NOVEL APPROACHES TO COVALENT MODIFICATION OF CARBON NANOTUBE BASED CHEMICAL FIELD EFFECT TRANSISTORS

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Our goal for this study is to develop carbon nanotube-based field effect transistor (CNT-FET) biosensors for detection of disease biomarkers. In this study, we utilised carbon nanotubes covalently functionalized using a 4-Carboxybenzene diazonium tetrafluoroborate salt, which grafted a carboxyphenyl moiety on the CNT sidewalls. The carboxyphenyl moieties on the CNTs were then covalently coupled to an amine modified oligonucleotide (aptamer). This coupling was achieved through formation of amide bonds between the carboxylic group of the carboxyphenyl moiety on the nanotubes and the amine group on the aptamer.

To prove the concept of the biosensor, an anti-thrombin aptamer-functionalized CNT-FET was successfully fabricated on a quartz substrate and characterized by atomic force microscopy (AFM). It has been demonstrated that the amine modified aptamer does not couple or adsorb to unmodified CNTs. The binding of thrombin to the aptamer on the CNT-FET has also been characterized by AFM. Optimization and characterization activities continue in our laboratory.

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PP-A-01

USE OF OFFGEL, SDS-PAGE, 2DE, AND MS TO EXPLORE POTENTIAL BIOMARKERS FOR ACUTE MYELOID LEUKEMIA (AML) IN HUMAN PLASMA

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Acute Myeloid Leukemia (AML) is a clonal disease of hematopoietic tissue, which represents about 15-20% of leukemia in children [1]. For AML diagnostic conventional exams, among them the blood count, are performed. These exams, however, do not use potential biomarkers that might be employed for early diagnostic and prognosis of diseases [2-4]. Techniques as OFFGEL and 2DE may be applied to search for biomarkers in different biological fluids [5,6]. In this work, we aim to find of biomarkers candidates for AML in human plasma using OFFGEL, 2DE analysis, and spots identification using MS. We evaluated different precipitation solvents: acetone, methanol, ethanol, and TCA/acetone (10% TCA), and the total protein quantification was carried out by Bradford assay. The average concentrations obtained in 100 μL in plasma were: 16.2 $mgmL^{-1}$ (acetone), 15.5 $mgmL^{-1}$ (methanol), 15.6 $mgmL^{-1}$ (ethanol) e 10.4 $mgmL^{-1}$ (TCA/acetone). After precipitation and quantification of proteins, the samples were evaluated by SDS-PAGE verifying the presence of a large amount of salts in samples precipitated with acetone, methanol, and ethanol, besides a large amount of albumin. The samples precipitated with TCA/acetone did not present salt interference, and were fractionated directly by OFFGEL (3100 Fractionator, Agilent Tecnologies) using 13 cm strips, pH 3-10 (GE Healthcare Life Sciences) and 12 cm strips, pH 4-7 (Agilent Tecnologies), according to standard protocol, by 24 h. The fractions obtained were collected and analyzed by SDS-PAGE and 2DE. We obtained a good separation of bands present in acidic regions and, at basic region,there was a large band of albumin for GE strip. With Agilent Tecnologies strips, pH 4-7, we observed a good resolution of bands for pH 4.0-5.5. New experiments will be performed to desalt samples by diaysis before the precipitation (acetone, methanol, and ethanol) and after analysis by SDS-PAGE, 2DE, and spots identification by mass spectrometry in healthy patients, and others diagnosed with AML, after optimization of all parameters.

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PP-A-02

DEVELOPMENT OF A MULTI-PURPOSE FLOW INJECTION ANALYTICAL PLATFORM COUPLED TO CAPILLARY ELECTROPHORESIS FOR SAMPLE MANAGEMENT AND BIDIMENSIONAL SEPARATIONS

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High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) represent two examples of well succeeded and established separation techniques. Despite of the high efficiency observed in these two analytical approaches, some new challenges of modern separation sciences demand even more resolution power due to sample complexity. One reasonable possibility is to use tandem- orthogonal separations. Many orthogonal separations are possible by the selection of suitable HPLC and CE separation mechanisms.

Flow injection analysis (FIA) and sequential injection analysis (SIA) represent not only important analytical tools, but a convenient manner to couple sample treatment procedures to several analytical techniques. FIA and SIA also present the advantage of reducing the sample handling to a minimum, which improves the precision and accuracy of the analysis.

Our research group has developed a versatile FIA-HPLC-CE analytical platform, capable to simultaneously manage sample treatment and HPLC-CE coupling for orthogonal bidimensional separations. The FIA is based on a 10-port rotatory valve (Valco Instruments Co. Inc., Houston, TX, U.S.A.) which is responsible, together with 15 solenoid valves, to select and distribute flow lines between HPLC, CE and a stir bar reactor vessel. Up to five accessory components may be chosen and attached to the 10-port valve. The HPLC system is composed by an autosampler System AS1000 (Thermo Separation Products, Fremont, CA, U.S.A.), an HPLC pump 510 (Waters Chromatography, Milford, MA, U.S.A.) and a portable UV-Vis detector Ocean Optics USB4000-UV-Vis (PASCO Scientific, Roseville, CA, U.S.A.). The CE system is constituted by a high voltage power supply CZE1000 (Spellman, Plainview, NY, U.S.A.), a capacitively coupled contactless conductivity detector (C4D), a temperature controller and a hydrodynamic injector, with all corresponding electronics and mechanisms home-built. Computer interfacing and equipment control is done by means of a USB-6341 and A USB-6501 data acquisition modules and a program developed with Labview software, respectively (all National Instruments, Austin, TX, U.S.A.). The built platform opens several possibilities, including sequential dilution for calibration purposes, labeling, chemical and enzymatic digestion, kinetic studies, and fraction collection from HPLC.

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PP-A-03

USE OF THERMAL MARKS IN THE ASSESSMENT OF ELECTROOSMOTIC FLOW VARIABILITY RELATED TO CAPILLARY PRECONDITIONING STUDIES IN THE CE SEPARATION OF AROMATIC AMINES

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Perhaps the most important cause of poor migration time repeatability in capillary electrophoresis (CE) is related to electroosmotic flow (EOF) variability [1]. This is particularly true for long eluting solutes in low pH buffers, where small changes of eof might cause severe deviations in migration times, compromising separation quality and method robustness.

Thermal marks are a convenient and elegant manner of monitoring the EOF [2]. These marks are obtained by variations of the transport number of ions by a punctual heating of the capillary channel. This strategy has been applied to capillary electrophoresis with conductivity detection (C4D) [2] and indirect UV-vis detection [3]. In indirect UV-vis detection schemes, the use of a chromophore as background electrolyte (BGE) aids the visualization of thermal marks. However, in direct UV-vis detection schemes, thermal marks are embedded in the baseline due to the low absorbance of the BGE components.

This work intends to compare different preconditioning strategies in the CE separation of 20 aromatic amines at low pH phosphate buffer using EOF stability as measured by thermal marks as a criterion for selection of the optimal procedure. In order to aid visualization of thermal marks and still allow the UV direct detection of analytes, the addition of low concentrations of a chromophore into a nonabsorbing BGE was attempted. A BGE composed of pH 2.5 phosphate buffer at 70 mmol L^{-1} concentration containing 0.5 mmol L^{-1} imidazole as chromophore was shown to be suitable for this purpose.

Double thermal marks were applied at different times during the run: at the beginning, after 10 min and after 20 min. Once the position of thermal marks was established and showed not to interfere with the migration of the aromatic amines, two different preconditioning procedures were contrasted: (1) deionized water and (2) 0.1 mol L^{-1} HCl, both applied for 10 min (950 mbar). The experiments showed that the EOFs at the beginning of the run were 6.30 TU and 6.00 TU, for procedures (1) and (2), respectively; after 10 min those values changed to 6.55 TU and 6.02 TU. These results showed that preconditioning (2) is appropriate.

In conclusion, it is possible to monitor EOF by thermal marks during direct UV detection of analytes by adding small amounts of a chromophore to the BGE. With this approach, a single wavelength can be used to monitor the analytes signal (directly) and thermal marks (indirectly). In addition, the variability of the EOF as measured by thermal marks can be used to discriminate preconditioning procedures without compromising the separation.

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PP-A-04

DETERMINATION OF AMINO ACIDS IN URINE BY CAPILLARY ELECTROPHORESIS TO CHARACTERIZE VESICOURETERAL REFLUX IN CHILDREN

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The vesicoureteral reflux (VUR) is defined as the retrograde flow of urine from the bladder into ureter and occurs in children. The most common clinical manifestation of VUR is an urinary tract infection (UTI). The association of VUR with UTI represents a threat to kidney tissue due the risk of pyelonephritis or urinary tract infection at the stage that it reaches the renal pelvis, which eventually evolves into kidney tissue healing. The risk of scarring is greater when pyelonephritis occurs during the first year of life and when the child has a high degree of reflux [1]. The scar tissue can cause renal failure, and modify the functioning of the kidneys.

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. They have been identified as biomarkers of many diseases and associated to several conditions, including kidney failure [2].

In this context, the versatility of capillary electrophoresis (CE) was explored in this work to develop several methods for the determination of primary aminoacids in urine to further characterization of VUR. An electrolyte consisted of 10 mmol/L 3,5-dinitrobenzoic acid buffered by 20 mmol/L triethylamine (TEA) at pH 10.86 was used to determine indirectly nonabsorbing aminoacids. Another electrolytes consisted of either pH 2.5 phosphate buffer or pH 1.8 formic acid were used to discriminate absorbing aminoacids and creatinine, which was further used to normalize the data. And finally, an electrolyte consisted of 100 mmol/L phosphate buffer at pH 7.0 was used to determine arginine.

A pilot study comprising 24 children, previously tested positive to VUR by voiding cystourethrography, and 15 healthy children has been devised. Selected urine samples from this set were preconcentrated and cleaned up by solid-phase extraction procedures (cation resin cartridges) prior to CE analysis. The validated methods are being applied to the determination of aminoacids. Furthermore, classification statistical procedures will be used to discriminate aminoacids contents in the selected urine samples as an attempt to establish the aminoacids role in the VUR condition.

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VALIDATION OF A STABILITY INDICATING METHOD FOR THE DETERMINATION OF METFORMIN HYDROCHLORIDE

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Metformin hydrochloride is a biguanide hypoglycemic agent used for diabetes mellitus type 2. This type of diabetes is a long-term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption. The aim of this study was to develop and validate a rapid, inexpensive and reliable capillary zone electrophoresis method (CZE) for the determination of metformin hydrochloride and its major degradation products. Separation was achieved using an uncoated fused-silica capillary of 75 µm i.d. with a total length of 40.2 cm, a running buffer solution containing 40 mmol L^{-1} triethylamine pH adjusted to 2.3 with orthophosphoric acid. Samples were injected hydrodynamically at 0.3 psi for 3 s, the detection was made by UV absorption at 200 nm, the capillary temperature was 25 ºC and the applied voltage was +22 kV. The proposed method showed good linearity over a concentration range from 240 µg mL^{-1} to 360 µg mL^{-1} with correlation coefficient higher of 0.99. The intra-day precision was lower than RSD 2 %. The precision of system was lower than RSD 1%. The specificity of the developed method was determined using forced degradation under hydrolysis (acid, alkaline, neutral and water), oxidation, photolysis and thermal conditions. Using this methodology six degradation products were detected with the following RMTs 0.89, 0.94, 1.06, 1.11, 1.14 and 1.45. The drug was stable against dry heat and photolysis conditions.The developed method is simple, precise, and specific. It is proposed for analysis of the drug and degradation products in stability samples be used in pharmaceutical preparation and quality control (QC) laboratories.

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PP-A-06

OPTIMIZATION OF CONDITIONS FOR OXYBUTYNIN AND N-DESETHYLOXYBUTYNIN ANALYSIS IN URINE BY CAPILLARY ELECTROPHORESIS

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Oxybutynin (OXY) is one of the drugs currently used to treat urinary incontinence, a symptom that affects mainly the elderly, bringing physical and mental impact on the quality of life [1]. Its half-life is short since OXY is extensively metabolized by the liver enzyme cytochrome P450, leading to its main active metabolite N-desethyloxybutynin (DEO) [2]. Capillary electrophoresis (CE) is a relatively new analytical technique that has never been used before for the analysis of OXY and DEO in biological samples. The aim of this study was to develop a CE method for the determination of OXY and DEO in urine using dispersive liquid-liquid microextraction (DLLME) for sample preparation.

CE experiments were performed on an Agilent CE system (model G1600A), with a photodiode array detector set at 204 nm. Some background electrolytes (BGEs) were evaluated under positive mode using a 50 µm ID fused-silica uncoated capillary. Resolution was achieved by the optimization of the experimental parameters: pH and concentration of the run buffer, voltage and temperature. For the DLLME stage, a mixture of many extraction and dispersive solvents were evaluated by injection into 5 mL of urine samples after their enrichment with OXY and DEO standards (0.5 µg/mL of urine) and alkalization until pH 10 with NaOH. An extraction solvent volume of 50 µL was isolated after centrifugation at 2260 g for 5 min, the solvent was evaporated to dryness under air flow and the residue was dissolved in water for quantification using the chosen CE method. Among the extraction solvents evaluated were: carbon tetrachloride, chloroform, dichloromethane, tetrachloroethylene and chlorobenzene and among the dispersive ones were: acetone, acetonitrile, methanol and ethanol.

Optimal condition was based on the best values for resolution (Rs), migration time (tm), tailing factor (TF) and symmetry. A successful separation (run time less than 12 min) was attained in 50 mmol/L triethylamine (pH adjusted to 3.0 with H_3PO_4 solution) with a constant voltage of +30 kV at 20 °C in an effective capillary length of 36.5 cm. For the DLLME method, the best results were obtained with carbon tetrachloride/acetonitrile mixture.

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FIELD EFFECT TRANSISTOR BIOSENSORS FOR DETECTION OF DISEASE BIOMARKERS

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This work focused on the development of a carbon nanotube-based field effect transistor (CNT-FET) biosensor. In this study, carbon nanotubes were drop-cast on an aminated silicon oxide surface, the amine groups in close proximity to the nanotubes were then modified with an anti-thrombin oligonucleotide (aptamer) through a glutaldehyde linkage. This linkage is possible through the amide bonds formed between the carboxyl group on either end of the glutalhdehyde and amine groups on the substrate and the amine modified aptamer. It has been demonstrated that the amine-modified aptamer does not couple or adsorb to unmodified CNTs hence the selective coupling of aptamer to the amine groups on the substrate is possible in the presence of CNTs. The CNT-FET was then characterized by atomic force microscopy (AFM) and fluorescence microscopy (FM) to demonstrate binding of thrombin to the aptamer in close proximity to the CNT. Optimization and characterization of this biosensor for real time conductance measurements of the binding of the antigen are also being explored in detail.

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PP-A-08

DEVELOPMENT OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE ASSAY OF ECONAZOLE AND ITS IMPURITIES IN THE PRESENCE OF PRESERVATIVES IN PHARMACEUTICALS

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Econazole nitrate (EN) is an antifungal drug of the imidazole class. It is used to treat skin infections such as athlete's foot, tinea, pityriasis versicolor, ringworm, and jock itch. Econazole nitrate is also used for the treatment of candidiasis. Regulatory requirements for the identification, qualification and control of impurities in drug substances and their formulated products are now being increasingly explicitly defined, particularly through the International Conference on Harmonisation. The present work was focused on developing a rapid and selective capillary electrophoresis (CE) method for the quantitation of econazole nitrate and its impurities (alpha-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol (DCE) and 4-chlorobenzyl alcohol (CBA)) in the presence of preservatives (methylparaben (MP) and propylparaben (PP)) in pharmaceutical topical creams. The electrophoretic separation was performed in an uncoated fused-silica capillary with 50 µm inner diameter x 30.2 cm total length (20 cm effective length). The optimized background electrolyte consisted of 10 mmol L^{-1} sodium tetraborate, and 50 mmol L^{-1} sodium dodecylsulfate (pH = 9.3). The UV detection wavelength was set at 200 nm, injection of the sample was hydrodynamically performed for 3 s at 0.5 psi, voltage was set at +18 kV and temperature was set at 25 ºC. Under these optimized conditions, the baseline separation of the analytes was obtained in about 3.3 min. The method was linear (R^2 better than 0.99) in the range of 30.0 to 72.0 µg mL^{-1} for EN, 2.0 to 4.8 µg mL^{-1} for DCE and CBA impurities, 15.0 to 36.0 µg mL^{-1} for MP and 8.0 to 19.2 µg mL^{-1} for PP preservatives. Detection and quantitations limits ranged from 0.146 to 2.823 µg mL^{-1} and 0.444 to 8.554 µg mL^{-1}, respectively. Intra-day precision was less than 1.8% RSD for all substances. Accuracy was performed by recovery test and 102.8 % was obtained for EN. After sample analysis, none of the impurities under evaluation were detected, meaning that the concentration of impurities was lower than the respective detection limit confirming the safety of marketed creams. Furthermore, no interference from cream excipients was observed.

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PP-A-09

APPLICATION OF PRESSURE CYCLING TECHNOLOGY (PCT) IN DIFFERENTIAL EXTRACTION

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Forensic DNA analysis has elevated the degree of confidence in the analysis and interpretation of evidence but the bottle neck that plagues the crime labs across the country is the tedious, time consuming protocols that require practice and expertise in analyzing mixtures. Organic differential extraction is the most commonly used method to isolate sperm DNA from sexual assault evidence. This two-step extraction procedure involves selective digestion of epithelial cells in the first step followed by isolation and digestion of sperm cell pellet. The major disadvantages of this technique are incomplete separation of sperm and non-sperm fractions, particularly in samples that are overwhelmed by large numbers of female epithelial cells relative to sperm cells and time-consuming nature of the process.

Pressure cycling technology sample preparation system (PCT SPS) involves the use of pressure pulses to disrupt tissues, cells and cellular structures enabling the recovery of their components. Barocycler® NEP2320, a commercially available instrument from Pressure BioSciences Inc, is equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 45000 psi resulting in the lysis of cells. The current study involves the application of pressure cycling technology in the selective digestion of sperm cells from evidence mixtures collected from different substrates with an emphasis on the role of buffer composition on sperm DNA yields. The cells were extracted into 1X PBS buffer (pH 7.4) with varying buffer compositions and subjected to 45000 psi pressure for 60 cycles . Samples were placed in specially designed MicroTubes or PULSE® tubes and introduced into the pressure chamber. This pressure treatment was followed by phenol chloroform isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with Promega Plexor® HY system.

According to our previous studies, high selectivity and improved recovery with the reducing agent, Tris (2-carboxyethyl) phosphine (TCEP) indicated the potential for highly selective detection of sperm cells in comparison to the addition of detergents or changes in temperature. These observations were applied to mixture studies of evidence obtained from various substrates such as swab and fabric. Preliminary data indicates that pressure cycling technology has application in differential extractions indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells in the presence of appropriate buffers.

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PP-A-10

A NOVEL ANTIMICROBIAL PEPTIDE DERIVED FROM PHYLOGENETIC ANALYSES AND PHYSICOCHEMICAL PROPERTIES

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Antimicrobial peptides (AMPs) have emerged as an alternative in the search for new treatments against pathogens. So far, nearly all the mechanisms of action are associated with sequence, secondary structure, and with perforating and breaking down bacterial membranes after a binding process. We studied a new AMP derived from phylogenetic analyses and physicochemical properties. The mechanism of action is deduced from a kinetic model and molecular dynamics simulation. We analyzed the sequence variability in cytokine and chemokine proteins in order to investigate whether these structurally proteins have a sequence useful in the development of new AMP. The cluster analysis allowed us to identify five groups with structure and sequence homology. The structure and function relationships of these groups, with physicochemical parameters, including length, sequence, charge, hydrophobicity and helicity, allowed us to select an AMP candidate. This peptide encompasses the C-terminal alpha-helix of chemokines (CXCL4/PF-457-70. Far-UV CD spectroscopy showed that this peptide adopts a random conformation in aqueous solution and 2, 2, 2 trifluoroethanol (TFE) is required to induce a helical secondary structure. The CXCL4/PF-457-70 peptide was found to have antimicrobial activity and very limited hemolytic activity. The mechanism of action was analyzed from a kinetic model and molecular dynamics. The kinetic model led to a reasonable assumption about a rate constant and regulatory step on its action mechanism. Using molecular dynamics simulations, the structural properties the CXCL4/PF-457-70 have been examined in a membrane environment. Our results show that this peptide has a strong preference for binding to the lipid head groups, increasing the surface density, decreasing the lateral mobility of the lipids and consequently alters its functionality.

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RAPID ANALYSIS OF CAFFEINE IN "SMART DRUGS " AND "ENERGY DRINKS " BY MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY

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In recent years, the use of "Energy Drinks" in association with alcohol has became a fairly popular trend, particularly among young people, because of the efficacy of caffeine to enhance alertness, hence limiting the sedative effects of alcohol [1,2]. Evidence of a clinical syndrome of caffeine abuse and/or dependence has been widely reported, as well as caffeine-related poisonings and even deaths [3-5]. Also, caffeine has been found in some of the so called "Smart Drugs ", *i.e.* "legal " alternatives to scheduled substances, which have become available through the Internet or in special shops (Smart Shops).

The aim of the present work was to develop a simple, direct and rapid method based on microemulsion electrokinetic chromatography (MEEKC) with diode array detection (DAD) for the quantitative determination of caffeine in Smart Drugs and Energy Drinks.

Separations were carried out in a 50 cm x 50 µm (ID) uncoated fused silica capillaries. The optimized buffer electrolyte was composed of 8.85 mM sodium tetraborate pH 9.4, SDS 3.3 % w/v, n-hexane 1.5 % v/v and 1-butanol 6.6 % v/v, the separation voltage being 20 kV. Injections were performed at 0.5 psi for 3 s, after sample dilution with buffer (1:2 for energy drinks and 1:10 for smart drugs). Diprofilline was used as the internal standard. The determination of the analytes was based on the UV signal recorded at 275 nm wavelength, corresponding to the maximum of absorbance of caffeine. Under the described conditions, the separation of the compounds was achieved in 6 min without any interference from the matrix.

Linearity was assessed within caffeine concentration range from 5 to 100 ug/ml. The intra-day (N= 6) and day-to-day precision (N= 5) coefficients of variation were below 0.37 % for migration times and below 9.86 % for peak areas. Very informative UV spectra could be recorded with the DAD, allowing confirmation of peak identity and purity.

The MEEKC-DAD method above described offers fast, easy and low cost analysis of caffeine in soft drink and Smart Drugs. Furthermore, preliminary tests performed on urine samples showed the possibility to apply the same method, with minor changes, to this biological fluid, only requiring urine dilution before injection.

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PP-A-12

DEVELOPMENT OF A STABILITY-INDICATING CAPILLARY ELECTROPHORESIS METHOD FOR DETERMINATION OF AN ATYPICAL ANTIPSYCHOTIC DRUG IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

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A simple, sensitive and reproducible capillary electrophoresis coupled with a photodiode array detector method was developed for the quantitative determination of risperidone in presence of its degradation products in pharmaceutical dosage forms (tablets). Electrophoretic separation was achieved with a Beckman PACE/MDQ capillary electrophoresis system and fused-silica capillary of 30 cm effective length x 75 µm i.d. The electrolyte was composed of 20 mmol mL^{-1} phosphate buffer containing 0.1 % triethylamine, at pH 2.5. Other conditions are: 28 kV applied voltage, hydrodynamic injection at 0.5 psi/3s, and ambient temperature. Nitrazepam was used as internal standard. Quantification was achieved with UV detection at 236 nm. The method was validated according to the present International Conference on Harmonization guidelines. The high determination coefficient (R^2) = 0.9990 value indicated clear correlation between the investigated compound concentrations and their peak areas within the test range. The detection and quantitation limits were 5.49 µg mL^{-1} and 16.7 µg mL^{-1}, respectively. The repeatability and intermediate precision, expressed by the RSD, were less than 1.5 %. The accuracy, resulting from recovery experiments, was between 98.84 and 101.2 % for two sample tablets. The drug was subjected to acid, alkaline and neutral hydrolysis and oxidative stress conditions. No interference was observed from concomitant substances normally added to the tablets neither from degradation products. The method can indicate stability and can be used for the routine analysis of production risperidone samples.

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CAPILLARY ELECTROPHORESIS USED TO FOLLOW THE KINETICS OF THE OXIDATION OF PHENOLIC COMPOUNDS BY A CO(II) SALEN COMPLEX

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Since its introduction, capillary electrophoresis (CE) has shown a great potential in biopolymer analysis, inorganic ion and drug analysis, among other areas [1] and has developed into a very attractive technique offering speed, small sample and solvent consumption, low cost and the possibility of miniaturization [2]. CE can be used to follow the kinetics of different reactions. Although this approach has gained attention in recent years, especially in the study of enzymatic reactions [3], it has not been used to other fields such as the development of heterogeneous catalysts. In this work, we have studied the kinetics for the catalytic oxidation of different phenolic compounds using a cobalt(II) Salen complex [4] immobilized on the walls of the sample vial. Specifically, this presentation will be focused on the effect of pH, phenol concentration and catalyst concentration on the kinetics of the reaction.

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CE-MS FOR LEISHMANIA ANTIMONY RESISTANCE EVALUATION

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Leishmaniasis threatens about 350 million people in 88 countries around the world. WHO estimates the worldwide prevalence to be approximately 12 million cases, with annual mortality of about 60,000 and around 1-2 million estimated new cases per year [1]. The treatment choice is essentially directed by economic considerations; therefore, for a large majority of countries, chemotherapy relies only on the use of cheaper antimonial compounds. Unfortunately, in most parts of the world, the frequency of parasite antimony resistance linked to treatment failure is unknown because of a lack of information on Leishmania antimony susceptibility. This information is crucial for addressing the risk of selection and transmission of drug-resistant parasites, particularly in areas where antimony is the only chemotherapeutic alternative.

Metabolomics aims at understanding biology by comprehensive metabolite fingerprinting. In particular antimony-sensitive and resistant *Leishmania infantum*, with and without antimony treatments were extracted with methanol/ethanol and extracts were analysed with capillary electrophoresis time of flight mass spectrometry (CE-TOF-MS). Fingerprints were overlaid and aligned, and features were filtered by frequency and submitted to multivariate analysis. Samples clustered in a PLS-DA model into three groups: antimony-sensitive with and without antimony treatment and antimony resistant. Differences in the last group due to the treatment did not appear, as it was expected, because resistance is kept by growing parasites in antimony containing medium.

Afterwards groups were compared by pairs to extract masses showing statistical differences in a t-test ($p<0.05$) and those masses were sent to databases for a tentative identification. Interestingly, several compounds in arginine pathway were found increased in the resistant group as compared to the sensitive non-treated one since L-arginine transport from the extracellular milieu is important in providing factors needed for parasite growth.

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CHEMOMETRIC TOOLS IN THE DEVELOPMENT AND OPTIMIZATION OF ANALYTICAL METHODS FOR CARBOHYDRATES DETERMINATION IN FOODS

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The development and optimization of analytical methods is a big challenge in instrumental food analysis, since several factors can affect chromatographic or electrophoretic separations. Moreover, when the aim is separating simultaneously a large diversity of compounds, the number of responses became higher, involving the resolutions between compounds that co-elute, the intensity of analytical signal, the intensity of the noise, and the runtime. Multivariate optimization employing central composite design, with only 17 experiments, as well as the Derringer and Suich function, were used to study the potential of carbohydrates separation by micellar electrokinetic chromatography with anionic surfactant. These compounds have structures very similar and represent a constant challenge concerning their separation. With the support of chemometric tools, the use of mathematical models to predict the optimal conditions allowed to obtain six methods to analyze different sets of carbohydrates found in six different food matrices. It was studied the effect of pH, electrolyte and surfactant concentrations on the compounds separation. The methods were applied successfully in samples of condensed milk (separating lactose, lactulose, epilactose, galactose and glucose), orange juice (saccharose, glucose and fructose), rice bran (glucose, arabinose, fructose and xylose), red wine (saccharose, galactose, glucose, arabinose, fructose, xylose and ribose), roasted and ground coffee (galactose, glucose, arabinose, mannose, fructose, xylose and ribose) and breakfast cereals (saccharose, lactose, lactulose, epilactose, maltotriose, maltose, galactose, glucose and fructose). Multivariate methods were of great importance to describe accurately the effects of each variable and the interactions among them, as well as to realize the mathematical prediction of the optimal conditions. This study demonstrated that these tools can assist in the development of analytical processes, saving analysis time and reducing the costs in the experiments.

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PP-A-16

METHOD DEVELOPMENT FOR THE SEPARATION OF 13 PHENOLIC COMPOUNDS FROM EXTRA-VIRGIN OLIVE OIL BY CAPILLARY ZONE ELECTROPHORESIS AND CHEMOMETRICS

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Extra-virgin olive oil is obtained from olives only by mechanical means, which allows the retention of several minor compounds, phenolic compounds being among them. Phenolic compounds have high antioxidant capacity and contribute to the health benefits that come from olive oil consumption. Characterization of these compounds in olive oil has not been completed yet, due to the diversity of structures and matrix complexity. The aim of this work was to optimize and validate a method for simultaneous separation and quantification of 13 phenolic compounds found in extra-virgin olive oil: tyrosol, hydroxytyrosol, oleuropein glycoside, ferulic acid, *p*-coumaric acid, cinnamic acid, *p*-hydroxybenzoic acid, gallic acid, caffeic acid, luteolin, apigenin, vanillic acid and 3,4-dihydroxybenzoic acid. An Agilent G1600AX Capillary Electrophoresis system with diode array detector (DAD) was used for the experiments. Multivariate statistical methods as central composite design and Derringer and Suich simultaneous optimization method were used to optimize the separation. The variables studied were boric acid concentration, pH and voltage. On-line preconcentration techniques were also evaluated to improve method detectability. Multivariate procedures were adequate for determination of the optimal separation condition, using peak-pair resolution and running time as responses. Using on-line preconcentration it was possible to reduce the consumption of sample and toxic organic solvents, such as hexane and methanol, by a factor of 7.5. The final method employed fused-silica capillary of 50 µm i.d. x 60 cm effective length with extended light path, electrolyte boric acid 50 mmol L^{-1}, pH 10.2, 25 ºC, injection of 50 mbar for 25 s with application of reversed voltage (-30 kV for 5 s) before setting running voltage (+30 kV), detection at 210 nm and running time of 12 min.

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SENSITIVE CAPILLARY ELECTROPHORESIS METHOD FOR THE ANALYSIS OF ERYTHROPOIETIN IN THE PRESENCE OF ALBUMIN BY THE USE OF ELECTROKINETIC INJECTION

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A capillary electrophoresis method was developed for the analysis of recombinant human erythropoietin (rhEPO) in final drug preparations in the presence of large amount of human serum albumin (HSA) which is used as a protein excipient. In spite of the similarity of the physical characteristics of rhEPO and HSA in solution, a complete separation of the two proteins was achieved by the addition of metal salt to the background electrolyte. Analysis of rhEPO was also achieved at low drug levels (2000 IU) by the use of the electrokinetic injection (EI). Precautions were taken to avoid the drawbacks of EI.

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PP-A-18

VALIDATION OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE QUANTIFICATION OF FREE SUGAR IN THE BRAZILIAN MENINGOCOCCAL GROUP C CONJUGATE VACCINE

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Neisseria meningitidis group C is an encapsulated bacterium that causes several diseases and is associated with high mortality rates becoming a serious public health problem. In Brazil some outbreaks related to this group were diagnosed and its incidence is approximately 71%. Bio-Manguinhos is developing a conjugate vaccine constituted by covalent attachment of capsular polysaccharide to tetanus toxoid, which is currently being evaluated in Phase II clinical studies in children between 1-9 years old. Some authors described in the literature the value of 20% as the maximum level of free sugar present in other conjugate vaccine, after the conjugation and purification steps. Excess of this free component can induce immunological tolerance, with no response after repeated exposure to this antigen, by unknown mechanisms. Thus, the aim of this study was to validate a quality control method appropriate to separate and quantify free polysaccharide present in the brazilian meningococcal group C conjugate vaccine, using capillary electrophoresis technique. The total free sugar was completely separated from the conjugate by free zone capillary electrophoresis under basic conditions, using 50 mM sodium tetraborate buffer, maximum voltage and optimum temperature of 40°C. In these conditions it was possible to determine the free polysaccharide content and validate the proposed method, which was linear in 0.047 to 0.164 mg/mL range, showed a matrix effect, and detection and quantification limits of 0.0154 mg/mL and 0.0454 mg/mL, respectively. The repeatability, intermediate precision and robustness were also studied. The developed and validated methodology will be used to evaluate the conjugate batch that will be submitted to Phase III clinical studies and in the routine quality control of the conjugate vaccine.

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PP-A-19

CAPILLARY ION ANALYSIS: APPLICATIONS IN FORENSICS

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In the past decades capillary electrophoresis (CE) has been introduced into many fields of clinical and forensic chemistry, showing a wide analytical spectrum, easy and low cost operation, high productivity and versatility, good sensitivity, accuracy, precision and excellent robustness. Among the many application modes of CE, capillary ion analysis (CIA) has shown a great potential for the separation and determination of inorganic ions in different biological and non-biological matrices. In fact, CIA offers a simple but highly sophisticated alternative to the traditional techniques, such as ion chromatography or selective electrodes, for the analysis of small inorganic and organic ions. Its nature of separation method applied in a microenvironment fits perfectly the needs of forensic analysis, where low volumes of samples and their degradation/contamination represent frequent analytical issues.

CE offers great flexibility since the same instrumental hardware used for other applications (*e.g.* drug or protein analysis) can easily be switched, without major modifications, to ion analysis in few minutes. Moreover, the possibility of using indirect UV detection for CIA makes standard CE instrumentation perfectly suitable for the determination of the majority of small inorganic and organic ions, which do not absorb UV radiation. Last-but-not-least, since CE separation mechanisms are substantially different from those of chromatography, CIA can be used as “orthogonal “ method to confirm the results from ion chromatography [1].

The present work will offer an overview of the methods developed in our laboratory for ion determination for forensic or clinical purposes. Original CIA methods were developed for the rapid determination of nitrate and nitrite, as gun shot residues (GSR) [2], and for analysis of potassium in the vitreous humour, for the estimation of the post mortem interval (PMI) [3]. Furthermore, methods have been developed for the determination of bromide and lithium ions in serum during therapeutic treatments [4-5].

Moreover, a more recent method aimed at the determination of cyanide and its direct metabolite, thiocyanate, in biological fluids will be discussed. This method exploits the on-line complex formation allowing for direct UV detection of cyanide with minimal sample pretreatment.

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PP-A-20

OPTIMIZATION OF AN ANALYTICAL METHOD FOR RESOLUTION OF FLUVASTATIN ENANTIOMERS USING CAPILLARY ELECTROPHORESIS

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Chiral analysis has been required by pharmaceutical regulatory agencies due to possible different therapeutic effects related to enantiomeric pairs. While one exhibits the desired therapeutic effect, the other might cause adverse effects or even no effect. Fluvastatin (FLV), a synthetic statin used as a first choice treatment of hypercholesterolemia, is marketed as a racemic mixture, however, the therapeutic activity is 30-fold higher for the (+)-3R,5S-FLV enantiomer [1]. Capillary electrophoresis (CE) is a relatively new and powerful analytical technique, which provides high resolution and efficiency, associated with a lower consumption of solvents or reagents. The aim of this study was to develop a CE method for chiral separation of FLV enantiomers.

A Beckman P/ACE MDQ capillary electrophoresis system equipped with a diode array detector set at 239 nm was used. Data acquisition and processing was performed using the software 32 Karat version 8.0. Different background electrolytes (BGEs) were evaluated, such as sodium phosphate, sodium acetate and sodium tetraborate buffers in several pH values and concentrations, using a fused-silica uncoated capillary with 75 µm internal diameter (ID), and 50.5 cm effective length. Other experimental parameters tested for the choice of the best analytical conditions were voltage, temperature, concentration and type of chiral selectors. Among the chiral selectors, we tested neutral and negative β-cyclodextrins. For sample injection, hydrodynamic injection mode was used, with a pressure of 0.5 psi applied for 7s.

The choice of best condition was based in the selection of the highest value of resolution and plates, evaluated together with the lowest migration time values. A successful separation of FLV enantiomers was obtained in 10.81 min for the first enantiomer, and 11.05 min for the second one, using a 50.5 cm effective capillary length with 75 µm ID, 50 mM sodium tetraborate buffer (pH 9.3) as a BGE, containing 20 mM 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) as a chiral selector, and a constant voltage of +20 kV at 15 °C. Temperature values higher than 15 °C and voltage values above +20 kV resulted in no resolution of the enantiomers or much higher migration times, and these findings agreed with those in the literature [2]. The choice of sodium tetraborate as BGE in alkaline pH was also predicted in literature: it is well known that borate ions have a fundamental role in the complexation between diols - an organic group presented in FLV chemical structure - and cyclodextrins [3-5]. Among all the cyclodextrins tested in our experiments, HP-β-CD was the only one that provided enantiomeric resolution.

The selected group of conditions using the capillary electrophoresis technique allowed us to obtain the separation of FLV enantiomers with high efficiency, resolution and suitable migration times.

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PP-A-21

PRECONCENTRATION, DERIVATIZATION, AND CAPILLARY ELECTROPHORESIS SEPARATION OF BIOGENIC AMINES

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Biogenic amines are compounds widely present in fermented foods and beverages. Overconsumption of biogenic amines can be toxic, especially if taking certain antidepressants, causing severe detrimental effects on the immune system and central nervous system. The goal of this project is to develop a high-throughput method of analysis that requires minimal time, reagents, and sample to identify and quantify the presence of biogenic amines in real samples. Capillary electrophoresis separation coupled with UV-absorbance detection (CE-UV) is an attractive platform for such an analysis. However, the quantification of these compounds is difficult due to the low concentration sensitivity of CE-UV, which is exacerbated by the low concentration of biogenic amines in foods and beverages and the lack of intrinsic UV-absorbance of many biogenic amines. To solve these challenges, a sample preparation procedure for preconcentrating and simultaneously derivatizing the biogenic amines with a UV-absorbing chromophore has been developed. Recent results will be presented, including the pitfalls of performing the sample preparation steps via an in-line microreactor.

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PP-A-22

DEVELOPMENT OF A CAPILLARY ELECTROPHORETIC METHOD USING VARIOUS CYCLODEXTRINS AS SELECTORS FOR DETERMINATION OF CHLOROPHENYLPIPERAZINE ISOMERS IN CONFISCATED PILLS

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Capillary electrophoresis (CE) is a versatile technique known for its capability of easy selectivity manipulations. One possibility is the addition of a selector into the background electrolyte, which interacts with the analytes selectively. Although the cyclodextrins (CDs) as separation agents are employed especially in chiral recognition, they are also effective in positional isomers resolution [1,2]. Chlorophenylpiperazines (CPP) belong to the group of piperazine derived designer drugs and according to the chlorine substitution on the phenyl ring three positional isomers (ortho, meta and para) exist [3]. During method development six neutral CDs (α-CD, β-CD, methyl-β-CD, dimethyl-β-CD, hydroxypropyl-β-CD and γ-CD) were tested in order to discriminate between the isomers. The method was also checked for interferences with other drugs possibly presented in confiscated pills such as amphetamine, methamphetamine 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 1-(3-trifluoromethylphenyl)piperazine (TFmPP), 3,4-methylenedioxy-N-ethylamphetamine (MDEA) and cocaine. The optimised background electrolyte consisted of 20 mmol/L phosphoric acid, adjusted to pH 2.5 with triethylamine and 10 mmol/L α-cyclodextrin. The analyses were performed in 60.2 cm (effective length 50 cm) versus 50 µm id uncoated fused-silica capillary maintained at 25 °C. The applied separation voltage was 25 kV and the wavelength used for UV detection was 236 nm. Procaine was selected as internal standard (IS). The analytical curves were linear (R^2= 0.9994 - 0.9995) in the range 10 - 200 µg/mL of oCPP/ mCPP and 20 - 200 µg/mL of pCPP. The limits of detection (LODs) were 2 µg/mL (oCPP), 2.5 µg/mL (mCPP) and 3 µg/mL (pCPP). The intraday precision at three concentration levels (10; 100; 200 µg/mL; n=18) was evaluated as RSD = 4.9% (calculated as the ratio of corrected area of analytes to corrected area of the IS). Several confiscated pills were analyzed and mCPP was found alone or in combination with pCPP, methamphetamine and even cocaine.

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STUDY OF INTERACTIONS OF SULPHONATED FOOD DYES AND BRIJ 35 MICELLES BY QSRR

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In this work, the interaction of sulphonated dyes commonly used in the food industry (Brilliant blue FCF, Indigo carmine, Sunset yellow FCF, Tartrazine, Erythrosine, Allura red AC, Ponceau 4R, Bordeaux S, Fast green FCF, Patent blue V, and Carmoisine) with Brij 35 nonionic micelles was investigated. Retention data was obtained with an electrolyte composed of 10 mmol L^{-1} TBS buffer at pH 10.1, containing 10% ACN, and varying Brij 35 concentrations, 0 to 40 mmol L^{-1} [1].

The retention behavior reflected changes in both solvation of the anionic dyes and hydrophobic interactions between the bulk and highly polarizable palisade region of the micelles. In the condition of maximum occupation, *i.e.* one dye anion per micelle, the differential contributions of solvation and hydrophobic interactions to the measured retention factor can be rationalized. The enhanced basicity of the sulphonate anion at the micelle as compared to the bulk accounts for the specific interaction descriptors that support the QSRR model. A H-bond interaction between the terminal hydroxyl in the detergent structure and the oxygen atom of the sulphonate dye is then invoked to explain the interaction mechanism.

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HUMAN URINE PROTEIN FRACTIONATION USING OFFGEL ELECTROPHORESIS COMBINED WITH MICROCHIP CE DETECTION

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Human urine plays an important role in clinical diagnostics. The use of urinary biomarkers, as proteins, to diagnose diseases is a long-standing practice. Urine from a healthy individual contains a significant amount of peptides and proteins, and the number of them identified is still increasing. The urine presents the advantage to be obtained in large quantities using non-invasive procedures, besides urinary peptides and lower molecular mass proteins are generally soluble and its content is relatively stable.Two-dimensional gel electrophoresis (2DE) is a widely used technique for the analysis of complex protein samples. Although this method allows high-resolution separations, it is technically demanding, tedious, time-consuming, and difficult to automate.Thus, there is the need for simpler methods that have the same resolution of 2DE. In this work, we combined OFFGEL electrophoresis, for separation of the proteins by isoelectric point, and capillary electrophoresis in microchip for separation by molecular weight (kDa). Third milliliters of urine from health patient were precipitated with acetone overnight. In the sequence, the sample was centrifuged, and the pellet collected, dried, and suspended in rehydration buffer (7 mol L^{-1} urea, 2 mol L^{-1} thiourea, 4%CHAPS). The sample was submitted to dialysis for 48 h against water, lyophilized, and resuspended in rehydration buffer again. Next, the sample was fractionated by OFFGEL electrophoresis. TheAgilent 3100 OFFGEL Fractionator with a 24-well setup and the Agilent 3100 OFFGEL low resolution kit, pH 3-10 were used according to the standard protocol. The 24 fractions obtained were subsequently analyzed using the Agilent 2100 Bioanalyzer with the Protein 80 assay, according to the standard protocol. We verified a good separation of bands in the Bioanalyzer equipment. The fractions presented concentrations between 0.112 to 32.96 µg. μL^{-1}, according to data generated by the Bioanalyzer.The most concentrated fraction is in pH range of 5.25 - 5.62. The fractions 1-10, which correspond to pH range of 3.0 to 7.88, presented the highest number of bands, which was expected since the proteins in the urine are more concentrated in the acidic range.The highest number of bands was observed in fraction 7, with pH between 5.25 and 5.62, which presented 22 bands. Combining OFFGEL and Bioanalyzer it was possible to get good analysis of proteins in much less time than traditional two-dimensional gel electrophoresis. Using both techniques, OFFGEL and Bioanalyzer, it might be possible to detect differences between healthy and ill patients, and it might be a useful tool for search of potential biomarkers for diseases in a more instrumental way than 2D-GE, which is still manual.

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CHARACTERIZATION OF NEUTRAL SUGARS FROM GUAVA PECTIN BY CAPILLARY ZONE ELECTROPHORESIS

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Guava (*Psidium guajava*) is a tropical fruit that can be consumed both fresh and industrialized as pulp, jelly, juices and others. It can be found in all Brazilian regions, and the State of São Paulo is one of the largest producers. Guava is very rich in pectin, and although is not the most usual source for its extraction, it can be an alternative source. Pectin is a group of polysaccharides rich in galacturonic acid, rhamnose, arabinose, and galactose. It is widely used as thickener, stabilizer and gelling, in the production of gums, jellies and dairy products. Extracted pectin from different sources has different characteristics such as degree of esterification, molecular weight and neutral sugar content, and thus different functional properties. This study evaluated the difference of neutral sugar content in pectin from guava fruits, cultured in the region of southern Brazil, by capillary zone electrophoresis (CZE). For extraction of pectin from guava acid extraction was performed in the presence of citric acid. The hydrolysis of pectin to obtain the neutral sugars was performed with 2 mol L^{-1} trifluoroacetic acid. The separation was performed on a P/ACE MDQ capillary electrophoresis equipment with direct UV detection at 270 nm, using a fused silica capillary with 75 um i. d. and 50/60 cm (effective length/total length). The electrolyte was 130 mmol L^{-1} $Na_2HPO_4.2H_20$ and 36 mmol L^{-1} NaOH at pH 13.2. The capillary was conditioned before each run with 1 mol L^{-1} NaOH, 0.1 mol L^{-1} NaOH, water and electrolyte. The samples were hydrodynamically injected at 5 psi for 3 s, and the electrophoresis were performed at 14 kV with the temperature maintained at 15 °C.The results show the presence of galactose, glucose, rhamnose, arabinose and xylose.

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PP-A-26

THE DEVELOPMENT OF NUCLEOTIDE -BASED GELS FOR APPLICATIONS IN BIOSEPARATIONS

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Our group has studied a body of work related to the properties and applications of gels formed from guanosine compounds (G-gels). The gels we are interested in use guanosine-5'-monophosphate (GMP) to bring other gelation agents into solution. Previous work shows that GMP can combine with guanosine in aqueous solution to create binary gels with interesting thermoresponsive properties as a function of the proportions of the two compounds (Yu, Y. PhD Dissertation, RPI, 2009.) Here we describe the gelation properties of binary mixtures of GMP with other nucleotide species, including CMP and AMP. Potential applications of these gels include chiral separations, sequence-based DNA and RNA separations, and separations of other proteins and other biomolecules in capillary gel electrophoresis and microfluidic chips.

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ENANTIOSELECTIVE FUNGAL BIOTRANSFORMATION OF RISPERIDONE AND ANALYSIS BY CAPILLARY ELECTROPHORESIS

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Risperidone is a benzisoxazole derivative class of atypical neuroleptic agent. The risperidone metabolism yields the formation of two chiral metabolites, 7-hydroxyrisperidone and 9-hydroxyrisperidone. The 9-hydroxyrisperidone is the main metabolite of risperidone, and it has a pharmacological action similar to risperidone. This led this metabolite to be marked as a new drug under the generic name paliperidone. Microbial transformations of compounds present vital roles in the preparation of new derivatives with biological activities. In addition, biotransformation is nowadays considered to be an economically competitive technology in searching of new production routes for fine chemical, pharmaceutical and agrochemical compounds. The biocatalysis with whole cells is advantageous because it can have high productivity process and the catalysis can be enantioselective. This mode of producing metabolites is very convenient, and the preparative mode can be applied to obtain metabolites with complex structures that are difficult to obtain from mammals or from chemical synthesis.

The aim of this work was to evaluate several fungi (endophytic and soil fungi) in the biotransformation of risperidone and to propose a new way to obtain its chiral active metabolite, paliperidone. The stereoselective separation of the risperidone metabolites was performed by capillary electrophoresis, employing pH 3.0 phosphate buffer at 100 mmol/L containing sulfated-α-CD 2.0% (w/v) and carboxymethyl-β-CD 0.5% (w/v) with a constant voltage of -10 kV. The temperature of analysis was 20 ^{o}C and the injections were performed hydrodynamically employing a pressure of 0.5 psi during 8 s. The analytes were extracted from the culture medium employing a microextraction technique, hollow fiber liquid phase microextraction (HF-LPME). The bioanalytical method was validated according to the FDA guidelines. The following parameters were evaluated: linearity, limit of quantification, stability, accuracy, precision and recovery. All parameters were in agreement with FDA guidelines. After fully validation, a biotransformation study was performed employing endophytic and soil fungi. The biotransformation study was conducted by incubating the fungi with 5 mg of risperidone during 144 hours at 30 ^{o}C. Among the evaluated fungi, the soil filamentous fungi *Mucor rouxii* demonstrated high ability to metabolize risperidone enantioselectively into its chiral active metabolite, (-)-9-hydroxyrisperidone. It was observed that this filamentous fungus promoted a stereoselective biotransformation of risperidone to 9-hydroxyrisperidone with an enantiomeric excess of 79.6% for the (-)-9-hydroxyrisperidone formation. This study suggests that fungi may be employed to obtain the active metabolites, in this case, paliperidone.

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PP-A-28

GOLD NANOPARTICLES -COATED CAPILLARIES FOR PROTEIN AND PEPTIDE ANALYSIS ON OPEN-TUBULAR CAPILLARY ELECTROCHROMATOGRAPHY

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We report a new method of immobilization of gold nanoparticles on a fused silica capillary though covalent binding. The resulting modified capillary was applied to an electrophoretic system to improve the efficiency of separation and the selectivity of selected solutes. The immobilization of AuNPs on the capillary wall was performed in a very simple and fast way without requiring heating. The surface features of an AuNPs-coated capillary column were determined using scanning electron microscopy. The chromatographic properties of AuNPs-coated capillaries were investigated through variation of the phosphate buffer pH from 4.0 to 8.0. The fused silica capillaries had a 75 µm i.d.x 41.2 cm total length (31 cm effective length). The running condition was 12 kV, 25 °C, with UV detection at 214 nm. Effective separations of synthetic peptides mixture were obtained on the AuNPs- coated capillaries. The method shows a remarkable stability since it was reused about 900 times. The capacity factor was duplicated. Therefore this modification is stable and can be applied to different separation purposes. A complex mixture of tryptic peptide fragments of HSA was analyzed in both the bare and the AuNPs-coated capillaries. Better electrophoretic peptide profile was observed when using the AuNPs-coated capillary.

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PP-A-29

ANALYTICAL CORRELATION BETWEEN CE AND COLORIMETRIC BIOASSAYS IN MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICES FOR DETERMINATION OF MELAMINE IN MILK

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Melamine is used as a total protein adulterant due to the high content of nitrogen, that counterfeits methods base on total N for protein quantitation. FDA has established a tolerance level for melamine between 1 to 2.5 ppm in food. The cases of adulteration in China in 2008, however, dairy products presented contamination above 3300 ppm, which caused kidney failure, and even death. These facts have triggered an increased interest in analytical methods for the determination of melamine in food and feedstocks.

Here we compare two analytical methods to detect melamine in milk. The Capillary electrophoresis method was developed to validate the colorimetric method we propose, and the quantification of the melamine itself. This work also describes the use of microfluidic paper-based analytical devices (μPAD) with gold nanoparticles (AuNPs) as colorimetric probes in bioanalysis. Colorimetric detection of melamine is achieved when AuNPs are functionalized with a thiol cianuric acid derivative (MTT, 1-(2-mercaptoetil)-1,3,5-triazine-2,4,6-trione) for specific interaction with melamine [1] producing a visible color change on the μPADs. The visible color change from the wine red to blue color in the presence of melamine is due to the strong surface plasmon resonance (SPR) of AuNPs at 519 nm.

Sample preparation conditions included sampling, mixing, ultrasonic extracting, centrifuging, and filtering. The μPADs were fabricated using a wax printer to define the microfluidic features of the paper device and create a hydrophobic barrier to direct fluid flow. The stabilization of AuNPs in μPADs were performed with surfactant Tween-20. Then, in each μPAD detection zone we spotted 0.5 μL of AuNPs-MTT; we used 10 μL of melamine standard to different amounts (1 - 250 ppm) for each μPAD device. Scanned images were analyzed in RGB mode using the red channel in Adobe Photoshop for quantitation. The pre-treatment with 10% tween-20 had a better homogeneous distribution in the hydrophilic surface of the paper, resulting in greater sensitivity and better color change in the μPADs. After optimization, blind samples were determined by both CE and μPADs with correlation better than 95%. Using this approach, we have demonstrated the versatility and the performance of μPADs for colorimetric detection of low amounts of melamine (1 ppm) in less than 1 min. Therefore, applications with these biosensors can detect concentrations as low as the limit allowed by the FDA in dairy products with melamine adulteration (up to 250 ppm), at very low cost.

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MELAMINE DETECTION WITH GOLD NANOPARTICLES (AuNPS) ON MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICES (µPADS) USING CONTACTLESS CONDUCTIVITY DETECTION

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The fabrication of microfluidic paper-based analytical devices (µPADS) for colorimetric and capacitively coupled contacteless conductivity detection (C4D) of melamine with Au nanoparticles (AuNPs) is described. Melamine is illegally used to falsify the protein levels in milk or dietary products due to the fact that it contains 66% nitrogen by mass. Ingestion of melamine above the safety limit (2.5 ppm in USA and EU, and 1ppm for infant formula in China) can induce renal failure and death in infants. Analytical methods that detect at ppb levels have been used to detect melamine, however, recent colorimetric methods employing AuNPs can show semi-quantitative and quantitative results when using additional detectors. µPADS are fabricated with a wax printer and a hot plate. This process forms a hydrophobic barrier on the paper for direct fluid flow and the formation of reaction areas. To apply different analytical methods, C4Dis applied to the µPADS for the first time for melamine detection.This technique involves the measurement of a capacitance between two electrodes and the solution in the paper microchannel defined by the wax. A signal is produced once the melamine with MTT interacts with the AuNPs in the detection region. This phenomena makes possible to obtain analytical curves and quantify melamine in milk samples. The potential of µPADs with AuNPs mediating C4D is unique, and further capabilities arise if electrophoretic separations is applied to this system.

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PP-A-31

SEPARATION OF NINE WATER-SOLUBLE VITAMINS BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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Vitamins have been defined as a group of organic substances present in minute amounts in natural foodstuffs, which are essential for normal metabolism. Methods based on capillary electrophoresis (CE) are used routinely in food laboratories for the analysis of vitamins of group B and vitamin C in beverages, milk, juice and flour. The two modes of CE most commonly used for analysis of vitamins in food are capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) [1]. However, as the heterogeneities of water-soluble vitamins include variations in charges and hydrophobicities, CZE may give limitated selectivity as compared to MEKC [2]. Therefore, in this work, the separation of nine water-soluble vitamins was assessed by MEKC using sodium dodecylsulfate (SDS), sodium tetraborate (pH 9.2) and methanol (15% v/v) as electrolyte and detection at 200 nm. The method presented good linearity $r^2 > 0.98$ in the concentration range from 20.0 to 50.0 mg L^{-1} for vitamin C and 10.0 to 40.0 mg L^{-1} for the B group vitamins. The limits of detection and quantification were in range from 1.25 to 5.62 mg L^{-1} and 4.16 to 15.1 mg L^{-1}, respectively. The repeatability for peak area was under 9.0 % RSD (n = 10). Application of the method for determination of vitamins in powder milk and lacteal flour samples after acid hydrolysis and preconcentration by solid-phase extraction is currently under investigation.

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PP-A-32

FORENSIC DNA SCREENING USING SHORT CHANNEL MICROCHIP ELECTROPHORESIS

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While analytical systems using multicapillary sequencers are well adapted for large-scale sample collection from blood or buccal swabs, these systems are neither portable nor flexible enough for use in applications such as border crossings or police stations where time to result is critical nor are they sufficiently portable for use in screening evidence at mass disaster sites. Currently such screening is done by fingerprinting, however DNA typing is a far more powerful tool and could replace or enhance the capability of border agents and police agencies across the world to rapidly identify persons of interest if sufficiently fast and portable systems could be developed.

These reasons, amongst others within the scientific community, have lead to great interest in the development of rapid microfluidic chip based screening and genotyping. A small subset of the full cadre of DNA markers could be used to quickly screen submitted samples for DNA. Stains containing non-probative DNA from the victim and other household members can be eliminated using this technique, saving time and valuable reagent costs. Another reason for interest in microfluidic genotyping is its portability. Portable genotying systems would be of great value in mass disasters for rapidly determining the identity of victims.

The goal of this project was to develop a method for the rapid high resolution genotyping of single-stranded DNA samples, using denaturing gel electrophoresis on a modified Agilent 2100 Bioanalyzer equipped with a thermal heat plate and dual laser detector. Because of its high speed, small footprint and portability, this system can be an effective tool to quickly screen and identify individuals detained at ports of entry, police stations and mass disasters. This poster describes our efforts to optimize the resolution and sizing precision of the microfluidic system. The system utilizes a 12-channel electrophoretic chip and associated software capable of performing genetic analysis of up to 7 multiplexed CODIS STR loci. Using elevated temperatures and a specially engineered polymer system we can resolve all components in the allelic ladder with a precision better than 0.15 bp. The system has the capability to distinguish individuals with a power of discrimination of greater than 1 in 10^5 with an electrophoretic separation time of under 80 s per sample, making it more than sufficient to presumptively identify individuals at border crossings and checkpoints.

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CAPILLARY ELECTROPHORESIS ENANTIOSELECTIVE ANALYSIS OF ZOPICLONE AND ITS IMPURITIES

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Zopiclone (ZO) is a non-benzodiazepine hypnotic drug indicated for the treatment of insomnia. ZO is a chiral drug administered as a racemic mixture [1]. However, a preparation containing only ((+)-(S)-ZO) has been approved by FDA [2]. The most important degradation products of ZO are RP 29307, RP 29753, RP48497 and RP 266953.Thus enantioselective methods for the analysis of ZO and its impurities in tablets are required as a tool in the study of degradation and racemization of this drug. A capillary electrophoresis method was developed and validated for the simultaneous quantification of ZO and its impurities (RP 29753 and RP 26695) in tablets. The separation was carried out with an uncoated fused-silica capillary (50 µm, 42 cm effective length, 50 cm total length) using 80 mM phosphate buffer pH 2.5 and 5 mM carboxymethyl- β-cyclodextrin. The analytes were detected at 305 nm. A tension of 27 kV was applied and the capillary temperature was 25 ºC. The analytes were extracted from tablets with acetonitrile. All enantiomers were analyzed within 8 min and linear calibration curves over the concentration range of 0.8 - 1.6 mg mL^{-1} for ZO and 0.8 - 1.6 µg mL^{-1} for its impurities were obtained. The coefficients of correlation obtained for the linear curves were greater than 0.99. The intra-day and inter-day accuracy and precision were lower than 5% for all analytes. This validated method [4] was employed in a study of degradation of ZO under stress conditions. The degradation of ZO demonstrated the importance of a stability-indicating assay method for this drug.

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PP-A-34

CAPILLARY ELECTROPHORESIS-ELECTROSPRAY IONIZATION MASS SPECTROMETRY IN STUDIES OF RADICAL ACETYLATION OF AMINO ACIDS

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Recently, we hypothesized that acetyl radicals produced by the diacetyl/peroxynitrite system may modify proteins and DNA thus threatening the functions of those cells experiencing nitrosative and carbonyl stress. In the present work, we provide methods of analyzing acetylated free amino acids via capillary electrophoresis-electrospray ionization mass spectrometry in tandem (CE-ESI-MS) and MS/MS with an ion trap analyzer. Twenty L-amino acid standard solutions (2.0 mMol L^{-1}) were studied under conditions favorable to radical acetylation. The reaction mixtures contained peroxynitrite (1.0 mMol L^{-1}) and diacetyl (2.0 mMol L^{-1}) in a 200 mMol L^{-1} phosphate buffer, pH 7.2 at 25 ^{o}C. All spent reaction mixtures analyzed by CE-ESI-MS revealed the presence of the corresponding acetylated amino acids plus 42 Da (mass of acetyl radical). Diacetyl, formic acid, phosphoric acid, ammonium formate, sodium tetraborate, sodium nitrite, Chelex-100, 2-methyl-2-nitrosopropane (MNP) and the amino acids (L-Gly, L-Ala, L-Val, L-Leu, L-Ile, L-Met, L-Phe, L-Tyr, L-Trp, L-Ser, L-Pro, L-Thr, L-Cys, L-Asn, L-Gln, L-Lys, L-His, L-arg, L-Asp and L-Glu were purchased from Sigma-Aldrich. Peroxynitrite was synthesized from sodium nitrite (0.60 Mol L^{-1}) and hydrogen peroxide (0.70 Mol L^{-1}) in a quenched-flow reactor according to [1] and quantified spectrophotometrically at 302 nm (μ = 1670 Mol L^{-1} cm^{-1}). A diacetyl (50 mMol L^{-1}) stock solution was prepared in a 200 mMol L^{-1} phosphate buffer. The radical acetylation reactions were conducted in medium of peroxynitrite (2.0 mMol L^{-1}) and added to diacetyl (2.0 mMol L^{-1}) and amino acid (1.0 mMol L^{-1}) in a medium containing a phosphate buffered solution (200 mMol L^{-1}, pH 7.4).

The separation and detection of the analytes in all spent reaction mixtures were performed with a Beckman Coulter, model MDQ capillary electrophoresis system (CA, U.S.A.) and a Thermo Finnigan mass spectrometer, model LCQ Ion Max Advanced with an ESI source and an ion trap analyzer (MA, U.S.A.). Electrophoreses were carried out in fused-silica capillaries from Polymicro Technologies (AZ, U.S.A.) with 50 µm I.D. x 70 cm total length. The electrolyte used for the CE/ESI-MS analyses was a 1.0 Mol L^{-1} formic acid solution. Before injection, the capillary was preconditioned by flushing 1.0 Mol L^{-1} ammonium hydroxide for 3 min followed by water for 2 min and finally, the electrolyte for 5 min. Reaction samples were injected under a 0.5 psi pressure for 5 s. The effective applied electric field was 260 V cm^{-1}, the capillary temperature was kept at 25° C, and the sheath-flow mode interface was used. The make up liquid was 50.0:49.5:0.5 water/methanol/acetic acid delivered at a flow rate of 5 µL min^{-1} using a Gilson Pump Model 402 (MI, USA). MS and MS/MS experiments were performed in the positive ion mode at a 4.5 kV ion spray voltage. Data acquisition was made by full scan and MS/MS scans at the most intense peaks with the normalized collision energy for MS/MS set at 30-35% and the isolation width of m/z 1.0. The results support our hypothesis of the chemical modification in protein and DNA by acetyl radicals leading to possible cell injury and protein modification.

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LECTIN-CHROMATOGRAPHY MEDIATED GLYCOPROTEOMIC ANALYSIS BY TWO-DIMENSIONAL ELECTROPHORESIS

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The field of glycobiology has grown considerably since the 80s, and now includes studies on glycosyltransferase defects in some forms of muscular dystrophy. The most studied defects in glycosylation are currently organized in groups known as congenital disorders of glycosylation (CDG), with the defects occurring in the stages of elongation (CDG type 1) or processing (CDG type 2) of oligosaccharides. Glycosylation defects include abnormalities in the synthesis of N-linked, O-linked, or both N- and O-linked glycoproteins. Glycoproteins are classified according to the type of binding that occurs between the oligosaccharide and the peptide in question. Its formation can occur either by an N-connection between the amide group of specific asparagine residues and N-acetylglucosamine (GlcNAc) residues, or by an O-linkage through hydroxyl groups, mainly of serine or threonine, via N-acetylgalactosamine (GalNAc), mannose (Man), xylose, and other monosaccharides [1].

Glycosylation pathways in eukaryotic systems are diverse and very important for proper metabolic development, as well as for communication between cells. Defects in glycosylation can affect protein interactions with other proteins interfering in signaling pathways, stability and conformation, thus altering the protein function in these systems [1].

Several techniques and methods have been developed and tested for analysis of glycoproteins. In the present work, we compared the results obtained from the analysis of total proteins and glycoproteins pre-purified by wheat germ aglutinin (WGA), from muscle tissue of C57Black6 mice. The separation technique used is the two-dimensional electrophoresis (2-DE), and the identification of proteins was made by mass spectrometry (MS) (both MALDI-TOF-TOF and ESI-FTICR-NLC). The elimination of the majority proteins (non-glycosylated proteins) from the extracts favors the detection of glycoproteins, especially those present in low concentrations, as it allows a greater amount of glycoproteins to be added to the isoelectric focusing strips. In the identification stage by MS, the absence of ions from highly abundant proteins ensures a considerable increase in the capacity of detection of ions related to the glycoproteins, allowing higher score values from these ions.

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PP-A-36

QUANTIFICATION OF FLAVONOIDS IN JELLIES FRUIT, A COMPARISON OF CAPILLARY ELECTROPHORESIS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Flavonoids are phenolic compounds with high antioxidant capacity and abundantly present in natural products. They have numerous biochemical, physiological and pharmaceutical activities, such as vasodilatatory, anti-inflammatory, antibacterial, immune-stimulating actions, and also the anti-allergic and the antiviral effects. This study aimed to compare High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE) on the efficiency of separation and quantification of seven flavonoids present in fruit jellies: (+)-catechin, (-)-epicatechin, kaempferol, myricetin, narirutin, quercetin and rutin. The two proposed methods were subjected to validation procedures and both were able to quantify six of seven flavonoids under investigation. Only the CE was able to quantify myricetin. The two methods showed acceptable selectivity, linearity, repeatability and recovery rates. Lower limits of detection and quantification were found in HPLC, but CE was faster, it had lower reagent consumption and less waste. Regarding samples analysis, the two techniques showed similar results.

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PP-A-37

CONCENTRIC ELECTRODES ON MICROFLUIDIC DEVICES FOR CONTACTLESS CONDUCTIVITY DETECTION

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In the last ten years, microfluidic devices featuring capacitively coupled contactless conductivity detection (C4D) have been widely employed for analytical determinations. The main advantage of C4D systems consists in the electrical insulation of the electrodes with respect to the electrolytic fluid, avoiding problems commonly present in faradaic electrochemical methods such as: i) passivation and contamination of the electrodes, ii) formation of bubbles, and iii) electrical interference between the detector circuit and the separation field applied in capillary electrophoresis (CE). On the other hand, its major disadvantage is related to the lower sensitivity (in general in the order of μmol L^{-1}) than conventional conductivity (contact-mode) and faradaic methods. This drawback arises from two phenomena, namely: i)absence of charge transference on electrode surface and ii) direct capacitive coupling between the electrodes, generating the stray capacitance. The ideal cell assembly concerning to the maximum sensing area consists of electrodes around the entire microchannel (concentric electrodes), as it happens in conventional CE. Compared to conventional capillaries, the limits-of-detection (LODs) for C4D microchips are *ca.* 1 order of magnitude higher. In order to overcome this main disadvantage, a new fabrication procedure to integrate the electrodes in C4D microchips was proposed. This method was based on the deposition of thin metal films on the both glass plates that compose the microchip. The dielectric layers were obtained covering the top and bottom plate electrodes with silicon dioxide and polydimethylsiloxane (PDMS) respectively. Experiments of flow analysis system were performed to compare the performance between the conventional planar electrodes and concentric electrodes. The LODs were improved in *ca.* five orders of magnitude compared to the levels obtained for planar electrodes. C4D systems provide the unique advantages associated to the electrical insulation of the electrodes, easy on-chip integration, and simple instrumentation. In addition, it is important to highlight that the sensitivity levels achieved herein can be further improved by integrating on C4D-CE microchips methods already described in literature. These include: i) high-voltage excitation signals (hundreds of volts); ii) Faraday shieldingto reduce the stray capacitance; iii) reduction of the baseline level; and iv) sealing by adhesive bonding with nano-thin film for insulation of the planar electrodes at the bottom plate.The development of high-performance C4D-CE microchips will extend the range of applications of this technique.

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PP-A-38

DEVELOPMENT OF A CAPILLARY ELECTROPHORESIS METHOD FOR EVALUATING AND MONITORING THE ADEQUATE ENCAPSULATION OF DOXORUBICIN IN LIPOSOMAL FORMULATIONS BY THE QUANTIFICATION OF SULFATE ION

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Doxorubicin (DXR) is a potent antineoplastic agent with activity against several human cancer diseases. DXR is a DNA-intercalating agent and a topo-isomerase II inhibitor. It is also known because of its ability to form free radicals which produce lipid peroxidation and can damage DNA, being these effects probably related to its mechanism of action. However, the administration of DXR can produce side effects such as vomiting, bone marrow suppression, alopecia, mucositis and drug induced dose limiting irreversible cardiotoxicity and myelosuppression.

Encapsulation of DXR into pegylated liposomes as drug delivery systems, modifies bioavailability, biodistribution, and reduces side effects. Therefore, achieving high encapsulation efficiency of DXR into liposomes is desirable. One strategy to load DXR into liposomes is the use of ammonium sulfate gradient. The principle is that the charged DXR base diffuses inside the liposome and it is retained within the liposome, and the ammonium diffuses outside. One way to measure the efficiency of encapsulation is to determine the concentration of inorganic sulfate, which produces the gradient, on both sides of liposomes membrane.

Optimization and validation of a capillary zone electrophoresis (CZE) method for the determination of inner and external inorganic sulfate from doxorubicin-loaded liposomes as a measurement of encapsulation efficiency is presented. Sulfate was determined using a fused silica capillary (50 cm x 75 μm ID) and background electrolyte (BGE) composed of 5 mM chromate with 1 mM of TTAB at pH 10.5 with indirect UV detection at 254 nm. The separations were achieved at temperature of 25 ºC, 5 kV of reverse applied voltage using a pressure of 0.5 psi for 5s for sample introduction. Sulfate quantitation from the outside of liposomes was obtained by simple dilution of the liposome sample but an extraction with a non-ionic surfactant and a mixture of methanol-chloroform-water was necessary to breakdown the liposomes for the quantitation of the total sulfate (inside and outside) with acceptable recovery percentages (96.3% to 102.6%). Parameters of validation were evaluated according to ICH guidelines such as specificity, linearity, LOD, LOQ, accuracy, precision and robustness. The proposed CE method resulted to be suitable for the inner and external sulfate analysis of doxorubicin-loaded liposomes for routine quality and stability control studies.

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PP-A-39

AMPEROMETRIC LACTATE BIOSENSOR BASED ON SCREEN PRINTED ELECTRODES MODIFIED WITH CARBON NANOTUBES

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The development of amperometric L-lactate biosensor based on screen-printed electrodes containing carbon nanotubes (CNTs), and its integration into a flow injection analysis system (FIA) is described herein. The biosensor was fabricated by immobilizing lactate oxidase (LOX) on a Prussian Blue (PB)-modified electrode. The lactate biosensor showed excellent performance under the selected experimental conditions (applied potential, pH, flow rate, linear range, detection limit, response sensitivity, and stability). The fabricated biosensor, that also displayed good selectivity, was applied to the determination of lactate in real samples.

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PP-A-40

DEVELOPMENT AND VALIDATION OF A MICELLAR ELETROKINETIC CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS SEPARATION AND QUANTIFICATION OF DRUGS IN IN VITRO PERMEATION STUDIES

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In vitro methods using animal intestinal epithelium are useful tools to evaluate drug permeability which is an important parameter of oral bioavailability. In this work a simple, precise and accurate micellar electrokinetic chromatographic (MEKC) method was developed and validated for simultaneous determination of zidovudine, lamivudine, acyclovir, phloridzin and metoprolol drugs in permeation studies using frog (*Rana catesbeiana*) intestinal membrane. The MEKC method was performed using a fused-silica capillary with 50.2 cm x 75 µm i.d. (effective length of 40 cm), a buffer solution containing 20 mmol L^{-1} sodium tetraborate (pH 9.2): 30 mmol L^{-1} sodium dodecyl sulphate and 10% (v/v) ethanol. The applied current was 80 µA, samples were injected hydrodynamically at 0.5 psi for 10 s and the capillary temperature was 25 °C. Direct UV detection at 214 nm led to an adequate sensitivity. All drugs were simultaneously separated in less than 20 min. The MEKC method was validated regarding linearity, detection and quantitation limits, precision and specificity. Analytical curves presented good coefficients of determination ($r^2 > 0.99$). Detection limits for zidovudine, lamivudine, acyclovir, phloridzin and metoprolol were 0.033 µg mL^{-1}, 0.051 µg mL^{-1}, 0.067 µg mL^{-1}; 1.3 µg mL^{-1} and 2.9 µg mL^{-1}, respectively. Quantitation limits for zidovudine, lamivudine, acyclovir, phloridzin and metoprolol were 0.10 µg mL^{-1}, 0.16 µg mL^{-1}, 0.20 µg mL^{-1}, 3.9 µg mL^{-1} and 8.9 µg mL^{-1}, respectively. Acceptable precision (RSD < 3%) was obtained. The specificity of the method was demonstrated by the absence of interference among the drugs and Ringer solution and intestinal membrane. The proposed MEKC method showed to be simple, precise and suitable to quantify drugs in *in vitro* intestinal permeation studies.

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PP-A-41

SENSITIVE AND FAST DETERMINATION OF SUDAN DYES IN CHILLI POWDER BY PARTIAL-FILLING MICELLAR ELECTROKINETIC CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Sudan dyes are a family of lipophilic synthetic organic colorants, characterized by a chromophoric azo-group, extensively used in industrial and scientific applications but banned as food colorants due to the carcinogenic effects. Sudan dyes have been illegally added to foodstuffs to enhance the red-orange color of products and easily used because of their low cost and wide availability [1]. A fast and sensitive method for the simultaneous determination of Sudan dyes (I, II, III, and IV) in food samples was developed for the first time using partial filling micellar electrokinectic chromatography with mass spectrometry (MEKC-MS). The partial filling technique was necessary to avoid the contamination of the ion source with non-volatile micelles. MEKC separation and MS detection conditions were optimized in order to achieve a fast and efficient separation of the four dyes with high sensitivity. Filling 25 % of the capillary with a MEKC solution composed of 40 mmol/L ammonium bicarbonate, 25 mmol/L SDS, and 32.5 % acetonitrile (v/v), a baseline separation of the four azo-dyes was obtained in 10 min. Under the optimized conditions, limits of detection from 0.05 to 0.2 μg/mL were obtained. The suitability of the developed method was demonstrated by the fast and sensitive determination of Sudan dyes (I, II, III, and IV) in spiked chilli powder samples.

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CHARACTERIZATION AND VISUALIZATION OF APTAMER-MODIFIED SURFACES AND THEIR AFFINITY PROTEIN CAPTURE

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We are studying the processes that govern affinity protein capture at aptamer-modified surfaces using an affinity MALDI-mass spectrometry (MALDI-MS) platform [1,2] in combination with imaging tools. Better fundamental understanding of protein capture at aptamer-modified surfaces will lead to improved design and reliability of devices and processes for protein analysis, separation, isolation and purification. Here we will describe studies of the thrombin binding aptamer (TBA), which consists of the DNA sequence 5 '-GGTTGGTGTGGTTGG-3' and binds specifically to the protein thrombin [3]. TBA forms a highly stable, "chair like " G-quadruplex structure consisting of two guanosine tetrads [4] that is necessary for thrombin binding. The affinity surfaces of interest include fused silica plates, fused silica beads, indium tin oxide (ITO)-coated glass slides, streptavidin-coated plates, and streptavidin-coated magnetic beads. Experiments include studies of the distribution and density of bound TBA at the various surfaces, optimization of these parameters for thrombin capture, and effects of experimental conditions such as protein concentration, buffer composition, pH, incubation time and temperature on protein capture.

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SEPARATION OF FOUR SAME-LENGTH BACTERIAL DSDNA FRAGMENTS BASED ON PRIMARY SEQUENCE USING GUANOSINE GELS IN CGE

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Capillary electrophoresis is widely used for the study of DNA. Sieving gels are most commonly used in DNA separation and have achieved high resolution and reproducibility. However, these kinds of gels can only resolve DNA strands of different lengths, while the analysis of complex bio-samples may require the distinction among different DNA sequences. Guanosine gels (G-gels) are self-assembled networks of guanosine nucleotide tetrads. Previous works have demonstrated their ability to separate single-stranded DNA 76-mers that differ by only a few A/G bases. The present work is focused on applying G-gels to the separation of denatured, double-stranded DNA in which only one strand is fluorescently tagged. DNA was collected from four different bacteria. Each strand is 193bp in length. DNA was denatured before use. G-gels in potassium phosphate or Tris buffer were used for the separation. Experimental conditions including temperature, pH, buffer concentration, salt concentration and additives were studied for optimization.

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