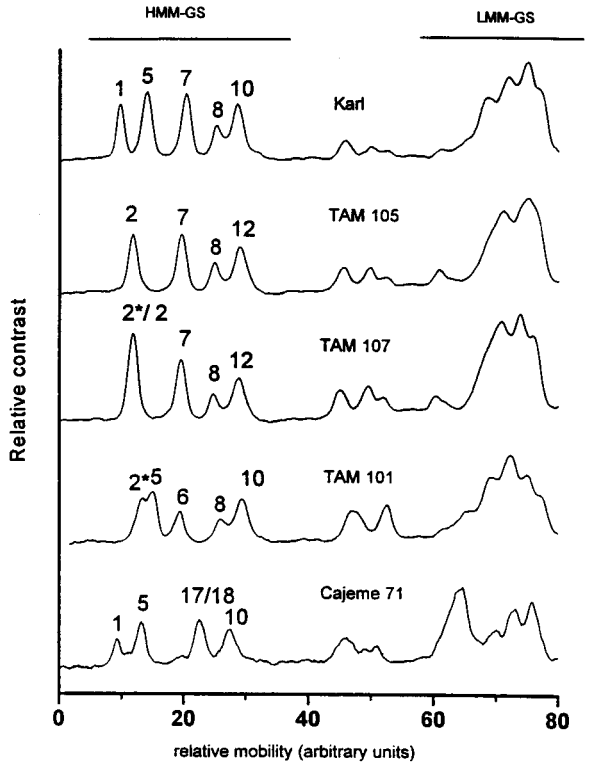

Figure 9. SDS−PAGE separations of glutenins from the wheat cultivars shown in Figure 8. Lane profiles were generated with Kodak Digital 1D software from the SDS−PAGE separations shown in (A). Profiles were generated for easier comparison to the SDS−CE separations shown in Figure 8 and were not intended to be used in a quantitative fashion.

different mobilities than those found on SDS−PAGE. Note that many of the differences found here between SDS−PAGE and the SDS−CE separations have been reported earlier by authors using the commercial polymer, ProSort (Werner et al., 1994; Weegels et al., 1995; Sutton and Bietz, 1997).

DISCUSSION

In part of a series of projects designed to find a rapid, high-resolution, reliable system for performing SDS−CE separations of wheat proteins, we compared four polymers that have been shown to effectively "sieve" proteins in the capillary format and have the ability to be used in uncoated capillaries. These four polymers included were a commercial reagent from Bio-Rad, dextran, PEO, and PAA. The main criteria used in evaluating how well the different polymer systems worked for the separation of wheat proteins was the ability of a given polymer to separate the HMM-GS within the test cultivar Karl (i.e., how well the polymer resolved the HMM-GS from each other). The resolution of the HMM-GS from the LMM-GS was also evaluated. These evaluations were done on a visual basis, i.e., were the HMM-GS resolved enough to be readily visible from each other and from the LMM-GS?

The commercial reagent from Bio-Rad contains a proprietary polymer to dynamically coat the inside surface of capillaries and reduce electroendosmotic flow (EOF) and protein-capillary interactions and can thus be used in uncoated capillaries (Zhang et al., 1996). Dextran is readily commercially available, fairly inexpensive, has good UV transmission properties, and has been shown to effectively "sieve" proteins (Ganzler et al., 1992). However, dextran binds poorly to silica (Gilges et al., 1994) and therefore does not provide EOF suppression and is typically used in coated capillaries (e.g. Ganzler et al., 1992). Recently a method was reported for using dextran in uncoated capillaries that were first deactivated by rinsing them with the Bio-Rad reagent (Zhang et al., 1996). Because of the high viscosity of the Bio-Rad reagent, larger diameter capillaries (i.e., 75 μm) had to be used. This was necessary to be able to fill the capillary with the reagent in a reasonable time frame. This was not the case when lower viscosity buffers such as those discussed below were used.

The third polymer tested, PEO, possesses good UV transparency properties, has relatively low viscosity (Ganzler et al., 1992; Guttman et al., 1993; Benedek et al., 1994), and has the benefit of binding to silica and suppressing EOF, thus coated capillaries do not have to be used (Iki and Yeung, 1996). However, PEO also binds SDS and should be considered as a charged polymer which may behave differently than other polymers that do not bind SDS (Takagi, 1997).

The final polymer, PAA, has been used as a crosslinked polymer in slab gels for many years (Shapiro et al., 1967) as well as being used as the first matrix for size-based separations in the capillary format (Wu and Regnier, 1992). Non-crosslinked PAA gels polymerized in situ (Cohen and Karger, 1987) and replaceable, fluid solutions of non-crosslinked PAA also effectively separated proteins in the capillary format (Widhalm and Schwer, 1991). However, because of the high UV absorbance of PAA (Ganzler et al., 1992), this sieving matrix has not been widely used with proteins. Purification of the polymer away from residual monomer and catalysts may somewhat reduce this problem (Chiari et al., 1992), but it still remains as a disadvantage when PAA is used as a sieving agent. Proteins were easily detected at 214 nm in this study, however.

Optimizing the separations in each polymer system produced interesting results. The commercial buffer from Bio-Rad and dextran both required the presence of ethylene glycol (or methanol) to provide baseline resolution of the HMM-GS. Adding ethylene glycol to the PAA buffer also improved resolution, but was not necessary for sufficient resolution of the HMM-GS. It was also interesting that, in these studies, the ethylene glycol was added to pre-existing buffers, which means that both the buffer concentration as well as the polymer concentration were effectively diluted, yet resolution was improved. The exact effect of ethylene glycol on the separation of wheat proteins in these types of separations is not yet known. Two ideas that have been suggested are the alteration of SDS-binding of the wheat proteins (W. Werner, personal communication), or alteration of the separation buffer viscosity (K. Sutton, personal communication). Experiments are in progress to try and determine the exact effect on wheat proteins.

Interestingly, addition of ethylene glycol to PEO reduced the resolution of HMM-GS separations. This was not the only difference noted between PEO and the other polymers. PEO produced substantially different separations of the wheat proteins than the other polymers, despite the fact that the marker proteins were well separated, for the most part, in all of the polymers. The exact reason for this is unknown, but could be related to the fact that PEO binds SDS, or could possibly indicate protein−polymer interactions. It was also interesting to note that the masses predicted for the

Table 3. Day-to-Day Repeatability of Migration Times and Peak Areas[a]

	migration time peak					corrected peak area[b] peak				
day	1	2	3	4	5	1	2	3	4	5
1	20.9	22.9	29.6	31.5	35.2	7461	7681	1612	4329	4610
2	20.7	22.8	29.5	31.3	35.1	7776	7135	1764	4112	4821
3	19.7	23.0	29.7	31.7	35.3	8378	7286	1637	4066	4835
4	19.8	23.1	29.8	31.7	35.4	7131	6214	1480	4077	4147
5	19.8	23.1	29.8	31.6	35.4	7747	6581	1630	4260	4173
av	20.2	23.0	29.7	31.5	35.3	7699	6979	1624	4085	4301
SD	0.57	0.11	0.13	0.15	0.13	460	582	101	118	338
RSD (%)	2.83	0.50	0.44	0.48	0.37	5.98	8.34	6.21	2.84	7.49

[a] Migration times and peaks areas measured from separations of glutenins isolated from the cultivar Karl. Separations were made on 5 different days over a 7 day period. Peaks selected for data analysis were the same as those used in Table 2. [b] Corrected peak area = peak area/migration time of peak (Altria, 1993).

wheat proteins in the PEO buffer were much different from the other polymers (see Table 1).

A comparison of the masses predicted for the various wheat proteins with each polymer produced interesting results (see Table 1). This is particularly interesting given that each polymer generally provided good resolution of the standard marker proteins, but varied somewhat in the separation of the wheat protein fractions. Each polymer predicted slightly different molecular masses and none matched the values typically accepted in the literature from SDS−PAGE, although the modified Bio-Rad reagent was the closest. For the HMM-GS, the values predicted from each of the polymers were much higher than the masses calculated from the cDNA. Note that SDS−PAGE also overestimates these values (see Table 1). This brings up two important conclusions, namely that molecular masses determined by these systems, including SDS−PAGE, should be used with caution. Also, the ability of a given polymer to separate all types of proteins is not enssured on the basis of its ability to separate standard marker proteins.

Note that some wheat proteins, in particular the HMM-GS, are known to have anomalous migration when separated by SDS−PAGE and thus may have abnormal migration in entangled polymer sieving systems. The exact reason for the anomalous migration on SDS−PAGE is not known, but several hypothesis have been proposed (Werner, 1995, and references therein). In some cases, proteins with lower molecular mass migrate slower than other HMM-GS of higher mass (Shewry et al., 1992). This problem can be corrected by including urea in SDS−PAGE gels (Shewry et al., 1992). The HMM-GS are also unusually resistant to denaturation by SDS and have been hypothesized to possess residual structure in the presence of SDS (Shewry et al., 1992). Whether these factors could influence the migration of the HMM-GS through the different polymers used in this study is not known.

Although each of the polymers used in this project produced acceptable results, we selected to further study the commercial reagent from Bio-Rad (modified with addition of ethylene glycol). This buffer was chosen primarily because of its commercial availability and ease of use. However, it has a high viscosity (somewhat reduced by the addition of 15% ethylene glycol) and may be difficult to use in some instruments, and requires larger bore capillaries for easy filling and emptying of the capillary. This buffer was found to have excellent repeatability, both of the migration times and of the peak areas (Table 2), something which is important if this technique is to be successfully used for quantitative studies. Day-to-day repeatability was somewhat higher than the run-to-run variability. Note that the run-to-run tests were conducted months apart and on separate capillaries with different lots of buffer, compared to the day-to-day tests, and both factors can influence the separation variability (Bietz and Lookhart, 1997). However, this also allows a comparison of the long-term repeatability because different capillaries and buffer lots will have to be used eventually in any laboratory. A comparison of Tables 2 and 3 clearly illustrates a shift in migration times from the first test to the second, most likely caused by the different capillary or buffer lot (Bietz and Lookhart, 1997). However, the day-to-day variability for samples analyzed under the same conditions was acceptable.

For long-term comparisons, a standard sample should be analyzed with every data set to enable normalization of the data (Sapirstein et al., 1989), to help reduce the variability caused by the use of different capillaries or buffer lots, both are conditions that will eventually occur in any laboratory analyzing large numbers of samples.

The modified Bio-Rad reagent produced good separations of all of the major storage protein classes. Resolution of the gliadins was lower than that obtainable with FZCE (Bean et al., 1998a), though it should be pointed out that gliadins are typically better resolved in acid A-PAGE, on the basis of differences in charge density, rather than in SDS−PAGE, where separations are based on size (Lookhart and Albers, 1988). Resolution was sufficient to distinguish the cultivars tested here on the basis of the gliadin patterns, however.

The modified Bio-Rad reagent also produced high-resolution separations of both the HMM-GS and the LMM-GS. A comparison of the electrophoregrams from several cultivars showed that the method also produced good separations of the more common allelic variants of HMM-GS found in U.S. wheats. It is also interesting to note that the migration order of the HMM-GS separated by CE differs from that found on the SDS−PAGE gels. This was also noted in earlier papers (Werner et al., 1994; Weegels et al., 1995; Sutton and Bietz, 1997) and was noted in a new, novel SDS−PAGE procedure (Kasarda et al., 1998). Several pairs of HMM-GS have reversed mobilities, for example HMM-GS 1 and 5, with 1 having a faster mobility than 5 on HPCE but slower on SDS−PAGE. This phenomenon also occurs with HMM-GS 8 and 10, HMM-GS 17,18, and 10, and HMM-GS 2* and 5. The reasons for this are not known at this time, but as mentioned earlier in this paper, the HMM-GS are known to have anomalous migrations on SDS−PAGE, and also to possess residual

structure and be incompletely denatured in the presence of SDS (Werner, 1995; Shewry et al., 1992). The addition of urea to SDS−PAGE corrects some of these problems (Shewry et al., 1992) and in fact, the migration order of some of the HMM-GS found in SDS−PAGE + urea was similar to that found in SDS−CE. Attempts to add 4 M urea to the Bio-Rad reagent/ethylene glycol mixture resulted in a complete loss of resolution when used in SDS−CE (data not shown). The reason for this is currently unknown.

CONCLUSIONS

Four different polymers were tested for their effectiveness in producing size-based separations of SDS−wheat protein complexes. Once optimized, all four polymers produced good separations of wheat proteins. As with earlier reports, the addition of organic solvents to the separation buffer improved the resolution of the HMM-GS, except when PEO was used as the polymer. The use of a commercial polymer from Bio-Rad modified by the addition of 15% ethylene glycol produced good separations of HMM-GS as well as total glutenin and gliadin protein fractions. Gliadins and glutenins from several different cultivars were separated and good resolution of most HMM-GS was found. Some allelic pairs could not be differentiated with this system, though the important 5 + 10 could be differentiated from 2 + 12. HPCE also provided high-resolution separations that could be easily quantified, something not easily achieved with SDS−PAGE.

The polymers selected for this study were used primarily because they could be used in uncoated or dynamically coated capillaries. Work is in progress to evaluate polymers used in coated capillaries. Future improvements in size-based separations of wheat proteins need to focus on reducing separation times, improving resolution (equivalent to that of gradient SDS−PAGE separations), gaining a better understanding of the effects of organic solvents on resolution, and continuing the screening of different polymers to find polymers with low viscosity and easy handling properties. The separation of unreduced polymeric proteins of wheat is also an area of size-based separations that needs to be developed. The separation of wheat proteins by size in the capillary format allows easy and rapid quantification of important proteins, e.g., HMM-GS. Future improvements in this technique should make it extremely useful to the field of cereal chemistry.

ACKNOWLEDGMENT

The authors would like to thank J. Bietz, Dr. M. Chiari, Dr. K. Sutton, and Dr. A. Bekkers for critical reading of this manuscript and helpful comments.

LITERATURE CITED

Altria, K. D. Essential peak area normalization for quantitative impurity content determination by capillary electrophoresis. *Chromatographia* **1993**, *35*, 177−182.

Bean, S. R.; Lookhart, G. L. Faster capillary electrophoresis separations of wheat proteins through modification to buffer composition and sample handling. *Electrophoresis* **1998**, *19*, 3190−3198.

Bean, S. R.; Bietz, J. A.; Lookhart, G. L. High-Performance Capillary Electrophoresis of Cereal Proteins. *J Chromatogr. A* **1998**, *814*, 25−41.

Bean, S. R.; Lyne, R. K.; Tilley, K. A.; Chung, O. K.; Lookhart, G. L. A rapid method for quantization of insoluble polymeric proteins in flour. *Cereal Chem*. **1998b**, *75*, 374−379.

Benedek, K.; Kalman, S.; Thiede, K. High-performance capillary electrophoresis of proteins using sodium dodecyl sulfate-poly(ethylene oxide). *J. Chromatogr. A* **1994**, *676*, 209−217.

Bietz, J. A.; Wall, J. S. Wheat gluten subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Cereal Chem*. **1972**, *49*, 416−430.

Bietz, J. A.; Lookhart, G. L. Wheat varietal indentification by capillary electrophoresis: an inter-laboratory comparison of methods. *Lebensm.-Wiss u-Technol*. **1997**, *30*, 210−213.

Bode, H.-J. The use of liquid polyacrylamide in electrophoresis. II. Relationship between gel viscosity and molecular sieving. *Anal. Biochem*. **1977**, *83*, 364−371.

Chiari, M.; Nesi, M.; Fazio, M.; Righetti, P. G. Capillary electrophoresis of macromolecules in "syrupy" solutions: facts and misfacts. *Electrophoresis* **1992**, *13*, 690−697.

Chrambach, A. Quantitative and automated electrophoresis in sieving media. *Electrophoresis* **1996**, *17*, 454−464.

Cohen, A. S.; Karger, B. L. High-performance sodium dodecyl sulfate polyacrylamide gel capillary electrophoresis of peptides and proteins. *J Chromatogr*. **1987**, *26* (397), 409−417.

Effenhauser, C. S.; Bruin, G. J. M.; Paulus, A. Integrated chip-based capillary electrophoresis. *Electrophoresis* **1997**, *18*, 2203−2213.

Ganzler, K.; Greve, K. S.; Cohen, A. S.; Karger, B. L. High-performance capillary electrophoresis of SDS-protein complexes using UV-transparent polymer networks. *Anal. Chem*. **1992**, *64*, 2665−2671.

Gilges, M.; Kleemis, M. H.; Schomburg, G. Capillary zone electrophoresis separations of basic and acidic proteins using poly(vinyl alcohol) coatings in fused silica capillaries. *Anal. Chem*. **1994**, *66*, 2038−2046.

Goetzinger, W.; Kotler, L.; Carrilho, E.; Ruiz-Martinez, M. C.; Salas-Solano, O.; Karger, B. L. Characterization of high molecular mass linear polyacrylamide powder prepared by emulsion polymerization as a replaceable polymer matrix for DNA sequencing by capillary electrophoresis. *Electrophoresis* **1998**, *19*, 242−248.

Graybosch, R. A.; Morris, R. An improved SDS−PAGE method for the analysis of wheat endosperm storage proteins. *J. Cereal Sci.* **1990**, *11*, 201−212.

Guttman, A. Capillary sodium dodecyl sulfate-gel electrophoresis of proteins. *Electrophoresis* **1996**, *17*, 1333−1341.

Guttman, A.; Horvath, J.; Cooke, N. Influence of Temperature on the Sieving Effect of Different Polymer Matrices in Capillary SDS Gel Electrophoresis of Proteins *Anal. Chem*. **1993**, *65*, 199−203.

Heller, C. Capillary electrophoresis of proteins and nucleic acids in gels and entangled polymer solutions. *J. Chromatogr. A.* **1995**, *698*, 19−31.

Hjerten, S.; Valtcheva, L.; Elenbring, K.; Liao, J. L.; Fast, high-resolution (capillary) electrophoresis in buffers designed for high field strengths. *Electrophoresis* **1995**, *16*, 584−594.

Hou, G.; Ng, P. K. W. Quantification of glutenin subunits by sequential acetone precipitation and by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) coupled with densitometry using a known quantity of glutenins as a standard. *Cereal Chem*. **1995**, *72*, 545−551.

Huang, D. Y.; Khan, K. A modified SDS−PAGE procedure to separate high molecular weight glutenin subunits 2 and 2*. *J. Cereal Sci*. **1998**, *27*, 237−239.

Huang, X. C.; Quesada, M. A.; Mathies, R. A. DNA sequencing using capillary arrays. *Anal. Chem*. **1992**, *64*, 2149−2154.

Iki, N.; Yeung, E. S. Nonbonded poly(ethylene oxide) polymer-coated column for protein separation by capillary electrophoresis. *J. Chromatogr. A* **1996**, *731*, 273−282.

Kasarda, D. D.; Woodard, K. M.; Adalsteins, A. E. Resolution of High Molecular Weight Glutenin Subunits by a New SDS−PAGE System Incorporating a Neutral pH Buffer. *Cereal Chem*. **1998**, *75*, 70−71.

Kenndler, E.; Poppe, H. Entangled, noncross-linked polymer solutions used as physical network in capillary zone electrophoresis. *J. Capillary Electrophor*. **1994**, *1*, 144−157.

Khelifi, D.; Branlard, G. A new two-step electrophoresis method for analysing gliadin polypeptides and high and low molecular weight subunits of glutenin of wheat. *J. Cereal Sci.* **1991**, *13*, 41−47.

Larroque, O. R.; Gianibelli, M. C.; Batey, I. L.; MacRitchie, F. Electrophoretic characterisation of fractions collected from gluten protein extracts subjected to size-exclusion high-performance liquid chromatography. *Electrophoresis* **1997**, *18*, 1064−1067.

Lookhart, G. L.; Albers, L. D. Correlations between reversed-phase high-performance liquid chromatography and acid- and sodium dodecyl sulfate-polyacrylamide gel electrophoretic data on prolamins from wheat sister lines differing widely in baking quality. *Cereal Chem.* **1988**, *65*, 222−227.

Parris, N.; Dickey, L.; Craig, J. Quantitative analysis of corn zeins by capillary electrophoresis. *Cereal Chem.* **1997**, *74*, 766−770.

Payne, P. I.; Corfield, K. G. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. *Planta* **1979**, *145*, 83−88.

Payne, P. I.; Lawrence, G. J. Catalogue of alleles for the complex gene loci, Glu-Al, Glu-Bl, Glu-Dl which code for high molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.* **1983**, *11*, 29−35.

Pollard, N. J.; Wrigley, C. W.; Bekes, F.; MacRitchie, F. Effective identification of grain-legume species and varieties; a comparison of five methods of analysing grain-protein composition. *Plant Varieties Seeds* **1998**, *11*, 187−200.

Reijenga, J. C.; Verheggen, T. P. E. M.; Martens, J. H. P. A.; Everaerts, F. M. Buffer capacity, ionic strength and heat dissipation in capillary electrophoresis. *J. Chromatogr. A.* **1996**, *744*, 147−153.

Righetti, P. G.; Gelfi, C.; Perego, M.; Stoyanov, A. V.; Bossi, A. Capillary zone electrophoresis of oligonucleotides and peptides in isoelectric buffers: theory and methodology. *Electrophoresis* **1997**, *18*, 2145−2153.

Ruiz-Martinez, M. C.; Berka, J.; Belenki, A.; Foret, F.; Miller, A.; Karger, B. L. DNA sequencing by capillary electrophoresis with replaceable linear polyacrylamide and laser-induced fluorescence detection. *Anal. Chem.* **1993**, *65*, 2851−2858.

Sapirstein, H. D.; Scanlon, M. G.; Bushuk, W. Normalization of high-performance liquid chromatography peak retention times for computerized comparison of wheat prolamin chromatograms. *J. Chromatogr.* **1989**, *469*, 127−135.

Scholz, E, Pogany, G, Ganzler, K, Laurie, G. W. Characterization of a basement membrane protein (BM180) using capillary electrophoresis. *J Capillary Electrophor.* **1997**, *4*, 287−292

Shapiro, A. L.; Vinuela, E.; Maizel, J. V. *Biochem. Biophys. Res. Commun.* **1967**, *28*, 815.

Shewry, P. R.; Halford, N. G.; Tatham, A. S. High Molecular Weight Subunits of Wheat Glutenin *J. Cereal Sci.* **1992**, *15*, 105−120.

Sutton, K. H.; J. A. Bietz. Variation among high molecular weight subunits of glutenin detected by capillary electrophoresis. *J. Cereal Sci.* **1997**, *25*, 9−16.

Takagi, T. Capillary electrophoresis in presence of sodium dodecyl sulfate and a sieving medium. *Electrophoresis* **1997**, *18*, 2239−2242.

Thomas, W. M.; Wang, W. W. In *Encyclopedia of Polymer Science and Engineering*; Kroschwitz, J. I., Ed.; Wiley: New York, 1985; Vol. 1, pp 169−211.

Weegels, P. L.; Hamer, R. J.; Schofield, J. D. RP-HPLC and capillary electrophoresis of subunits from glutenin isolated by SDS and Osborne fractionation. *J. Cereal Sci.* **1995**, *22*, 211−224.

Werner, W. Ferguson plot analysis of high molecular weight glutenin subunits by capillary electrophoresis. *Cereal Chem.* **1995**, *72*, 248−251.

Werner, W. E.; Demorest, D. M.; Stevens, J.; Wiktorowicz, J. E. Size-Dependent Separation of Proteins Denatured in SDS by Capillary Electrophoresis Using a Replaceable Sieving Matrix. *Anal. Biochem.* **1993**, *212*, 253−258.

Werner, W. E.; Wiktorowicz, J. E.; Kasarda, D. D. Wheat varietal identification by capillary electrophoresis of gliadins and high molecular weight glutenin subunits. *Cereal Chem.* **1994**, *71*, 397.

Widhalm, A.; Schwer, C. Capillary Zone Electrophoresis with a Linear, Non-Cross-Linked Polyacrylamide Gel Separation of Proteins According to Molecular Mass. *J. Chromatogr.* **1991**, *549*, 446−451.

Wu, D.; Regnier, F. E. Sodium dodecyl sulfate-capillary gel electrophoresis of proteins using non-cross-linked polyacrylamide. *J Chromatogr.* **1992**, *608*, 349−356.

Zhang, Y.; Lee, H. K.; Li, S. F. Y. Separation of myoglobin molecular mass markers using non-gel sieving capillary electrophoresis. *J. Chromatogr. A.* **1996**, *744*, 249−257.

Zhen, Z.; Mares, D. A simple extraction and one-step SDS−PAGE system for separating HMW and LMW glutenin subunits of wheat and high molecular weight proteins of rye. *J. Cereal Sci.* **1992**, *15*, 63−78.

Received for review April 27, 1999. Revised manuscript received July 8, 1999. Accepted July 19, 1999. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned. U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

JF990413N