Journal of Chromatography A, 652 (1993) 215–224
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 204

Analysis of a recombinant granulocyte macrophage colony stimulating factor dosage form by capillary electrophoresis, capillary isoelectric focusing and high-performance liquid chromatography

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ABSTRACT

The analysis of a recombinant granulocyte macrophage colony stimulating factor (GM-CSF) dosage form by free solution capillary electrophoresis (FSCE), capillary isoelectric focusing (cIEF), and high-performance liquid chromatography (HPLC) is described. The quantitative use of capillary electrophoresis, whether FSCE or cIEF, will always be prone to special problems, such as sensitivity to differences in salt concentrations between the standard and sample, and can not match the ruggedness of HPLC. The usable quantitative linear range for both HPLC and FSCE surpass that achieved for cIEF methods by a factor of 10 or greater. The FSCE system, utilizing an octyl bonded/Brij-35 coated capillary, did not work for all proteins examined. This is probably due to an interaction of the protein with the bonded phase or the adsorbed Brij-35. In contrast, the cIEF method worked well for all proteins tested thus far, yielding high efficiency and resolution comparable to slab gel isoelectric focusing. This paper addresses the potential for using free solution capillary electrophoresis and capillary isoelectric focusing as a quantitative analytical tool. Also, the effect of salt in the dosage form on quantitation, reproducibility, and efficiency of capillary electrophoresis methods is also discussed.

INTRODUCTION

The use of capillary electrophoresis (CE) for the analysis of pharmaceutical products has not yet reached a level where it is considered routine. There are many examples or application notes provided by instrument companies where CE can potentially be used, but these examples fail to discuss the quantitative aspect in the methods development process. The reasons for this are varied, but the detailed methods development and validation process is a time consuming event. The development and validation of quantitative methods is critical if CE is to be truly used, on a daily basis, for the analysis of pharmaceutical products. Analysis of a pharmaceutical dosage form has been reported by Guzman *et al.* [1] and other applications in the pharmaceutical industry have been reported by Hurni and Miller [2] and Compton [3]. This paper discusses the analytical use of free solution capillary electrophoresis (FSCE) and capillary isoelectric focusing (cIEF) in comparison with HPLC for the analysis of granulocyte macrophage colony stimulating factor (GM-CSF), a recombinant protein, in a dosage form.

The potential for using FSCE and/or cIEF as a quantitative analytical tool is now emerging as more applications are being developed. Protein separations in FSCE have been reported using both acidic and basic extremes of the pH range in uncoated capillaries [4,5]; however, undesirable problems sometimes occur. For certain proteins, the use of a pH 2.5 separation buffer causes protein aggregation, and therefore the

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0021-9673/93/$06.00

method is not an accurate representation of the protein composition in the dosage from. Generally, proteins should be separated and quantitated within one pH unit of physiological pH. However, to prevent protein adsorption on the capillary walls, the capillary surface must be chemically modified. Numerous buffer modifiers and wall chemistries have been described, such as ethylene glycol [6], a hydrophilic bonded surface [7], a polyethylene glycol (PEG) modified surface [8] as well as PEG as a protein modifier [9], carbohydrate [10] and polyethyleneimine [11] modified surfaces. Also, buffers containing high concentrations of zwitterionic salts [12] have been used. Success with GM-CSF was achieved using a coated/bonded capillary system developed by Towns and Regnier [13,14].

Capillary isoelectric focusing (cIEF) developed by Hjertén and co-workers [15–18] employed an acrylamide bonded capillary to reduce the electroosmotic flow. The application of cIEF to recombinant tissue plasminogen activator glycoforms [19] and other proteins [20,21] has been described. However, these cIEF methods consisted of a two-step process. The first was the focusing of the proteins in the capillary and the second required a change of either the cathode or anode buffers with a salt solution to mobilize the proteins past the detection window. Improvements and optimization of the cIEF separation parameters have included the use of tetramethylethylenediamine (TEMED) to adjust the pH range as described by Yao-Jun and Bishop [22], alternate mobilization agents, and the addition of non-ionic surfactants such as reduced Triton X-100 to minimize protein precipitation as described by Zhu *et al.* [23]. These improvements still required a salt mobilization of the focused bands for detection. This mobilization step and a lack of stable capillary wall chemistries have made cIEF unattractive for routine testing. Wu and Pawliszyn [24–26] discussed cIEF improvements for protein detection using a concentration gradient detector and imaging system, while Wang and Hartwick [27] used whole column absorbance detection.

Recently, cIEF bonded/coated chemistries developed by Mazzeo and Krull [28–35], without the need for salt mobilization of the focused protein bands past the detection window, have taken cIEF from a basic investigational tool to a readily automated method to study proteins. This procedure is successful for the analysis of GM-CSF, interleukin-3, and other recombinant proteins studied at Sandoz Research Institute. The cIEF method can be used to quantitative impurities and to study deamidation of proteins. In all cases, the stability of the capillary bonded/coated chemistries directly effect the reproducibility of the final analytical method.

If CE and cIEF methods are to succeed in the pharmaceutical industry, several issues must be addressed. These issues include reproducibility of migration time, peak area and peak height, within a capillary and from capillary to capillary. Also, parameters of ruggedness and capillary longevity must be fully evaluated. This paper addresses these issues in addition to others, such as ease of use and shorter analysis times with respect took both CE, other cIEF analytical methods and an existing HPLC method used for the analysis of GM-CSF.

EXPERIMENTAL

The octyl bonded capillaries were purchased from Supelco (Bellefonte, PA). The Pharmalyte 3-10 was purchased from Pharmacia LKB Biotechnology (Piscataway, NJ, USA). The Brij-35 was purchased from Aldrich (Milwaukee, WI, USA). The hydroxypropylmethylcellulose (HPMC), 4000 cP, as well as the sodium phosphate monobasic and sodium phosphate dibasic, were purchased from Sigma (St. Louis, MO, USA). The TEMED was purchased from Bio-Rad (Hercules, CA, USA); trifluoracetic acid (sequencing grade) was purchased from Pierce (Rockford, IL, USA). The capillary electrophoresis experiments described were carried out on the Beckman P/ACE 2050 (Fullerton, CA, USA). All data were collected and processed using Waters ExpertEase software (Milford, MA, USA).

The FSCE of GM-CSF was accomplished using a slightly modified version of the method described by Towns and Regnier [13]. A Beckman capillary cartridge was fitted with C_8

bonded capillary (the CElect H150 from Supelco) which had a total length of 37 cm (30 cm to the detector) and an internal diameter of 50 μm. The capillary was rinsed with a 0.5% (w/v) solution of Brij-35 for 2 h, then rinsed for 20 min the running buffer. The running buffer was 50 m*M* sodium phosphate (pH 6.8)–0.05% Brij-35. The effective field strength was 400 V/cm (14.8 kV overall). The polarity was negative. Detection was carried out at 200 nm. The capillary cartridge was maintained at a temperature of 23°C. The capillary was rinsed with running buffer for 2 min between each injection. The GM-CSF lyophilizate (0.4 mg/vial) was reconstructed with 1.0 ml sterile water-for-injection (WFI) and injected directly onto the capillary for 4 s under positive pressure. The total analysis time was 20 min.

The cIEF was accomplished using a method described by Mazzeo and Krull [28]. For the cIEF analysis, the Beckman cartridge was once again fitted with a CElect H150 capillary with a total length of 47 cm (40 cm to the detector) and an internal diameter of 50 μm. The volume of the capillary was 923 nl and the mass of GM-CSF loaded was 23 ng. The capillary was rinsed for 1 h with 0.5% (w/v) HPMC followed by water for 5 min. The GM-CSF lyophilizate was reconstituted as described above and diluted 1:1 with a 2× concentrate of the cIEF-ampholyte mixture which consisted of 970 μl of deionized water, 800 μl of 1% HPMC, 200 μl of Pharmalyte 3–10, and 30 μl of TEMED. The final running concentration of each of the components of the cIEF-ampholyte mixture was 0.2% HPMC, 2% Pharmalyte 3–10, and 0.75% TEMED. The capillary was rinsed for 4 min with 10 m*M* phosphoric acid and then filled with the sample preparation for 2 min using the high-pressure rinse capability of the Beckman P/ACE. The running field strength was 300 V/cm (14.1 kV overall) and the polarity again was negative. Detection was carried out at 280 nm; the capillary cartridge was maintained at a temperature of 23°C. The catholyte, 20 m*M* NaOH, was placed at the inlet and the anolyte, 10 m*M* phosphoric acid, was placed at the outlet. The total analysis time was 14 min.

The analysis of GM-CSF by reversed-phase HPLC was carried out on a Nucleosil C-4 column (Machery–Nagel) with a pore size of 300 Å and a particle size of 5 μm: The column dimensions were 10 cm × 4.6 mm I.D. Mobile phase A consisted of 0.1% (v/v) trifluoroacetic acid (TFA) in distilled-deionized water while mobile phase B consisted of 0.1% (v/v) TFA in acetonitrile–water (90:10, v/v). The protein was eluted from the column using a linear gradient from 38 to 58% B in 20 min; the flow-rate was 1.2 ml/min. The detector wavelength was 214 nm and the injection volume was 50 μl, corresponding to 20 μg of GM-CSF.

The UV spectrophotometric analysis was performed on a Perkin-Elmer Lambda 2 UV–Vis spectrophotometer. The GM-CSF was analyzed first at 280 nm at concentrations of 15.94, 7.97, 3.98 and 1.99 mg/ml using a 0.1 cm quartz cell. The absorptivity at 280 nm was found to be 1.15 ml/cm mg. The GM-CSF was then analyzed at 210 nm at concentrations of 1.594, 0.797, 0.398 and 0.199 mg/ml using the same quartz cell. The absorptivity at 210 nm was found to be 18.52 ml/cm mg.

RESULTS AND DISCUSSION

Free solution capillary electrophoresis

The free solution approach using the octyl bonded/Brij 35 coated phase shows good peak symmetry and an efficiency of 51 000 theoretical plates as shown in Fig. 1. The electropherogram shows adequate resolution between the GM-CSF and the human serum albumin (HSA). The HSA peak exhibits three major components with minor shoulders on the first and last components, also as shown in Fig. 1.

Both electrokinetic and pressure injection processes were examined. As shown in Table I, the assay results were found to be 16.3 and 9.3% of the labeled value for the 0.7 and the 0.4 mg/vial dosage forms, respectively, when employing the electrokinetic injection process. The assay results utilizing the pressure injection technique were 99.9 and 94.5% for the 0.7 and the 0.4 mg/vial, respectively. The reference standard, supplied in liquid form, was diluted in sterile WFI, while the GM-CSF lyophilizates were reconstituted with 1.0 ml of sterile WFI.

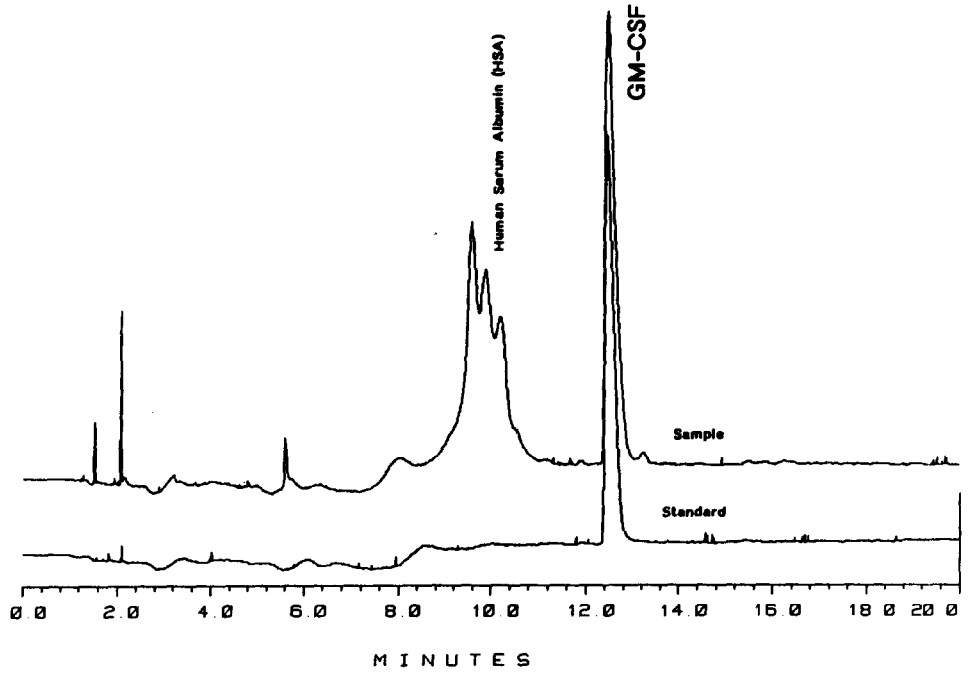

Fig. 1. Electropherogram of a GM-CSF standard and a GM-CSF sample with human serum albumin (HSA) by FSCE. Conditions: capillary, CElect H150, 37 cm × 50 μm I.D.; buffer, 50 m*M* NaH_2PO_4 (pH 7)/0.05% Brij 35; electric field strength, 400 V/cm (reverse polarity); detection, 200 nm; temperature, 23°C; injection 4 s pressure.

TABLE I

RESULTS FOR FSCE OF GM-CSF BY ELECTROKINETIC AND HYDROSTATIC INJECTION TECHNIQUES

Assay percent of two dosage forms using both electrokinetic and pressure injection.

Dosage form	% of Label	
	Electrokinetic injection	Hydrostatic injection
0.7 mg/vial	18.9	99.9
	15.1	99.4
	14.9	100.5
Mean	16.3	99.9
R.S.D. (%)	13.8	0.6
0.4 mg/vial	8.9	94.9
	9.4	94.0
	9.7	94.7
Mean	9.3	94.5
R.S.D. (%)	4.3	0.5

However, the GM-CSF lyophilizate contains a high salt concentration when reconstituted.

The effect of salt concentration of the sample on quantitation was examined for both the electrokinetic and pressure injection techniques, as shown in Fig. 2. These results indicate that the difference in salt concentration between the standard and the sample is critical for accurate

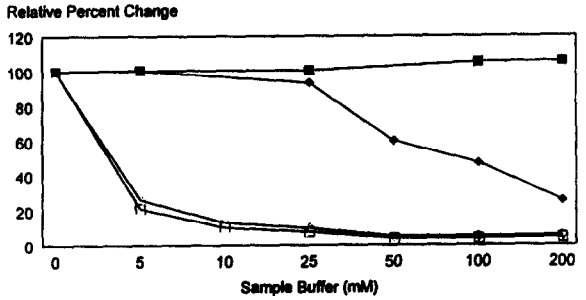

Fig. 2. The effect of salt concentration of peak area and peak height for both electrokinetic and pressure injections. □ = Electrokinetic injection, peak area; ■ = pressure injection, peak area; ◇ = electrokinetic injection, peak height; ◆ = pressure injection, peak height.

quantitation when employing the electrokinetic injection process. When the pressure injection technique was employed, these differences in salt concentration did not appear to effect peak area. The lack of change in peak area above 25 m*M* salt concentration was the result of severe peak broadening as shown in Fig. 3. In contrast, the peak heights decreased quickly as the salt concentration of the sample or standard approached that of the separation buffer. For these reasons, the ruggedness of the pressure injection technique is preferred. To maintain good efficiency, the salt concentration of both the sample and standard should be no greater than half that of the separation buffer.

Initially, the method was developed using a 75 μm I.D. capillary. The GM-CSF dosage form contains a high salt concentration when reconstituted. To achieve a stacking effect during the injection process, the sample must be of a lower ionic strength than that of the separation buffer. Therefore, the only way to achieve a stacking effect would be to increase the ionic strength of the running buffer. This approach was not possible due to the high current observed in a 75 μm I.D. capillary. Decreasing the capillary I.D. to 50 μm allowed the use of higher ionic strength separation buffers, 50 *versus* 10 m*M* phosphate buffer, in order to achieve stacking or injection plug compression. Linearity was observed from 1.0 mg/ml to 30 μg/ml with a correlation coefficient of 0.997, encompassing all dosage form strengths.

Capillary isoelectric focusing

The work of Mazzeo and Krull [28] demonstrated that a salt mobilization step was not necessary because a reduced electroosmotic flow exists allowing the focused protein bands to migrate past the detector window. Both the focusing and mobilization steps occur simultaneously, but the focusing step occurs more rapidly than mobilization. Mazzeo and Krull [28] first showed this work using a 60 cm unbonded capillary with 40 cm from the inlet to the detector and 20 cm from the detector to the outlet. The inlet reservoir contained the anolyte (10 m*M* phosphoric acid), the outlet contained the catholyte (20 m*M* sodium hydroxide), and forward polarity was employed. Adjusting the amount of TEMED in the sample allows all proteins to focus in the 40 cm capillary section before the detector and to drift towards the outlet with the electroosmotic flow past the detector window. This resulted in analysis times of 17 to 24 min.

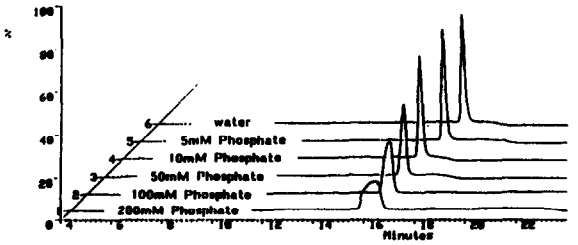

Fig. 3. The effect of salt on peak shape in FSCE.

Mazzeo and Krull [28] improved this method by employing reverse polarity, thus switching the anodic and cathodic reservoirs, using an octyl bonded capillary, and adjusting the amount of TEMED. Using this configuration, the TEMED now blocks the 40 cm from the inlet to just past the detector window. The proteins focus in the 20 cm past the detector window and the electroosmotic flow moves in the reverse direction towards the inlet. The proteins then drift past the detector window resulting in reduced analysis times of 4 to 6 min.

The improved cIEF configuration described above was used, with the addition of an octyl bonded/HPMC coated capillary, to quantitate the GM-CSF dosage forms as shown in the electropherogram in Fig. 4. Employing reverse polarity and adjusting the TEMED concentration, allowed over 40 cm (from the inlet to just past the detector) of a 47 cm capillary to be blocked, as schematically shown in Figs. 5 and 6. The pH gradient from 3 to 10 was located in the 7 cm from the detector to the outlet. The GM-CSF focused in a section of the 7 cm of capillary from the detector window to the outlet. The reduced electroosmotic flow in the octyl bonded capillary allowed the focused proteins, GM-CSF and HSA, to drift back towards the inlet reservoir past the detector window. The shortest capillary length obtainable from the detector to the outlet is a function of the manufacturer's instrument design. It appears that a short capil-

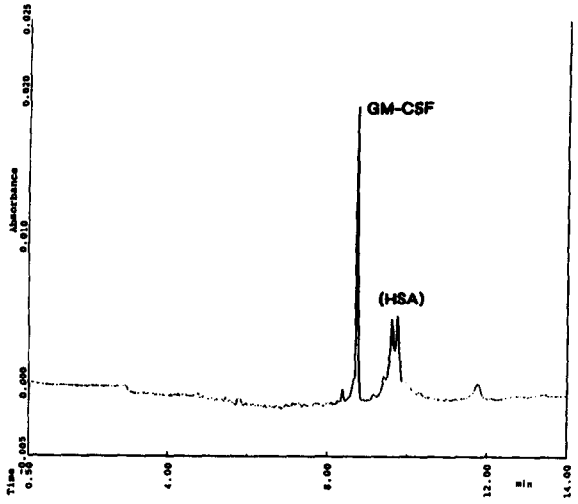

Fig. 4. A cIEF electropherogram of GM-CSF dosage form.

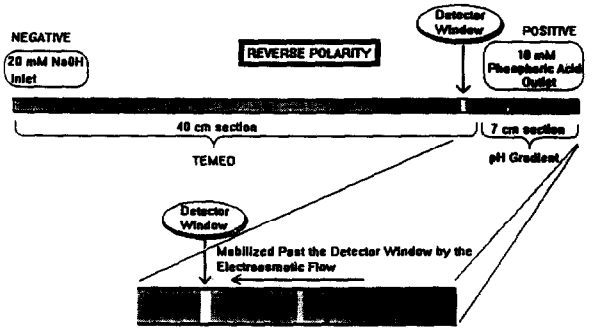

Fig. 5. A schematic of the capillary isoelectric focusing process showing position of focused bands on the outlet side of the detector.

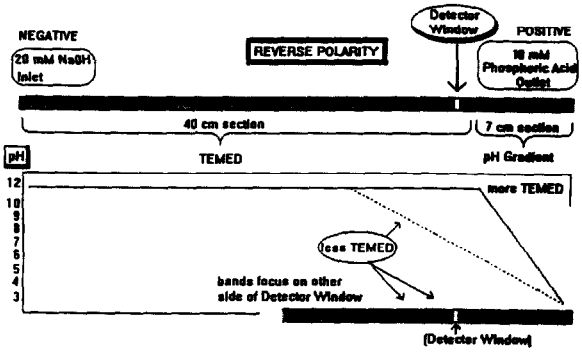

Fig. 6. A schematic of the capillary isoelectric focusing process showing the adjustment of TEMED on the location of the pH gradient and the problem of focused bands on the opposite side of the detector when too little TEMED is used.

lary section from the detector to the outlet (*i.e.* 7 *versus* 20 cm) is advantageous since this results in a shorter analysis time for a given electric field strength and should expose the protein to less capillary wall during the mobilization process resulting in a minimization of protein-wall interactions. The width of a focused band derived by Svensson [36] is given by the following equation:

$$x_i = \pm\left[\frac{D}{E(-\mathrm{d}\mu/\mathrm{d}(\mathrm{pH}))(\mathrm{d}(\mathrm{pH})/\mathrm{d}x)}\right]^{1/2} \quad (1)$$

where x_i is the width of the focused band, D is the diffusion coefficient, E the electric field strength, $\mathrm{d}\mu/\mathrm{d}(\mathrm{pH})$ the rate of change of mobility with pH, and $\mathrm{d}(\mathrm{pH})/\mathrm{d}x$ is the pH gradient. The experimental parameters which determine the final width of a focused protein band are the range of ampholytes, the amount of TEMED, and the applied electric field gradient. Since the pH gradient is forced into a smaller section of the capillary (*i.e.* 7 *versus* 20 cm), the protein focuses in a narrower band which results in lower detection limits. However, since a higher concentration of the protein is achieved, precipitation may be observed earlier than if this section of capillary was longer.

The HSA contained in the dosage form exhibited 5 to 6 major components which were easily separated from the GM-CSF as shown in Fig. 4. The linear range for GM-CSF was observed from 10 to 40 μg/ml using a 47 cm capillary (overall length). GM-CSF concentrations from 60 to 250 μg/ml showed anomalous peaks which may represent precipitation [23] of the GM-CSF in the capillary. Concentrations above 250 μg/ml showed no peaks at all and again may represent protein precipitation. Another recombinant protein, interleukin-3, exhibited a linear range from 10 to 250 μg/ml without protein precipitation. The range of protein concentrations which may be used in cIEF vary from protein to protein and must therefore be optimized.

The capillary length represents an injection loop similar to HPLC. Since the overall length of the capillary represents the injection volume, shortening the capillary reduces the mass of protein loaded. Above a sample concentration of

40 μg/ml for GM-CSF, additional peaks were observed due to protein precipitation [23]. Precipitation occurs when a certain mass is exceeded for a given length of capillary as depicted by Fig. 7. The linear dynamic concentration range can be extended by shortening the length of the capillary. Shortening the capillary length to 27 cm (overall) allowed a higher concentration of sample, 50 to 100 μg/ml (less sample dilution) to be loaded while maintaining the same protein mass in the capillary, as shown in Fig. 8. Since the GM-CSF dosage form contains a large amount of salt, a higher concentration of the protein results in higher salt concentrations which effects migration time and efficiency. Therefore, a 47 cm capillary length is preferred for the GM-CSF method.

Detection in cIEF is performed at 280 nm due to the absorbance of the ampholytes below a wavelength of 235 nm. The absorptivity of GM-CSF at 280 nm is 16 times less than at 210 nm. To obtain a quantitative detection signal at 280 nm, the concentration of the focused protein bands must be much greater than the sample concentration which was loaded into the capillary. The focusing process results in a large increase in the sample band concentration. In FSCE analysis of GM-CSF, detection is performed at 200 nm. A comparison of the efficiency and band width was made. The efficiency for GM-CSF in cIEF is 270 000 theoretical plates while that achieved in FSCE is 51 000. The spatial width of the sample band, w_s, with units of length, in FSCE is calculated using the following equation as discussed by Huang *et al.* [37]:

$$w_s = \left(\frac{l_d}{t_m}\right) w_t - w_d \quad (2)$$

where l_d is the length of the capillary to the detector, in mm; w_t is the temporal peak width, in s, measured at the baseline; t_m is the migration time, in s; and w_d is the width of the detector window. The GM-CSF peak width in FSCE is 12 mm. The efficiency in cIEF for GM-CSF is approximately 5 times that observed in FSCE, the peak width in cIEF is assumed to be 1/5 that observed in FSCE. Therefore, the width of the band in cIEF is approximated to be 2 mm which corresponds to 4 nl. Since the capillary length represents an injection loop, filling the capillary with GM-CSF at a concentration of 25 μg/ml, yields 23 ng of protein which is loaded and focused. The protein concentration of the focused band is on the order of 5.8 mg/ml. Detection at 280 nm in FSCE is not possible for GM-CSF at 25 μg/ml due to the small injection volume typical in FSCE necessary to maintain good peak efficiency.

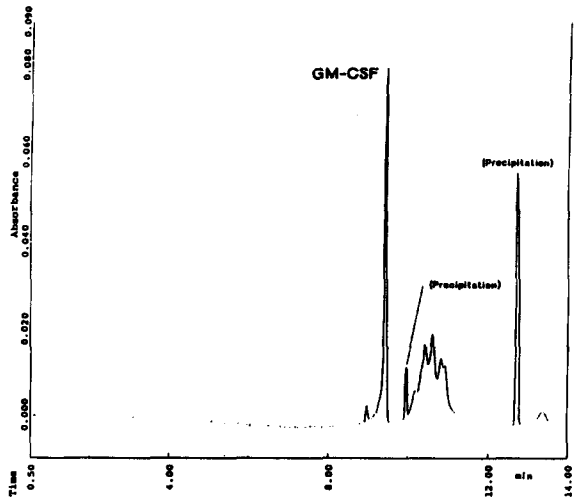

Fig. 7. A cIEF electropherogram of GM-CSF dosage form above the quantitative linear range showing precipitation peaks.

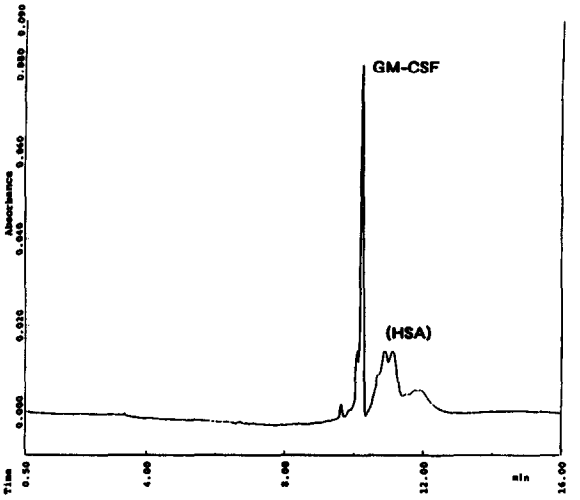

Fig. 8. A cIEF electropherogram of GM-CSF dosage form using a 27 cm capillary.

An additional peak, shown in Fig. 4, always eluting last in every cIEF electropherogram, may represent a small portion of the sample at the outlet of the capillary in contact with the anodic solution. This portion of the sample is not accessible during the focusing step and is eluted with the electroosmotic flow. This peak is used as a marker to indicate the end of the electropherogram and to calculate the electroosmotic flow. Using equation 1 yields a spatial width w_s of 1.5 mm, which corresponds to 3 nl. Using this approach the concentration of the focused band is 7.7 mg/ml. A third approach to estimate the concentration of the focused band is to measure the absorptivity. The absorptivity of GM-CSF was determined to be 1.15 ml/cm mg using a Perkin-Elmer Lambda-2 UV–Vis spectrophotometer. The GM-CSF peak in Fig. 4 shows an absorbance of 0.02 AU. Using Beer's law $\{A = abc$, where A is the absorbance, b is the optical pathlength (cm), a is the molar absorptivity [ml/(cm · mg)], and c is the sample concentration (mg/ml)}, and an optical pathlength of 50 μm, and solving for c yields a concentration for the focused band of 3.5 mg/ml.

Due to the relatively low absorptivity of proteins at 280 nm, detection can be achieved in cIEF at 280 nm because of the high concentration of the focused bands. When GM-CSF at 50 μg/ml is injected into a 47 cm capillary, the focused band has a concentration of 11.4 mg/ml. Above these concentrations, it is understandable that protein precipitation occurs, particularly due to the fact that the proteins are at their pI, the point of least solubility. The cIEF method concentrates the original sample of 25 μg/ml to 5.7 mg/ml, which is 228 times the original sample concentration.

The amount of TEMED used to extend the pH range, as described by Yao-Jun and Bishop [22], must also be considered, as well as the range of ampholytes and the concentration of HPMC in the development of the method. Migration time variability from capillary to capillary was observed and is caused by differences in electroosmotic flow. This may represent different degrees of capillary wall modification achieved by the manufacturer. Improvements of bonding procedures and/or the use of different mobile phase additives may reduce this variability. The purity of ampholytes influenced the resolution and resulted in the appearance of additional peaks.

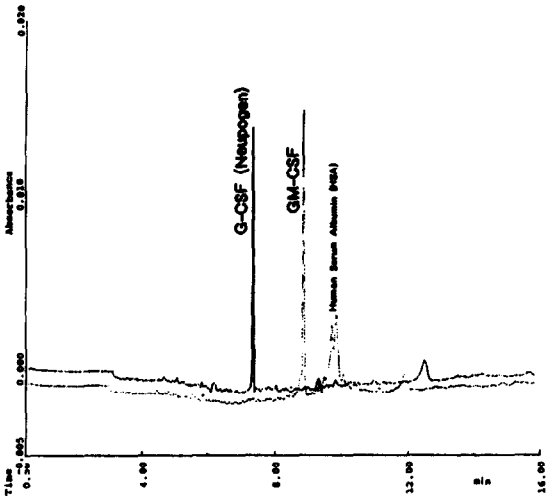

Fig. 9. A cIEF electropherogram of GM-CSF and G-CSF (Neupogen) showing different pI values as a method to distinguish product identification.

Although cIEF is being used to check GM-CSF concentration of dosage forms, it also offers the opportunity to distinguish different recombinant proteins of different pI values. Quick identifications can be accomplished as shown in Fig. 9 which distinguishes recombinant granulocyte colony stimulating factor (G-CSF), trade name Neupogen (Amgen) from GM-CSF. Assignment of pI to a protein for identification or estimation of pI values for degradation products can be achieved much more rapidly using cIEF than conventional slab gel isoelectric focusing as

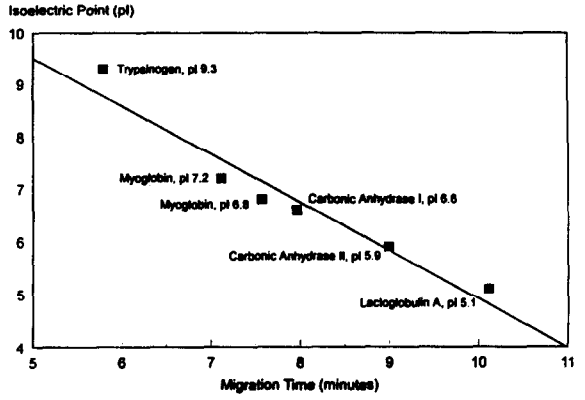

Fig. 10. Standard protein calibration curve showing migration time *versus* pI.

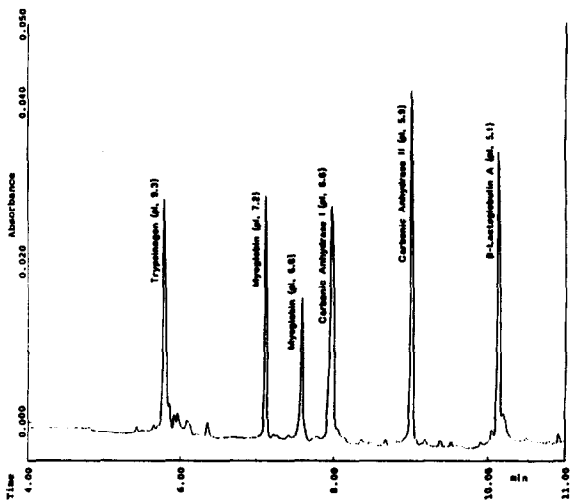

Fig. 11. Electropherogram of a protein standard mixture.

previously mentioned [28]. A reference standard curve is shown in Fig. 10 and the corresponding electropherogram in Fig. 11.

The cIEF methods have problems which again are protein dependent. It was observed that after analyzing GM-CSF, other protein standards did not separate in the same capillary as they had previously; the migration times were longer and the efficiency was substantially less. GM-CSF may be adsorbing to the capillary wall, changing the wall characteristics. Designating individual capillaries for a particular method or protein may prevent this problem.

Method comparison

A comparison of the quantitative results for HPLC, FSCE and cIEF is shown in Table II. The results are within acceptable ranges for all methods. The use of FSCE and cIEF methods for the testing of different dosage forms shows promise and offers to reduce analysis times and consumption of solvents. Method developments in FSCE and cIEF are prone to special problems such as sensitivity to salt differences between the standard and sample, or loss of separation due to high salt concentration in the sample. In general, HPLC is a more rugged technique and less prone to these difficulties. However, once these difficulties are overcome, the routine operation of a CE method appears to offer several advantages. These include the elimination of the problems associated with HPLC pump check values and plunger seal leaks, reduction in solvent consumption, reduced analysis times, and ease of use for daily operation.

TABLE II

COMPARISON OF ANALYTICAL RESULTS FOR FSCE, cIEF AND HPLC FOR GM-CSF DOSAGE FORMS

Sample	RP-HPLC	FSCE	cIEF
1-SFG-303	98.45	105.37	102.44
0-SFG-303	98.73	99.80	104.32
0-SFG-304	104.63	102.22	105.21

CONCLUSIONS

The usable quantitative linear range for both HPLC and FSCE surpass that achieved for cIEF methods by a factor of 10 or greater. The quantitative use of capillary electrophoresis, whether FSCE or cIEF, will always be prone to special problems, such as sensitivity to differences in salt concentrations between the standard and sample, and can not match the ruggedness of HPLC. Continued development of capillary wall chemical modifications in conjunction with solution additives continues to be a major avenue for uncovering new protein applications. However, the stability and reproducibility of these capillary wall and solution chemistries are the keys to solve the problem of reproducible electroosmotic flow observed from capillary to capillary.

The octyl bonded/Brij-35 coated capillary developed by Towns and Regnier [13] does not work for all proteins. The standard test mixture of proteins shown by Towns and Regnier [13], the GM-CSF, and some monoclonal antibodies used in our laboratory worked well with this system. However, other monoclonal antibodies, interleukin-3 and interleukin-6 showed poor efficiency. This is probably due to an interaction with the hydrophobic bonded phase or the adsorbed Brij-35, and may be dependent upon the hydrophobic or hydrophilic surface of the protein.

In contrast, cIEF worked well for all proteins tested thus far, yielding high efficiency and resolution comparable to slab gel isoelectric focusing. On a comparative basis to slab gel

isoelectric focusing (work which is presently being studied), cIEF offers reduced analysis times, direct transfer of data to a computer data base, and is 10 times more sensitive, on a concentration basis, than Coomassie stained gels. It is also remarkable that the original sample concentration is amplified by a factor of 228, resulting in a final detectable concentration of 5.7 mg/ml. Precipitation of the focused bands appears to be the major pathway leading to spurious peaks in cIEF. cIEF methods may be potentially interfaced with other spectroscopic techniques, such as light scattering, to determine the extent of aggregates present in focused bands. Also, since the protein concentration is so high, cIEF may be coupled to off-line techniques, such as sequencing, which is not practical for FSCE.

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