# Poly(diallyldimethylammonium chloride) as a Cationic Coating for Capillary Electrophoresis

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## Abstract

**A novel cationic polymer coating that exhibits a fast anodal electroosmotic flow (EOF) has been developed for capillary electrophoresis. In the first approach, poly(diallyldimethylammonium chloride) is chemically bonded onto the interior capillary wall; in a second approach, the polymer is physically adsorbed onto the inner wall of the capillary. Capillaries modified by both approaches exhibit an anodal EOF in the pH range of 2.2–8.8, with a relatively pH-independent EOF (approximately $-5.5 \times 10^{-4}$ $cm^2/V$ s) over the pH range of 2.2–5.5. The application of the novel EOF-reversed phase is demonstrated by the improved separation of basic proteins and beta-adrenergic blocking drugs. Separation efficiencies ranging from 50,000 to 200,000 plates per meter are observed for proteins. The relative standard deviation (RSD) of migration times for multiple injections of test proteins is less than 0.65%. The reproducibility of capillary synthesis is 2.3% RSD for capillaries synthesized on three different days. The lifetimes of both the bonded and physically coated capillaries exceed 40 h of continuous use at 240 V/cm at pH 4.**

## Introduction

Capillary electrophoresis (CE) has become a routine analytical separation technique that offers advantages in terms of high efficiency, resolving power, and small sample volumes (1–3). However, a limitation of the routine application of CE to proteins and other macromolecules remains that of solute adsorption onto the capillary walls. This adsorption phenomena produces band-broadening and asymmetry, nonreproducible migration times, and, in the extreme case, low recovery or irreversible absorption of analytes onto the capillary walls. Many kinds of interactions may contribute to this phenomena, such as ionic, hydrogen bonding, van der Waals forces, hydrophobic interactions, etc. Ionic interactions play the most significant role in basic protein adsorption onto the bare fused-silica capillary surfaces, although others also contribute to the absorption problem. These interactions are sometimes observed with small molecules (e.g., beta blockers); however, the interactions increase with the molecular weight. Thus, the separation of proteins has been a challenge to CE practitioners.

Various approaches have been taken to eliminate or minimize such solute–wall interactions. Among them are (*a*) capillary surface modifications which include physical and chemical coatings (4–16, Q. Liu, F. Lin, and R.A. Hartwick. Free solution capillary electrophoretic separation of basic proteins and drugs using cationic polymer coated capillaries. *J. Liq. Chrom. & Rel. Technol.*, in press); (*b*) manipulation of background electrolytes, which includes the use of buffer additives to dynamically coat the capillary surface (6,17,18 Q. Liu, F. Lin, and R.A. Hartwick. Capillary electrophoretic separation of basic proteins and drugs using guaran as a buffer modifier. Submitted for publication in *Anal. Let.*) and control of buffer pH and ionic strength (11,12,19–21); and (*c*) use of an additional radial electric field (22). For bare fused-silica capillaries, operating at a pH greater than the pKa value of the sample (19) leads to mutual repulsion of anionic solutes and the ionized silanols, producing excellent efficiencies. Similarly, operating at a low pH (11,12) will ionize basic proteins, and on a coated (nonadsorptive) capillary, will produce suitably high efficiencies. A problem with neutral-coated capillaries, of course, is the loss of electroosmotic flow (EOF), which reduces the operating flexibility when configuring a separation. The use of high concentrations of salt in the background electrolyte (20) and the use of nonionic or zwitterionic buffers (21) have also proven effective in reducing the solute adsorption problem. The use of high ionic strength buffers, however, also increases the Joule heating, and is thus limited by a lower operating electric field strength or a restrained capillary's inner diameter dimensions. The use of an additional radial potential field is a powerful way to control the surface charges and to reduce the solute–wall interactions, but its routine use is hindered by its complex instrumentation. Of the various approaches taken, it was felt that either the physical adsorption or covalent bonding of cationic groups to the capillary would offer significant advantages to the separation of basic solutes. First, when operating at pH values less than the pH of the pro-

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tein, mutual ionic repulsion of the solute from the walls should occur, improving efficiency. Secondly, the charged capillary walls will produce a significant EOF, allowing separation configurations similar to those used in "conventional" CE using bare fused-silica capillaries at alkaline pH values. Finally, for a coating with either permanent cations or with groups with fairly high pKa values, the EOF might be expected to be relatively constant at pH values 2–3 units lower than the coating functional groups.

Physical adsorption of a modifier onto the capillary walls prior to a separation has proven successful in reducing the solute–wall interactions by physically blocking adsorption sites (typically silanol-type structures) on the capillary surface. Various substances have been applied in this mode, such as polyethyleneimine (4), polymethylglutamate (5), chitosan (6), polyarginine (7), poly(vinyl alcohol) (8), etc. All of these coatings have proven effective in reducing the solute adsorption.

Covalently bonding modifier substances onto the capillary walls has attracted great attention and has proven to be the most flexible method to minimize the solute adsorption problem. A number of covalently bonded surfaces have been developed, such as poly(acrylamide) (9,10), poly(vinylpyrrolidinone) (11), poly(ethylene glycol) (12), polymers containing quaternary ammonium groups (13–15), poly(2-aminoethyl methacrylate) (PAEM) (Q. Liu, F. Lin, and R.A. Hartwick. Free solution capillary electrophoretic separation of basic proteins and drugs using cationic polymer coated capillaries. *J. Liq. Chrom. & Rel. Technol.,* in press), maltose (16), and many others. Among the listed coatings, polyethyleneimine (PEI), quaternary ammonium groups, and PAEM reversed the charges and zeta potential on the capillary walls, producing a reversed EOF. However, these coatings exhibit low or pH-dependent EOFs, resulting in long analysis times and narrow pH operating ranges.

The use of buffer additives to achieve dynamic coating during the separation has also been demonstrated to be a useful method to reduce the basic solute absorption problem. Chitosan (6), fluorosurfactant (17), guaran (Q. Liu, F. Lin, and R.A. Hartwick. Capillary electrophoretic separation of basic proteins and drugs using guaran as a buffer modifier. Submitted for publication in *Anal. Let.*), poly(diallyldimethylammonium chloride) (PDADMAC) (18), and many other substances have been employed as dynamic reagents and added to the buffer solution.

For the separation of proteins, it is particularly important that the capillary is able to afford a wide pH range because a protein's charge and its migration characteristics strongly depend on the pH. Thus, the modified capillary surface should be stable over a fairly wide pH range.

In this paper, we reported a novel capillary modification method to produce capillaries that exhibit a fast and slightly pH-dependent anodal EOF over a pH range of approximately 2–6. The novel coating was used for the improvement of separations of basic proteins and beta-adrenergic blocking drugs. Efficiencies were typically from 50,000 to 200,000 plates per meter for basic proteins with a migration time relative standard deviation (RSD) of 0.65%.

## Experimental

### Instrumentation

All separations were performed on a laboratory-constructed CE instrument enclosed in a plexiglas box consisting of a high-performance CE high power supply (Spellman, Plainview, NY), a high-voltage power supply controller (Chamonix Industries, Johnson City, NY), a Spectra 100 ultraviolet (UV) detector (Thermal Separations, Freemont, CA), and an LCI 100 integrator (Perkin–Elmer, Norwalk, CT). Injection was accomplished hydrodynamically by elevating the sample reservoir to a height of approximately 5 cm above the other buffer reservoir for 5 s.

### Reagents and materials

A fused-silica capillary with a 75-µm i.d. was purchased from Polymicro Technology (Phoenix, AZ). Protein standards, ammonium persulfate, and N,N,N',N'-tetramethylethylenediamine (TEMED) were purchased from Sigma (St. Louis, MO). All beta blockers used in this study were gifts from Professor Roman Kaliszan (Medical Academy and University of Gdansk, Poland). Diallyldimethylammonium chloride (DADMAC) was obtained from Aldrich Chemicals (Milwaukee, WI). Methacryloxypropyltrimethoxysilane was obtained from Huls America, Inc. (Bristol, PA). All other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals were used as received with no further purification.

### Capillary modifications

*Covalently bonded capillaries*

Fused-silica capillaries were first conditioned with 1M sodium hydroxide for 1 h, then rinsed with deionized water for 30 min. A solution of 1% bifunctional compound, methacryloxypropyltrimethoxysilane (adjusted to pH 3.5 with acetic acid), was forced by pressure through the capillaries and allowed to react for 2 h at room temperature. The capillaries were then rinsed with water. A solution of an appropriate concentration of DADMAC monomer containing 1 µL/mL TEMED and approximately 1 mg/mL ammonium persulfate was then drawn into the capillaries and allowed to react with the bifunctional compound until polymerization was complete. The excess unbound polymer and monomer were removed from the capillary by rinsing with water and left to dry at 30–40°C overnight.

*Coated Capillaries*

Fused-silica capillaries were first conditioned with 1M sodium hydroxide for 1 h, then rinsed with deionized water for 30 min. A solution of an appropriate concentration of PDADMAC was drawn into the treated capillaries. After 5 min, the excess polymer was removed with a stream of nitrogen. The capillaries were then dried at 30–40°C overnight.

## Results and Discussion

### Capillary modifications

Figure 1 shows the schematic for the polymerization of DADMAC for covalently bonded capillaries. The reaction of

the bifunctional compound (methacryloxyltrimethoxysilane) with the inner surface of a fused-silica capillary was discussed by Hjerten (9). Ammonium persulfate was used as a free radical initiator, and TEMED served as the catalyst. The mechanism for polymerization of DADMAC was discussed by Boothe and coworkers (23). In their work, ethylenediaminetetraacetic acid (EDTA) was used as an additive in the polymerization system. It was observed that EDTA was not required in this work.

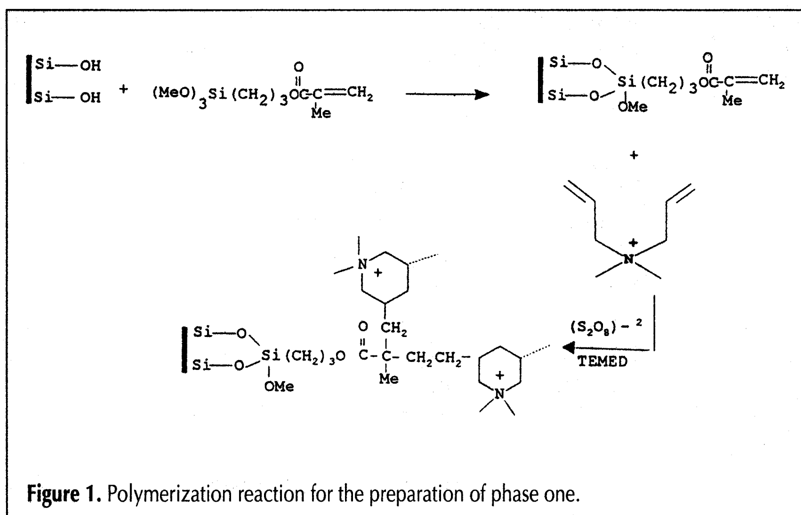


**Figure 1.** Polymerization reaction for the preparation of phase one.

## EOF

The optimization of separation parameters in CE routinely includes changing the strength and composition of the electrolyte. These changes can significantly affect the zeta potential of analytes, resulting in changes in their electrophoretic mobility. Varying electrolyte pH changes not only the solute zeta potential, but also the degree of dissociation of silanol groups on the interior wall of the bare capillary, indirectly altering the EOF. Therefore, in many instances, it can be a tedious and time-consuming process to optimize the separation conditions required when using bare fused-silica capillaries.

Ideally, a basic polymer (e.g., PDADMAC) coated on the interior wall of the capillary should exhibit a relatively pH-independent EOF over a large pH range. Figure 2 shows the observed relationship between the measured EOF and the buffer pH of a PDADMAC chemically modified capillary. As observed, the EOF remained relatively constant in the acidic pH range. At pH values greater than 5.5, the increased dissociation of the residual silanol groups most likely contributed to the decreased anodal EOF. Capillaries physically coated with PDADMAC exhibited a pH dependency nearly identical with that shown in Figure 2.

The highest EOF for the modified capillaries at pH 2 was determined to be $-5.9 \times 10^{-4}$ cm$^2$/V s, which, in absolute value, was approximately two thirds the maximum EOF of a bare-silica capillary. Previously reported cationic coatings (15) have only achieved anodal EOFs in the range of $-4 \times 10^{-4}$ cm$^2$/V s to $-2 \times 10^{-4}$ cm$^2$/V s. The fast anodal EOF of the new modified capillaries led to short separation times.

### Stability and reproducibility of coatings

To test the stability of the chemically bonded coatings, capillaries that were chemically modified with PDADMAC were used extensively for one week (applied electric field, approximately 240 V/cm, in different electrolytes for 20 h). Afterwards, capillaries were subjected to a continuous electric field of 240 V/cm for 20 h at pH 4.0 (33mM acetate buffer). The measured EOF value varied 5% during the period of testing, whereas the run-to-run RSD (six replicates) for EOF determinations remained less than 0.6%.

The stability of capillaries that were physically adsorbed with PDADMAC was measured by continuously applying an electric field of 250 V/cm (pH 3.0, 10mM phosphate buffer). The EOF for the tested capillary remained within approximately 5% for the first 20 h, falling to about 10% loss in flow after 40 h of continuous exposure, as shown in Figure 3.

To test the reproducibility of capillaries that were chemically coated with PDADMAC, the EOFs of capillaries modified on dif-

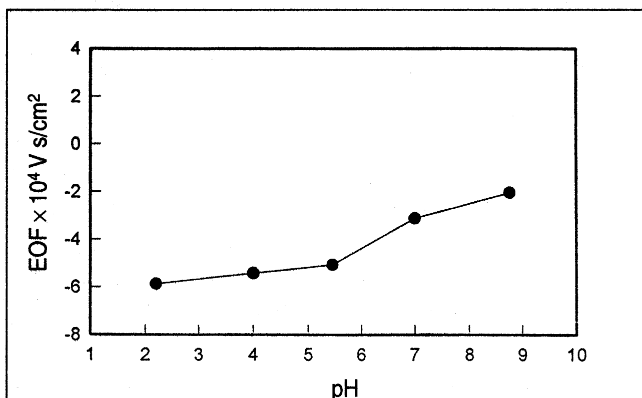


**Figure 2.** Plot of EOF in phase one as a function of run buffer pH. PDADMAC chemically coated capillary: 50.5 cm length (36.0 cm to detector), 75-µm i.d., 360-µm o.d.; hydrodynamic injection; phenol or acetone as the EOF marker; detection wavelength at 210 nm (phenol) or 205 nm (acetone); 15 kV applied voltage.

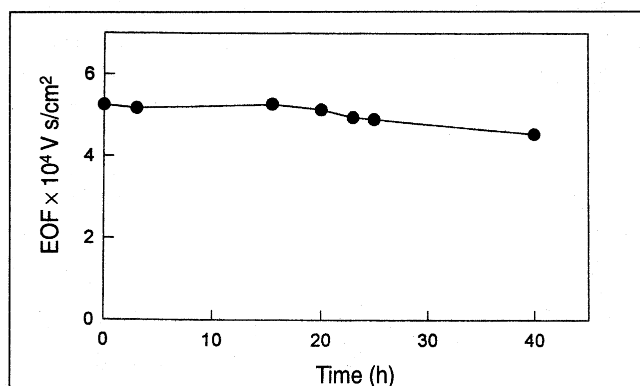


**Figure 3.** EOF of phase two as a function of high voltage application time. PDADMAC physically coated capillary: 48 cm total length (37 cm to detector), 75-mm i.d., 360-mm o.d; run buffer: 10mM phosphate, pH 3.0; 12 kV applied voltage; hydrodynamic injection by elevating the sample vials at the height of approximately 5 cm over the buffer vial for 5 s.

ferent days were measured at pH 5.5. The anodal EOFs for three independently prepared capillaries were $5.0 \times 10^{-4}$, $5.0 \times 10^{-4}$, and $4.8 \times 10^{-4}$ cm²/V s, respectively (RSD = 2.3%).

### Applications of the new coating phases

The fast anodal EOF phases should find practical applications for the separation of cationic macromolecules (e.g., basic proteins) and for various applications in micellar electrokinetic capillary chromatography (MEKC) using cationic micelles.

Figure 4 shows the CE separation of a mixture of seven proteins at pH 4.0 using the PDADMAC chemically modified capillary. The high EOF allowed for a shorter analysis time compared with the separation of a similar protein mixture on a reversed-EOF capillary reported in the literature (15). The positively charged interior wall of the modified capillary repelled basic proteins, greatly improving the symmetry, efficiency, and resolution of the peaks. The migration precision was excellent, exhibiting a migration time run-to-run RSD less than 0.65% for the protein samples (five runs). The efficiencies of these samples ranged from approximately 100,000 to 500,000 plates, normalized to a 100-cm effective length. While these efficiencies were not as high as those observed with anionic solutes, the fact that they could be separated at all without severe tailing and adsorption makes their absolute efficiencies less important.

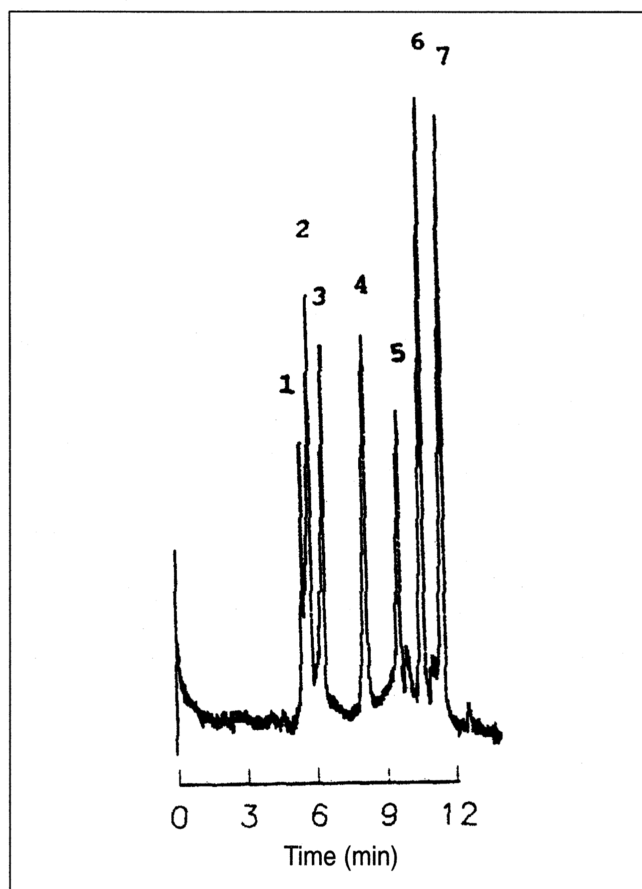


**Figure 4.** Electropherogram of a mixture of seven proteins. PDADMAC chemically coated capillary: 50.5 cm total length (36.0 cm to detector), 75-mm i.d., 360-mm o.d; run buffer: 33mM acetate, pH 4.0; 15 kV applied run voltage; detection wavelength: 210 nm; hydrodynamic injection. Solutes: 1, insulin; 2, α-lactalbumin; 3, β-lactoglobulin B; 4, ribonuclease A; 5, myoglobin; 6, cytochrome C; and 7, lysozyme. All protein concentrations were approximately 0.2 mg/mL.

The tertiary amino group on beta-adrenergic blocking drugs, also known as beta blockers, is cationic at normal operating pH ranges, and subsequently, this class of compounds generally exhibits poor peak efficiency and symmetry in conventional CE (unpublished data from this lab). As a result, it is usually difficult to separate beta blockers effectively using underivatized capillaries. Figure 5 shows the separation of five beta blockers using the PDADMAC chemically coated capillaries. Highly efficient (more than 100,000 plates per meter) and symmetrical peaks were obtained.

### Capillary efficiencies

Figure 6 shows the relationship between peak efficiency and applied voltage for α-lactalbumin on the PDADMAC chemically

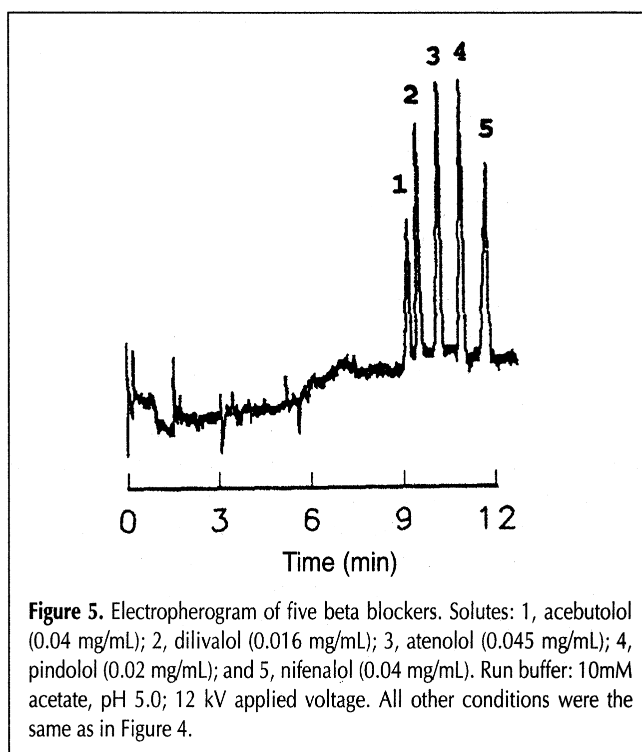


**Figure 5.** Electropherogram of five beta blockers. Solutes: 1, acebutolol (0.04 mg/mL); 2, dilivalol (0.016 mg/mL); 3, atenolol (0.045 mg/mL); 4, pindolol (0.02 mg/mL); and 5, nifenalol (0.04 mg/mL). Run buffer: 10mM acetate, pH 5.0; 12 kV applied voltage. All other conditions were the same as in Figure 4.

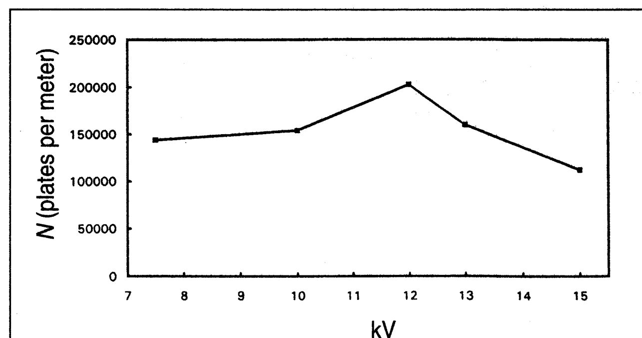


**Figure 6.** Plot of peak efficiency as a function of applied electric field strength for α-lactalbumin (0.2 mg/mL). PDADMAC chemically coated capillary: 75-mm i.d., 360-mm o.d., 50.5 cm total length (36.0 cm to detector). Run buffer: 33mM acetate, pH 4.0, hydrodynamic injection.

modified capillary. Efficiencies on the order of 200,000 plates per meter were obtained at an optimum field strength of 200 V/cm. In the presence of a weaker field, diffusion processes contributed to lower observed efficiencies. In contrast, higher field strengths led to reduced peak efficiencies as a result of joule heating.

## Conclusion

The applicability of capillaries that are either chemically bonded or physically coated with PDADMAC has been demonstrated. The EOF remained strong enough within the acidic pH range to permit rapid separations of basic proteins and beta blockers with good reproducibility. Although covalent bonding of capillary coatings would seem preferable in terms of durability, similar robustness was observed for both types of coatings in this particular case. It is summarized that the stability of the physically coated capillaries is due to the strong interactions of the multiple basic sites on the polymer with the silica wall, which is the typical source of problems when attempting to separate basic solutes. Thus, the polymer might be thought of as a localized, highly concentrated "competitor" for the silanol groups, which effectively shields the wall, while the excess ionized groups on the polymer contribute to the EOF.

Coated or bonded cationic polymers are quite useful due to the relative pH insensitivity of the resulting EOF. This constancy of flow is beneficial to separation method development because the ionic state of the solutes can be manipulated to improve a separation without the problems of simultaneously altering the capillary EOF. The polymer coating in itself also seemed to diminish hydrophobic adsorption, which, in addition to the ionic repulsion, produced acceptable efficiencies and peak symmetries for basic solutes. Another key advantage of these types of capillaries is the absence of high concentrations of additives to the samples, which reduces complexity and sources of impurities and permits sample recovery as well as interfacing to mass spectrometry.

## Acknowledgment

The authors thank Professor R. Kaliszan at the Medical Academy and University of Gdansk, Poland for his gift of the beta-adrenergic blocking drugs.

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Manuscript accepted October 24, 1996.