THE EFFECT OF MATRIX ORDER IN DNA CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

The effect of order in separation media on DNA capillary zone electrophoresis is studied experimentally. A microfluidic technique based on colloidal self-assembly is employed to generate structures with different degrees of order. A model, SEM, and thermoporosimetry of the structures are employed to characterize media properties. The DNA mobility and dispersion coefficient is quantified in each structure through fluorescent detection. The result shows that higher ordered structures are favored because of greater mobility and less dispersion. This shows DNA separation can be improved further by introducing ordered materials in dense porous matrix.

KEYWORDS

DNA electrophoresis, order, silica particle, self-assembly

INTRODUCTION

We have developed colloidal self-assembly of crystalline arrays of nanoparticles within microfluidic channels as a powerful tool to fabricate nanoporous media, both highly ordered [1], and with systemically controlled degrees of disorder [2]. Separation media with different degrees of disorder are easily fabricated by doping one particle size with another using colloidal self-assembly (CSA). This approach allows study of the effect of order and disorder in nanoporous media on DNA separation. Two-dimensional asymmetric pulsed field electrophoresis (APE) of DNA molecules within ordered and disordered arrays has shown a non-monotonic dependence of the resolution on the media order, with an optimum for short-range ordered structures. [2]

Theory is contradictory regarding the influence of order in DNA electrophoresis, but agrees that the effect of order depends crucially on the separation mechanism. However, due to the difficulty in fabrication of structures with different degrees of order, there is a lack of experimental data to test models. With the advantages of CSA structures, this experimental study explores the effect of order in one-dimensional DNA capillary zone electrophoresis (CZE) systematically, lying in the Ogston regime instead of the reptation regime employed by APE. The degree of order is quantified as well as pore size distribution, and the two major indicators, electrophoretic mobility and dispersion coefficient, are investigated to identify the effect of matrix order.

EXPERIMENT

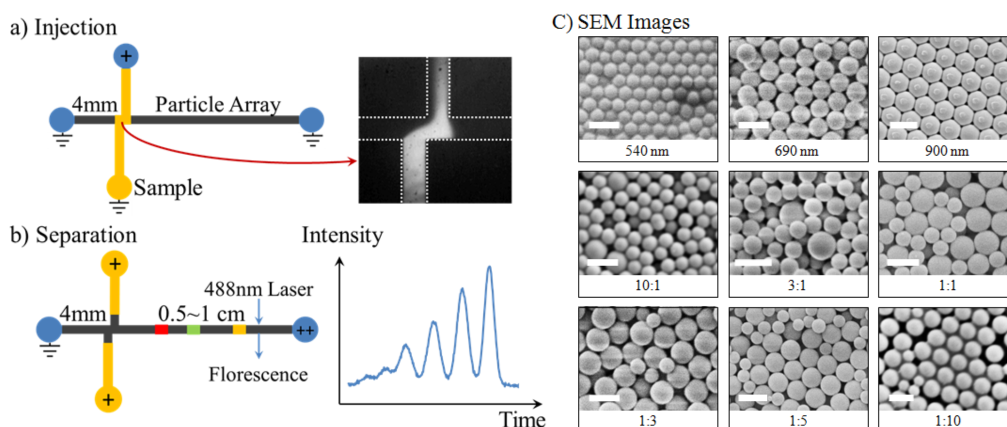

Figure 1. a)&b) A schematic view of DNA CZE microchip, channels are filled with self-assembled nanoparticle arrays. c) SEM images of monodisperse or binary packed CSA. Scale bars represent 1 μm.

DNA CZE was conducted in PDMS chips fabricated using a standard soft lithography technique, with a double T injector (Figure 1a,b). Aqueous suspensions of silica colloids (Bangs Laboratories, Fishers, IN) of 540, 690, and 900 nm diameter were used to form the self-assembled nanoparticle array inside the microchips. Monodisperse suspensions gave highly ordered separation media with polycrystalline, hexagonal close-packed structures. Binary mixtures of 540 and 900 nm particles yielded structures with degrees of disorder controlled by the mixing ratio notes as the volume fraction of 900 nm particles χ_{900} in total particle volume. Level of order was quantified by an orientational order parameter (ψ) calculated from SEM images (Figure 1c). Pore size distribution of structures was examined through a theoretical model, [3] and by thermoporosimetry of water's freezing point depression [4] using a differential scanning calorimeter (Q1000, TA Instruments), with a ramping rate of 0.05 °C/min.

A low DNA mass ladder (Invitrogen, Life Technologies) and 4 kbp and 10 kbp DNA (Fermentas, Thermo Fisher Scientific) were stained by YOYO-1 (Molecular Probes) with a dye-to-base ratio of 1:10. The fluorescence image shown in Figure 1a represents the junction of the injection channel and separation channel. Electric potentials were applied using platinum electrodes, generating an effective field of 18.6 V/cm in all experiments. DNA samples were

excited by a 488-nm argon ion laser beam, and the fluorescent emission was collected by a sensitive CCD camera (Astrovid) associated with a lab-built epifluorescence microscope composed of a 550DRLP dichroic mirror, 515 nm long-pass filter, and a 40x planachromat objective (0.6 N.A., LDN, Carl Zeiss). A 4x objective (0.1 N.A., Olympus) was used for imaging under low-magnification. Videos were captured by VirtualDub (1.9.11, Avery Lee) and analyzed by ImageJ (1.44p, NIH, USA).

RESULTS AND DISCUSSION

The variation of ψ was calculated with respect to χ_{900} of the binary packing (black squares in Figure 2a) forms a U shape, with a maximum disorder in structure near χ_{900}=0.5. In monodisperse packing (red circles in Figure 2a) the degree of order is relatively high, even though particle size variations and defects prevent achieving value of 1. The change of ψ with χ_{900} shows different degrees of disorder can be introduced and controlled systemically, where ψ provides a descriptive parameter of matrix order. The model developed by Dodds [3] was used to calculate the pore size, and the resulting average pore size is plotted against χ_{900} for the 540/900 nm in Figure 2a, increasing monotonically with the volume fraction of the larger particle, giving a(DM). The average pore radius a(DSC) and peak radius R(peak) of the pore size distribution measured by DSC are shown in Figure 2b&c, both increasing with a(DM). The standard deviation, kurtosis, and skewness of the logarithm distribution are all linearly correlated with a(DM). (Data not shown) All the descriptive parameters shown have a monotonic correlation with the modeled factor, a(DM), making it valid and expedient to regard Dodds' average pore size, as representative of this group of media properties regarding pore size distribution.

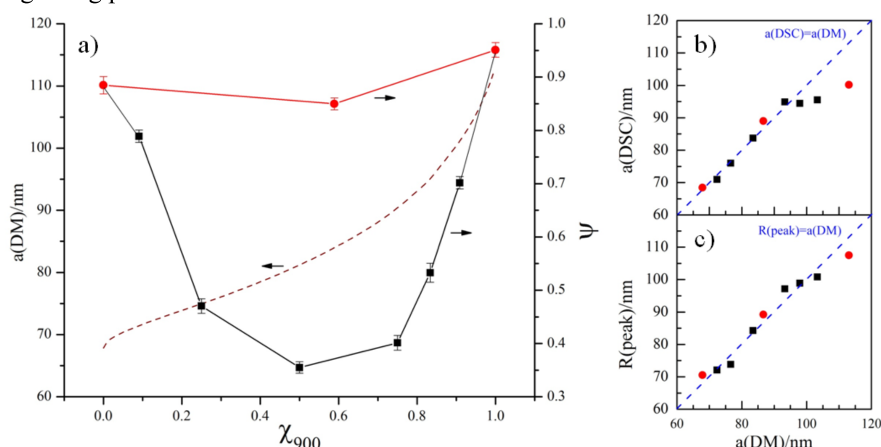

Figure 2. a) Average pore size a(DM) and orientational order parameter ψ calculated for each structure, and correlation between the b) average pore size a(DSC) and c) peak radius R(peak) measured by DSC with a(DM).

Another important factor of separation media is porosity. In Figure 3a, Dodds' model of porosity follows a slightly concave trend similar to the literature, but all data are consistently smaller than 0.26—the porosity of fcc or hcp close packing of uniform particles, because the model considers only the smallest pores, neglecting octahedral voids, for example. The porosity from the DSC measurement, however, is always larger than 0.3, indicating the presence of defects and perhaps a systemic error from expansion on freezing. While on different levels, both of the two traces of porosity are almost flat. This relative uniformity means that the binary mixing will affect the average porosity far less than it affects the degree of order.

The fraction of accessible volume f for DNA is plotted against a(DM) in Figure 3b. The f value from DSC is much larger than the one modeled for every DNA size, given the same reasons discussed before. All fractions are

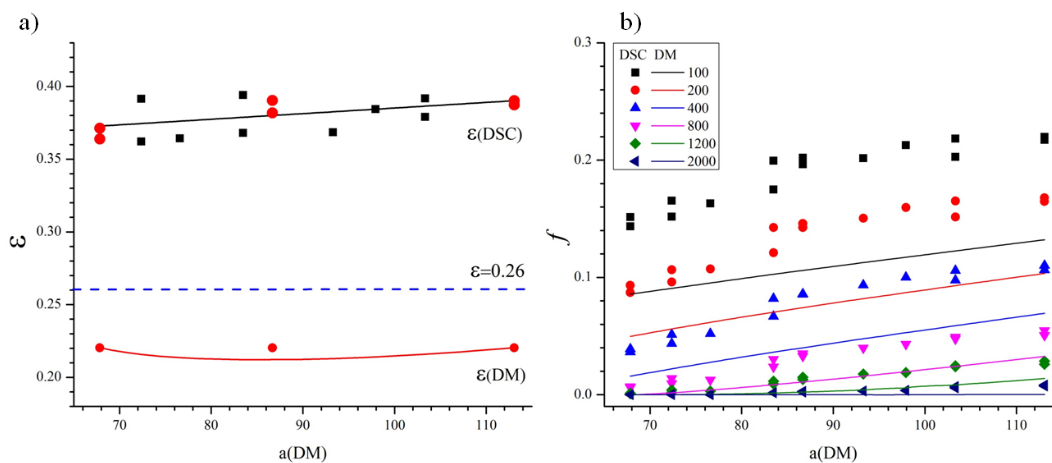

Figure 3. a) Porosity from DSC ε (DSC) and Dodd's model, ε(DM), red circles represent the monodisperse structures, the dashed line is the ideal porosity of fcc or hcp packing. b) Accessible volume fraction f from DSC (dots) and Dodds' model (lines), with different color for each DNA size.

smaller than about 0.2 in general, which means the packing, is much denser than in gels. The fraction for 4000 and 10000 bp is always zero. The accessible fraction increases for smaller DNA size and larger pore size, and is above zero up to 2000 bp of DNA size, the upper bound of the Ogston regime.

With DNA size, average pore size and matrix order as major variables, observed DNA mobility μ and dispersion coefficient D_E were analyzed by multivariable regression (Table 1). Data correspond to 4000 and 10000 bp DNA is not included, since the molecule it too large to in the Ogston regime. 100 bp data are also excluded because free solution mobility becomes size dependent for molecules below 200 bp. Both of the regressions are efficient and statistically significant at the 95% confidence level. The fitted free-solution mobility $\mu_0=(1.3_5\pm0.1_2)\times10^{-4}$ is comparable with literature.[5]

It is found that DNA size and average pore size of the media are the dominant variables, together attributing to 89.9% of the total variance of the mobility and 88.9% of the dispersion coefficient. The fraction of contributions from matrix order ψ to mobility and dispersion are 5.3% and 5.8% respectively, indicating the influential power of order. The positive coefficient shows mobility is higher when the structure is more ordered, in accord with Slater's simulation. [6] There is a decrease of dispersion as order (ψ) increases, since the slope is significantly negative.

Table 1. The regression model and results of mobility and dispersion coefficient.

$\log\mu = -k_1\frac{N^{k_2}}{a^{k_3}} + k_4\psi + \log\mu_0$		$\log D_E = k_1 a + k_2 N + k_3\psi + k_4$	
$k_1=(1.7_4\pm0.5_8)\times10^2$	F=61.28>F (95%)=2.28	$k_1=0.0141_4\pm0.0003_9$	F=585.18>F(95%)=2.28
$k_2=0.274\pm0.01_9$	$R^2=0.982$	$k_2=-(1.71_9\pm0.08_6)\times10^{-4}$	$R^2=0.931$
$k_3=1.7_0\pm0.1_2$	$R^2(N,a)=0.899$	$k_3=-0.22_5\pm0.02_6$	$R^2(N,a)=0.889$
$k_4=0.26_7\pm0.01_2$	$R^2(\psi)=0.053$	$k_4=-8.06_8\pm0.03_9$	$R^2(\psi)=0.058$

CONCLUSIONS

Our results demonstrate that, in addition to the well-known role of average pore size and molecular size as major determinants, the degree of order is also a considerable factor in DNA CZE in dense media. The electrophoretic mobility increases, while the dispersion coefficient decreases as the level of order goes up, holding other factors constant. Hence, in our study, coherent, ordered structures give more efficient DNA separation than random porous media, which agrees with our APE results.[2] Our observation is consistent with Slater's simulation in the Ogston regime showing the ordered structure has greater mobility,[6] and also support Locke's standpoint that the dispersion coefficient in porous media depends on the obstacle arrangement.[7] Our results contrast with the simulation of Patel and Shaqfeh [8] suggesting disorder was better for separation than order in sparse media. Since the effect of order is proved experimentally, this encouraging signal opens the door to improve electrophoresis under Ogston sieving mechanism further through tuning the media architecture towards greater levels of order in a dense porous matrix.

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