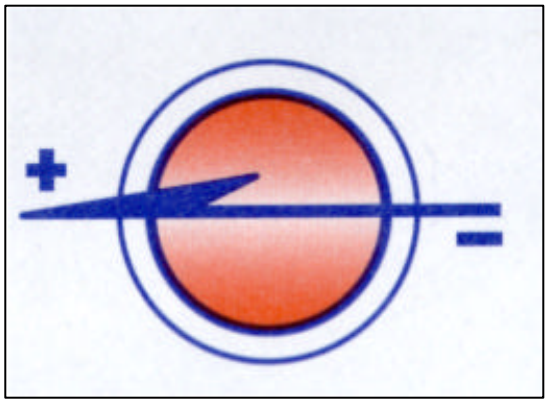

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Poster Abstracts

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AES-1

Blood Typing in a Dielectrophoretic Microdevice

Prashant Reuben Daggolu and Adrienne R. Minerick. Dave C. Swalm School of Chemical Engineering, Mississippi State University, Mississippi State, MS.

Other than conventional pathological techniques, medical analysis laboratories utilize flow cytometers for many standard blood tests. This relatively expensive equipment ranges from $30,000 to $150,000 [1]. The fee for each test to be done on these cytometers ranges above $50 with medical costs rising annually [2]. Eventually, it could be possible to design an inexpensive portable microdevice that can be used to perform multiple blood tests. The microdevice is expected to cost not more than 25 USD each. These microdevices could also find good application in understanding and identifying sickle cell anemia. The present cost of each sickle cell anemia kit which work using electrophoresis, have ranged about $69- $175 each [3,4]. Specialized microdevices have potential to be user-friendly, cost effective medical diagnosis kits. In the present work, dielectrophoresis is used to study the human blood of types A+, B+, AB+, and O+ and quantify them. Dielectrophoresis, a type of electrokinetics, is the use of a non-uniform AC field to manipulate and characterize cells [5,26. This tool has potential use in medical diagnosis applications, particularly those involving cell analyses.

The present work describes a multifold approach that includes experimentation, analysis, and correlation with existing physiological data. In Experimentation, the dielectrophoretic field is generated within the microdevice using platinum electrodes positioned 250 microns apart in a perpendicular configuration to create a non-uniform AC field [7]. Whole blood samples are diluted using Phosphate Buffer Saline in 1:60 V:V ratio and introduced into the microdevice via sample ports. An AC current of 1 MHz and 5 Vpp (volts peak to peak) is applied. The red blood cells polarize in the electric field and interact with each other to form pearl chains along the electric field lines. The preferential movement of these cells is dependent on blood type, as each blood type has a different surface property. The movement of the erythrocytes is recorded via video microscopy at 10-second intervals for 4 min. In the Analysis, the images are processed to get the X, Y position, cell radius, cell area, bound width and bound height of the cells using Axiovision 4.5 software. Based on the difference in optical intensity of the cells and the background in the image, the software selects the cells or conglomeration of cells. Then, the cells are properly separated and identified to get accurate position coordinates. The images have shown characteristic differences in their movement and this move-ment up to 120 seconds is studied. In Interpretation, the images are divided into 6 equal sized sextants to analyze the cell position distribution in the field using a MATLAB program. The sextants are made across the mid field line and two vertical trisection lines. Then the number of cells in each sextant at each time interval is normalized with the initial number of cells. Then plots are made for this normalized number of cells in each sextant as a function of time for each blood type separately. In order to check for consistency in data, experi-ments are performed for 3 different days. The data showed signature trends for each positive blood type. The characterization of the blood types using this technique would lead to portable medical diagnostic device capable of detecting blood type. This is useful in times of medical emergency for quick blood transfusion.

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AES-2

"Comparison of microfluidic protein patterning methods onto waveguide surfaces" *Scott Lynn, Colorado State University, Fort Collins, CO*

Protein patterning is an essential element to the creation of sensing motifs that rely on receptor-ligand binding for selectivity. Microfluidic devices have the potential to greatly aid in the development of simple, robust methods for deposition of biological capture agents. Here we present the characterization of two methods static microfluidic patterning (sMFP) and flow focused microfluidic patterning (ffMFP), for deposition of biological receptors on a waveguide surface. These focusing techniques are used to create localized regions of immobilized agents onto a SiNx waveguide. The receptor agents increase the cross section of the waveguide core, and thus modify the local evanescent field. Therefore the immobilized patch, as well as any secondary binding events, can be detected by monitoring the change in the localized evanescent field. The impact of different flow types (hydrodynamic versus electrokinetic) and microfluidic material (native versus modified) on immobilization density and spot size will be presented.

AES-3

How is it possible to sequence 600 bases of DNA in 6.5 minutes? The central role of carefully engineered polymer networks and coatings in microchip electrophoresis *Christopher P. Fredlake and Annelise E. Barron, Northwestern University, Chicago, IL*

Electrophoretic DNA sequencing remains the only technology that can deliver the long reads that are necessary for the sequencing of complex mammalian and plant genomes. We report on our work to create enabling technologies and materials for microfluidic chip electrophoresis sequencers, which promise to replace capillary array electrophoresis for high-throughput DNA sequencing. Low-viscosity DNA sequencing matrices are advantageous for microchannel electrophoresis systems since these polymer solutions can be easily pumped into the channels at relatively low applied pressures in only a few minutes. Dynamic (adsorptive) polymer coatings are also ideal for microfluidic platforms; they are much easier to apply and give virtually 100% yield of functional coatings, which are substantially more stable than covalently linked coatings. We have previously demonstrated sequencing reads of over 500 bases in 5-6 minutes using a poly(N,N-dimethylacrylamide) (pDMA) polymer matrix in short, 7.5-cm chip channels dynamically coated with poly(N-hydroxyethyl-acrylamide) (pHEA). These sequencing times are much shorter than any previous chip sequencing result (the previous speed record for > 500 base reads was 15-18 minutes). Through further development of polymer and matrix properties, we have now achieved four-color sequencing reads up to 600 bases in only 6.5 minutes (with 98.5% accuracy of base-calling), which is the longest sequencing read ever obtained in such a short separation distance. We show that this surprisingly fast sequencing is obtained by a hybrid separation mechanism, which combines both DNA reptation and transient entanglement coupling, explaining the faster and better sequencing performance of our pDMA networks relative to commercially available matrices. We discuss the DNA and polymer dynamics responsible for the excellent performance of these pDMA matrices and discuss the results in terms of the separation mechanism of DNA in different types of polymer solutions we have studied, making direct comparisons with what was previously the gold standard matrix for chip-based sequencing, linear polyacrylamide. Insights gained from these studies are being used to further optimize matrix properties and separation conditions for the further development of microchip-based DNA sequencing systems.

AES-4

Lessons Learned from "Not Entirely Successful" Attempts to Use Room Temperature Ionic Liquids (R T I Ls) for Electrochemical Separations *Ricardo Condemarin, Ashutosh Jha, and Paul Scovazzo. Department of Chemical Engineering, University of Mississippi, University, MS*

Room Temperature Ionic Liquids (RTILs) are organic salts that are liquids at ambient conditions consisting entirely of ions. RTILs possess a number of unique properties for electrochemical processes including: negligible vapor pressure, high thermal stability, non-flammability, and high ionic conductivity. It was this last property, we hoped to exploit in our work to develop an Electrochemically Modulated Complexation (EMC) separation system for CO2. EMC is a process that combines a reversible set of redox reactions with absorption/desorption steps to achieve selective separation of gas mixtures. In this paper, we will discuss our efforts and failures in selecting, designing, and using RTILs for EMC. For example, all attempts to increasing the redox-active carrier solubility in the RTIL resulted in increased solution viscosity. Therefore, we were faced with a trade-off selection process. Furthermore, in the reduction-to-practice, the carrier concentration needs to be greater than 0.5 mol/liter. In complex solvents, like RTILs, this is a mole-fraction of at least 0.15. This leads to a number of complications due to dynamic changes in voltage control, carrier solubility, viscosity etc. Consider

the difficulty of voltage control (i.e. reference electrode meaning) in a system were 15 mole-% of the solution changes oxidation state during the process. We also encountered changes in carrier solubility and increased viscosity as the oxidation state of 15 mole-% of the solution changed. The widely-studied imidiazolium-based RTILs initially were our choice for the EMC system. We will discuss how other non-imidiazolium-RTILs can alleviate some of the above complications.

AES-5

On-Chip ITP and IEF of Proteins: Experiment and 2D Simulation *Huanchun Cui[1], Prashanta Dutta[2], and Cornelius F. Ivory[1] (1) Chemical Engineering and (2) Mechanical Engineering, Washington State University, Pullman, WA*

This paper reports both the experimental application and two-dimensional simulation of nonlinear electrophoresis (ITP/IEF) of proteins in a microfluidic chip. A two-dimensional ITP model has been developed from a one-dimensional model and simulated using the Nernst-Planck equations. The concentration stacking and separation features of ITP are explored by simulations of three virtual proteins. Experiments of ITP demonstrated that a mixture of three fluorescent proteins were concentrated and stacked into three adjacent protein zones under a constant voltage of 100 V over a 2 cm long microchannel. The self-sharpening behavior of ITP zones dispersed by a T-junction was clearly demonstrated both by experiments and simulations. Two dimensional simulation of isoelectric focusing of proteins has been first developed by using existing solvers in Femlab. 8 carrier ampholytes which have pIs from pH 3 to pH 10 were employed in this simulation to generate a pH gradient of 3-10 in a 300micron x 2cm microchannel. Each ampholyte has three charge states of -1, 0 and 1 which are simulated as three individual components, but related to each other by finite reactions. A virtual protein with 7 charge states was simulated to demonstrate the character-istics of isoelectric focusing of proteins. Simulation revealed that increase of current density results in a stepwise pH gradient from pH 3 to pH 10 and more ampholytes would generate more linear pH gradient. The simulation also demon-strated that the protein is being focused via the well-known double-peak approach to its pI position. Experiments of IEF demonstrated that several fluorescent proteins were focused in a 2 cm long microchannel in 3-10 min using broad-range ampholytes at electric field strengths ranging from 25 to 100 V/cm.

AES-6

Nanostructure Based Biomimetic Interfaces for Bioelectronic Applications *Devesh Srivastava, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI*

Nanosized functional particles represent an area of great research interest. In the field of biosensors and biocatalysis, nanosized materials offer the potential for extremely high surface area to volume ratio, thus allowing immobilization of large amounts of biomolecules per unit projected area. Carbon nanoparticles, such as carbon nanotubes (CNTs), and exfoliated graphite nanoplatelets (xGnPs), provide high conductivity and surface area without excessive diffusional resistance. These nanoparticles are widely used to prepare solid electrode systems and supporting substrates in electrochemical biosensors, due to their high chemical inertness and wide range of working potentials with low electrical resistance. Nanoparticles are conventionally dispersed using ultrasonication but study of dispersion of nanoparticles into various solvents will also be done using a nanomixer from Primix Corporation, Japan. Due to the enhanced high shear forces, the dispersed nanomaterials have superior stability in solvents. We also try to exploit redox capabilities of enzymes to generate an electric signal which can be interpreted as analogous to presence of a certain analyte of interest. One of the self assembly techniques to immobilize enzymes and nanostructures onto the electrodes is layer-by-layer deposition, commonly abbreviated as LBL deposition technique.

AES-7

Microfluidic electroporation based on constant DC voltage and its applications to drug/gene delivery *Hsiang-Yu Wang and Chang Lu, Chemical Engineering, Agriculture and Biological Engineering, Purdue University, West Lafayette, IN*

Electroporation has been widely used to load impermeant exogenous molecules into cells. Rapid electrical lysis based on electroporation has also been applied to analyze intracellular materials at single cell level. There has been increasing demand to implement electroporation in a microfluidic format as a basic tool for applications ranging from screening of drugs and genes to studies of intracellular dynamics. Generally, a specialized pulse generator is required to conduct the electroporation. In this study, we demonstrated the electroporation of Chinese hamster ovary (CHO-K1) cells on a microfluidic device using simple techniques and DC power supply. In our design, electroporation only happened in a defined section of a microfluidic channel due to the local field

amplification by geometric variation. The change in the cell morphology during electroporation was observed in real time. We determined that electroporation of CHO-K1 cells occurred when the local field strength was increased to around 400 V/cm. The influence of the operational parameters of the device on cell viability was determined, too. A large percentage of cells remained viable after electroporation when the parameters were tuned. We also studied rapid cell lysis when the field intensity was in the range of 600-1200 V/cm. The rupture of cell membrane happened within 30 ms when the field strength was 1200 V/cm. Channels designed equivalent to single or multiple pulses were used to study the effects of number of pulses on the electroporation. The delivery of small molecules into the CHO-K1 cell was confirmed by the uptake of SYTOX Green. We also demonstrated the transfection of CHO-K1 cells using the pEGFP-C1 plasmid. Given the simplicity, high throughput, and high compatibility with other devices, this microfluidic electroporation technique may provide the basis for microfluidics-based screening of drugs and genes.

AES-8

Rapid Chip-scale Bio-Particle Detection by a Micro-Spiral Flow and Surface Enhanced Raman Scattering (SERS) *Diana Hou, Siddharth Maheshwari, Hsueh-Chia Chang, Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN*

We report a new chip-scale bio-particle concentration technique that can rapidly (< 15 min) produce strong surface-enhanced Raman (SERS) signals from a dilute 0.2 ml sample with low pathogen counts (~ 104 CFU/ml). This concentration technique relies on convection by a long-range converging micro-spiral flow to rapidly concentrate pathogens into a packed mound on the bottom of the liquid chamber. This micro-spiral flow, a discharge driven vortex, is generated by an ionic wind imparting force on the interface of the liquid which drives the vortex. Inertial effects in the bulk liquid create a secondary toroidal flow such that the liquid resembles a fast rotating downward spiral with a converging flow stagnation point at the bottom of the chamber. Pathogens suspended in the liquid are swept to the stagnation point by convective forces and trapped by external body forces, such as gravity. Latex particles, yeast, and E. coli suspensions in concentrations as low as 104 CFU/ml, can form packed mounds at the stagnation point, increasing the local concentration to orders of magnitude higher than the initial suspension concentration. A laser can then be focused at the packed mound to measure the intensity and the Raman shift of the dilute sample. Since this concentration technique relies on convection, trapping can occur within minutes of the initiation of the spiral flow.

An additional electrophoretic trap is fabricated on the bottom of the liquid chamber to enhance the capture of bacteria and other submicron pathogens. The micro dimension of the pathogens enhances Brownian effects limiting the concentration and stability of the packed mound, which is necessary for accurate Raman spectroscopy. Coupling an electrophoretic trap with the converging stagnation flow hence aids in the trapping and stabilization of the pathogens onto a substrate, providing consistent Raman spectra for them. In contrast to optical tweezers, this form of trapping is non-obtrusive and hence is not detrimental to the viability of the pathogens, thus allowing for further differentiating between live and dead pathogens. It is hence possible to rapidly obtain spectra for suspensions of pathogens at low concentrations using SERS. These advantages of rapid concentration and detection can lead to the advancement of a field applicable diagnostic kit.

AES-9

Electrolyte Dependent Aggregation or Separation of Micron Sized Particles in Low Frequency AC-Electric Fields *James D. Hoggard, Paul J. Sides, and Dennis C. Prieve, Carnegie Mellon University, Pittsburg, PA*

It has been previously observed that colloidal particles near a planar electrode can be made to move tangentially along the electrode surface in the presence of other particles by applying an ac-electric field directed normal to the electrode surface. At low frequencies were Faradaic reactions occur (< 1000 Hz), particles have been shown to aggregate in certain electrolytes and separate in others. Previous work has shown that an electrolyte dependent phase angle between the applied electric field and the particle height could account for this electrolyte dependent behavior. In a model, Fagan et. al. showed that when this phase angle is less than 90º, separation will occur, and aggregation will result when the phase angle is greater than 90º. In this contribution these phase angles were measured on single particles in numerous electrolyte-electrode combinations. The separation distances were measured on isolated particle pairs as a function of time. In all instances, the experimental observations agree with the particle motions, either aggregating or separating, as predicted by the phase angle measurements. These isolated particle pair experiments provide insight into the particle behavior of multi-particle systems in different electrolytes.

AES-10

Fabrication, Optimization, and Application of a Micro-Free Flow Electrophoresis Microfluidic Chip
Bryan Fonslow, Department of Chemistry, University of Minnesota, Minneapolis, Minnesota

Free flow electrophoresis (FFE) is a continuous preparative separation technique that introduces a sample stream into a planar separation chamber pumped continuously with separation buffer. An electric field is applied laterally and analytes are separated based on electrophoretic mobility differences.

There has been increasing demand to implement addition, purified samples can be collected into fractions at the outlet of the separation channel. The main drawback of FFE is sample stream broadening due to many bulk phenomena, decreasing purified sample yields. Micro-FFE (m-FFE) is the miniaturization of FFE for analytical applications which minimizes the effects of Joule heating and eliminates thermal convective mixing. From FFE to m-FFE, the separation channel volume is decrease from ~25 mL to 5 mL. The microfluidic chip was fabricated using anodic bonding of two etched and electrode deposited wafers. To eliminate electrolysis product formation, four-fold deeper channels were etched for the electrodes to facilitate a 16 times greater linear velocity. Two-dimensional modeling was used to determine the best channel geometry for parallel buffer flow in the two-depth chip. The removal of electrolysis products increased resolution of fluorescent standards by a factor of 1.3 under similar separation conditions. Also, the new design allowed for a four-fold increase in applied electric field to 586 V/cm before Joule heating conditions persisted. Experimental variance data from the fluorescent analyte standards allowed for determination of a migration distance squared dependence on band broadening under non-diffusion-limited conditions. From this phenomenon, minimization of migration distance, possibly through suppression or modification of electroosmotic flow, allows for the highest resolution of analytes and efficiency of separations. Also, empirical equations were derived to describe total peak variance, plate height and number, optimum linear velocity, peak capacity, and resolution. Linear velocity emerged as an important variable for optimization. Similarly, previously reported equations allow for precise positioning of analyte streams in the separation channel based on linear velocity and applied electric field. Separations of biomolecules have been performed to show the improved functionality of the m-FFE through knowledge gained from the fundamental studies.

AES-11

Spatially Controlled Chemistry Using Remotely Guided Nanoliter Scale Containers *Timothy Leong, The Johns Hopkins University, 3400 N. Charles St. Baltimore, MD*

We describe a new pico-nanoliter scale chemical encapsulation and delivery system in the form of 3D metallic containers with controlled surface porosity. The advantageous attributes of the containers are a parallel fabrication process with versatility in sizes and shapes; precise and monodisperse surface porosity; and the ability for remote guidance using magnetic fields. We demonstrate the isotropic and anisotropic release of reagents from the containers, remotely guided chemical release, and spatially controlled chemical reactions in microwells. The arbitrary chemical patterns that can be generated are not limited by flow profiles in conventional microfluidics that must often be formed downstream from a channel inlet. These containers suggest a new strategy for precisely engineering spatially localized chemical reactions with sub-nanoliter scale control.

AES-12

Proteome analysis of Pseudomonas putida during biodegradation of high concentration of benzoate: activation of the meta pathway and physiological responses *Kai Chee Loh* and Bin Cao (presenter). Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore*

Previous research has reported that a switch in the biodegradation pathway of benzoate by Pseudomonas putida P8 (ATCC 49451) could be initiated via a change in the initial substrate concentration. With benzoate up to 200 mg/L, the substrate was degraded via the ortho pathway. However, cells grown on high enough concentrations (=300 mg/L) were shown indirectly to activate both the ortho and the meta pathways. It is anticipated that this pathway-switch could result in a variety of physiological changes within the cells. In this study, conventional proteome approach was employed to investigate the metabolic pathways and the cellular responses of an environmental bacterium P. putida P8 during growth on benzoate. Protein extracts from 100 mg/L (L) and 800 mg/L (H) benzoate-grown P. putida cells were analyzed by 2-DE method using gel patterns from succinate-grown cells (S) as reference. The correlation coefficients (R2) for biological and analytical gel replicates were 0.74 ± 0.03 and 0.85 ± 0.05, respectively. By applying threshold (1.5-fold) criteria (H vs. S), 55 spots were selected and excised from the gels. Among them, 32 protein spots were found differentially

expressed (Student's ttest, $p < 0.05$) in the 800 mg/L benzoate-grown versus the succinate-grown cells, indicating the effects of different carbon sources on the proteome profiles. In addition, although the expression of most of the 55 spots was found unchanged, 9 proteins showed differential expression ($p < 0.05$) in response to the different initial benzoate concentrations (H vs. L). All the 55 spots were positively identified as 51 different protein species with 4 species represented by multiple isoforms/spots on the 2-D gel. Of particular significance were the 5 newly induced protein spots in 2-D gels from cells grown on 800 mg/L benzoate which were identified as key enzymes in the meta degradation pathway. Additionally, enzyme assay results showed that the activity of catechol 2,3- dioxygenase was only detected in cell lysates obtained from high benzoate concentration. To our knowledge, these results are the first to provide confirmation of the activation of the meta degradation pathway at high benzoate concentration directly at the protein level. Furthermore, the induction of stress response proteins and other adaptation phenomena in response to benzoate at different concentrations were also inferred based on the protein spots that were also identified.

AES-13

A Continuous Microfluidic Blood/Plasma Separation Unit with Electrokinetic Stirring and Cross-Flow
Shramik Sengupta, Zachary R. Gagnon, and Hsueh-Chia Chang. Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN

Many biomarkers of clinical significance are found in the plasma. However, before the plasma can be analyzed to assay for their presence, the blood cells need to be removed. Many point-of-care diagnostic procedures require that this operation (blood/plasma separation) be performed continuously or intermittently over a sustained duration (at least a few hours) without the need for human intervention and/or replacement of parts. The task is further complicated by the requirement that no more than a few cc of blood can be drawn from the patient per day but diagnostic reading should be made frequently (in some cases, once every 5 minutes) using micro-liter sized samples. The microfluidic device being presented provides a means to achieve this goal.

A conceptually simple technique of removing the blood cells is to filter them out using porous structure such as a monolith. However, in a dead-end filtration mode, the pores get clogged after a very short time, and the device fails. In our system, a porous monolith is placed in the vertical arm of a T-junction network right next to the intersection of the two arms. Through one end of the horizontal arm, suspension (blood with cells) is introduced at a controlled flow rate. Through the other end of the horizontal arm, suspension is withdrawn at a controlled flow rate. The key is that that latter (withdrawal) flow-rate is smaller than the former (infusion) flowrate and the balance is made up by the flow of filtrate (plasma) through the porous monolith and down the vertical arm of the T channel network.

This system is able to operate much longer than dead-end filtration systems because the blood cells excluded at the filter are swept away by the cross-flow of the fluid. Additionally, patterned electrodes at the junction are used to induce an AC electro-osmotic flow that drives the cells excluded by the filter towards the center of the cross-flow, and thereby enables the device to operate for hours without failure.

AES-14

Quantitative Preparative Native Continuous Polyacrylamide Gel Electrophoresis
Bernd Kastenholz, Research Center Juelich, Institute of Chemistry and Dynamics of the Geosphere, Institute Phytosphere (ICG-III), Juelich 52425. Germany

QPNC-PAGE (Abbr.: quantitative preparative native continuous polyacrylamide gel electrophoresis) is a new state-of-the-art polyacrylamide gel electrophoresis procedure for isolating native metalloproteins in complex biological matrices. In these processes protein molecules are separated according to their individual isoelectric points. The QPNC-PAGE procedure is accomplished in a special electrophoresis chamber (Model 491 Prep Cell from Bio-Rad) at 4° C by using a continuous buffer system (20 mM Tris-HCl, 1 mM NaN3, pH 10.00). Due to the specific properties of the electrophoresis buffer, most proteins (MW > 200 - 5 kDa) of a biological system are negatively charged and migrate from the cathode to the anode in the electrical field. In order to obtain a fully-polymerized gel for a PAGE run, the polyacrylamide gel is polymerized for a time period of 69 hr at room temperature. As a result, the prepared gel is homogeneous, mechanically stable and free of monomers. The pore sizes are very large. For these reasons interactions of the gel with the biomolecules to be isolated can be neglected. The separated metalloproteins are not dissociated into apoproteins and metal cofactors. The bioactive structures (native conformation) of the isolated protein molecules do not undergo any conformational changes by using QPNC-PAGE. Consequently, quantitative amounts of native metal cofactor-containing proteins and protein isomers are eluted in different PAGE fractions. The isolated metalloproteins may be analyzed by NMR spectroscopy for elucidating their chemical structures.

AES-15

Lab-on-a-Chip Sample Preparation for Subcellular Analysis: a Technique to Rapidly Rupture Erythrocytes in a Dielectrophoretic Microdevice
Adrienne R. Minerick and Sheena Reeves. Dave C. Swalm School of Chemical Engineering, Mississippi State University, Mississippi State, MS

Laboratories-on-a-chip and micro Total Analytical Systems (microTAS) are seen as one of the key growth industries for the 21st century. These systems are attractive due to the promise of raw unprocessed samples entering the device with quantitative analysis results as an output from the device. Because of their small size, these microdevices require small sample volumes and conceivable can return the results rapidly (within a matter of minutes). Such devices also have the potential to decrease the cost of analysis for applications such as medical testing, do not require technician operation, and can be adapted as point of care devices for use at home to monitor vital indicators for diseases.

Electrokinetic tools have been identified as the most promising forces to manipulate and quantify analytes within the microdevices. This work examines the use of special electric fields to reliably and rapidly rupture erythrocytes for subsequent subcellular analysis. Dielectrophoresis (DEP) is the movement of particles in a non-uniform alternating current field. Under the influence of a non-uniform AC field, charged particles become polarized. By tuning the AC frequency, membrane instabilities can be created thus causing the cell to lyse.

The fresh human blood was obtained via venipuncture by a certified phlebotomist; samples were stored in K2 EDTA anticoagulant at 5?C. The blood was diluted with a 0.143M sodium phosphate saline buffer (PBS) just prior to experimentation in a microdevice consisting of a glass slide constructed out of perpendicularly positioned 100 micron platinum wire. Dielectrophoretic fields were applied via the microdevice electrodes. A Zeiss Axiovert 200M inverted light microscope with a high resolution Axiocam camera was used to record images of the experiment every twenty seconds. The frequencies were varied from 1 kHz – 5 kHz and the field intensity for a series of dependence experiments was varied from 1Vpp/200?m to 6Vpp/200?m. The total number of RBC in each image was analyzed manually in 1 minute intervals over the 8 minute experiment. These counts were tabulated in a spreadsheet and graphed using a number fraction (number of RBC at a specific time/number of RBC at time=0) as a function of time.

Results from this process show that there exists a relationship between the age of the blood sample and the applied field. The optimal field density for rupturing human blood was 5Vpp/200?m. At this field density, 50% of the cells were ruptured within 220 seconds and complete sample rupture was accomplished after only 360 seconds. Varying the frequency of the process showed that it indeed had an effect on rupturing. The age dependency of the rupturing for a single sample was conducted over a five day period to reveal any statistical dependencies on age.

AES-16

Role Of Tilted Angle and Electrical Field Magnitude In An Electrokinetic Cell Of Cylindrical Geometry: Solute Aspects
Antonio Martínez[1], Mario Oyanader[1], and Pedro Arce[2] 1. Dept. Chemical Engineering Universidad Católica del Norte, Avda. Angamos 0610, Antofagasta, Chile 2.Department of Chemical Engineering, Tennessee Tech Univerisity, , Cookeville, TN

In a previous work, competing driving forces that affect the motion of the solute species* has been identified in electrokinetics processes. The principles of pressure, buoyancy and electrostatic forces are the basis to understand the transport phenomena taking place in an electrokinetic cell. Altogether, this driving forces need to be studied under certain conditions that resemble normal field operations. In particular, certain technologies such as “Lasagna” befit the use of vertical and horizontal arrangement of cells. The implications of this practice and how it affects the hydrodynamic have not been yet studied.

This contribution concentrates on the study of the different driving forces controlling the electrokinetic process in a cell under the most representative tilted positions. In particular, this work values the influence of magnitude of the applied electrical field and how it may promote distinct flow regimes. Afterwards, the information is used to predict optimal times of separation of macromolecules of different properties. In this contribution uses a cylindrical capillary model and based on the selection of values of the parameter space presents and discusses several limiting cases related to the flow regimes reported in an earlier contribution.

* Oyanader, M., P. Arce, and A. Dzurik, “Avoiding Pitfalls In Electrokinetic Remediation: Robust Design And Operation Criteria Based On First Principles For Maximizing Performance In A Rectangular Geometry”, Electrophoresis, 2003, 24, 3457

AES-17

Optimization of Chip-Based Infusion Nanoelectrospray Tandem Mass Spectrometry for the Rapid Analysis of Complex Proteomes *Ana G. Pereira-Medrano[1], Alistair Sterling[2], Ambrosius P. Snijders[1], Kenneth F. Reardon[3], and Phillip C. Wright1. (1) Department of Chemical and Process Engineering, The University of Sheffield, Sheffield, United Kingdom, (2) Advion Biosciences Ltd, Norwich, United Kingdom, (3) Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO*

In high-throughput proteomics, accuracy, coverage and analysis time are crucial requirements. Sample introduction via automated nanoelectrospray infusion has definite advantages when compared to liquid chromatographic (LC)-electrospray ionization ESI sample delivery, including high-throughput operation, automation, no carry-over or cross-contamination, and a constant sample matrix. We have systematically evaluated and optimized the use of a chip-based nanoelectrospray device (Advion Nanomate) compared to LC-ESI, each interfaced with a QStarXL Q-TOF mass spectrometer, for the development and optimization of sample preparation and mass spectrometry identification protocol. Analysis were carried out on tryptically digested samples: cytochrome C and a six-protein mixture (containing cytochrome C, lysozyme, alcohol dehydrogenase, bovine serum albumin, apo-transferrin, and b-galactosidase) at concentrations from 5 to 500 fmol/µl. We quantified the effects on the identification and peptide coverage of MS and MS/MS scan times, Zip-Tip C18 sample clean-up, in-gel digestion residues and sample concentrations. Different survey (1-10 seconds) and MS/MS (1-10 seconds) scan times were evaluated, and spectra were searched with Mascot against generic and species specific databases. Each infusion experiment consisted of 22 cycles in the mass spectrometer, and each cycle consisted of 1 survey and 8 MS/MS scans. Infusion results were compared to those from nanoflow LC-ESI-MS/MS, which was run isocratically to maintain a quasi-uniform sample introduction. The results from the isocratic LC-ESI-MS/MS suggest that the chip-based infusion yields greater coverage, with marginally lower MOWSE scores. The optimal MS method, selected to take advantage of the extended infusion without losing the high-throughput requirement, includes a 10 second survey scan and 8 cycles of 3 second MS/MS scans. Each isocratic LC experiment, from injection to injection, took approximately twice as long as each chip-based experiment. These results were in close agreement, for the 50 and 500 fmol/µl protein mixtures, to LC-ESI results, but in half the time. A shorter run employing a five-second survey scan time and a three-second MS/MS time (10.6 min total experiment time) was also sufficient to identify all of the proteins, although scores, coverage and reproducibility were lower. NanoES yielded up to 300% greater peptide coverage percentage compared to LC-ESI. We discuss the feasibility of extending this methodology to more complex proteomes, such as those obtained from the model cyanobacterium, Synechocystis sp. PCC6803, as we have extensive experience with this organism [1]. Furthermore, we discuss the role of this configuration in aiding identification of post-translational modifications.

[1] Gan CS, Reardon KF, Wright PC, Comparison of protein and peptide prefractionation methods for the shotgun proteomic analysis of Synechocystis sp. PCC 6803, Proteomics, 5(9), 2005, 2468-2478.

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Applying Multivariate Statistical Tools to 2D-PAGE Data *Dave Bramwell, Anna Kapferer, Ian Reah, Dave Miller, Christian Andersson Ståhlberg, Nonlinear Dynamics, Durham, NC*

In order to extract relevant information from proteomics experiments that reflect the complex interactions taking place within the cell, multivariate statistics has established itself as a powerful analysis tool. However, current workflows to statistically evaluate images from 2DGE are restricted due to the missing value problem, which limits the application of higher lever statistics. Missing values may arise from inadequately matched or undetected spots - a problem that is reinforced by the inability to sufficiently align spot patterns. Furthermore, the number of missing values increases in proportion to the number of images included in the experiment, thus encouraging the use of far fewer replicates than would be desirable for achieving statistical significance.

By employing an advanced image alignment step coupled to improved analysis workflows we are able to produce statistically robust 2DGE data with no missing values. Consequently, we are now in a position to extract the most meaningful information using a variety of multivariate data analysis tools, including Principal Component Analysis (PCA), Discriminant Analysis (DA), Multidimensional Scaling (MDS), and Correlation Analysis.

The data presented will show the applicability of these tools on a range of complex real world data sets.