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High-performance capillary electrophoresis for in-process control in the production of antithrombin III and human clotting factor IX

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Abstract

Antithrombin III (ATIII) and factor IX (FIX), two proteins from the clotting cascade, were investigated in parallel experiments, using capillary gel electrophoresis and capillary isoelectric focusing. The results from these experiments were compared with the results obtained from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and slab gel isoelectric focusing. In the case of ATIII, capillary gel sieving showed comparable results to SDS-PAGE with the added advantage of the shorter time required for analysis. By optimizing capillary isoelectric focusing (cIEF), a separation of the ATIII isoforms was achieved. In the case of FIX, capillary gel electrophoresis (SDS-CE) of a FIX preparation gave similar results to those obtained by size-exclusion high-performance liquid chromatography and SDS-PAGE, but turned out to be less sensitive in detecting protein impurities at low concentrations. The microheterogeneity of this protein was shown by using cIEF.

Keywords: Process monitoring; Antithrombin; Factor IX; Proteins

1. Introduction

The production of therapeutic proteins from human plasma by recombinant mammalian cells has given new impulses to accompanying analytical methods for in-process control and quality control [1,2]. Recombinant plasma proteins such as tissue plasminogen activator [3], clotting factors VIII [4] and IX [5] and antithrombin III [6] are either already on the market or will soon be available once the approval has been given by the registration authorities. The criteria defined for the quality control of recombinant proteins are also increasingly being applied to the same proteins isolated from human plasma [7].

Apart from the methods used for the measurement of activity, the most frequently applied analytical methods for the quality control of plasma proteins include HPLC and several electrophoretic and immunochemical methods, in particular sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF) and immunoblot. In addition, capillary electrophoresis (CE) is one of the chosen methods since it combines the quantification and handling benefits of HPLC with the separating power of conventional electrophoretic techniques [8,9]. The method permits automatization, and analyses are therefore performed very fast, i.e. in less than 30 min. However, difficulties concerning reproducibility and detection have prevented CE from becoming a general analytical method for monitoring protein production and purification [10].

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As compared with HPLC, CE offers an important advantage in that it is much easier to switch between methods, thus allowing a relatively wide variation of analytical conditions, such as buffers, additives or gels, during method development [11].

Two glycoproteins from human plasma, antithrombin III and clotting factor IX were chosen as model proteins for the use of CE in in-process control and quality control. Both proteins are found in rather low concentrations in the starting material. They are glycosylated and show microheterogeneity, to which the techniques used for their purification [12–15] are contributing factors. The commercially available plasma-derived ATIII preparations are usually produced from the respective fractions of the Cohn procedure [19,20]. In a subsequent step, ATIII is isolated by affinity chromatography with immobilized heparin [20,21]. Some preparations contain human serum albumin (HSA) as stabilizer.

The plasma-derived preparations of FIX currently available on the market are produced either by anion-exchange chromatography combined with heparin affinity chromatography [21], by metal-chelating chromatography [24] and by immunoaffinity chromatography with immobilized anti-FIX antibodies [25].

Both FIX and ATIII are also produced by recombinant mammalian cells [5,6].

2. Experimental

2.1. Chemicals

Pharmalytes, PD-10 columns prepacked with Sephadex G-25, Ampholine PAGplates p*I* (isoelectric points) 4.0–5.0 and p*I* 4.0–6.5 and IEF marker proteins were purchased from Pharmacia Biotech (Uppsala, Sweden). Biolytes (ranges from p*I* 3–10 and 3–5), TEMED (N,N,N′,N′-tetramethylethylenediamine), 2-mercaptoethanol, SDS-PAGE and SDS-CE molecular mass markers, IEF mobilizer, 20 m*M* phosphoric acid and 40 m*M* sodium hydroxide were purchased from Bio-Rad (Hercules, CA, USA). CElect-H150 capillaries were obtained from Sulpelco (Bellefonte, PA, USA). Glutamic acid, lysine, β-alanine and HPMC (hydroxypropylmethylcellulose) were obtained from Sigma (St. Louis, MO, USA). Triethanolamine and citric acid were from Merck (Darmstadt, Germany).

2.2. Proteins

The FIX concentrates Octanyne and Octanine F from human plasma were from Octapharma (Vienna, Austria). The FIX concentrate Mononine was obtained from Armour Pharma (Eschwege, Germany).

The Octanyne final container was additionally separated by size-exclusion chromatography on Fractogel EMD BioSec (S) (Merck) as described elsewhere [16]. Briefly, the 900×100 mm I.D. column (Merck) packed with Fractogel EMD BioSec (S) was equilibrated with 200 m*M* NaCl, 15 m*M* Tris·HCl (pH 7.4). A total of 30 000 I.U. of FIX (500 mg of protein) in 100 ml mobile phase was applied to the column. The elution profile showed two peaks. The second peak, which contained the active FIX, was further analysed in SDS-PAGE and SDS-CE.

Cryopoor plasma, depleted of vitamin K-dependent coagulation factors, was used as starting material for the purification of ATIII. A total of 10 ml of Heparin Sepharose FF (Pharmacia Biotech), Heparin Hyper D (Biosepra, Marlbough, MA, USA), Fractogel EMD Heparin 650 (S) (Merck) were packed into a XK16-column (Pharmacia Biotech), giving a bed height of 5 cm. After equilibration with a buffer consisting of 20 m*M* sodium citrate, containing 0.15 *M* NaCl (pH 7.4), 500 g of plasma were pumped over the column at a linear flow-rate of 120 cm/h. Loosely bound substances were washed out with 20 m*M* sodium citrate, containing 0.5 *M* NaCl (pH 7.4). The pure ATIII was eluted with 20 m*M* Tris·HCl, containing 2 *M* NaCl (pH 7.4). The following commercially available ATIII concentrates from human plasma were investigated: ATenativ (Pharmacia, Stockholm, Sweden), Thrombhibin (Immuno, Vienna, Austria) and Athimbin HS (Behring, Marburg, Germany)

2.3. Methods

2.3.1. SDS-PAGE

SDS-PAGE was carried out under reducing conditions according to the Laemmli method [17] using a Hoefer system (Pharmacia Biotech). For separation, 7.5–15% and 4.5–15% gradient gels were

used. The amount of protein applied was 2 μg per lane. Protein bands were stained using the silver staining method according to Heukeshoven and Dernick [18].

2.3.2. Molecular sieving in capillaries

Capillary SDS-CE was performed on a BioFocus 3000 instrument (Bio-Rad). Uncoated 50 μm I.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) and 25 cm/21 cm total/effective length were used. Samples containing 0.5–1 mg/ml protein were diluted 1:2 with sample buffer from Bio-Rad. 2-Mercaptoethanol and internal standard solution (Bio-Rad) were both added to a final concentration of 5% and the samples were boiled for 10 min. Samples were injected under pressure of 100 p.s.i/s (1 p.s.i.=6894.76 Pa) and separated at 15 kV. Detection was performed by measuring *A* at 220 nm.

2.3.3. Isoelectric focusing Ampholine PAGplate

IEF Ampholine PAGplates (p*I* 4.0–5.0 for FIX and p*I* 4.0–6.5 for ATIII) were used. A total of 10–15 μl of samples, containing 0.5–1.0 mg protein/ml in water, were applied at the cathodic end of the gel after a prefocusing step (700 V, 12 mA, 8 W, 20 min). Focusing settings were used as recommended by the manufacturer. After fixation in ethanol–water–acetic acid (50:40:10, v/v) overnight, the gels were silver stained according to the method of Heukeshoven et al. [18]

2.3.4. Capillary isoelectric focusing

cIEF was also performed on a BioFocus 3000 (Bio-Rad) instrument.

ATIII: the linear polyacrylamide (LPA)-coated BioCap capillaries from Bio-Rad, self assembled to 21-cm effective length, were used. ATIII samples were desalted in water by gel filtration on PD 10 columns. Biolytes p*I* 3–5 and p*I* 3–10 were used at a final concentration of 1.6% and 0.4%, respectively. Additionally, 0.5% TEMED and 0.02% HPMC were added. After filling the capillary with sample by high pressure injection for 60 s, focusing was performed for 300 s at 18 kV, using 20 m*M* H_3PO_4 as anolyte and 100 m*M* triehanolamine as catholyte. The sample was mobilized by changing the catholyte against the Bio-Rad mobilizer at a constant voltage setting of 18 kV.

Between the runs, the capillary was purged by high-pressure injection of water for 60 s, followed by one flush for 60 s with Biolytes (1.6% p*I* 3–5 and 0.4% p*I* 3–10) and 0.02% HPMC in water.

FIX: coated CElect-H150 capillaries from Sulpelco, self assembled to 21-cm effective length, were used. FIX samples were desalted with PD-10 columns in 5 m*M* sodium citrate buffer (pH 7.4). The final protein concentration was 25 I.U./ml. Ampholytes were used at a final concentration of 1% consisting of 40% 2.5–5, 40% 4.2–4.9 (Pharmacia) and 20% 3–10 (Bio-Rad). All samples contained 0.01% HPMC. The anolyte and catholyte during focusing were 20 m*M* citric acid and 0.1 *M* lysine, respectively. The sample was injected at high pressure for 60 s and focused at a constant voltage of 15 kV for 180 s. Mobilizing was performed with the mobilizing reagent from Bio-Rad. The separation was achieved at a constant voltage setting of 15 kV. In all experiments the capillary was thermostated at 22°C.

Between the runs, the capillary was purged by high-pressure injection of water for 60 s, followed by one flush for 60 s with Biolytes (0.4% 2.5–5, 0.4% 4.2–4.9 and 0.2% 3–10) and 0.01% HPMC in water.

3. Results

3.1. Antithrombin III

The purity of ATIII concentrates from different manufacturers was investigated in SDS-PAGE and SDS-CE. Fig. 1a shows SDS-PAGE of different ATIII preparations. ATenativ (lane 2) and Thrombhibin (lane 3) are stabilized with HSA, whereas Athimbin HS contains only ATIII, without proteineous stabilizers (lane 4).

In SDS-PAGE, ATIII yielded a band with a lower apparent molecular mass than HSA. This agrees with the data found in the literature according to which the molecular mass of ATIII is 55 000 [11], compared to 67 000 of HSA. As expected, the preparation Athimbin shows only one band with an apparent molecular mass of 55 000 (lane 4). In the case of Thrombhibin, an additional band with an apparent

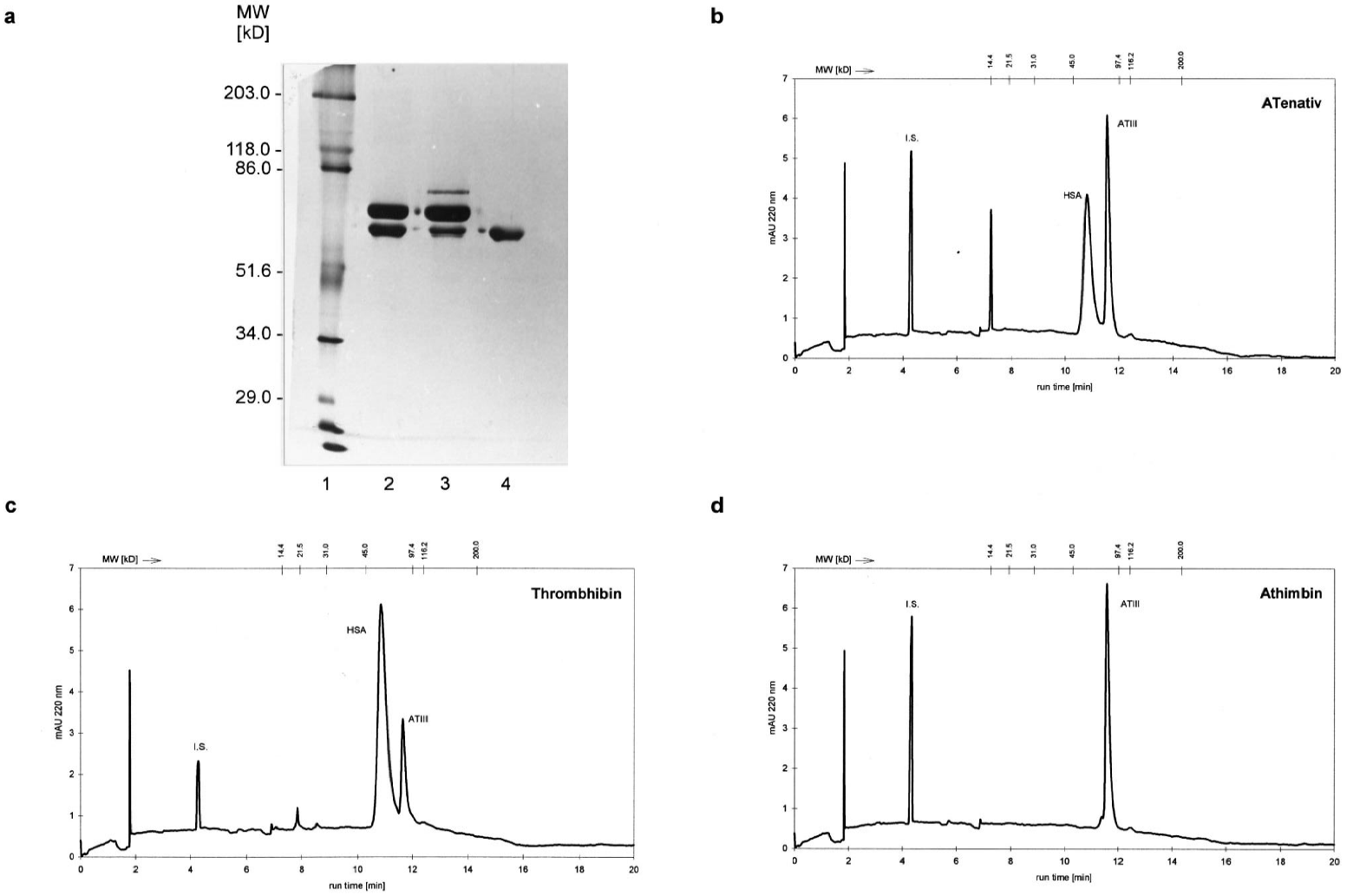

Fig. 1. (a) SDS-PAGE of different human ATIII concentrates. A 7.5–15% gradient gel under reducing conditions was used. A total of 2 μg protein was applied to each lane: (1) molecular mass marker of Bio-Rad, (2) ATenativ, (3) Thrombhibin and (4) Athimbin HS. (b to d) Electropherograms of SDS-CE under reducing conditions of different ATIII concentrates from human plasma. (b) ATenativ, (c) Thrombhibin and (d) Athimbin were injected at concentrations of 0.3 mg/ml. The internal standard used appears as a peak at 4.2 min. ATIII migrates more slowly than HSA, despite its lower molecular mass (55 000 for ATIII versus 64 000 for HSA). MW=molecular mass; kD=kilodalton.

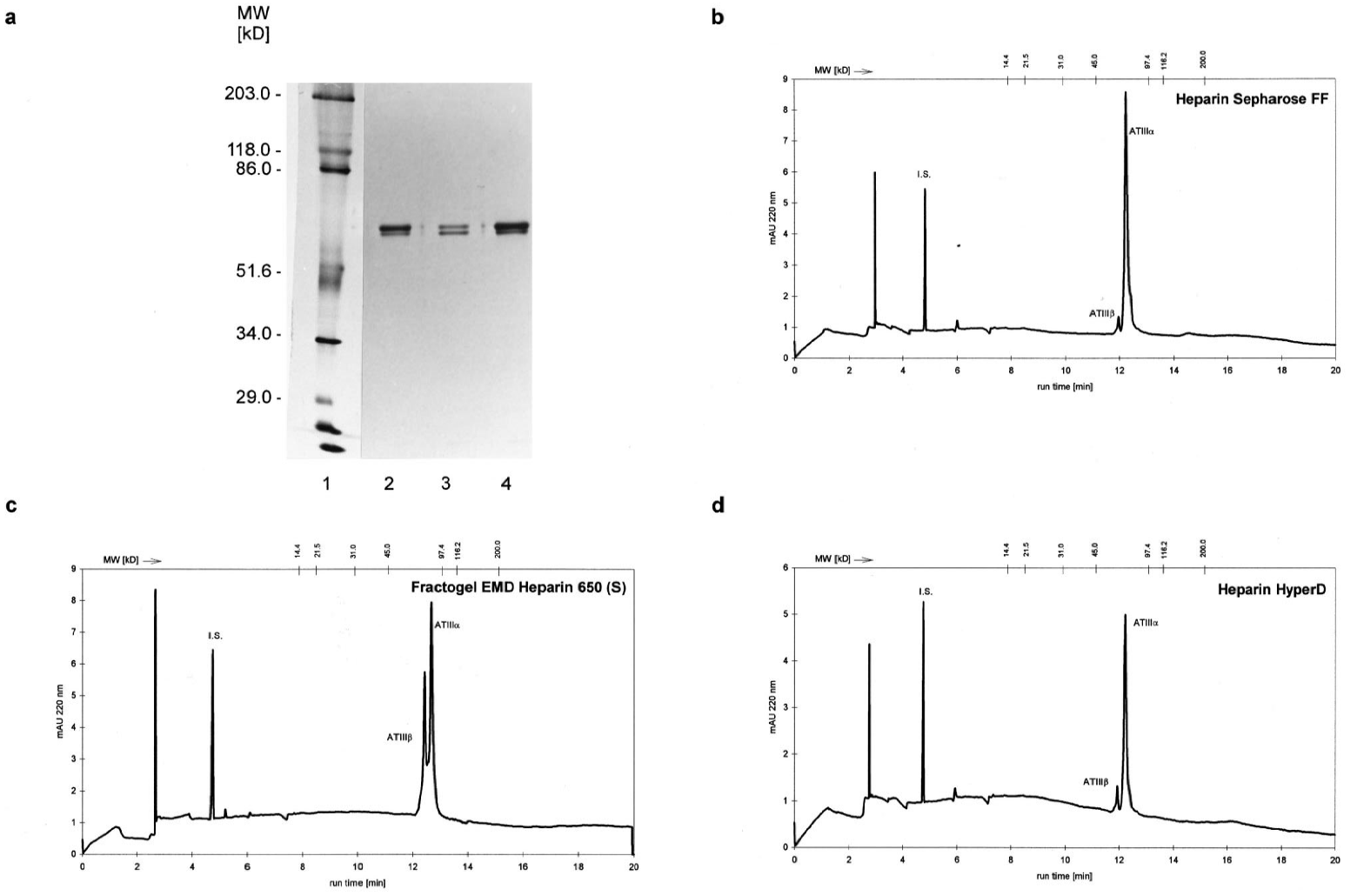

Fig. 2. (a) SDS-PAGE of ATIII, purified from human plasma using different heparin resins. A total of 2 μg protein was applied to each lane. Lane (1) molecular mass marker of Bio-Rad, ATIII purified by lane (2) Heparin Sepharose FF, lane (3) Fractogel EMD Heparin 650 (S), lane (4) Heparin HyperD. (b to d) Electropherograms of molecular sieving in capillaries under reducing conditions of ATIII purified from human plasma using different heparin resins. ATIII was purified by (b) Heparin Sepharose FF, (c) Fractogel EMD Heparin 650 (S) and (d) Heparin HyperD. The ATIII preparations had a protein concentration of 0.3 mg/ml.

molecular mass between 70 000 and 80 000 can be identified (lane 3), which is probably caused by contamination from the stabilizer HSA. SDS-CE also splits the ATIII preparations stabilized with HSA into two separate peaks (Fig. 1b and c). The fact that ATIII appears after HSA in spite of its lower molecular mass is contradictory. Fig. 2 shows samples of ATIII which have been obtained by the use of different commercially available affinity gels with immobilized heparin. All the experimental batches from our process development give a double band with apparent molecular masses ranging from 53 000 to 55 000 (lanes 1 to 3). The corresponding electropherograms are represented in Fig. 2b–d.

IEF of ATIII in the gel and the capillary is illustrated in Fig. 3. The gel clearly shows six main bands and three to four weak ones ranging from p*I* 4.3–5.2. CE-IEF also yields six different peaks, though less well resolved.

3.2. Clotting factor IX

FIX has a molecular mass of 57 000–72 000 [14,26] depending on the method used for its determination. SDS-PAGE and molecular sieving CE of a FIX preparation (Octanyne), containing considerable amounts of contaminants, is represented in Fig. 4. In SDS-PAGE, FIX gave a band at M_r 70 000, and the impurities are recognizable at approx. M_r values of 300 000, 80 000, 50 000 and 45 000 (Fig. 4b). In SDS-CE, two peaks, FIX and the main protein contaminant can be identified (Fig. 4b). The FIX preparations with high specific activity, like the product purified by immunoaffinity chromatography (Mononine), yielded a strong band at around 70 000 and some minor bands in the low molecular mass range (Fig. 5a). The same band pattern appears for the other purified preparations (Octanine F and FIX peak isolated from Octanyne after SEC). In molecu-

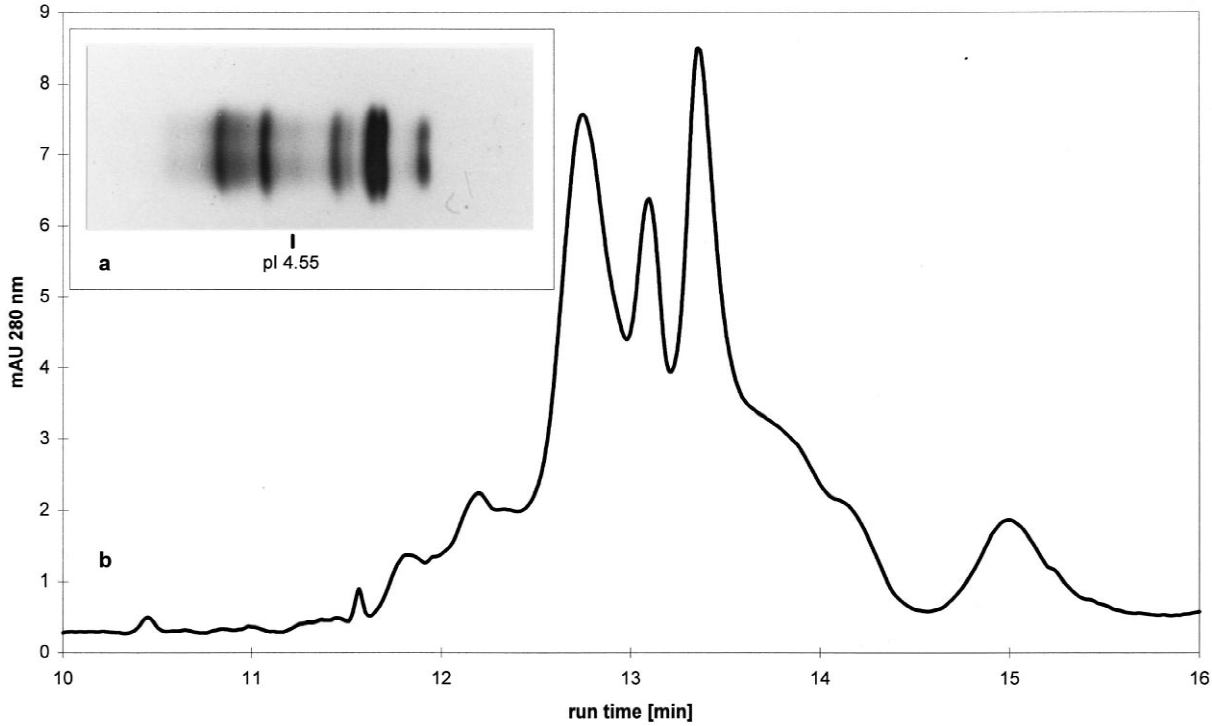

Fig. 3. Pattern of ATIII (purified from human plasma using Heparin HyperD) in cIEF versus IEF in PAGplate (pH 4.0–6.5). In cIEF, 0.6 mg/ml of ATIII were injected under pressure for 60 s. On the IEF PAGplate, 10 μl of ATIII at a concentration of 1.0 mg/ml were applied to the gel.

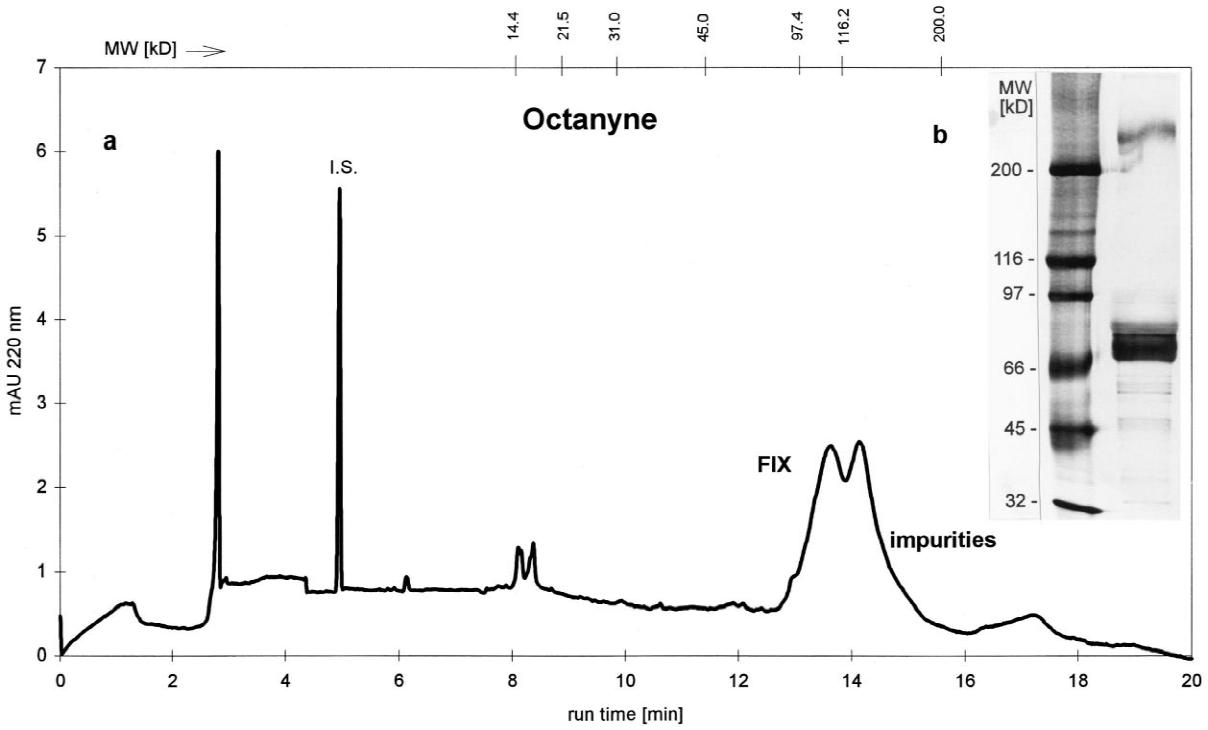

Fig. 4. (a) Capillary gel SDS electrophoresis of Octanyne under reducing conditions. The sample (540 μg/ml) was injected under pressure (100 p.s.i./s.) and then a voltage of 15 kV was applied for separation. Detection was performed at 220 nm. (b) SDS-PAGE of the FIX concentrate Octanyne. A 4.5–15% gradient gel under reducing conditions was used. A total of 2 μg protein were applied to each lane. The left part shows a molecular mass marker (Bio-Rad); the right one the FIX concentrate Octanyne F. This FIX preparation shows beside the main FIX band also some bands derived from impurities.

lar sieving CE, only one peak is detected (Fig. 5b to d).

The results of IEF in slab gel are illustrated in Fig. 6a and those in capillary are shown in Fig. 6b and c. The microheterogeneous structure of the protein can be demonstrated by the appearance of four bands or peaks, respectively, and an isoelectric point between 4.1 and 4.5.

4. Discussion

4.1. SDS-PAGE and SDS-CE from ATIII and FIX

4.1.1. ATIII

Molecular sieving of ATIII in the presence of SDS and under reducing conditions gave similar results to those obtained by SDS-PAGE (Figs. 1 and 2). In SDS-CE, HSA (peak at 10.8 min) was successfully separated from ATIII (peak at 11.6 min) in both ATenativ and Thrombhibin, but could not be separated by size-exclusion high-performance liquid chromatography (HPSEC). All the samples, with or without HSA, yielded only one peak in this separation (not shown here, cf. [23]). It is remarkable that ATIII in the separation by CE has a higher apparent molecular mass than HSA. This possibly results from the rather high degree of glycosylation of ATIII in contrast to the nonglycosylated HSA [27]. The peaks in the low molecular mass range (between 7 and 8 min), found in the ATIII preparations containing HSA, are probably caused by low molecular mass stabilizers from albumin. The band with an apparent molecular mass between 70 000 and 80 000, which appears in SDS-PAGE of Thrombhibin (band 2 in

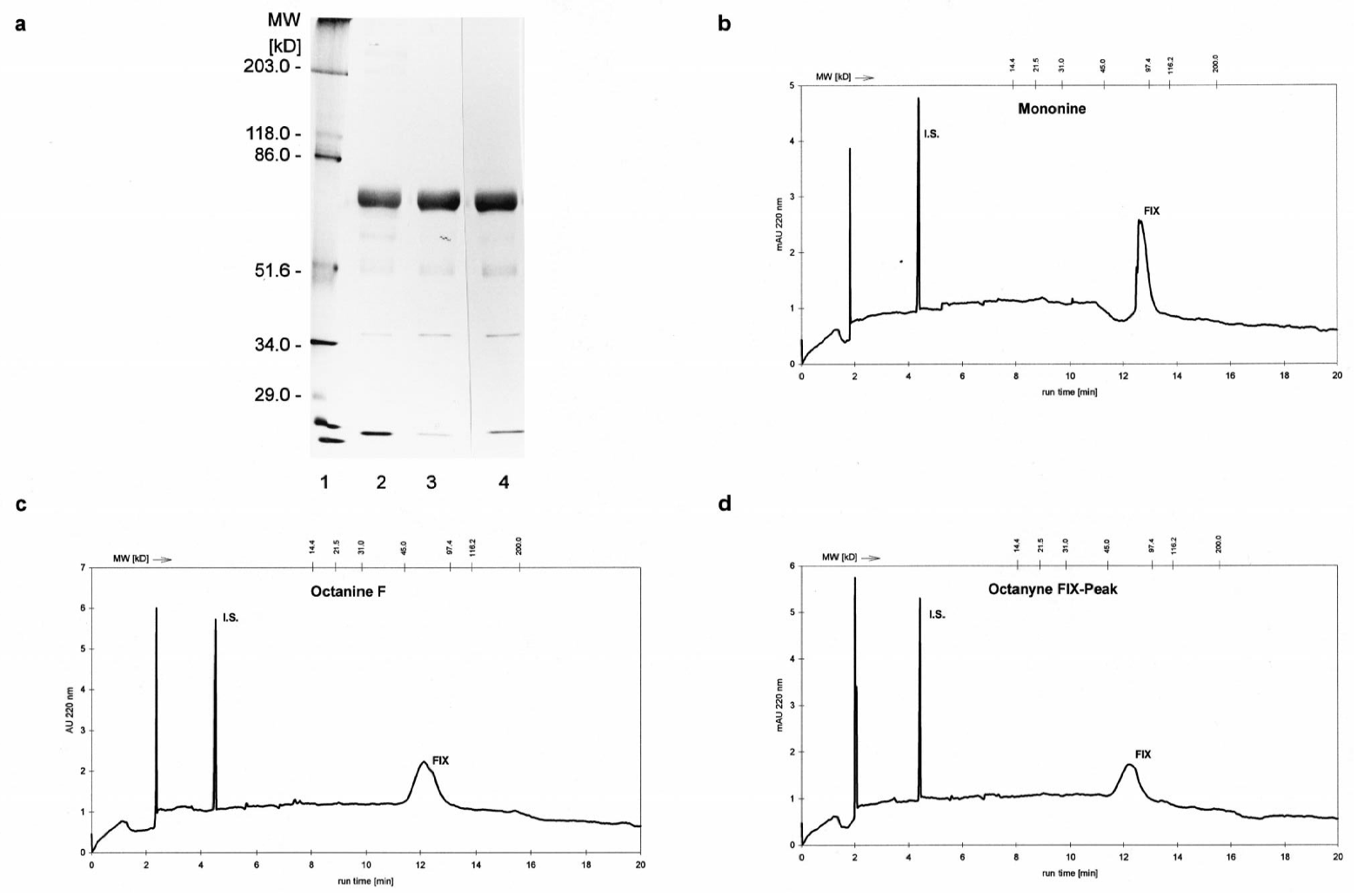

Fig. 5. (a) SDS-PAGE and SDS-CE of different FIX concentrates. A 7.5–15% gradient gel under reducing conditions was performed. A total of 2 μg protein were applied to each lane. Lane 1 shows Mononine, lane 2 Octanine F and lane 3 FIX isolated from Octanyne by SEC. (b to d) SDS-CE of FIX concentrates Mononine (b), Octanine F (c) and Octanyne FIX peak (d). FIX preparations were injected at the following concentrations: (b) 60 I.U./ml, (c) 60 and (d) 70 I.U./ml.

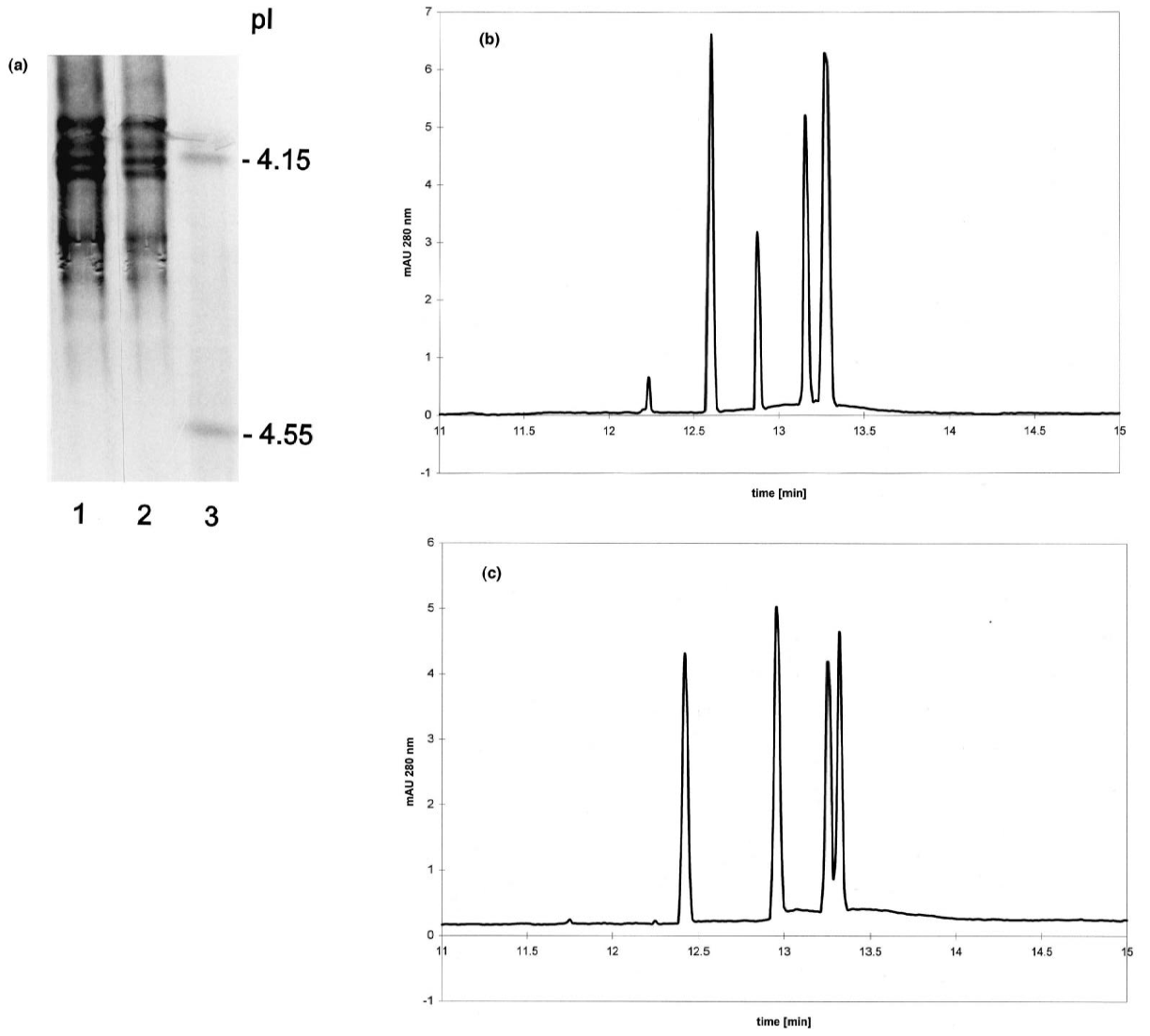

Fig. 6. (a) Slab gel IEF and cIEF of different FIX concentrates performed on a Ampholine PAGplate p*I* 4–5. Lane 1 shows Octanine F (1.8 I.U. per lane), lane 2 Mononine (1.3 I.U. per lane) and lane 3 p*I* protein standards (Sigma). Both FIX preparations show a similar pattern of microheterogeneity having bands between pH 4.1–4.5. (b) cIEF of Mononine, a FIX concentrate isolated by immunoaffinity chromatography. The sample was injected for 60 s at a FIX concentration of 25 I.U./ml. Separation conditions are described in Section 2.3. (c) cIEF of FIX concentrate Octanine F (cf. also [24]). The sample was injected for 60 s at a FIX concentration of 25 I.U./ml. Separation conditions are described in Section 2.3.

Fig. 1), has no corresponding peak in CE. This peak is probably concealed in the tailing of the HSA peak.

In the case of the ATIII samples obtained by affinity chromatography using different heparin gels, two peaks appeared in CE (see Fig. 2), similar to the results obtained in SDS-PAGE. The pattern of the electropherogram was influenced by the type of gel used for isolation. When Heparin Sepharose FF or Heparin Hyper D were used, the content of material with a somewhat lower apparent molecular mass was rather low. When Fractogel EMD heparin was used, the amount of this material in the eluate was almost one third (Fig. 2).

This different binding behaviour is very likely due to a different composition of the oligosaccharides in this glycoprotein. ATIIIβ shows a higher affinity to heparin than ATIIIα and is lacking glycosylation at Asp 135 [22]. The ratio of ATIIIα/ATIIIβ depends on the type of gel used for isolation. One reasonable explanation for this phenomenon is that heparin from different sources is used in the production of heparin gels.

4.1.2. FIX

The separation of FIX (Octanyne) from protein impurities is shown by SDS-PAGE and SDS-CE (Fig. 4). The main accompanying protein contaminant of FIX has a molecular mass of ~80 000 and its position in the SDS-gel is very close to FIX itself (Fig. 4b). These two bands are well resolved in the slab gel. In SDS-CE, two peaks also appeared, though not baseline separated (Fig. 4a), in contrast to HPLC, where baseline separation was achieved (not shown here, cf. Ref. [16]).

However, in SDS-PAGE, additional bands are visible, one at M_r ~300 000 and several others below 70 000. These bands are not detected in SDS-CE.

Fig. 5 illustrates the separation of different FIX preparations with high specific activity. These concentrates are highly purified and therefore less contaminated by other proteins. In SDS-PAGE, bands between 70 000 and 34 000 are notable (Fig. 5a). In contrast, SDS-CE yielded only one peak which corresponded to the main FIX band at 70 000 (Fig. 5b). Again, low concentration impurities or FIX fragments were not detected.

The rather broad quality of the FIX peaks in the electropherograms is probably due to the microheterogeneity of FIX. Glycoproteins migrate too slowly in SDS electrophoresis, since the sugar moiety does not bind to SDS [27]. On the basis of these results it can be concluded that as far as the analysis of FIX is concerned, SDS-PAGE cannot be replaced by molecular sieving CE, since accompanying proteins at low concentrations are not detected.

4.2. IEF in gel and in the capillary of ATIII and FIX

The separation conditions were developed by optimizing following parameters: first we tested the LPA-coated BioCap capillaries from Bio-Rad and the CElect-H150 capillaries from Sulpelco. In the case of ATIII, no satisfactory separation was achieved by using CElect capillaries, since this protein showed very broad peaks with low resolution and long retention times. In contrast, the separations of FIX were more reproducible in CElect capillaries, despite the fact that the IEF pattern was similar to that obtained in BioCap capillaries.

The use of uncoated capillaries was abandoned since ATIII appeared as a broadened and unresolved peak with long retention times and FIX was not eluted at all after 50 min. This behavior of our very acidic testing samples ATIII (p*I* 4.3–5.2) and FIX (p*I* 4.1–4.5) is in line with the results of Mazzeo et al. [28], who report that in uncoated capillaries the magnitude of the electroosmotic flow (EOF) is a function of pH, and that EOF decreases dramatically at a pH below 7, for example by 50% when the pH is lowered to 4–3. As a result, only basic and neutral proteins can be separated with good peak sharpness.

4.2.1. ATIII

To optimize separation conditions, we tested 20 m*M* phosphoric acid, 10 m*M* acetic acid and 20 m*M* citric acid as analyte and 40 m*M* sodium hydroxide, 0.1 *M* triethanolamine and 0.1 *M* lysine as catholyte. The best resolution of isoform separation was achieved when we applied 20 m*M* H_3PO_4 as anolyte and 0.1 *M* triethaolamine as catholyte. The ampholytes were tested at concentrations ranging from 0.5 to 4%. Low range ampholytes were tested from Bio-Rad (ranges 4–6, 3–5), Serva (4–5), Sigma (3–5) and Pharmacia (2.5–5, 4.2–4.9). Biolytes of p*I* 3–5 and 3–10 at a final concentration of 1.6% and

0.4%, respectively, were found to be superior to all other combinations of ampholytes of the different manufacturers in terms of peak shape and retention time. TEMED at a final concentration of 0.5% had to be added to prevent ATIII from passing the detector during focusing.

The sample was focused for 300 s at a voltage of 18 kV. We had to apply this voltage to make the current drop to 10% of the starting value. The sample was then mobilized with the mobilizing reagent from Bio-Rad, which contained a zwitterionic agent in basic solution. Zwitterionic mobilization is based on two mechanisms as described by Zhu et al. [29]. The mobilization of acidic proteins is greatly enhanced by the presence of a p*I* 3.2 zwitterion. The use of additives like Triton X-100, bicine, CHAPS and polyethylene glycol (PEG), which among others, have been recommended in literature [30], was examined. These additives may improve the resolution of neutral and basic proteins, but did not work with the acidic proteins ATIII and FIX.

By using IEF in the slab gel, we could determine the ATIII isoforms in a p*I* range from 4.3–5.2. These results are in agreement with the work of Daly et al. [31], who found in the same p*I* range three major and three minor bands and one doublet. The separation of ATIII in cIEF yielded six main peaks. cIEF of recombinant ATIII (rATIII), produced in baby hamster kidney cells, was reported by Reif and Freitag [32]. They also separated six isoforms, even if their peak pattern looks different to that shown here. So far, there is no clear evidence that rATIII is correctly glycosylated, which means that the isoform patterns need not necessarily be congruent. Further, the different conditions that we applied in our experiments have an influence on the separation results.

4.2.2. Factor IX

To optimize FIX separation, we worked with the same conditions as those used for ATIII. With 20 m*M* citric acid as anolyte and 0.1 *M* lysine as catholyte, the separation yielded several peaks, whereas the other anolyte and catholyte combinations generated only one or two peaks.

Protein concentrations exceeding 150 I.U./ml caused poor resolution or even no peaks at all. The best results in terms of peak number and resolution were achieved with ampholytes at a final concentration of 1% consisting of 40% 2.5–5, 40% 4.2–4.9 and 20% 3–10, while ampholyte concentrations higher than 2% caused the capillary to clog after some runs. The use of all other above-described ampholytes and their combinations led to a loss in peaks, and were therefore not suggested.

Reproducibility was improved by adding 0.01% HPMC. Higher concentrations, however, turned out to have no further effect on the separation.

The isoform patterns of Mononine and Octanine F in slab gel are congruent, showing four main bands for each preparation (Fig. 6a), and the respective four peaks appeared in the IEF electropherograms (Fig. 6b and c). While time intervals for the appearance of all four peaks were very similar for both FIX preparations, retention times varied from peak to peak. One likely explanation for this phenomenon is that the IEF pattern is influenced by the sample matrix. In cIEF, we observed that the addition of p*I* marker proteins, e.g. β-lactoglobulin, changed the IEF pattern in terms of retention time and elution profile (data not shown). Suomela et al. [14] report that FIX focuses between pH 4.04 and 4.65 and that separation usually yields four isoelectric components. Although their peaks were obtained by using a LKB 8101 column, we too could detected the four respective main isoform bands and peaks in a similar p*I* range by using IEF in gel and in capillary.

5. Conclusions

Molecular sieving and isoelectric focusing for the analysis of physiologically active proteins from human plasma, such as ATIII and FIX, provided a welcome addition to the established methods such as SDS-PAGE, immunoblot and HPSEC. CE was superior to conventional electrophoretic techniques in terms of shorter time required for analysis, but turned out to be less sensitive in detecting proteins at low concentrations in SDS-CE. Protein impurities in FIX concentrates could not be detected in contrast to SDS-PAGE. The separation of ATIII and FIX isoforms with cIEF showed promising results and this technique can therefore be regarded as an additional

analytical method for quality control of the final products.

In our case, the decision as of whether to apply CE methods for routine analysis must therefore be taken on a case-to-case basis, depending on the particular separation problem involved.

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