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World Analytical Chemistry & Mass Spectrometry

&

World HPLC, Separation Techniques & Pharmacovigilance

August 29-30, 2018 | Toronto, Canada

Poster Presentation

Alina Vasilescu, J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
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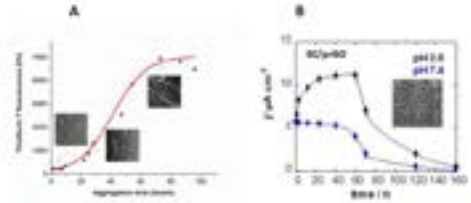
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Electrochemical sensors coated with prGO coated enable to capture different aggregation behaviors of proteins and peptides

Alina Vasilescu
International Centre of Biodynamics, Romania

Statement of the Problem: Protein instability due to misfolding and aggregation is of big concern for protein-based therapeutics because it impacts the bioavailability and immunogenicity of such drugs. Simple and cost-effective analytical methods, indicating the presence or absence of protein aggregates, are consequently of high importance.

Methodology & Theoretical Orientation: Porous reduced graphene oxide (prGO) coated electrodes have been investigated for the early and sensitive identification of protein aggregation. The detection principle lies in following the change in the oxidative current of the proteins. The sensor architectures studied included glassy carbon electrodes with drop cast prGO and disposable, screen printed electrodes modified with prGO using the layer-by-layer deposition technique. The studies focused on the protein lysozyme and the pharmaceutical polypeptide calcitonin having the ability to form aggregates in different conditions of pH and temperature. Parallel experiments were performed by fluorescence with thioflavin T, size exclusion chromatography and Atomic Force Microscopy Imaging.



Findings: Comparing the oxidation peak of lysozyme by differential pulse voltammetry for different electrode architectures allowed validating the higher sensitivity of the prGO-coated interfaces versus bare ones. Moreover, the modified electrodes allowed detecting in a fast and reliable manner the changes in the protein structure occurring at pH 2 and pH 7.4, as per processes leading to the formation of amyloid and amorphous aggregates, respectively (Fig.1). Screen printed electrodes modified with prGO enabled to differentiate between the amyloid-type aggregation of calcitonin (2 mg mL-1) in citrate buffer and no amyloid formation in acetate buffer. These electrodes were also applied to the analysis of a pharmaceutical drug product of low potency, Miacalcic (8.3 µg mL-1 calcitonin), where no aggregation was observed.

Conclusion & Significance: Electrochemical sensors coated with prGO coated enable to capture different aggregation behaviors of proteins and peptides and represent a complementary tool for biopharmaceutical analysis.

Biography

Alina Vasilescu is an analytical chemist with expertise in the development and validation of novel analytical methods. Her experience encompasses both academic research and analytical research in the pharmaceutical industry. She currently works as a Senior Researcher at the International Centre of Biodynamics in Bucharest, where she coordinates several research projects, focussing on the development of (bio)sensors for practical applications. Study of protein aggregation is a primary research area and she collaborates with other groups for including nanomaterials in the development of novel sensors.

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Notes:

Nives Galic et al., J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

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Structural investigation of Fe(III) and Ga(III) complexes with aromatic hydrazones by ESI MS/MS

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Aroylhydrazones can act as neutral, monoanionic or dianionic ONO tridentate ligands [1]. The coordination abilities of aromatic hydrazones derived from nicotinic acid hydrazide and differently substituted 2-hydroxybenzaldehydes towards Fe^{3+} and Ga^{3+} will be discussed. Different techniques, like UV-Vis, vibrational spectroscopy and mass spectrometry were used for structural investigation of the complexes in solution and in the solid state. In this work, the ESI MS and MS/MS spectra, including fragmentation pathways of Fe(III) and Ga(III) complexes with aroylhydrazones are presented.

Biography

Nives Galic was born in Zagreb, Croatia. She received a Ph.D. degree in Analytical Chemistry (1999). In 2016 she was elected to the position of Full Professor. During the period 2011-2017 she was the Head of the Division of Analytical Chemistry. She is a leader of the project funded by Croatian Science Foundation (IP-2014-09-4841).

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August 29-30, 2018 | Toronto, Canada

A new marker system for neem oil

Caijun Zhang
Terramera Inc., Canada

Statement of the Problem: Neem oil contains more than 100 biologically active compounds (limonoids). Azadirachtin as a marker for neem oil is well adopted in the biopesticide industry. However, this marker is insufficient to distinguish whether the neem oil is contaminated with other botanical oil because azadirachtin (A and B) represent only the polar and hydrophilic limonoids, very small portion of bioactive ingredients in neem oil.

Methodology & Theoretical Orientation: Terramera develop a finger-print HPLC method which can detect most bioactive ingredients in neem oil. According to the HPLC profile, a new marker system was developed in Terramera. This new marker system includes a wider spectrum of active ingredients and can protect from the adulterated neem oil because any foreign peaks can be easily detected under other limonoids region

Conclusion & Significance: The new marker system for neem oil provides a much better representation of the quality of neem oil than measuring azadirachtin alone.

Biography

Caijun Zhang has built up his expertise in analytical chemistry support to pharmaceutical industrial for more than 20 years. He recently brings his knowledge to Terramera Inc, an agriculture biotech company and support the product development from prove- concept stage to the commercialization of finished product.

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What about dried blood spot for cannabinoid quantification?

Lounès Haroune
Pharmacology Institute of Sherbrooke, QC

While there has been a growing interest in understanding the pharmacological and physiological properties of cannabinoids in the last decades, analytical methodologies including sample preparations, remain one of the most challenging topics for their quantification in biological matrices. Moreover, the low sample weight or volume coupled to the complexity of biological samples (i.e. whole blood, plasma, etc.) could overwhelm the analyst expectations.

In this study, we explored different possibilities to quantify a mixture of 8 natural phytocannabinoids present in biological samples (cannabinol, cannabigerolic acid, cannabinochromene, cannabigerol, cannbidiolic acid, tetrahydrocanninol, tetratracannabinolic acid and cannabidiol).

The evaluation was carried-out using plasma and whole blood samples using different usual extraction protocols (solid phase extraction, liquid-liquid extraction, protein precipitation and blood spot sampling). Stability of tested molecules was also evaluated in several matrices (plasma, serum, *ex vivo* and pharmacokinetic profiles).

The results showed a moderate matrix effect resulting by signal suppression (≤30%) and acceptable recoveries (≥60%) for most of the different tested extractions and matrices, except for whole blood when using acetonitrile for protein precipitation, which appears to be the less efficient approach for cannabinoid extraction, with a recovery lower than ≤40%.

The applicability of tested methodologies was also applied for the determination of pharmacokinetic profiles and showed that dried blood spot sampling (DBS) could become an interesting alternative for *in vivo* studies. DBS is a rapid, acute and minimally invasive technic based on a single blood drop (10µL–25µL) that reduces handling and quantity of blood to be sampled, which consequently also reduces the cost of analysis. According to these aspects, DBS could become a reference methodology for *in vivo* pharmacokinetic experiments.

Biography

Lounès Haroune is manager of the bioanalytical platform of the pharmacology institute of Sherbrooke University. After 5 years as analytical development manager in an analytical laboratory and graduated in analytical chemistry, he is working on the development of analytical methodology and sample preparations for the detection and quantification of biomolecules in biological matrices. He also works on the metabolomics and peptidomics methodologies in complex matrices. He also focuses on the development and implementation of new analytical workflow for molecular characterizations (biocatalysis, physico-chemistry reaction, etc).

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Notes:

Sabrina Saibi, J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

A simple analytical method for the detection and quantification of a pharmaceuticals and pesticides in complex environmental matrices

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Université de Sherbrooke, QC

During the last years, the use of sewage sludge as soil amendments for crops has gain in interest. It limits the addition of fertilizers, it reduces the land occupation and it promotes waste valorization. However, the presence of organic compounds such as pharmaceuticals and pesticides (PhPCs) may cause the transfer of these contaminants to the soil and to the groundwaters. In this work, an analytical method for the simultaneous extraction of 70 compounds from complex matrices was developed and validated using an experimental design plan. Firstly, the targeted compounds were extracted by an optimized QuEChERS approach using ethyl acetate/water (4/1, v/v) as the extraction solvent and a dispersive solid phase extraction (dSPE) with C18/Na_2SO_4. Then, the analytes were quantified using a LC-MS/MS methodology. The method was validated in terms of accuracy and precision. The results obtained showed a strong matrix effect resulting by signal suppression. Therefore, the solvent matched calibration approach was chosen for the quantification. The applicability of the method for different matrices was demonstrated through the analysis of biosolids samples from Magog (Qc) waste water treatment plant, sediment samples from Massawippi (Qc) and Montjoie (Qc) Lakes and benthic organisms (cheronomedae and oligochaete). The recoveries were higher than 50% for most of the targeted compounds in all tested matrices. 10 compounds (acetaminophen, caffeine, carbendazim, naproxen, carbamazepine, atrazine, ibuprofen, fenofibrate and ketoprofen metolachlor) were quantified in the samples at concentration ranging from ≈ 5 ng.g-1 to ≈ 40 ng.g-1.

Biography

Sabrina Saibi is PhD student at université de Sherbrooke. She is working on the development of analytical methodology and samples preparations for the detection and quantification of contaminants of emerging concern (CEC) in complexes matrices (biological, environmental). She also works on the monitoring and occurrence of the CEC in the environment. She also focuses on the development and implementation of new biotechnologies process for the removal of organic contaminants (fungi, bacteria, enzyme catalysis).

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Notes:

Isil Yasa et al., J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
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August 29-30, 2018 | Toronto, Canada

Platform Size Exclusion Chromatography (SEC) method development for a broad range of monoclonal antibodies

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Bristol-Myers Squibb, USA

Understanding of size heterogeneity in biotherapeutic proteins is essential since it is one of the Critical Quality Attributes (CQA) due to its impact on safety and efficacy. The size heterogeneity covers the product related species/impurities that includes fragments, monomers and aggregates. Size exclusion chromatography (SEC) has been widely used to separate aggregates, monomer and fragments of monoclonal antibodies (mAbs) that might form during manufacturing, storage and shipping. The progress in biologics pipeline and the urgency to reach first-in-human (FIH) has provided an opportunity to develop a generic method that can serve as a platform method for monoclonal antibodies. This study describes platform SEC method development for monoclonal antibodies using commercially available highperformance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) SEC columns. For this purpose, several antibodies covering a broad range of isoelectric points (pI) and hydrophobicity were analyzed. Initial comparison was performed using six different mAbs to understand the impact of mobile phase and organics on separation of aggregates and fragments.

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August 29-30, 2018 | Toronto, Canada

LC-MS analysis of intact amino acids on a novel mixed-mode HPLC column

Itaru Yazawa
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There are four established methods for analyzing amino acids: pre-labeled, post-labeled, ion-pairing reversed phase and normal-phase, but each of these methods has disadvantages. The pre-labeled method has problems with derivitization efficiency and cost, while the post-labeled method is usually not compatible with LC-MS due to non-volatile mobile phases. The ion-pairing reversed-phase method has difficulty separating polar amino acids; on the other hand, the normal-phase mode has problems separating all the compounds, especially the Leu and Ile isomers. We have developed a novel amino acid separation column for LC-MS (MS) which can separate all 20 amino acids in protein using a mixed-mode stationary phase structure. We have also estimated separation and detection characteristics using LC-MS instruments. We found two methods to successfully analyze the complete array of 20 amino acids: 1) high throughput separation with Leu/Ile separation in 5min and 2) simple gradient separation. We also found that detection can occur not only in single MS mode but also in triple MS mode. In addition, no derivitization is required and a standard LC-MS (MS) system is sufficient for the analysis. This novel HPLC method will be a powerful tool for amino acid LC-MS(MS) analysis in many different biochemistry applications.

Biography

Itaru Yazawa has co-founded a company, Imtakt Corporation in 1999 at Kyoto Japan to focus on separation technology providing his own designed and manufactured HPLC columns to the global market. He used to work for Shimadzu Corporation (instrument development) and YMC Co.Ltd (column development) and then he wanted to supply his own unique column products such as RP+AX+CX muli-mode ODS column "Scherzo C18 Family" and 2um Non-porous ODS column "Presto FF-C18" etc. which are based on his own technical idea. Now he will introduce a next-generation novel amino acid analysis column for LC-MS "Intrada Amino Acid" for this conference.

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August 29-30, 2018 | Toronto, Canada

Comparison of immune cells subsets, *ex-vivo* and *in-vivo* expression of T cell activation and memory marker between LNC and corresponding PBMC from Calves Exposed to Natural *Mycobacterium bovis* Infection

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Cell-mediated immunity and development of necrotic granulomas in *Mycobacterium bovis (M. bovis)* infected lymph node (LN) is pathognomonic for bovine tuberculosis (BTB). This delayed hypersensitive host response involves a complex interaction of cellular and immune mediators within systemic circulation and LN. Hence, tuberculosis immunological response should be independently investigated at the peripheral blood and LN tissue level. The objective of this study was, therefore, to compare the cell surface and cytokine expression between immune cell from peripheral blood and lymph node cells (LNC) from calves on BCG efficacy trial. Twenty pairs of peripheral blood mononuclear cells (PBMC) and) LNC from M. bovis naturally infected calves during BCG vaccine experiment trial were isolated and investigated in two phases of the flow-cytometry experiment. In the first phase of a flow-cytometry experiment the proportion of *ex-vivo* CD25+ expressing cells was significantly higher ($P<0.05$) in CD4+ and CD8+ T node than that of peripheral blood. However, such difference in CD25+ expression was not observed in WC1 γδ T cells. Contrary to CD25+ *ex-vivo* expression, *in-vitro* IFN-γ and TNF-α producing cells were greater ($P<0.05$) in T cells of the peripheral blood than T cells of lymph node after PMA + ionomycin stimulation. This difference in IFNγ and TNFα responses was also statistically significant between a vaccinated and non-vaccinated group. An IL-4 producing cell was not evident in PBMC and LNC. During the second phase of flow-cytometry experiment additional surface marker; CD2, CD21, CD205, CD335 and CD1W2 were included to add more panels for immune cell subset. The second experiment revealed that PBMC CD4–WC1+ and CD8–WC1+ γδ T cells and CD205+D1W2+ DC subset exhibited lower percentage than γδ T cells and DC of LNC respectively ($p=0.0001$, $p=0.0061$). However, PBMC CD335+CD2+ NKT cells subset exhibited a higher percentage than NKT cells of LNC ($p=0.0129$). No difference was observed between groups in the percentage of the rest of T-cell and B-cell ($p>0.05$). Findings of this study suggest the existence of phenotypic immune compartmentalization between the two tissue compartments.

Biography

Fekadu Desta is a veterinarian with a dream and responsibility to control and prevent the existing endemic and emerging new diseases of livestock and companion animals, Ethiopia. For the last 6 years, he has been doing his PhD in Tropical Infectious Diseases with a PhD dissertation entitled "Comparison of Immune Cell Subsets, *ex-Vivo* and *in-Vitro* Expression of Activation and Memory Marker Between LNC and the Corresponding PBMC from Calves Exposed to Natural Mycobacterium bovis Infection in BCG Efficacy Trial" which is one of a research priority of the country. Currently, he is on data analysis, interpretation and result dissemination stage. He has published one paper on molecular epidemiology of M. bovis and preparing 3 more manuscript on bovine immunology.

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August 29-30, 2018 | Toronto, Canada

Effects of policosanol in older patients consuming nitrates vasodilators

Julio C Fernandez[1], **Jose Illnait, Lilia Fernandez, Sarahi Mendoza, Rafael Gamez, Rosa Mas, Luis E Lopez**[2], **Juan A Gutierrez** and **Meilis Mesa**
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Introduction: Policosanol is a cholesterol-lowering drug with concomitant antiplatelet effects. The efficacy and safety of policosanol have been investigated in clinical studies and post-marketing surveillance. Policosanol is very safe and no drug-related Adverse Events (AE) have been demonstrated, even in population subsets with high consumption of concomitant therapy, indicating that the potential risk of Drug-Drug Interaction (DDI) for policosanol is low. Vasodilators are used in geriatric populations mainly to treat congestive heart failure and acute decompensating of heart failure, although associated with other anti-hypertensive are also used to manage arterial hypertension. Vasodilators, however, have a considerable risk of drug-related toxicity, the most frequent symptoms being those derived from excessive vasodilation and hypotension, such as nausea, vomiting, loss of consciousness and reflex tachycardia. Vasodilators show important DDI derive from pharmacodynamic interactions with several drugs, those associated with the concomitant use of other vasodilators and diuretics being the most relevant. Considering such facts, the interest to study putative DDI between policosanol and vasodilators is supported.

Objective: To investigate whether policosanol administered to older patients consuming vasodilators induces any specific disturbance on safety indicators and/or increase the frequency or severity of AE in such patients.

Methods: This report was based in the analysis of the records of all patients (185) taking nitrates vasodilators included in a prevention study in the elderly randomized to policosanol 5 mg/d or placebo for 3 years. The analysis was by Intention-to-treat.

Results: Baseline characteristics were well balanced in both groups. After one year on treatment, policosanol lowered significantly Low-Density Lipoprotein-Cholesterol (LDL-C) (20.9%), Total Cholesterol (TC) (15.9%) and triglycerides (19.3%), whereas raised High-Density Lipoprotein-Cholesterol (HDL-C) (8.3%). Policosanol effects persisted, even increased, during the 3 years treatment. At the end of the study, policosanol reduced LDL-C (35.0%), TC (25.0%), triglycerides (19.3%) and raised HDL-C (16.7%). Of 185 randomized patients taking vasodilators, 44 (23.8%) withdrew from the trial. The frequency of withdrawals in placebo (31/95; 32.6%) was greater ($p<0.01$) than in the policosanol group (13/90; 14.4%). Overall, 26/185 (14.1%) patients discontinued due to some AE: 23 placebo (24.2%) and 3 policosanol patients (3.0%) ($p<0.01$). Policosanol did not impair safety indicators compared with placebo but induced additional decreases in systolic pressure compared with placebo. The frequency of policosanol patients experiencing Serious Adverse Events (SAE) (3/90; 3.3%) was lower ($p<0.01$) than in the respective placebo (23/95; 24.2%). Likewise, the frequency of policosanol patients who experienced some mild or moderate AE during the study (10/90; 11.1%) was lower ($p<0.05$) than in matched placebo (28/95; 29.5%).

Conclusions: Policosanol was well tolerated in older subjects with high coronary risk-taking vasodilators, not impairing safety indicators or increasing any AE respect to placebo. Policosanol, however, produced additional decreases of arterial pressure and reduced the frequency of SAE compared with placebo. Cholesterol-lowering efficacy of policosanol was persistent and consistent with that expected. These results indicate that policosanol can be administered to older patients taking vasodilators without risk of relevant adverse DDI.

Biography

Julio Cesar Fernandez Travieso is a Senior Investigator in Clinical Trials Unit, National Centre for Scientific Research, Havana, Cuba. He has completed his BSc in Pharmaceutical Sciences from Havana University, Cuba in 1996. He was awarded with PhD in Pharmaceutical Sciences in 2003. He has published more than 130 publications and presented more than 100 papers in various scientific events. His research interest mainly focuses on clinical trials phase I-IV of different natural products: Policosanol, Abexol, Prevenox and Palmex.

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Concomitant use of policosanol and antiplatelet drugs in older patients

Julio C Fernandez[1], **Jose Illnait, Lilia Fernandez, Rosa Mas, Sarahi Mendoza, Rafael Gamez, Luis E Lopez**[2] and **Meilis Mesa**
[1]National Centre for Scientific Research, Cuba
[2] Surgical Medical Research Centre, Cuba

Introduction: Policosanol is a cholesterol-lowering drug with antiplatelet action. Policosanol effects have been investigated in clinical trials and post-marketing surveillance, its efficacy and safety are demonstrated. Policosanol has resulted very safely, even when administered to special populations with high consumption of concomitant drugs, without demonstration of drug-related Adverse Events (AE). Accordingly, the potential risk of Drug-Drug Interaction (DDI) with policosanol appears to be low. DDI generally comes from pharmacokinetic or/and pharmacodynamic actions. Experimental data show that DDI with policosanol derived from pharmacokinetic interactions is not very probable. Nevertheless, DDI based on pharmacological interactions needs to be investigated. Antiplatelet drugs are widely used in middle-aged and geriatric populations mainly to prevent recurrent coronary or cerebrovascular events. Experimental and small clinical studies have shown that policosanol enhances the antiplatelet effects of aspirin in an additive manner. Hence, the interest to study putative DDI between policosanol and antiplatelet drugs in a population sensitive to drug-related effects, as the elderly, is supported.

Objective: The objective of the present analysis as a part of a prevention study, we investigated whether policosanol administered to older individuals taking antiplatelet drugs supposes concern regarding a potential risk for adverse drug-drug interactions.

Methods: We randomized 1470 elderly patients at high coronary risk to policosanol 5 mg/day or placebo for 3 years. For this analysis, the records of all patients (334) taking antiplatelet drugs were included. The analysis was by intention-to-treat.

Results: After one year, policosanol decreased significantly Low-Density Lipoprotein-Cholesterol (LDL-C) (21.0%), total cholesterol (16.9%) and triglycerides (19.6%), while raised High-Density Lipoprotein-Cholesterol (HDL-C) (6.2%). Policosanol effects were maintained, even improved, during the follow-up. At study completion policosanol lowered LDL-C (34.3 %), total cholesterol (23.9%), triglycerides (22.2%) and raised HDL-C (14.5%). Sixty patients (41 placebo, 19 policosanol, $p<0.01$) withdrew from the study, 33 (23 placebo, 10 policosanol) ($p<0.01$) due to some adverse event, all serious. Policosanol did not impair safety indicators and did not increase any adverse event with respect to placebo.

Conclusions: The policosanol can be administered to older patients taking antiplatelet drugs without risk of relevant adverse drug-drug interactions.

Biography

Julio Cesar Fernandez Travieso is a Senior Investigator in Clinical Trials Unit, National Centre for Scientific Research, Havana, Cuba. He has completed his BSc in Pharmaceutical Sciences from Havana University, Cuba in 1996. He was awarded with PhD in Pharmaceutical Sciences in 2003. He has published more than 130 publications and presented more than 100 papers in various scientific events. His research interest mainly focuses on clinical trials phase I-IV of different natural products: Policosanol, Abexol, Prevenox and Palmex.

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Manufacturing and optimization of a microfluidic device LEGO portable for optical detection-Photonic lab on a chip

Maria Ramos Payan[1], **Adrian Munoz**[2] and **Andreu Llobera**[3]
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LEGO building blocks have been patterned with laser so as to obtain photonic elements which can easily be combined into a Photonic Lab on a Chip (PhLoC). As opposite to the monolithic integration, the building block architecture enable to modify only specific regions of the PhLoC. In addition, it allows to replace damaged parts without a complete breakdown of the system. In this context, different laser writing speeds and conditions have been tested so as to achieve light guides of 500um in width and 500um in depth on transparent LEGO building blocks. Once the optimal writing conditions were achieved, they were used to implement absorbance-based filters using transparent but colored building blocks. Here, it has been obtained stopbands higher than 30dB for blue transparent building blocks, which is the maximum dynamic range of the Maya Spectrometer (Ocean Optics) used. Finally, an experimental set-up was implemented by using a building block as a cuvette and measurements in absorbance and fluorescence were pursued by placing lightguides either at 180° or at 90° from the input light guide. Measurements in absorbance showed a Limit of Detection (LoD) of 0'0171ppm using XXXXX as target analyte. When measuring fluorescence, two different compounds were tested: Fluorescein (LoD of 5.22ppm) and Norfloxacine (22.82ppm). The results presented herein allows confirming the possibility of defining PhLoC building blocks for absorbance and fluorescence measurements.

Biography

Maria Ramos Payan has expertise in improving sample preparation techniques focused on microfluidic-chip devices as miniaturization. The novelty of her microfluidic devices offers more advantages than the existing methodologies. Maria has worked at different institutions (the University of Seville, University of Huelva, University of Lund, University of Copenhagen, University of North Carolina, USA, Microelectronic National Center of Barcelona and University of Autonoma Barcelona). Currently, she works at the University of Seville with the aim of implementing optical detection into microfluidic devices for multiple different applications.

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Validation and development of an optical detection microfluidic device for the determination of antibiotics in environmental waters

Maria Ramos Payan[1], **Andreu Rosell**[2] and **Andreu Llobera3**
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This work consists of the development and calibration of a microfluidic device for direct fluorescence detection. First, the microchip was evaluated and tested using fluorescein, a substance of high fluorescence, soluble in methanol and widely used as a marker in methods of measuring other non-fluorescent substances. This microfluidic device was applied to the determination of an antibiotic commonly used for people and animals: norfloxacin. The measurements were carried out using two optical fibers, spectrometers and optical potentiometers at a wavelength of 278 y 445 nm. Finally, the microfluidic device was calibrated for norfloxacin with detection limits and quantitation limits of 0.07 mg/L and 0.24 mg/L, respectively. This device was satisfactorily applied in environmental samples, specifically to waters from the Llobregat River passing through Manresa, Barcelona. This has been demonstrated to be a low-cost device offering the short time of analysis and low detection and quantification limits. Additionally, this device is reusable and easy sampling to use.

Biography

Maria Ramos Payan has expertise in improving sample preparation techniques focused on microfluidic-chip devices as miniaturization. The novelty of her microfluidic devices offers more advantages than the existing methodologies. Maria has worked at different institutions (the University of Seville, University of Huelva, University of Lund, University of Copenhagen, University of North Carolina, USA, Microelectronic National Center of Barcelona and Universitat Autónoma de Barcelona). Currently, she works at the University of Seville with the aim of implementing optical detection into microfluidic devices for multiple different applications.

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A quantitative description of the kinetic and concentration regularities of bioanalytical techniques

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Statement of the Problem: The high affinity and specificity of biological receptors create both the demand and the intensive development of analytical systems based on their use. Therefore, the development of theoretical concepts of such systems' functioning, studies of quantitative regularities for the reactions occurring within them and the interrelations between the parameters of bioreceptor reactions and analysis with their use, have become key fundamental tasks of bioanalytical chemistry. Although several proposed mathematical models have described various bioassays and biosensors, most of those models consider bioreceptor interactions in the approximation of equilibrium conditions. Due to this limitation, various effects that arise under nonequilibrium conditions remain outside existing studies.

Methodology & Theoretical Orientation: Any bioanalytical technique is based on the affine recognition reaction ($A+R \leftrightarrow AR$), which obeys the laws of the reversible kinetics of a bimolecular reaction. An analytical solution of the differential equation of the complexation rate gives the function, which is presented in Figure 1. In a multistage analysis, an analytical description of the processes requires more parameters and additional simplifications for efficient operation. We have shown that, within a high-affinity interaction ($kd<0.0001$), the approximation of an irreversible binding is adequate for describing the analytical system.

Conclusion & Significance: The presented equation is suitable for describing the elementary stages of bioanalytical techniques. This equation provides both the kinetic dependence (if the interaction time (t) is the variable parameter) and the calibration dependence (if the initial concentration of the analyte $[A]_0$ varies). The proposed approaches will be useful for developers of bioanalytical methods as instruments for assessing the influence of various factors on the parameters of analysis and their targeted optimization.

Biography

Dmitry V Sotnikov received his M.S. education in Chemistry in 2007 at the Dmitry Mendeleev University of Chemical Technology, Moscow, Russia. From 2008 to 2012, he was a Ph.D. student at the A.N. Bakh Institute of Biochemistry of the Russian Academy of Science. From 2015 to the present, Dmitry V. Sotnikov is a research associate of the Federal Research Center (Fundamentals of Biotechnology) of the Russian Academy of Sciences. After completing his dissertation "Detection of specific antibodies by immunochromatography: Principles and practical applications," Dmitry V. Sotnikov was awarded a Ph.D. in biochemistry in 2016. His current research is focused on the kinetics of antigen-antibody interaction, its influence on the sensitivity and specificity of immunoassays and the development of novel immunoanalytical techniques.

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August 29-30, 2018 | Toronto, Canada

Accepted Abstracts

J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Parameter variation of crystal violet degradation on tio_2-fibrous clay material

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Organic dyes are an important source of environmental contamination, as they are toxic and mostly no biodegradable. Crystal violet (CV) is a basic dye very used for the coloring of paper, fibers, leather etc., but this dye is no-biodegradable and environmentally persistent. A wide range of methods have been developed for the removal of CV from the wastewaters such as biological methods membrane filtration, coagulation–flocculation, adsorption and advanced oxidation processes (AOPs). Among these techniques, photocatalysis constitutes one of the emerging technologies for the organic pollutants degradation. With the catalyst that was synthesized in the laboratory based on modified fibrous clays of central Tunisia and by relying on the photocatalysis method, a large proportion of crystal violet in solution is disappearing. In conclusion by more advanced research in the chemical field we can eliminate some dyes harmful to our environment even at less expensive and that what we do in our work.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

High-Performance Liquid Chromatography: A boon for routine and advanced chromatographic separations

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Chromatography was first discovered in 1903 by Twsett for isolation of chlorophyll. In 1960s HPLC was developed as an analytical tool. Liquid chromatographic approaches cover a wide range of applications today. It is closely relevant in scientific studies, which provides a powerful identification of certain components that will be effective for curing specific diseases. Especially important, LC methods can assist us in the isolation and purification of therapeutic drugs that benefit the development of the medical industry. As the development of liquid chromatography, the single effective component within a drug can be separated and purified under extreme purity requirements from a biological system with great complexity and abundance. However, improvement of LC methods is still urgently needed for better application of this method to benefit our lives. It has also been widely used in the separation of herbal drug molecules. Routine analysis of herbal drug substances and formulations has also become possible to meet the regulatory requirements. The current study shows review literature of certain advances in chromatographic techniques.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Linear propulsion of gold-nickel-platinum nanojet steered by dual off-center nanoengines

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In this paper, a novel nanojet with dual off-center nanoengines consisting of Au, Ni and Pt is designed, as shown in Figure 1. Au and Ni are shaped as concentric disks with 12 μm in diameter. The thicknesses of Au- and Ni-disks are 0.2 and 0.1 μm, respectively. Two identically off-center Pt nozzle nanoengines form cylindrical chambers and are symmetrically distributed on the base of the Au-Ni disk. The diameter, bottom-thickness, wall-height and wall-thickness of the nozzle nanoengines are 3, 0.3, 1.5 and 0.3 μm, respectively. The propulsion mechanism for the Au-Ni-Pt nanojet. Without the presence of hydrogen peroxide (H_2O_2), the nanojet suspended in deionized (DI) water is stationary. After the addition of H_2O_2 into DI water, oxygen (O_2) bubbles are generated at the Pt-surface (the nanojet and O_2 bubbles have a joint velocity of v_1). The generated O_2 bubbles grow bigger (growing state in Figure 2(a)). At this state, the nanojet and O_2 bubbles have a same velocity of v_2. When O_2 bubbles reach a certain diameter, they detach from the surface of the nanojet (detaching state in Figure 2(a)). The nanojet has a velocity of v_3, while O_2 bubbles have a different velocity of v_0. According to the Momentum Conservation Law and the Momentum Theorem, a driving force F'drive is generated, resulting from momentum change induced by the detachment of O_2 bubbles, to thrust the nanojet propelling forward. The nanojet is equipped with two identically and symmetrically distributed off-center nanoengines, resulting in the total driving force Fdrive is well aligned with the drag force Fdrag. Hence, the Au-Ni-Pt nanojet propels forward linearly. At steady state, the nanojet will continuously propel forward at a speed of v.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

The effect of glycerol on shape, size and growth rate of *Escherichia coli*

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Carbohydrate is a decisive factor in the growth of bacteria. Studies showed the importance of glucose as the most common carbon source of bacteria in the bacterial growth. However, the role of glycerol on bacterial growth remained to be clear. In the current study, we examined the effects of glycerol on the two strains (HF19, MC1061) of *Escherichia coli* growth. To this aim, we measured the optical density and colony formation unit per milliliter of cultured media. In addition, the cell dry mass of the cultured media has been assessed. Furthermore, in order to directly study the effect of glycerol on the *E. coli* cell proliferation, we used hemocytometer. Here we found strong correlation between optical density and CFU/ml in the all incubation period in the control groups. Surprisingly, our data showed very weak correlation between optical density and CFU/ml in the glycerol-treated group after 24-hour incubation. Our data also indicated that glycerol increased the optical density, colony formation unit and cell dry mass of the sample. Although our data indicated strong relation in the control groups, in all incubating period, in the glycerol-treated group, we observed strong relation only in 6-hour and 12-hour incubation period, not in 24-hour incubation. This data suggest that in long time glycerol incubation, glycerol induced changes in shape and/or size of *E. coli* in both strains.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Occurrence and levels of potentially harmful elements (PHEs) in natural waters of the gold mining areas of the Kette-Batouri Region of Eastern Cameroon

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This study was designed to determine the extent to which alluvial and bedrock gold mining activities in the Kette-Batouri region of Eastern Cameroon is responsible for the discharge of potentially harmful elements (PHEs) into natural water bodies. This is considered to be of great public concern, since elevated levels of PHEs in these water bodies, which are widely used for domestic purposes, could have adverse health and environmental effects on the population and nearby ecosystems. The investigation into the levels of PHEs in natural water bodies of this area was conducted to ascertain the toxicity posed by mine waste stockpiles as data from the literature on this subject are scarce. Fourty two water samples from the region were analyzed for some 60 PHEs by ICP-OES, of which 22 that were not below the detection limit were considered for further data analysis. Maximum total concentrations in water of As, Cr, Pb and V and Zn are above the World Health Organization (WHO) maximum allowable concentrations (MAC) levels and are as follows (µg l–1): As (21.90-50.9); Cr (1.80-57.30); Pb (0.50-34.70) V (24.70-77.20) and Zn (3.10-481.70). This information is consistent with our recent research efforts which have indicated moderate pollution by heavy metals in the soils with a slight deterioration of site quality in this region. The data generated from this investigation is important in the formulation of water management strategies and recommendations for remediation of water bodies at abandoned mine sites for meeting water quality standards.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
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World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Characterization and distinction of species the genera *Gnaphalium* and *Achyrocline* using chromatographic profiles (HPLC-DAD) and pattern recognition techniques

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Plants of the genera *Gnaphalium* and *Achyrocline,* belonging to the family *Asteraceae* are used in various parts of the world as medicinal plants due to their recognized applications in the therapeutic treatment of various pathological conditions. Additionally, it has been found that its extracts have activities such as antioxidants, anti-inflammatory, antimicrobial and even anti-tumor. Several species belonging to these genera have similar morphological characteristics which lead to sometimes confused between these genera, there are, however, significant differences in the class and quantity of flavonoids and other molecules with biological potential present in them. In this work a chromatographic study was performed by HPLC-DAD, for the leaf and flower ketone extracts in the species *Gnaphalium elegans, Achyrocline satureioides* and *Achyrocline bogotensis*, through which it was possible to establish signal patterns, specific for each one of them, thus allowing to have a tool for its rapid identification and that it can be used to evaluate the biological potential of other species the family *Asteraceae*. Through pattern recognition techniques, it was established that these profiles show significant differences between them, which allows a quick and unambiguous characterization of these species. The methodology developed for the establishment of chromatographic profiles, which includes a gradient with 2% acetic acid and methanol, on a RP-18 column, It also allows the identification and quantification of at least 10 flavonoids that may be present in these species. These results contribute to the optimization of time and resources around the investigations that will lead to the establishment of therapeutic treatments from extracts of these plants.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Determination of enrofloxacin and its metabolite in eggs by capillary electrophoresis together with Fabric Phase Sorptive Extraction(FPSE)

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Enrofloxacin is a kind of fluoroquinolones, widely used in the veterinary treatment of diseases. Its residues in eggs pose a risk to consumers. Fabric Phase Sorptive Extraction (FPSE), a novel sorptive microextraction technique, was used to reduce the interference of the sample matrix and the loss of analytes in the sample pretreatment process. The quantitative analysis of enrofloxacin and its metabolite ciprofloxacin was carried out by capillary zone electrophoresis. The separation conditions were optimized: the running buffer solution was a 40 mM phosphate solution at pH 7.6, the detection wavelength at 254 nm, the separation voltage at 10 kV, the injection time 15s and the sample solvent was a buffer solution diluted 100 times. The linear range of enrofloxacin and ciprofloxacin was 0.5-10 μg/ml and the detection limits were 0.33μg/mL and 0.17 μg/mL, respectively. The recoveries of the samples were between 94.4-114.3%. This study extends the application of FPSE in capillary electrophore ciprofloxacin analysis.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
&
World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Quantification of water soluble vitamins in natural health products and dietary supplements using Liquid Chromatography: Mass Spectrometry

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A liquid chromatography triples quad tandem mass spectrometry (LC-MS/MS) method has been developed for the quantitative determination of water-soluble vitamins in a dietary supplement and dietary dosage forms (tablets, softgels and powders). The water-soluble vitamins include thiamine, riboflavin, niacinamide, niacin, pantothenate, pyridoxine, folic acid, biotin, methylcobalamin and cyanocobalamin. This method consisted of initial extraction with methanol or methanol: water (50:50 v/v) containing 10 mm ammonium formate and 0.1% butylated hydroxyl toluene in an ultrasonic water bath for 30 min depends on the sample matrixes. This method involves the following simple pre-treatment procedures, centrifugation and filtration after an extraction step, whereas extract samples were diluted prior to injection. Chromatography separation was performed on a Waters Acquity BEH C18, 2.1×100 mm, 1.7 µm column. Mobile phases consist of (A) 0.1% formic acid, 5 mM ammonium format in and (B) methanol. Mobile phase flow was 0.35 mL/min at 40 °C with a run time of 5 min. The amount of compounds present is determined with a calibration curve consisting of sample extracts from a dietary supplement that were spiked at three different level including 75%, 100% and 125% of the label claim. The spike recovery (n=3) for the three levels ranged from 70-120 (RSD 11.5-20.5%). The effect of the matrix on the ionization process in ESI was evaluated by analyzing the extracted sample, spiked extract sample and standard solution at the same concentration and the absolute matrix effect was from 80-120%. The method was applied to identify and quantify the vitamins in commercial natural health products and dietary supplement.

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J Anal Bioanal Tech 2018, Volume 9
DOI: 10.4172/2155-9872-C1-028

18th International Conference on
World Analytical Chemistry & Mass Spectrometry
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World HPLC, Separation Techniques & Pharmacovigilance
August 29-30, 2018 | Toronto, Canada

Photoacoustics spectroscopy: The less explored non-destructive spectroscopy for multicharacterization

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Optical spectroscopy remains a widely used and most important tool for investigating and characterizing the properties of matter. The energy used in optical spectroscopy exists in the form of optical photons or quanta, with a wavelength ranging from less than 1A˚ in the x-ray region to more than 10 6A˚ in the far-infrared. It is highly versatile, widely ranged and non-destructive in nature. Optical Spectroscopy has been a scientific tool for over a century and a half and it has proven invaluable in studies on reasonably clear media, such as solutions and crystals and on specularly reflective surfaces. There are, however, several instances where conventional transmission spectroscopy is inadequate even for the case of clear, transparent materials. Such a situation arises when one is attempting to measure a very weak absorption, which in turn involves the measurement of a very small change in the intensity of a strong, essentially unattenuated, transmitted signal. Although this problem occurs for all forms of matters, it has received particular attention in the case of transparent gas mixtures containing minute quantities of an absorbing species or pollutant. Various techniques develop to overcome this difficulty, such as derivative spectroscopic, have proven to generally inadequate. In addition to weakly absorbing materials, there are a great many nongaseous substances, both organic and inorganic, that are not readily amenable to the conventional transmission or reflection modes of optical spectroscopy. These are usually highly light-scattering materials, such as powders, amorphous solids, gels, smears and suspensions. Other difficult materials are those that are optically opaque and have dimensions that far exceed the penetration depth of the photons. Over the years, several techniques have been developed to permit optical investigation of highly light – scattering and opaque substances. The most common of these are diffuse reflectance, attenuated total reflection (ATR) and internal reflection spectroscopy and Raman scattering. All these techniques have proven to be very useful, yet each suffers from serious limitations. In particular, each method is applicable to only a relatively small category of materials, each is useful over a small wavelength range and the data obtained are often difficult to interpret. The modern scanning and tunneling microscopic techniques in spite of their versatility are having inherent inadequacies and economically very costly. The photoacoustic spectroscopy strikes a balance between the optical spectroscopy and the modern microscopic techniques in that it is relatively cheaper, highly efficient over a wide range of wavelengths, applicable for any type of material. The newly developed electronics technology is highly assisting the versatility of the photoacoustic spectroscopy (PAS), Ultrasonic photoacoustic microscopy and Piezoelectric Photoacoustic microscopy (PPAM) to study the thermal and optical characteristics of any type of materials in the micro and nanoscales. In this work, we present the thermal diffusivity measurement of Poly (methyl methacrylate) (PMMA) - montmorillonite (MMT) clay nanocomposite by PPAM and compare it with the X-ray diffraction studies.

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