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Low Voltage Capillary Electrophoresis Using High Conductivity Agarose Nano-Platinum Composites

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Micro-channel based electrophoretic separation systems are commonly used in gene sequencing and analysis. A significant amount of research has focused on the development of fast and high resolution separation systems as part of miniaturized genotyping platforms. These devices are not suitable for field deployment due to their high voltage requirements for micro-channel based electrophoresis. In order to address this issue, we have developed and tested a new agarose gel doped with nanoplatinum for conducting capillary electrophoresis in micro capillaries at low electric field values. The platinum nanoparticles reduce the gel resistance of the agarose gels by 3–4 fold. We have further observed a faster movement of DNA band in this novel gel material allowing a reduction of the fractionating electric field within Poly dimethyl Siloxane (PDMS) capillaries. Impedance studies performed on this new gel material indicate a 1.34 times increase in the dielectric constant of the medium as a result of doping by nano-particles and a 37% reduction in the resistance. We believe that the higher conductivity of the medium and an increased dielectric constant of the medium result in increased ionic mobility within this material. We have also compared the stain mobility values in glass PDMS capillaries of a 1 kbp ladder, and a 750 bp dS DNA segment and have successfully obtained electrophoresis within the micro-channels at a lower electric field (25 V/cm).

Keywords: Capillary, Nanoplatinum, DNA, PDMS, Electrophoresis, Mobility.

RESEARCH ARTICLE

1. INTRODUCTION

Technologies to enable miniaturized DNA electrophoresis within fused silica capillaries (50–75 microns ID) using extremely high electric fields have been developed to achieve higher DNA separation speeds at better resolutions in micro-channel systems.[1] These techniques can be applied for micro-fluidic integrated gene analysis systems[2–3] demonstrating an overall reduction in size, reduced use of reagents, increased speed and accuracy of analysis. The field applications of such devices, however, are limited by power requirements imposed by highly resistive capillary columns. Generally speaking, DNA separation requires an electric field strength of 300–800 V/cm and an applied voltage of the order of 1–3 KV in electrophoresis

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applications.[4] Since speed and resolution of DNA separation have been the main focus of research in previous work, the ability to size fractionate at lower voltages has received less attention. Fu et al.[4] designed a separation channel partitioned into smaller separation zones using closely spaced electrode pairs. In this work, a traveling electric field was generated using sophisticated controls by sequential energizing of electrode pairs placed very close to each other. They could achieve a good separation at 300 V/cm (1.2 KV). Several other groups have demonstrated measurable flow control within microfluidic devices by using of 100–150 V/cm external electric field applied to closely-spaced multiple electrode architectures.[5–9] Although this technique is successful to electrophorese at low external voltages, it involves complex device architectures and instrumentation. To the best of our knowledge, no exploratory research has indicated the use of conducting separation matrices for low voltage capillary electrophoresis. A significant amount of work has already been reported in the area of gelled polymer electrolytes, although it has never been applied to capillary electrophoresis in micro-channels. Muszynska et al.[10] have demonstrated an overall increase in conductivity of a polyethylene oxide matrix using Al filler nanoparticles in concentration range of 0.25–5 wt%. Beyond this miniscule range, a decrease in conductivity is observed. Oh et al.[11] have reported about the increased conductivity of Polyethylene oxide (PEO), which cross-links with Polyethylene glycol dimethacrylate (PEGDMA) to form a gelled polymer electrolyte using lithium salts. Here, the ionic conductivity is enhanced with an increase in the lithium content. Morita et al.[12] have demonstrated an increase in the conductivity of the polymeric matrix (comprised of PEO-PMA and $Li(CF_3SO_2)_2N$) by addition of ceramic filler materials like α and γAl_2O_3 respectively. The increase in conductivity by addition of filler nanoparticles is attributed to an increase in the highly conductive amorphous phase on the grain matrix border the thickness of which determines the overall conductivity of the matrix by leading to an easy flow path for current which is concentrated in the percolation paths formed by the grain shells.[13] Hong et al.[14] have reported earlier a novel PDMS capillary filled with agarose matrix for DNA separation using an electric field of 71.4 V/cm. In our work, we have used agarose matrices doped with filler metal nanoparticles in PDMS micro-capillaries for size fractionating nucleic acids with electric fields as low as 25 V/cm. This is about a third of the values reported by Hong et al.[14] who have demonstrated agarose based capillary electrophoresis in micro-capillaries using a fractionating electric field of 71.4 V/cm. Impedance measurements performed on the new gel material shows a 1.34 times increase in the gel dielectric constant and a 37% resistivity decrease. We hypothesize that the mobility enhancement can be attributed to this increase in gel dielectric constant and reduction in resistance.

2. MATERIALS AND METHODS

2.1. Preparation of Agarose Gels with Filler Platinum Nanoparticles

The new gel material has been developed by doping agarose matrices with externally synthesized platinum nanoparticles.[15] The details of this work is reported in Ref. [16]. The doped agarose films show an ionic conductivity increase by a factor of five and a resultant increase in mobility of nucleic acids. Platinum nanoparticle hydrosols at two different concentrations were prepared by reducing an aqueous solution of potassium platinum (II) tetrachloride (K_2PtCl_4, 5.8 mM and 11.6 mM) with sodium borohydride (137.2 mM and 274.4 mM) in the presence of mercapto succinic acid (MSA, 26.7 mM and 53.4 mM) in a Schlenk flask under an argon atmosphere.[15] Any further solute loading beyond this concentration may lead to nanoparticle hydrosols which are less stable and coagulate with time. The agarose platinum mix was prepared by heating 2% agarose powder in a solution made up of 1× TAE buffer and nanoplatinum hydrosol mixed in a 1:4 volumetric ratio.

2.2. Impedance Analysis of Doped Gel Materials

The gel dielectric constants and resistance were measured using impedance spectroscopy techniques wherein a set of interdigitated micro-fabricated Pt electrodes over a silicon

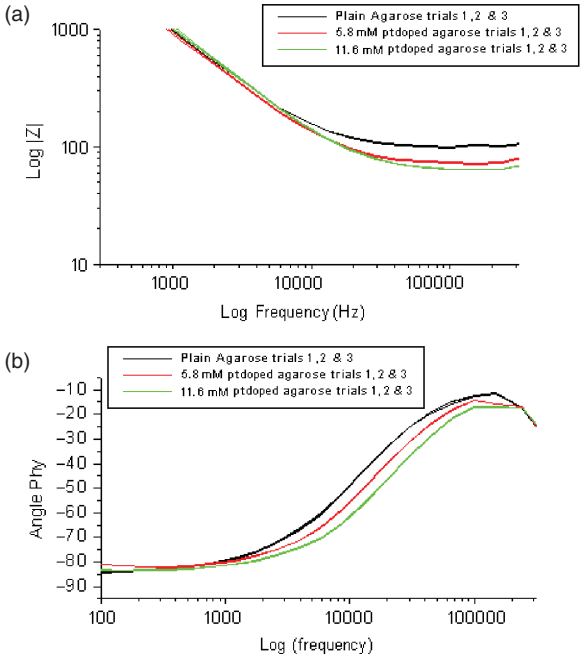

Fig. 1. (a) Logarithmic plot of modulus of impedance with frequency.[16] (b) Logarithmic plot of phase angle with frequency. Reprinted with permission from [16], S. Bhattacharya et al., Manuscript under review, Analytical Chemistry.

wafer (mounted with a fixed volume PDMS well) is used to determine the impedance of the medium.[15] The impedance values were acquired using a pair of micromanipulators to probe the electrodes and the impedance/phase angle values were digitally acquired over a frequency range 100 Hz–100 KHz (Figs. 1(a) and (b)). The data was fitted using a Matlab code with a parallel RC circuit model and the gel dielectric capacitance and resistance was iteratively determined (Figs. 2(a) and (b)).

2.3. Fabrication and Loading of the Glass/PDMS Microchannel Capillaries and DNA Mobility Studies

The micro-capillaries were realized in PDMS (poly dimethyl siloxane) and glass using standardized soft lithography and replica molding processes.[17] The separation channels molded in PDMS using a SU-8 mold were 225 micron (thick) × 1000 micron (wide) × 25.40 mm (long) and had a taper of 500 micron in an inch. The

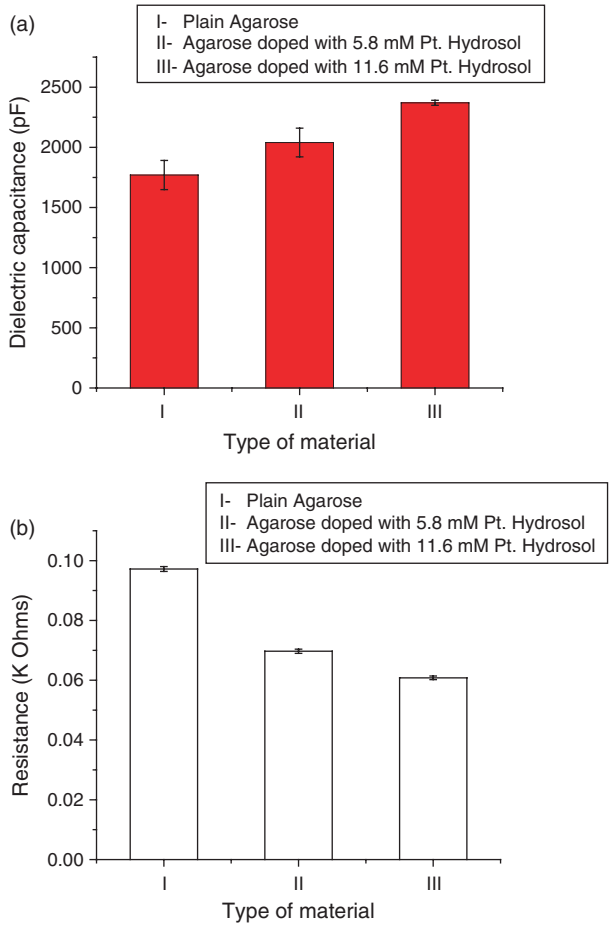

Fig. 2. (a) Comparison of gel dielectric capacitance for (I) Plain agarose, (II) Agarose doped with 5.8 mM Pt Hydrosol and (III) Agarose doped with 11.6 mM Pt Hydrosol. (b) Comparison of gel resistance for data obtained on I, II and III. (Data obtained by parameter extraction method).

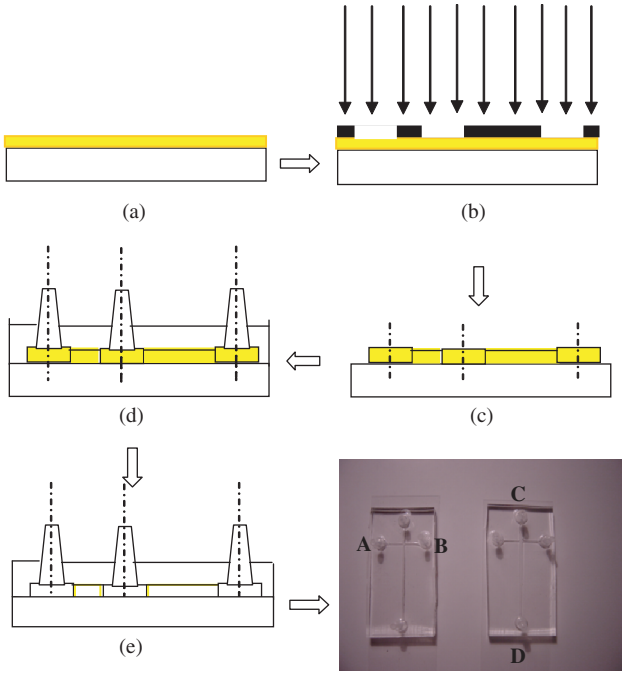

Fig. 3. Fabrication flow chart (a) spin SU8 photresist, (b) selectively expose using a black and white transparency mask printed at 3200 dpi resolution, (c) Develop and etch away the unexposed portions, (d) mount inlet/outlet ports and pour the PDMS (and heat cure at 85 deg. C for 40 mins.), (e) bond irreversibly to another piece of glass.

PDMS microchannels were irreversibly sealed to another clean glass substrate after both surfaces were exposed to oxygen plasma.[18] Figure 2 gives a fabrication flowchart for the device. The cross channel "A–B" was used to inject the DNA sample orthogonally into the main separation capillary "C–D" (refer Fig. 3) and was sealed permanently. Then, the molten agarose with and without platinum nanoparticles was injected separately in two different capillaries from the ends C and D thus burying the sample DNA trapped in between channels A–B and C–D as a plug. The gel is allowed to cool off within the capillary in this condition. Two platinum wires were inserted into the inlet/outlet ports of the separation capillary as electrodes and a high voltage DC power supply was connected to them. The chip was mounted on a UV trans-illuminator and a Kodak digital camera interfaced with a computer was used for imaging purposes. The voltage applied through the platinum wires size fractionated a 1 kbp DNA ladder (M/S promega) within a plain agarose filled capillary. The horizontal distance of traverse of the DNA stains inside the capillary was measured and the time of its movement was recorded. The one-dimensional mobility model for the DNA stains was applied in these capillaries. Simultaneously the mobilities were observed in standardized slab gels using the same setup and the stain mobility was studied in the agarose gel doped with highest platinum concentration (11.6 mM) and plain agarose samples. The mobilities are enhanced 1.5 folds in these new gel samples.

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3. RESULTS AND DISCUSSION

The detailed analysis on nanoplatinum synthesis and its characterization are reported in Ref. [16]. Here, we briefly provide the important highlights. TEM images showed spherical platinum nanoparticles with a mean diameter of 13.16 ± 3.93 nm. An UV-Vis absorbance spectra of the platinum hydrosol indicated a sharp peak in 216 nm region indicating the presence of platinum nanoparticles.[19] A thin film of platinum doped agarose gel spun on glass substrates was visualized further with a field emission scanning electron microscope (SEM). A well-distributed array of platinum nanoparticles (200–250 nm) buried inside the agarose matrix was visualized and characterized by electron dispersive spectroscopy. The high peak around 2.09 KeV obtained from the "Mα platinum lines" indicated a strong presence of platinum metal. A 527 bp DNA segment was successfully detected using standardized gel electrophoresis technique and an enhancement in its mobility was observed with addition of platinum nanoparticles. Figure S1 [supplementary material] shows images of agarose and platinum doped agarose (using the higher platinum concentration hydrosol) taken at 10, 15, 20 and 25 mins. at a DC potential of 200 V. Similar images taken at 150, 100 and 50 volts were also analyzed. The mobility values of the DNA stains as calculated using the one dimensional mobility equation ($\mu = v/E$)[20] are plotted with the electric fields (supplementary material, Fig. S2). An increase in the mobility of the DNA stain from 6.6E-5 $cm^2/V \cdot sec$ to 9.3E-5 $cm^2/V \cdot sec$(1.5 times) was observed in the platinum doped composite at 8 V/cm electric field using the standard gel electrophoresis method. At fields below this, the mobility of different DNA strands does not show any remarkable difference in both gels. The difference in mobility values increase at higher field values of 16 V/cm. To understand the mechanism of increased DNA mobility in this new gel material, we performed impedance spectroscopy on the different gel materials and found a decrease of the characteristic impedance in the range of 100 Hz–100 KHz. The corresponding range where the bulk solution resistance dominates (peak in the phase angle plot (Fig. 1(b))) was also shifted to higher frequency indicating its decrease which can be attributed to an increase in ionic concentration.[21] Figures 2(a) and (b) summarizes the gel dielectric capacitance and resistance by the curve fitting and parameter extraction methodology discussed earlier. The dielectric capacitance as can be seen from Figure 2(a) increases from $(1.77 \pm 0.12)E + 3$ pF to $(2.37 \pm 0.02)E + 3$ pF which is an increase of 1.34 times with increased platinum concentration. Simultaneously, the solution resistance goes down from $(9.72 \pm 0.08)E - 02$ K ohms to about $(6.08 \pm 0.06)E - 02$ K ohms which is a 37% decrease. (refer Fig. 2(b)) Naka et al.[22] have reported an increase in dielectric constant of poly(vinyl chloride) using poly(2-methyl-2-oxazoline) coated gold nanoparticles which are made with a similar reduction

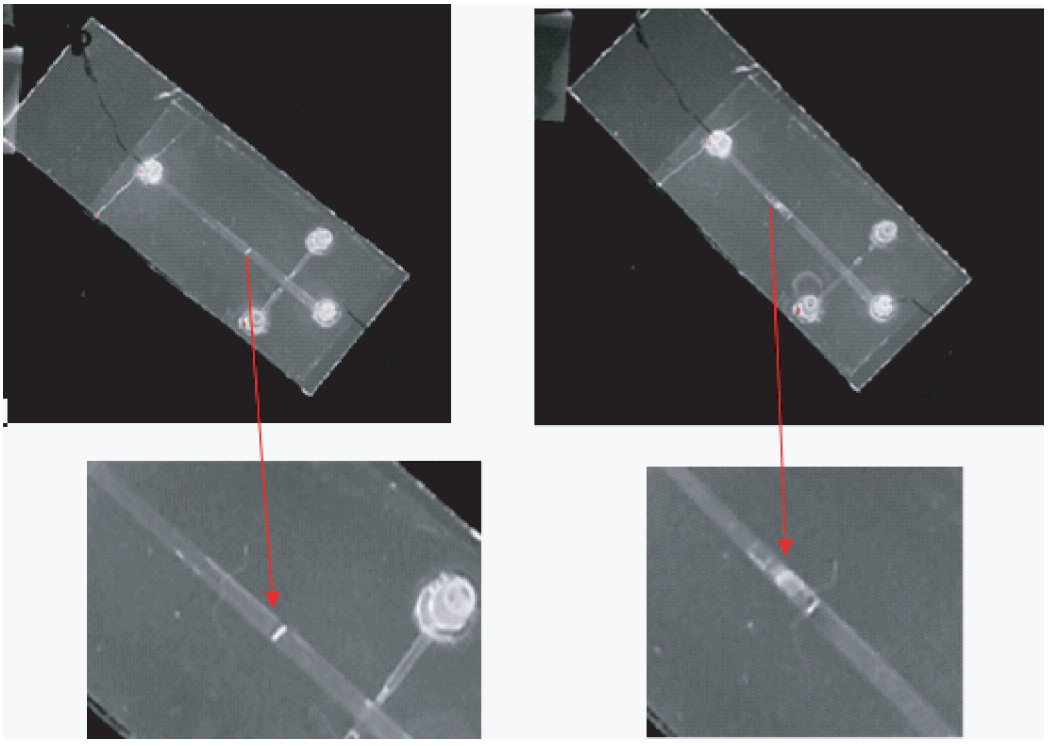

Fig. 4. (a) Movement of a 100–1000 bp gene marker captured in an UV detection setup after 25 secs. (b) Electrophoresis of gene marker between 45 and 50 secs (300 V DC is applied).

chemistry. NaCl and KCl salts were obtained in our platinum hydrosols[15] (causing increased ionic strength). This should have resulted in reduced stain mobilities as reported earlier by Stelwagen et al.[23] due to the screening of DNA molecules by an increase in counter-ions in the surrounding ion atmosphere. One of the main reasons for enhanced electrophoretic mobility can be attributed to the increased dielectric constant of the medium. The stain mobility in electrohphoretic gels is also described by the equation, $u = \varepsilon\varepsilon_0\zeta/\eta$ where, $u =$ mobility of the ion, ε is the dielectric constant of the medium, ζ is the zeta-potential of the ion and η is the viscosity of the medium.[24] The viscosity of the medium typically depends on the percentage of agarose, which remains unaltered in our case in both plain and doped agarose. The DNA molecule being highly negatively charged does not have an alteration in its zeta potential value because of a change in surrounding ion atmosphere.[20] Therefore, the increased mobility may be attributed to the enhancement of dielectric constant. The slope of the plot of mobility versus electric field doubles in case of the doped agarose showing possibility of capillary electrophoresis at lower electric field values. The newly formed gel samples were fractionated in micro-fabricated capillaries and their electrophorescing voltages were recorded. We used several different agarose concentrations ranging from 1.0 to 2.5% and found that the agarose took excess curing times at lower concentrations and had a greater chance of bubble formation disrupting the separation process. At higher concentrations (2.5%), the gelling time is very short as compared to the loading time and thus the capillary gets discontinuously filled and does not show any electrophoretic behavior. We found 1.5∼2% (by weight) loading to be effective in demonstrating separation repeatedly. Also, various operating voltages were used for checking the size separation and we found 300 V (Electric field = 85–100 V/cm) to be sufficient for electrophorescing the sample. We could not obtain any substantial electrophoresing effects below this voltage. A plug like movement of a 100–1000 bp gene marker was observed (Fig. 4(a), picture taken at the end of 25 secs) for approximately 45 secs. This was followed by the size fractionation immediately at the end of 50 secs (Fig. 4(b)). We performed the mobility analysis of the marker during the plug like behavior of the stain and calculated the mobility as $9.101E-4$ cm^2/V · sec. (Stain velocity = 0.078 cm/sec., Electric field = 85.7 V/cm). We also electrophoresed a 750 bp segment using similar capillaries and found out the mobilities to be $(7.84 \pm 0.2)E-4$ cm^2/V · sec. (Fig. 5(a)). This high mobility value of the 100–1000 bp ladder can be attributed to the contribution of the smaller segments of DNA present in the injected volume. We know that the shorter segments move faster than the longer segments in any conventional electrophoresis process.[20] In case of the 750 bp segment, the entire injected volume of the sample contains a higher segment length (750 bp). The ladder comprises of different sizes (most of them less than 750 bp) and thus the overall mobility increases (consider a weighted average of the individual stain mobilities of all segments 100–1000 bp) during the plug like motion. Finally, we have used platinum-doped agarose separation matrices to electrophore a 750 bp segment. The stain was formulated by using an external voltage of 65 V (corresponding to an electric field of 25 V/cm). The polarity of the electrodes was changed and the stain was further moved in the reverse direction at the same operating voltage (Figs. 5(b) and (c)). Our current research emphasizes on finding out the mobility enhancement within these micro-capillaries by using these filler gel composites. The new gel materials show similar separation resolution as agarose.

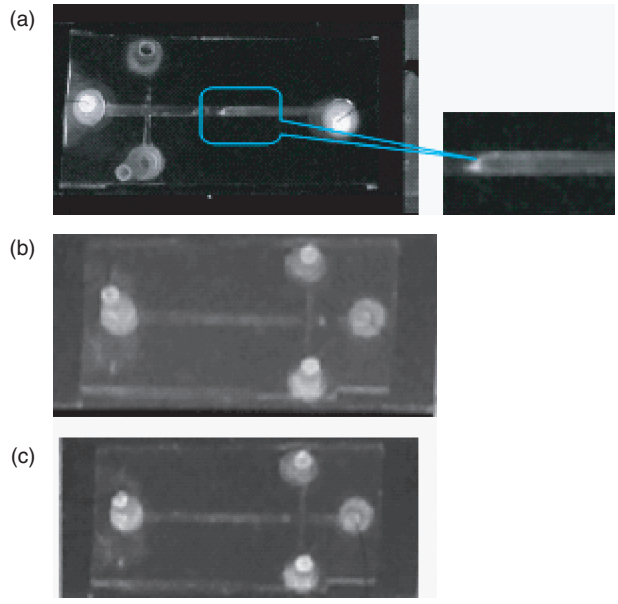

Fig. 5. Capillary Electrophoresis of a 750 bp nucleic acid segment in (a) in pure agarose (300 V), (b) in agarose with filler nano-particles. (65 V, Electric field = 25 V/cm), (c) in agarose with filler nanoparticles. (65 V, Electric field = 25 V/cm) with electrode polarity reversed.

4. CONCLUSION

We have developed a new nanoplatinum agarose composite material with enhanced sample mobility and increased conductivity. The sample mobility in the composite increases from $6.6E-5$ cm^2/V · sec to $9.3E-5$ cm^2/V · sec (1.5 times) at low (8 V/cm) field values. The slope of the mobility versus electric field characteristics increase by a factor of 2 whereas the conductivity of the new composite is found to increase 3–4 fold. We believe that the mobility increase comes from a decreased gel resistance (37%) and an increase in the dielectric constant of the medium (1.34 folds). This novel gel material has been used to perform low voltage capillary electrophoresis in glass PDMS micro-channels using 25 V/cm electric field and 65 V external voltage.

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SUPPLEMENTARY INFORMATION

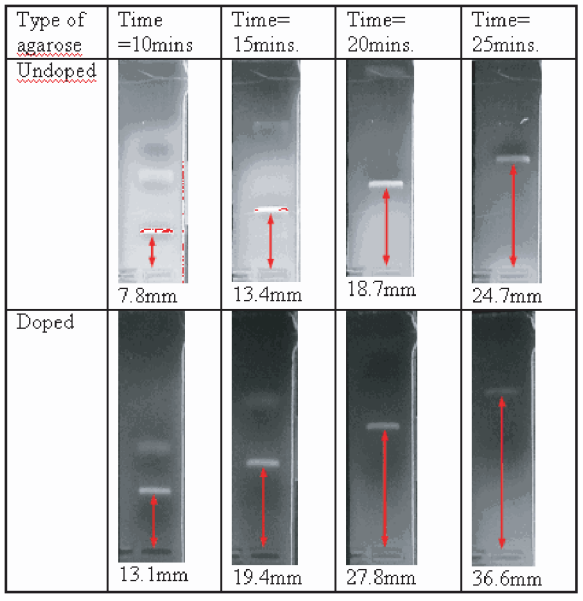

Fig. S1. Images of the fluorescent band in plain and platinum doped agarose taken at different times for 200 V applied voltage. Reprinted with permission from [16], S. Bhattacharya et al., Manuscript under review, Analytical Chemistry.

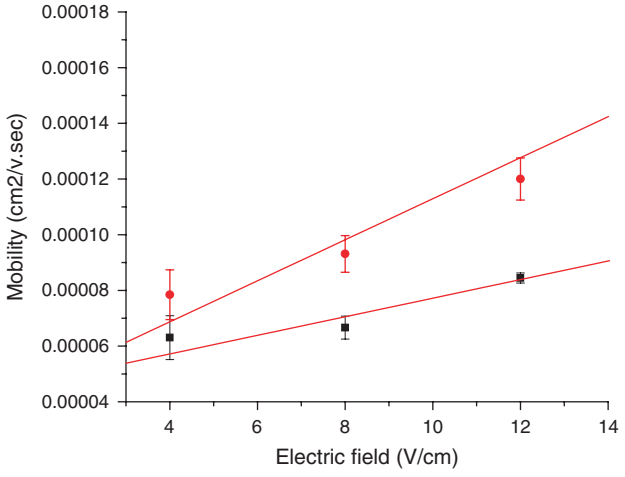

Fig. S2. Mobility plots for plain and doped agarose (closed red circles-platinum doped agarose, closed black squares-plain agarose). Reprinted with permission from [16], S. Bhattacharya et al., Manuscript under review, Analytical Chemistry.

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