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# HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS OF HISTONES

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## Abstract

A high performance capillary electrophoresis (HPCE) system has been developed for the fractionation of histones. This system involves electroinjection of the sample and electrophoresis in a 0.1M phosphate buffer at pH 2.5 in a 50 µm x 35 cm coated capillary. Electrophoresis was accomplished in 9 minutes separating a whole histone preparation into its components in the following order of decreasing mobility: (MHP) H3, H1 (major variant), H1 (minor variant), (LHP) H3, (MHP) H2A (major variant), (LHP) H2A, H4, H2B, (MHP) H2A (minor variant) where MHP is the more hydrophobic component and LHP is the less hydrophobic component. This order of separation is very different from that found in acid-urea polyacrylamide gel electrophoresis and in reversed-phase HPLC and, thus, brings the histone biochemist a new dimension for the qualitative analysis of histone samples.

## Introduction

The separation of histones by zone electrophoresis in a starch gel matrix was first introduced by Neelin and Connell over 30 years ago [1]. Since that time, the gel electrophoresis of histones, particularly in polyacrylamide gels, has been widely practiced [2-12] because it is inexpensive to implement and simple to operate [13]. On the other hand, the gel preparation is tedious, the electrophoresis is time-consuming, and quantification involves staining, destaining, and scanning densitometry which is labor-intensive, time-consuming, and semiquantitative [13].

The advent of high-performance capillary electrophoresis (HPCE) [14, 15] potentially offers a new opportunity to raise the electrophoretic fractionation and analysis of histones to a performance level superior to that of gel electrophoresis. The advantages of HPCE should include high resolution, rapid analysis time, on-line detection, and computer-based quantification. Most importantly, HPCE has the potential for analysis of very small samples in the femtomole range [15]. In our laboratory, the ability to perform analysis on such small samples is attractive because it would facilitate the analysis of histones from the small numbers of cells acquired by flow cytometry cell sorting. For these reasons, we have initiated a program to develop HPCE methods to fractionate nucleoproteins. This report describes the development of the separation of histones by zone electrophoresis in free solution using low pH buffers in coated silica capillaries.

## Experimental

### Cell Cultures and Histone Preparation

Chinese hamster cells (line CHO) were grown exponentially in suspension culture as described by Tobey et al. [16]. Chromatin was prepared from whole-cell homogenates as described by Gurley et al. [17]. Histones were extracted from the chromatin with 0.4N sulfuric acid, recovered by acetone precipitation, and stored as a lyophilized powder at -70°C until used for HPCE [17].

To prepare purified histones for HPLC, $3 \times 10^8$ nuclei were prepared from CHO cells using the detergents Nonidet P-40 and sodium deoxycholate in hypotonic solution as described by Gurley, et al [18]. The nuclei were then dissolved for two hours in 1 ml of reagent containing 6M guanidine•HCl, 0.2% TFA, 0.155M NaCl, 0.026 M HCl, and 0.025% dithiothriotol according to the method of Jackson and Gurley [19]. Undissolved residual nuclei were sedimented by centrifugation at 8000 x g for 10 minutes. The supernatant fluid containing soluble DNA and proteins was decanted into 9 ml of $H_2O$ containing 0.2% trifluoroacetic acid (TFA). The residual undissolved nuclei were dissolved in 1 ml of dissolving reagent for one hour and then centrifuged as before. Only a trace of nuclear material remained undissolved. The supernatant fluid was decanted into the

$H_2O$/0.2% TFA containing the first decantate. The DNA in the solution was precipitated overnight at 4°C due to the acid conditions of the solution. The DNA was sedimented by centrifugation at 8000 x g for two hours. The 11 ml of supernatant fluid, containing the total complement of proteins from the cell nuclei, was stored at -70°C. When used for HPLC this solution was thawed and centrifuged again for two hours at 8000 x g to remove any residual DNA clouding the sample.

HPLC of Histones

The guanidine-soluble DNA, salts and other chemical reagents in the dissolved nuclei samples were separated from the nuclear proteins by size exclusion HPLC. This was accomplished as described by Jackson and Gurley [19] using a Waters HPLC system equipped with a Bio•Sil TSK guard column followed by a Bio•Sil TSK size exclusion column (Bio•Rad Laboratories). The sample was injected into the guard column and was eluted through the two columns with $H_2O$/0.2% TFA at a flow rate of 0.5 ml/min. The guard column adsorbed any soluble DNA remaining in the sample and the proteins were separated from the salts and other chemical reagents by size exclusion on the Bio•Sil TSK column [19]. The effluent protein fraction was either lyophilized to a dry powder and stored at -70°C for future use or it was injected directly into an HPLC reversed-phase µBondapak CN column (Waters Associates) [20] which adsorbed the proteins on the top of the column [21].

The histone proteins were fractionated and purified by reversed-phase HPLC on the µBondapak CN column using an acetonitrile gradient elution containing 0.2% TFA as described by Gurley et al. [22]. This column fractionated the histones into the five basic types: H1, H2A, H2B, H3, and H4; and the H2A and H3 histones were subfractionated into two variants of each, the less hydrophobic (LHP) H2A and (LHP) H3 and the more hydrophobic (MHP) H2A and (MHP) H3 [20-22]. Each of these seven individual histone fractions were collected and either lyophilized to dryness or evaporated to dryness while centrifuging in a vacuum chamber (Speed-Vac) and stored at -70°C [22] until used to identify the individual histones fractionated by HPCE.

HPCE of Histones

HPCE was performed using a Model HPE-100 High Performance electrophoresis system manufactured by Bio•Rad Laboratories, Richmond, CA. Experiments were performed using 20 cm x 25 µm, 35 cm x 50 µm, and 50 cm x 50 µm capillary tubes whose inside surfaces were chemically modified to produce a patented hydrophilic coating. Coatings of this nature have been reported by several laboratories to eliminate electroendoosmosis and protein adsorption [23-25]. Cathode, anode, and capillary buffers were 0.1M sodium phosphate, pH 2.5 (Bio•Rad Laboratories) unless otherwise specified. Detection of the separated proteins traversing the capillary was accomplished by in-tube monitoring of the peptide bond absorbance at 200

nm with a spectrophotometer setting of 0.005 absorbance units full scale. To evaluate the performance of this instrument a standard mixture of nine polypeptides was subjected to electrophoresis. This mixture contained bradykinin, angiotensin II, ∝ MSH, TRH, LHRH, (2-5) leucine enkephalin, bombesin, methionine enkephalin, and oxytocin (Bio•Rad Laboratories).

Samples were prepared for HPCE by dissolving lyophilized histone powder in water containing 0.2% TFA at a concentration of 0.1 µg/µl. Poly-L-lysine hydrobromide, Type VI, 13,000 molecular weight (Sigma Chemical Co.) was added to some of these solutions at a concentration of 0.05 µg/µl to serve as an internal mobility marker during electrophoresis. These samples were loaded into the capillaries by electroinjection [26]. To accomplish this, the cathode chamber and capillary were filled with 0.1M phosphate buffer and the anode chamber was filled with water. Then 5µl of sample was injected into the loading chamber at the anode end between the capillary and the water. Power was applied to the capillary at 10 kV (constant voltage) and ~38 µamps for 10 seconds. This actively carries the cations (including the positively charged protonated proteins) into the capillary. Under these low ionic strength sample conditions, the proteins are concentrated and stacked in the capillary during this loading step [27]. The excess sample in the loading chamber and the water in the anode chamber were then replaced with 0.1 M phosphate buffer and electrophoresis was performed by applying power to the capillary at 10 kV (constant voltage) and ~41 µamps. Electrophoresis normally took 10 minutes using the 35 cm x 50 µm coated capillary.

## Results

### 20 cm x 25 µm Capillary

The performance of the HPE-100 High Performance Electrophoresis instrument is typically evaluated by subjecting a mixture of nine polypeptides to zone electrophoresis in a 20 cm x 25 µm coated capillary using a 0.1M phosphate buffer at pH 2.5. In our laboratory repetitive electrophoresis of this standard produced fractions whose peak-heights varied by only 3.0% (coefficient of variation) and whose mobilities varied by 7.2%. This high performance led us to choose these operating conditions for our first attempts to separate histones by HPCE. We found that when whole histones were dissolved in water containing 0.2% TFA (as they are when recovered from an HPLC column) we could electroinject them into the capillary in 10 seconds at 10 kV. Electrophoresis at 10 kV produced a single peak of unresolved proteins (Figure 1A). By decreasing the electrophoresis voltage (Figure 1, B-F) we found that this single peak contained the various histone fractions which had mobilities ranging over six minutes at 1 kV (Figure 1F), but they could not be resolved under these conditions.

It was thought that perhaps protein-protein interactions in the capillary might cause a degradation of resolution. To prevent this, NaCl was added to

the electrophoresis buffer. Preliminary experiments indicated that when NaCl concentrations greater than 0.12M was used, the electrophoresis was inhibited due to the elevated conductivity of the buffer. However, when 0.12M NaCl was used, histone electrophoresis was accomplished (Figure 1, G-I). Histone resolution at 4 kV was better in the presence of 0.12M NaCl (Figure 1H) than it was without NaCl (Figure 1D). However, it was not adequate for analytical use.

50 cm x 50 µm Capillary

Another possible cause for the poor resolution might be protein interactions with the capillary walls. To reduce the magnitude of this effect we performed the electrophoresis in a larger diameter capillary having the dimensions of 50 µm x 50 cm long. After electroinjection at 10 kV, the histones were subjected to electrophoresis at four different voltages ranging from 4-10 kV (Figure 2, A-D). We found that the resolution of the histones was far superior in this 50 µm capillary (Figure 2D) when compared to the same operating conditions in the 25 µm capillary (Figure 1A). Reducing the electrophoresis voltage from 10 kV to 6 kV (Figure 2B) appears to improve this resolution. However, close inspection indicates that while the peaks are spread further apart at 6 kV, the longer electrophoresis time permitted diffusion to spread the bands so that no significant increase in resolution was accomplished.

NaCl was added to the electrophoresis buffer in the 50 µm x 50 cm capillary. There was some small increase in the resolution when the NaCl concentration was increased from 0 M to 0.12 M (Figure 3, A-D). The most noticeable effect of NaCl was on the largest fraction which was latter identified as H2B. When the electrophoresis voltage was reduced from 10 kV to 7 kV in the presence of 0.12 M NaCl, the H2B peak was observed to undergo partial resolution into more fractions (Figure 3, E-H). Unfortunately, the longer electrophoresis time at 7 kV permits too much band broadening due to diffusion thus destroying the overall resolution of the whole sample (Figure 3H).

35 cm x 50 µm Capillary

Since diffusion appeared to be a significant factor in the degradation of the electropherogram, we used a shorter capillary (35 cm long x 50 µm diameter) to reduce the time of the run. With the shorter capillary, the histones migration took less than eight minutes at 10 kV (Figure 4B) while with the 50 cm capillary they took 15-17 minutes (Figure 4A). Under these conditions, it was necessary to increase the chart recorder speed from 1 cm/min to 3 cm/min (Figure 4C) or 6 cm/min (Figure 4D) in order to adequately visualize the resolution of histones which passed the detector between seven and eight minutes. This capillary size appeared to give the best resolution of the three examined, so we moved on to investigate other operational parameters in this capillary.

## pH of the Electrophoresis Buffer

All the previous experiments were performed at pH 2.5. To determine if this pH was the optimum condition for resolving the histones, the pH was varied in the 35 cm x 50 µm capillary from pH 2.0 to pH 5.0 (Figure 5). We found that histones ran the fastest at pH 2.0 (Figure 5A) and the slowest at pH 3.5 (Figure 5E). The best resolution was obtained at pH 2.5 (Figure 5B). Higher pH runs produced greater overlapping fractions and above pH 3.5 the electropherogram became a broad unresolved smear. Thus, pH 2.5 appears to be the optimum condition for this set of proteins.

## Identification of the Histone Fractions

Our first attempt to identify the individual histone fractions in the electropherograms was made while using the 50 cm x 50 µm capillary. Purified histone fractions (H1, H2A, H2B, H3, and H4) were obtained from CHO nuclei by HPLC (Figure 6A). Each of these five fractions were subjected to electrophoresis at pH 2.5 (Figure 6, C-G) and compared to a whole histone preparation (Figure 6B) and a 0.2% TFA/$H_2O$ blank, the solvent in which the samples were dissolved (Figure 6H). The fractions were found to be pure by HPCE, H1 and H2B migrating as a single peak, H2A migrating as four variants which are known to exist for this histone, histone H4 migrating as a single peak with a presumptive acetylated form following it, and H3 migrating as a single peak with its presumptive acetylated form following it. The fractions had migration times varying from 16 to 20 minutes (Figure 6, C-G). The whole histones had migration times from 17 to 20 minutes. We pointed out at the beginning of this paper that there was a 7.2% variability in the mobility of standards. This variability made it impossible to use the mobility of the individual histone fractions to identify the histone fractions in the whole histone electropherogram in Figure 6B.

In some experiments polylysine was added to samples as an internal standard in the hope that this mobility variation could be normalized as a ratio of the mobility of the fractions to that of polylysine (Figure 4D). However, this did not reduce the variability sufficiently to provide unequivocal assignments based on the individual fraction mobilities.

This problem was overcome by adding individual histone fractions to the whole histone sample (a method called "spiking"). Seven individual histone fractions were isolated by HPLC (Figure 7A). Each of these fractions was mixed with a separate sample of whole histone containing polylysine and loaded into a 35 cm x 50 µm capillary for 10 seconds at 10 kV. The samples were then subjected to electrophoresis at 10 kV for 10 minutes. Each faction in the electropherogram of the whole histone sample was then located by the increase in the fraction's peak height (Figure 7, B-H). From these data the order of mobility of each histone fraction was determined (Figure 8). From the fastest to the slowest, they are: (MHP) H3, H1 (major variant), H1 (minor variant), (LHP) H3, (MHP) H2A (major variant), (LHP) H2A, H4, H2B, (MHP) H2A (minor variant). Under these conditions all the

fractions move past the detector in a time span of one minute. Two unidentified fractions are detectable in the whole histone electropherogram, one between the (LHP) H2A and H4 and one between the H4 and H2B (Figure 8). The whole histone preparations also contain a cluster of unidentified high mobility minor components which precede the histones at seven minutes and a cluster of unidentified low mobility minor components which follow the H2B at eight minutes (Figure 8).

## Discussion

The experiments reported in this paper outline the development of the first application of HPCE to histone analysis. The optimum conditions that we have found to date involve sample electroinjection for 10 seconds at 10 kV and electrophoresis at 10 kV under acid conditions at pH 2.5 in 0.1 M phosphate buffer in a 50 μm x 35 cm coated capillary. Some of the criteria for high performance are met by this system. Small sample volumes (5 μl) can be used and electrophoresis is accomplished in a short time (9 minutes). In-capillary detection by UV absorption at 200 nm is possible provided the sample is concentrated enough (0.1 μg/μl). This permits the use of computers to perform on-line data analysis of the electropherograms. This procedure also fulfills our need to be able to perform analysis on the histones from a small number of cells, such as those acquired by flow cytometry cell sorting. For example, a 5μl sample at 0.1 μg/μl is the amount of histones we can obtain from 55,000 CHO cells.

The order in which the histone fractions migrate in HPCE is very different from that found in acid-urea polyacrylamide gel electrophoresis (8, 12) or in reversed-phase HPLC (Figures 4A, 5A). For this reason, HPCE brings the histone biochemist a new dimension for the qualitative analysis of histone samples. It also provides a quick method of quality control for evaluating the purity of isolated individual histone fractions. For example, we demonstrated that histone fractions isolated by reversed-phase HPLC were quite pure (Figure 6).

The apparent greater amount of H2B compared to the other histone fractions (Figure 8) may reflect heterogeneity in that band. When we added 0.12 M NaCl to the electrophoresis buffer and dropped the voltage to 7 kV this fraction broadened and appeared to develop a slower moving component as a trailing shoulder (Figure 3H). Further work will be required to determine what this extra component is. Also two other unknown components were observed in whole histone preparations, one preceding H4 and one following H4. These also need to be identified. Since they are readily separated by HPCE, we will be able to use this method as an assay for them. This will enable us to search chromatograms for their presence and then verify their purity during isolation.

The system has two problems that need to be dealt with further; that of narrowly spaced bands and broadly diffused bands. These problems are not restricted to the HPCE of histones, for we have encountered them with the

HPCE of plasma proteins as well. Since we do not encounter these problems when performing HPCE on small peptide standards, they appear to be problems associated strictly with macromolecular proteins. These problems interfere most seriously during quantitative analysis. For example, at the beginning of the Results Section we pointed out that we could obtain peak heights that varied only 3.0% using small peptide standards. This high precision has not been obtainable for histones or other proteins, however.

To improve the HPCE of histones, a system needs to be found that will separate the bands further apart in a shorter time. At present, all the histone fractions pass the detector in the time-span of one minute. The use of longer capillaries did not increase their resolution because it also increased the time taken to traverse the capillary thus broadening the bands due to diffusion. However, the close bands would not be such a problem if the protein bands were narrow like those of small peptides. Thus, the most serious problem to solve is that of band boadening.

It may be that the broader protein bands result from incomplete stacking of the proteins during the electroinjection step. It has been suggested that protein-protein interactions may cause such an interference. Our experience with polylysine argues against this reasoning, however. We found that polylysine, having a molecular weight of 13,000, produced a broad band like a protein (Figure 8). We would not expect this highly-positively charged macromolecule to produce significant intermolecular interactions. An alternative suggestion is that the hydration shell around the proteins may be much greater than that around the small peptides thus preventing the high degree of concentration from occuring during the stacking process. If this is the case, the HPCE of proteins may have a difficult time producing the narrow bands observed with peptides and we will be forced to find a system that produces bands spaced further apart in order to increase resolution.

Although the HPCE of proteins has several shortcomings at this early stage of development, capillary electrophoresis still offers the potential for further development into an important high performance analytical system for the histones. This report lays the groundwork and experience for further experiments to solve the resolution and quantification problems associated with the application of this new technology to protein biochemistry.

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Figure 1. HPCE of whole histones in a 20 cm x 25 μm coated capillary at various voltages. All samples were electroinjected 10 seconds at 10 kV. Electrophoresis was performed at 10 kV (A), 8 kV (B), 6 kV (C), 4 kV (D), 2kV (E), and 1 kV (F) in 0.1M phosphate buffer, pH 2.5 (A-F). Electrophoresis was also performed at 8 kV (G) 4 kV (H), and 2 kV (I) in the same buffer containing 0.12 M NaCl (G-I). Resolution was monitored at 200 nm with a detector response time of 1 sec and a chart speed of 1 cm/min.

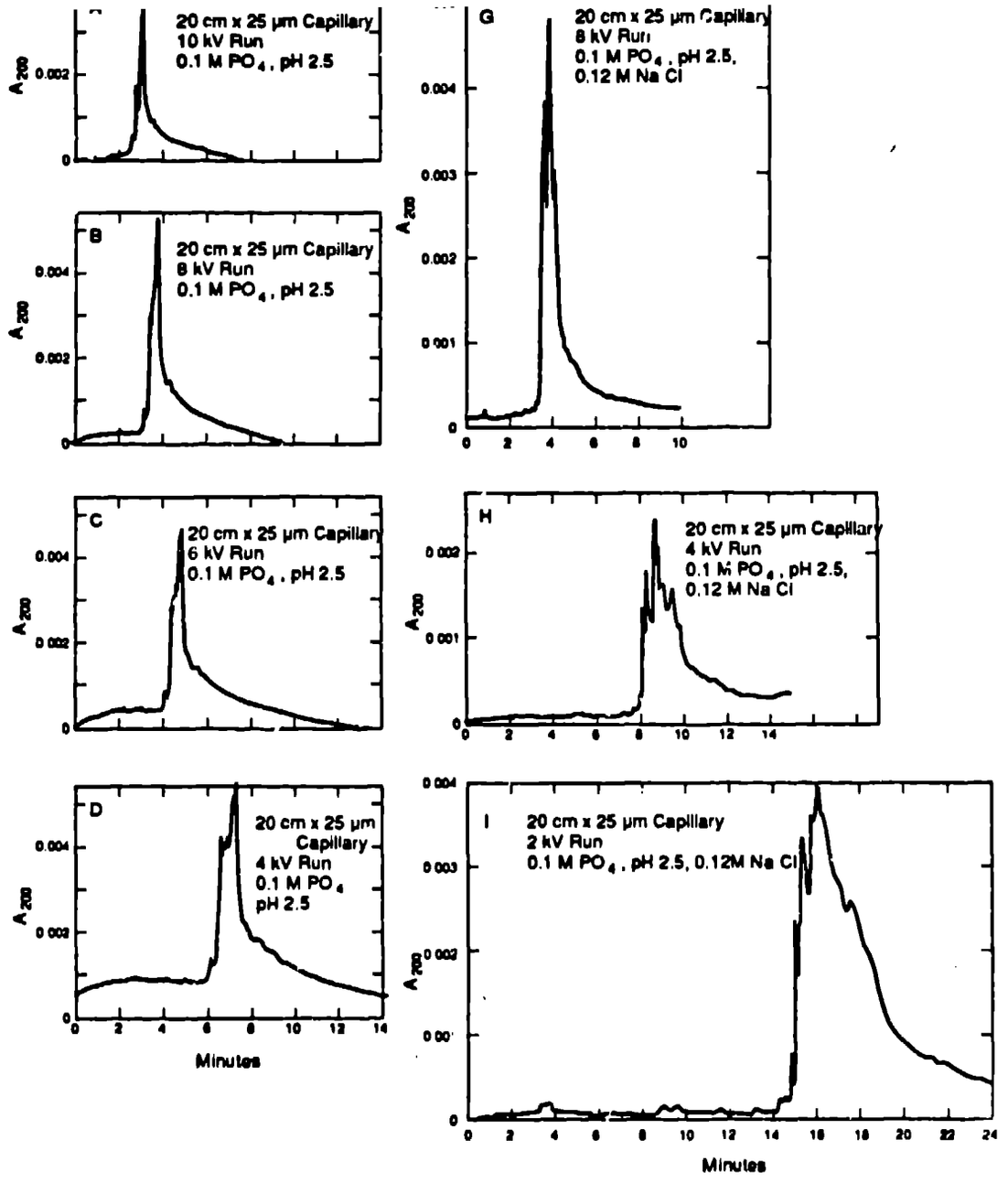

A
20 cm x 25 µm Capillary
10 kV Run
0.1 M PO4, pH 2.5
B
20 cm x 25 µm Capillary
8 kV Run
0.1 M PO4, pH 2.5
C
20 cm x 25 µm Capillary
6 kV Run
0.1 M PO4, pH 2.5
D
20 cm x 25 µm Capillary
4 kV Run
0.1 M PO4
pH 2.5
Minutes
E
20 cm x 25 µm Capillary
2 kV Run
0.1 M PO4, pH 2.5
F
20 cm x 25 µm Capillary
1 kV Run
0.1 M PO4, pH 2.5
G
20 cm x 25 µm Capillary
8 kV Run
0.1 M PO4, pH 2.5,
0.12 M Na Cl
H
20 cm x 25 µm Capillary
4 kV Run
0.1 M PO4, pH 2.5,
0.12 M Na Cl
I
20 cm x 25 µm Capillary
2 kV Run
0.1 M PO4, pH 2.5, 0.12M Na Cl
Minutes
A200

Figure 2. HPCE of whole histones in a 50 cm x 50 μm coated capillary at various voltages. All samples were electroinjected 10 sec at 10 kV. Electrophoresis was performed at 4 kV (A), 6 kV (B), 8 kV (C), and 10 kV (D) in 0.1 M phosphate buffer, pH 2.5. Resolution was monitored as in Figure 1.

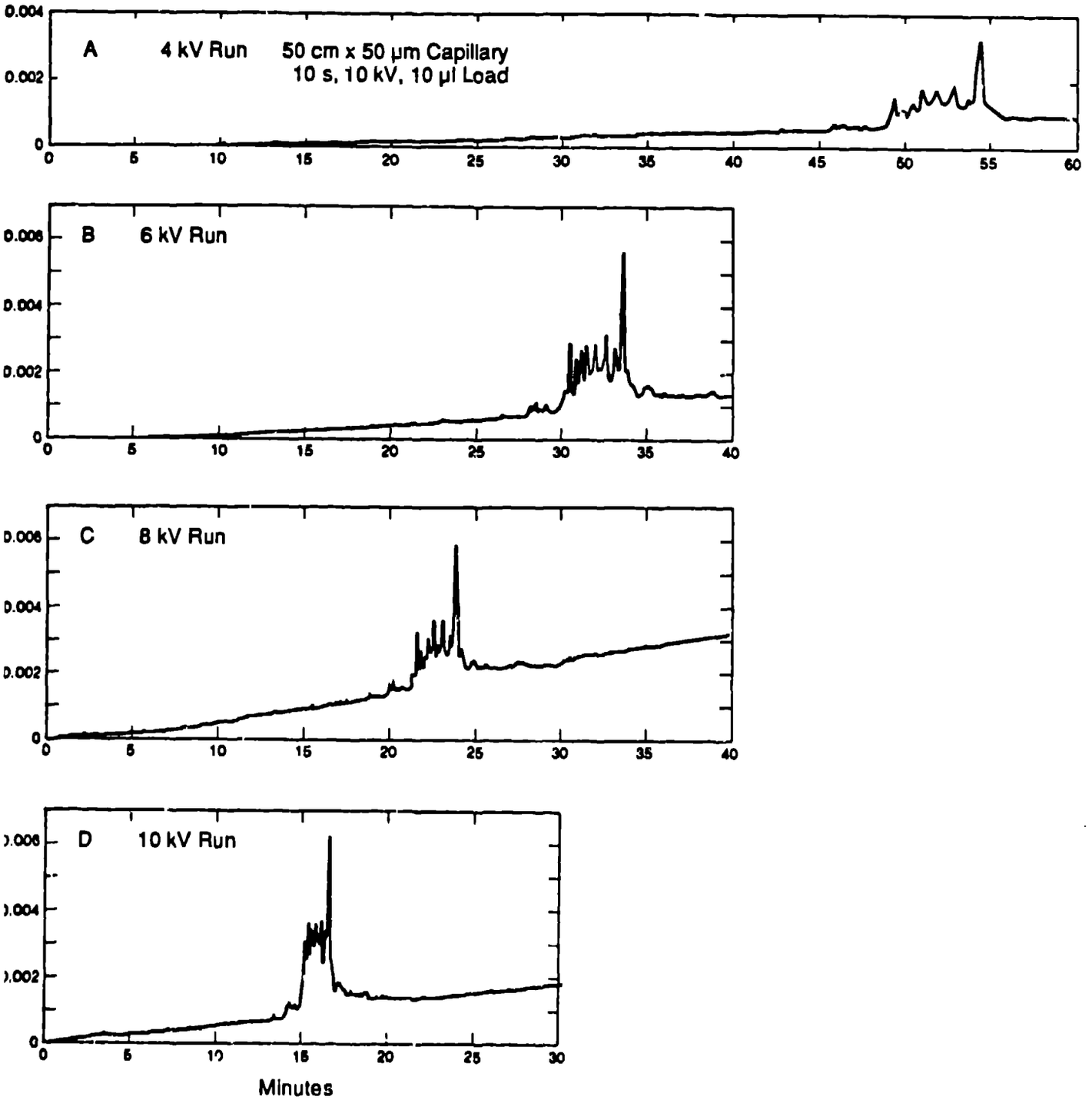

A
4 kV Run
50 cm x 50 µm Capillary
10 s, 10 kV, 10 µl Load
B
6 kV Run
C
8 kV Run
D
10 kV Run
Minutes

Figure 3. HPCE of whole histones in a 50 cm x 50 µm coated capillary at various NaCl concentrations and at various voltages. All samples were electroinjected 10 sec at 10 kV. Electrophoresis was performed at 10 kV (A-D) in 0.1 M phosphate buffer, pH 2.5 containing 0 M NaCl (A), 0.06 M NaCl (B), 0.09 M NaCl (C), and 0.12 M NaCl (D). Electrophoresis was also performed in 0.1 M phosphate buffer, pH 2.5 containing 0.12 M NaCl (E-H) at 10 kV (E), 9 kV (F), 8 kV (G), and 7 kV (H). Resolution was monitored as in Figure 1.

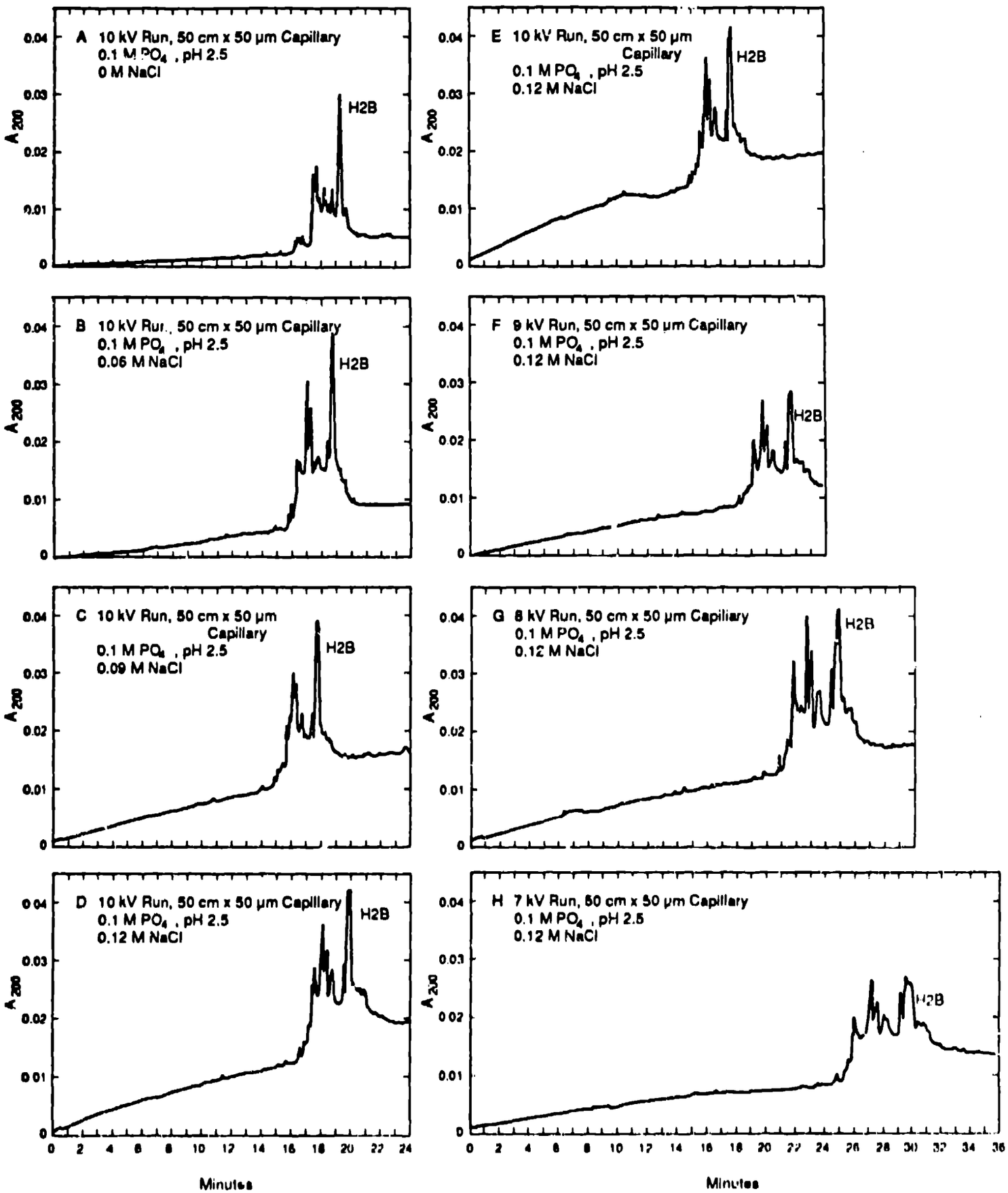

A 10 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0 M NaCl
H2B
B 10 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.06 M NaCl
H2B
C 10 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.09 M NaCl
H2B
D 10 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.12 M NaCl
H2B
E 10 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.12 M NaCl
H2B
F 9 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.12 M NaCl
H2B
G 8 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.12 M NaCl
H2B
H 7 kV Run, 50 cm x 50 µm Capillary
0.1 M PO4 , pH 2.5
0.12 M NaCl
H2B
A200
Minutes
Minutes

Figure 4. HPCE of whole histones in a 35 cm x 50 μm coated capillary. All samples were electroinjected 10 sec at 10 kV and electrophoresed at 10 kV. A comparison is shown between electrophoresis in a 50 cm x 50 μm capillary (A) and a 35 cm x 50 μm capillary (B). Resolution was monitored as in Figure 1 except that the chart speed of 1 cm/min (B) was increased to 3 cm/min (C), and 6 cm/min (D) in order to facilitate visualization of the resolution of the closely spaced bands in the 35 cm x 50 μm capillary.

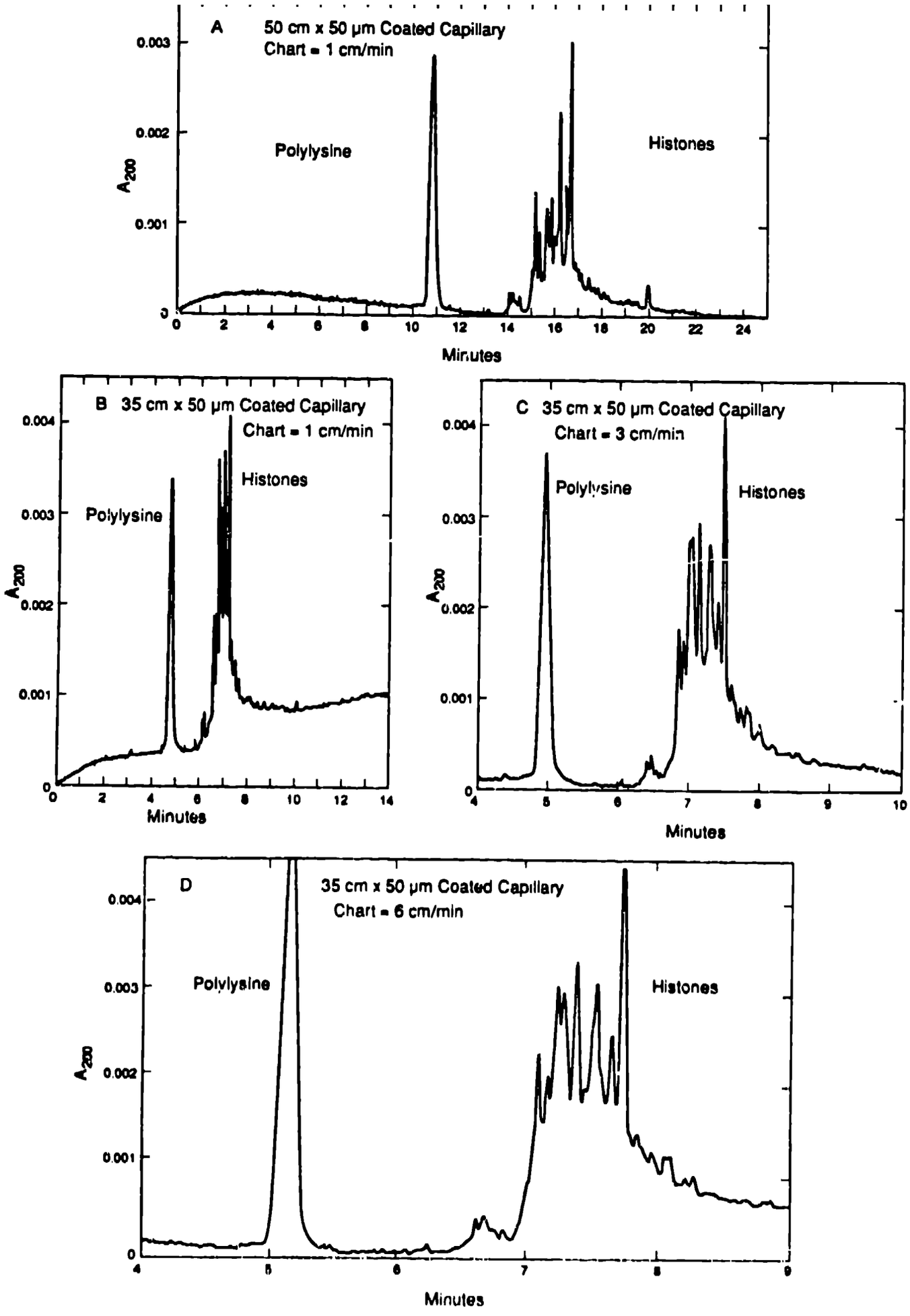

A
50 cm x 50 µm Coated Capillary
Chart = 1 cm/min
Polylysine
Histones
A200
0.003
0.002
0.001
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Minutes
B 35 cm x 50 µm Coated Capillary
Chart = 1 cm/min
Polylysine
Histones
A200
0.004
0.003
0.002
0.001
0
0 2 4 6 8 10 12 14
Minutes
C 35 cm x 50 µm Coated Capillary
Chart = 3 cm/min
Polylysine
Histones
A200
0.004
0.003
0.002
0.001
0
4 5 6 7 8 9 10
Minutes
D
35 cm x 50 µm Coated Capillary
Chart = 6 cm/min
Polylysine
Histones
A200
0.004
0.003
0.002
0.001
0
4 5 6 7 8 9
Minutes

Figure 5. HPCE of whole histones in a 35 cm x 50 μm coated capillary at various pH values. All samples were electroinjected 10 sec at 10 kV. Electrophoresis was performed in 0.1 M phosphate buffer at pH 2.0 (A), pH 2.5 (B), pH 2.75 (C), pH 3.0 (D) and pH 3.5 (E). Resolution was monitored at a chart speed of 3 cm/min.

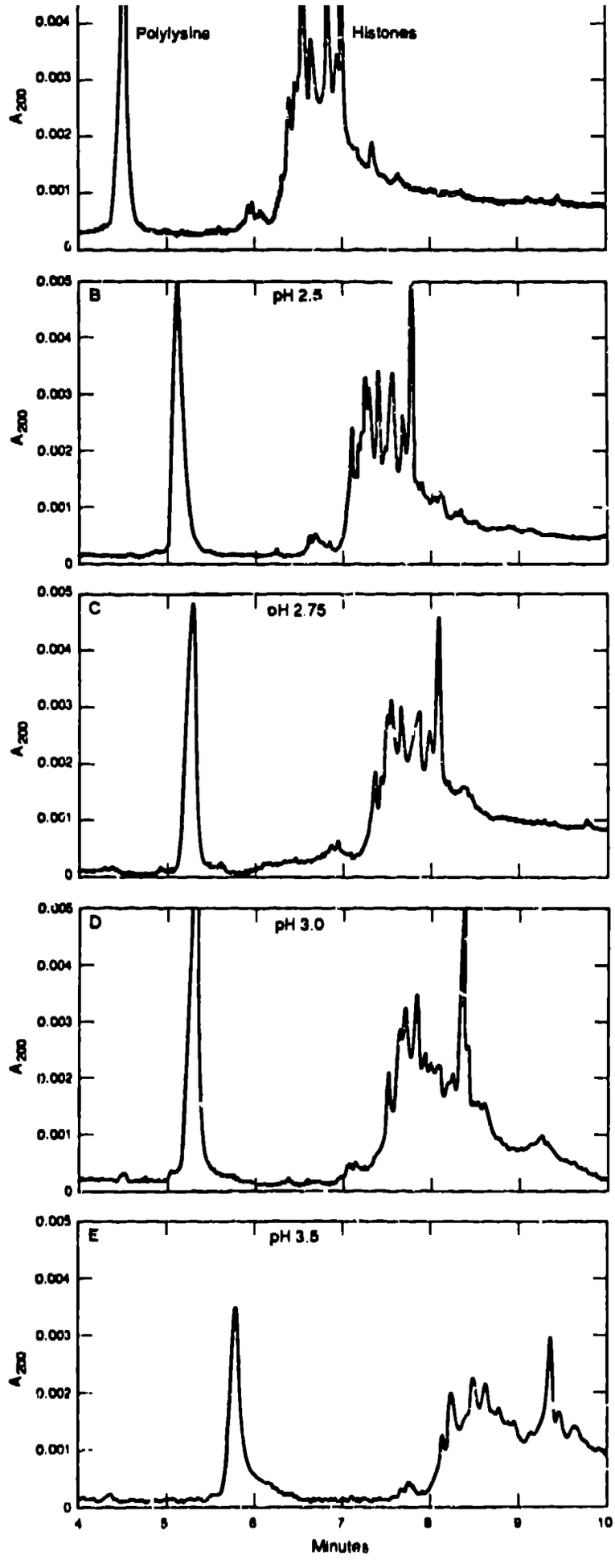

Polylysine
Histones
B
pH 2.5
C
pH 2.75
D
pH 3.0
E
pH 3.5
A200
0.004
0.003
0.002
0.001
0
0.005
4
5
6
7
8
9
10
Minutes

Figure 6. HPCE of histone fractions in a 50 cm x 50 μm coated capillary. The histones of CHO nuclei were fractionated into the five histone classes (H1, H2B, H2A, H4, and H3) by reversed-phase HPLC (A). Whole unfractionated histones (B), H1 (C), H2B (D), H2A (E), H4 (F), H3 (G), and a 0.2% TFA blank solution in which these histones were dissolved (H) were subjected to HPCE in 0.1 M phosphate buffer, pH 2.5 at 10 kV. Resolution was monitored as in Figure 2.

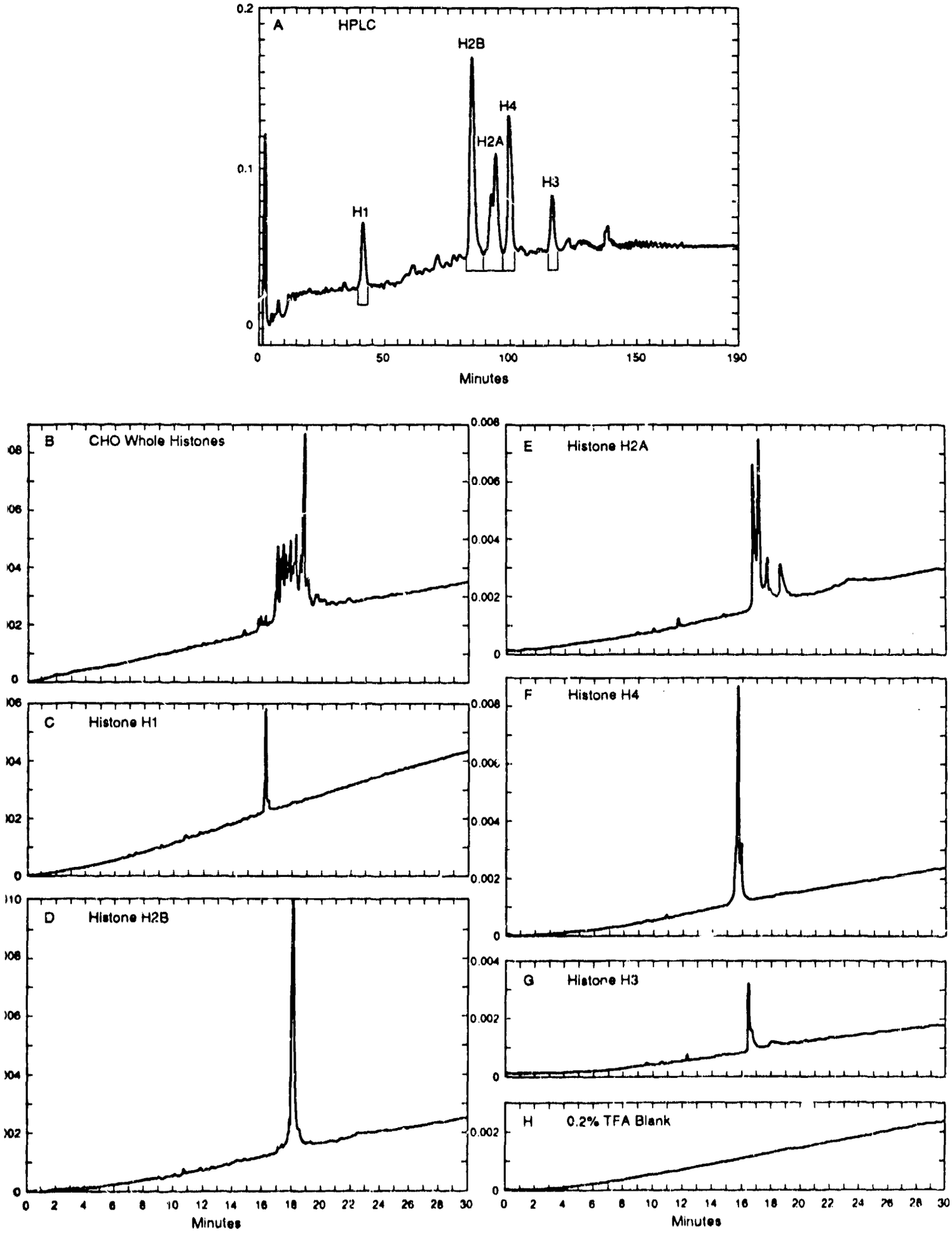

A HPLC
H2B
H4
H2A
H3
H1
0.2
0.1
0
0
50
100
150
190
Minutes
B CHO Whole Histones
C Histone H1
D Histone H2B
E Histone H2A
F Histone H4
G Histone H3
H 0.2% TFA Blank
0.006
0.004
0.002
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Minutes

Figure 7. HPCE of whole histones "spiked" with individual histone fractions using a 35 cm x 50 μm coated capillary. The histones of CHO nuclei were fractionated into the five histone classes (H1, H2B, H2A, H4, and H3) and two variants each of H2A and H3 which are indicated as the less hydrophobic (LHP) and more hydrophobic (MHP) forms (A). A sample of each of these fractions was evaporated to dryness and added to a separate whole histone preparation along with polylysine (an internal standard) and subjected to HPCE in 0.1 M phosphate buffer, pH 2.5 at 10 kV (B-H). Resolution was monitored at a chart speed of 6 cm/min. Histone H1 (B) H2B (C), LHP-H2A (D), MHP-H2A (E), H4 (F), LHP-H3 (G), and MHP-H3 (H) were identified in the whole histone electropherograms by the increase in the peak height of an individual component.

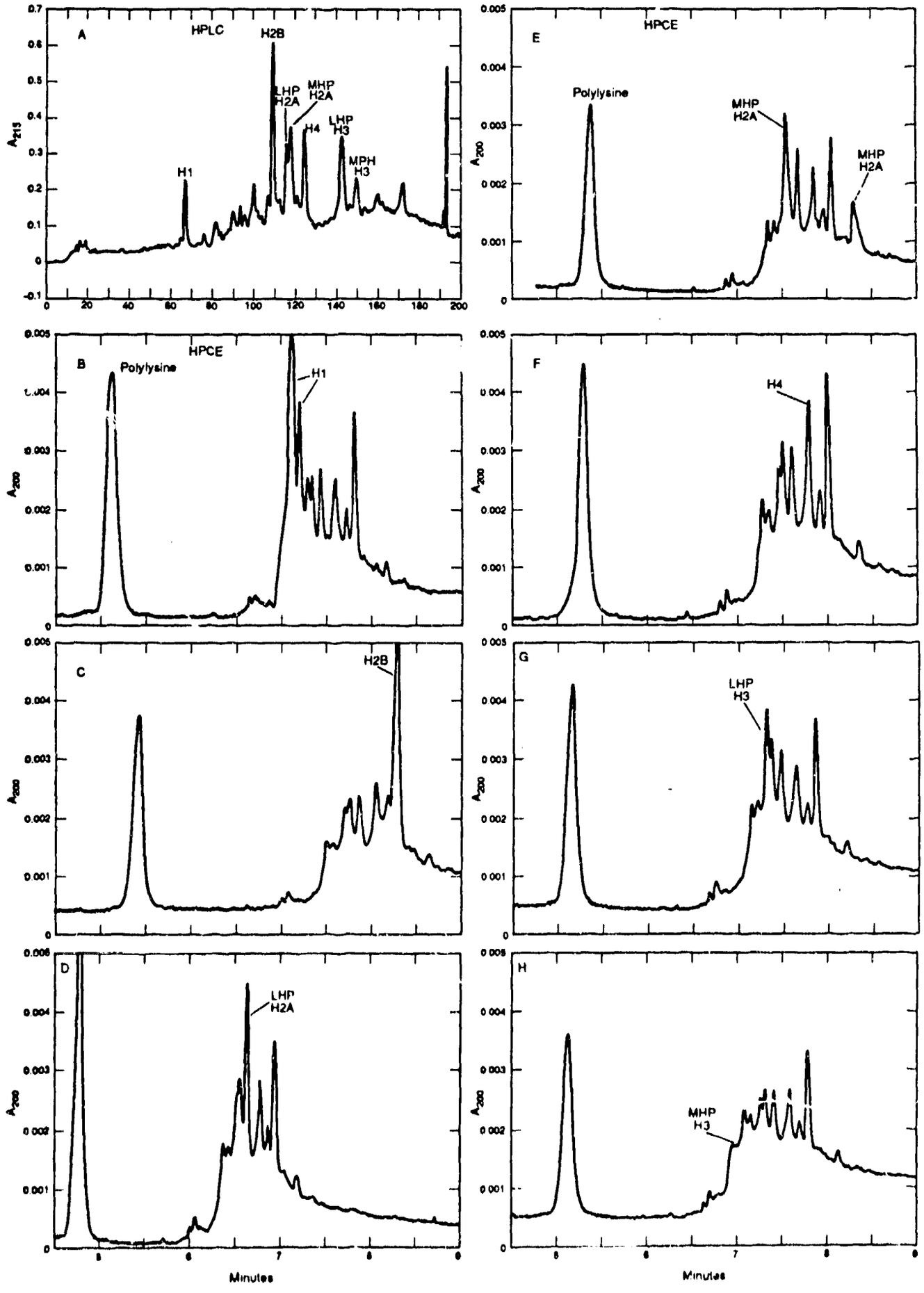
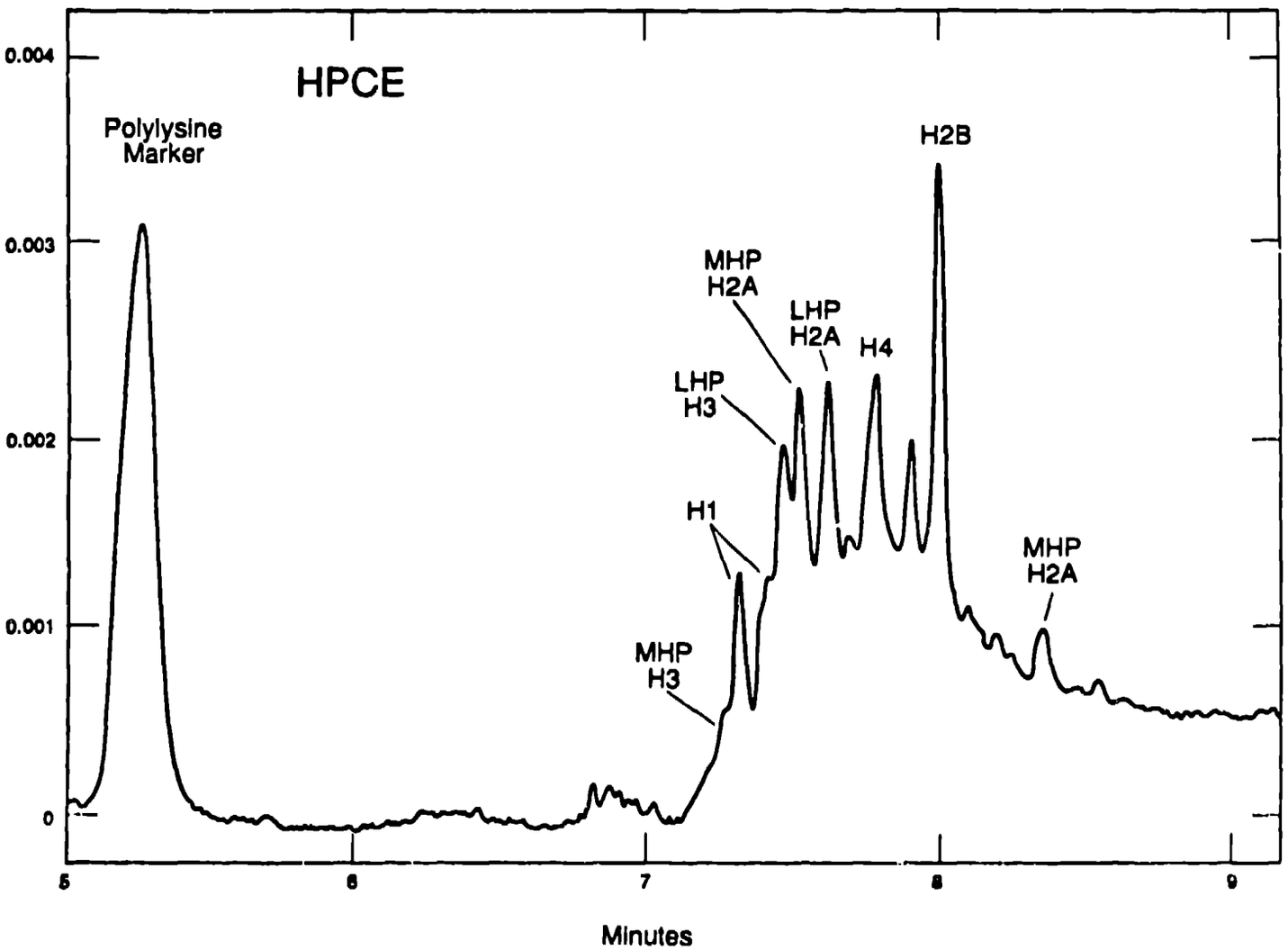

A
HPLC
H2B
LHP H2A
MHP H2A
H4
LHP H3
MPH H3
H1
$A_{215}$
B
HPCE
Polylysine
H1
$A_{200}$
C
H2B
D
LHP H2A
Minutes
E
HPCE
Polylysine
MHP H2A
MHP H2A
F
H4
G
LHP H3
H
MHP H3
Minutes

Figure 8. Assignment to histone fractions to the HPCE electropherogram of a whole histone sample. HPCE was performed in a 35 cm x 50 µm coated capillary. The sample was dissolved in 0.2% TFA and electroinjected 10 seconds at 10 kV. Electrophoresis was performed at 10 kV in 0.1 M phosphate buffer, pH 2.5. Resolution was monitored at 200 nm with a detector response time of 1 sec and a chart speed of 6 cm/min. Polylysine was added to the sample as an internal mobility standard.


HPCE
Polylysine Marker
H2B
MHP H2A
LHP H2A
H4
LHP H3
H1
MHP H2A
MHP H3
0.004
0.003
0.002
0.001
0
5
6
7
8
9
Minutes