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P01 Chemometric optimization of a UPLC-MS/MS-based plasma assay for diagnosis of APRT deficiency and therapeutic drug monitoring

Unnur A Þorsteinsdóttir [1]Margrét Þorsteinsdóttir [1, 2], Hrafnhildur L Runólfsdóttir [3], Viðar Ö Eðvarðsson [1, 3], Runólfur Pálsson [1, 3]

[1] University of Iceland, Reykjavik, Iceland
[2] ArcticMass, Reykjavík, Iceland
[3] Landspitali University Hospital, Reykjavík, Iceland

Introduction

Adenine phosphoribosyltransferase (APRT) deficiency is an inborn error of adenine metabolism which results in excessive urinary excretion of poorly soluble 2,8-dihydroxyadenine (DHA), causing nephrolithiasis and chronic kidney disease (CKD). A significant number of patients are asymptomatic at diagnosis and in some cases end-stage kidney failure is not recognized until after a failed kidney transplant. Treatment with the xanthine oxidoreductase (XOR) inhibitors allopurinol or febuxostat reduces DHA excretion and prevents or slows CKD progression. A reliable method for both diagnosis and therapeutic drug monitoring (TDM) is lacking, as some patients have recurrent kidney stones or progression of CKD despite treatment.

The aims of the study were to optimize an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)-based assay for simultaneous quantification of DHA, adenine, allopurinol, oxypurinol and febuxostat in human plasma, for diagnosis of APRTd and monitoring of pharmacotherapy in patients with APRTd, utilizing the chemometric approach design of experiments (DoE).

Methods

The UPLC-MS/MS quantification method was optimized, employing the chemometric software MODDE Pro 13 (Sartorius Stedim Data Analytics, Umeå, Sweden). Fractional factorial (FF) design was used to reveal significant factors influencing retention time, resolution and peak area for all analytes, using partial least square (PLS) regression. Plasma samples obtained from patients before and after treatment and from healthy controls were analyzed with the optimized UPLC-MS/MS assay.

Results

The FF design revealed significant factors and factor interactions affecting the peak response and resolution. The coefficient plots from the FF design revealed that all seven main experimental factors were significant for at least one of the responses selected for the model. Thus, following the FF design, additional complementary experiments were included at the axial points to investigate potential curvature in the model. Following optimization of the assay, the sensitivity was improved and chromatographic resolution between the purine analytes was achieved. Preliminary data revealed that the median (range) plasma DHA concentration was 249 (123-1315) ng/ml in the untreated patients and below limit of quantification (BLQ) in those on treatment with either allopurinol or febuxostat. DHA was not detected in the plasma samples from healthy controls. Today the UPLC-MS/MS assay is used for diagnosis and TDM in patients with APRT deficiency, leading to an improvement in the clinical management of patients.

Conclusion

A UPLC-MS/MS assay for quantification of DHA, purine metabolites and XOR inhibitors in human plasma was successfully optimized using DoE.

P02 Flawed or Flawless? A deep dive into real validation practices

Jokin Ezenarro [1]Daniel Schorn-García [2]

[1] University of Copenhagen, Frederiksberg C, Denmark
[2] Stellenbosch University, Stellenbosch, South Africa

Chemometric models are becoming essential in the analysis of near-infrared spectroscopy (NIRS) data, particularly for food analysis, where they facilitate the rapid, non-destructive prediction of physicochemical properties. However, their usefulness relies on a proper validation [1]. This systematic review critically examines validation strategies employed in regression models for NIRS-based food analysis, highlighting the prevalent use of cross-validation and external validation, alongside key figures of merit (FoM). Through an extensive literature review of 200 randomly selected studies from 2015 to 2025, the review identifies dominant trends, with partial least squares (PLS) regression emerging as the preferred modeling approach due to its robustness with collinear spectral data.

Despite the recognition of external validation as the gold standard, a significant number of studies rely solely on cross-validation, risking overestimation of model performance. Moreover, the inconsistent application and definition of FoM, such as RMSE, R^2, and RPD, impede comparability across studies. The review also highlights a lack of methodological transparency, with many studies failing to adequately report validation protocols and FoM calculations. This inconsistency can lead to challenges in assessing the true predictive capabilities and reliability of chemometric models, especially when models are applied to different datasets or real-world scenarios.

The review focuses on the various cross-validation techniques, including leave-one-out, *k*-fold, and random resampling methods, noting significant variability in their implementation and reporting. Similarly, external validation strategies, such as the use of independent datasets and structured data splitting methods (e.g., Kennard-Stone and SPXY algorithms), are examined to assess their impact on model generalisability. The findings suggest that while cross-validation provides valuable insights during model development, it should not replace external validation when assessing model performance on unseen data.

Furthermore, the study reveals that a substantial proportion of reviewed articles do not explicitly define or justify their choice of validation methods, nor do they provide detailed descriptions of FoM calculations. This lack of standardisation hampers the reproducibility of results and the ability to perform meta-analyses or comparative studies across different research works. The frequent omission of the deffinition of ambigiuous metrics, such as bias and limits of detection, further complicates the evaluation of model validation.

The findings underscore the necessity for rigorous, transparent validation methodologies, advocating for the combined use of cross-validation and external validation, comprehensive reporting of FoM with explicit definitions, and the inclusion of visual performance assessments. By addressing these gaps, the reliability and reproducibility of chemometric models in NIRS-based food analysis can be significantly enhanced, fostering their broader application in quality control and regulatory environments. Ultimately, this review aims to provide a foundation for improving validation practices, thereby strengthening the scientific integrity and practical utility of NIRS-based predictive models in the food industry.

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P04 Exploring Feature Extraction Methods for Raman Spectroscopy: A Comparative Study

Jamile Jafari [1, 2], Thomas Bocklitz [1, 2]

[1] Leibniz Institute of Photonic Technology, , Germany
[2] Institute of Physical Chemistry (IPC) and Abbe Center of Photonics (ACP), , Germany

Exploring Feature Extraction Methods for Raman Spectroscopy: A Comparative Study

Raman spectroscopy is a powerful, nondestructive technique widely used to investigate the chemical composition, structure, and molecular interactions of materials. However, Raman spectral data's high-dimensional and complex nature necessitates effective feature extraction methods to reduce data volume and enhance analysis.

This study aims to investigate various feature extraction approaches and explore strategies to optimize their performance for Raman spectroscopy applications. Specifically, we examine methods such as Principal Component Analysis (PCA), Multivariate Curve Resolution (MCR), Independent Component Analysis (ICA), and Non-negative Matrix Factorization (NMF) to create low-dimensional representations of spectral data.

For this investigation, we utilize Raman spectra collected from six bacterial species, each cultured independently in nine replicates. After preprocessing, these feature extraction techniques are applied, and their performance is benchmarked through classification tasks using Linear Discriminant Analysis (LDA) and Support Vector Machine (SVM). A leave-one-replicate-out cross-validation strategy ensures robust evaluation, integrating the feature extraction methods into the cross-validation loop [2]. Our analysis focuses on comparing the effectiveness of these approaches in preserving data structure, improving classification accuracy, and enhancing the interpretability of the extracted features.

Preliminary results indicate that, in Raman spectral data, PCA effectively preserves variance and reconstruction accuracy, whereas MCR provides interpretable spectral components and benefits from incorporating prior information. ICA and NMF are also evaluated for their ability to enhance data representation. Our findings highlight the trade-offs between these methods regarding classification performance, efficiency, and interpretability.

In conclusion, by benchmarking multiple feature extraction approaches, we provide insights into their relative advantages and limitations for Raman spectroscopy. Future work will focus on refining these methods to optimize their performance in spectral analysis.

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P05Spatial Transcriptomics and MSI Uncover ER Stress Mechanisms in Prostate Cancer Progression

Abhibhav Sharma [1]Maximilian Wess [1, 2], Maria K. Andersen [1, 3], Morten B. Rye [1, 4], Sebastian Krossa [1], Elise Midtbust Sandholm [1, 3], Trond Viset [5], Øystein Størkersen [5], Antii Kiviaho [6, 7], Matti Nykter [6, 7], Alfonso Urbanucci [6, 7], Julia Debik [1], May-Britt Tessem [1, 3], Guro F. Giskeødegård [1]

[1] Norwegian University of Science and Technology, Trondheim, Norway
[2] ELIXIR, , Norway
[3] Clinic of Surgery, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway
[4] Clinic of Laboratory Medicine, St.Olavs Hospital, Trondheim University Hospital, Trondheim, Norway
[5] Department of Pathology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway
[6] Tampere University, Tampere, Finland
[7] TAYS Cancer Center, Tampere, Finland

Prostate cancer (PCa) is a clinically and biologically heterogeneous disease, making its prognosis particularly challenging. The lack of reliable prognostic biomarkers for PCa recurrence arises from a limited understanding of the molecular mechanisms and the complex crosstalk among heterogeneous prostatic tissue regions that underlie cancer progression.

In this study, we spatially investigate the multiomic characteristics of prostatic epithelial regions by integrating spatial transcriptomics and mass spectrometry imaging (MSI) profiles of PCa patients with varying biochemical recurrence and/or metastasis status.

We identified differential characteristics of endoplasmic reticulum (ER) stress responses, where the canonical ER stress pathways and ER stress related apoptosis were less pronounced in recurrent PCa tissues compared to non-recurrent PCa.

Notably, ER stress activation exhibited a spatial pattern in recurrent PCa, decreasing with distance from the cancer region. The MSI data further revealed a higher abundance of phospholipid metabolites in tissue regions of recurrent PCa, mirroring the spatial distribution of ER stress activation.

We validated the prognostic relevance of ER stress activity in publicly available clinical cohorts.

Our integrative multi-omics data analysis approach suggests that tumor cells in aggressive PCa utilize stress-resolving mechanisms to maintain ER homeostasis and proliferation while evading ER stress-related apoptosis.

P06 Hyperspectral imaging discriminated the viscoelastic properties of cellulose textile fibers

Ella Mahlamäki [1]Mikko Mäkelä [1], Inge Schlapp-Hackl [2], Michael Hummel [2], Marja Rissanen [2]

[1] VTT Technical Research Centre of Finland , Espoo, Finland
[2] Aalto University, School of Chemical Engineering, Department of Bioproducts and , , Finland

Chemical recycling of waste textiles into new regenerated cellulose fibers helps to meet the growing demand for textile fibers. The viscoelastic properties of dissolved cellulose fibers are important for chemical recycling, as they influence the spinning and regeneration behavior of the dope and need to be controlled during recycling. Traditional laboratory methods to estimate the viscoelastic behavior of textile fibers based on intrinsic viscosity are, however, time-consuming and expensive.

In our recent publication and patent, we presented a novel non-invasive and fast approach to estimate intrinsic viscosity of cellulose-based textile fibers using near-infrared (NIR) imaging combined with supervised pattern recognition. The sample set consisted of two distinct material types of cotton. Our method employed spectral images of these samples determined in the wavelength region 1000-2500 nm and took advantage of averaging over individual pixel spectra to enhance the stability of the sample spectra for robust feature extraction and classification.

Our linear discriminant analysis (LDA) classifier discriminated the textile samples into three different viscosity ranges. The results showed overall classification accuracies of 84–97% based on the training and test sets, and wavelength analysis indicated that the relevant spectral features were related to water, cellulose, and cellulose crystallinity. Based on these findings, we then hypothesized that a decrease in the intrinsic viscosity of cotton fibers was associated with changes in cellulose crystallinity and water adsorption. This hypothesis was supported by X-ray and vapor sorption measurements, which showed that samples with higher intrinsic viscosity had on average lower relatively crystallinity and adsorbed more water under higher relative humidity.

Our findings highlight the potential for non-invasive estimation of the degree of polymerization and the suitability of cotton materials for chemical recycling across different material types. By accurately determining the intrinsic viscosity and related characteristics, we can better control the behavior of these materials during the recycling process. In the future this would allow tailoring the pretreatment conditions for chemical recycling of textile wastes.

P07 In the quest for catechin polymers using fluorescence spectroscopy and PARAFAC

Helene F. S. Halberg [1, 2]Mads Rønnow Staffe [1], Åsmund Rinnan [1]

[1] University of Copenhagen, Copenhagen, Denmark
[2] Foss Analytical A/S, Hillerød, Denmark

The quality and sensory perception of red wine are greatly affected by phenolic compounds such as tannins. Tannins consist primarily of flavanols. As flavanols polymerize, the sensory expression of the wine changes from bitterness to a much more desirable astringency [1,2].

High Performance Liquid Chromatography is commonly used to assess the tannin composition and polymer size [3]. This study explores whether fluorescence Excitation-Emission Matrices (EEMs) in combination with Parallel Factor Analysis (PARAFAC) [4] can serve as a much faster alternative to study flavanol polymerization. Catechin, the most common flavanol in red wine, was used as a model compound. As the fluorophore, catechin, only experience minor structural changes during polymerization, the fluorescence properties may not be altered a lot. At the same time, the chemical complexity of the sample increases with a multitude of polymers of different sizes. All this complicates the analysis.

Catechin polymerization was monitored in-line with fluorescence spectroscopy, measuring EEMs over approximately two days. Liquid Chromatography coupled with Mass Spectrometry (LC-MS) served as a reference analysis for polymer characterization. The EEMs produced a dataset with unique challenges for PARAFAC, including local minima, Raman scatter correction, and model constraints. Additionally, when the chemical complexity of the sample is unknown, determining the appropriate PARAFAC model complexity is not trivial, and it requires careful validation through resampling and comparisons of excitation and emission loadings.

This study highlights the potential of PARAFAC for investigating subtle, yet important changes in the fluorescence EEMs, even when the chemical complexity of the sample increases. These findings contribute to the development of a fluorescence-based method for studying flavanol polymerization in wine.

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P08 Discovery Proteomics Analysis of Endothelial Cells in Response to Catecholamine Stimulation

Arnar Ingi Vilhjálmsson [1, 2]Óttar Rolfsson [1, 2]

[1] Center for Systems Biology, Reykjavik, Iceland
[2] University of Iceland, Reykjavik, Iceland

Catecholamines are widely recognized for their critical role in maintaining vascular homeostasis, regulating blood pressure and preserving perfusion in acute critically ill (ACI) patients [1]. The short-term effects of the sympathoadrenal system are essential for survival. However, prolonged exposure to high circulating catecholamines becomes detrimental to the patient [2]. In about a quarter of ACI patients, sustained high catecholamine levels contribute to the development of Shock-Induced Endotheliopathy (SHINE), a condition characterized by prolonged catecholamine exposure, loss of vascular homeostasis and severe vascular damage, leading to endothelial dysfunction. The mortality rate among patients with SHINE is approximately 50% and has remained unchanged for decades [3]. Despite the well-established role of catecholamines in vascular function, their direct effects on endothelial cells in the context of endothelial dysfunction remain poorly understood.

Our research focuses on elucidating the endothelial response to catecholamines. Using discovery proteomics, we aim to characterize proteomic alterations in endothelial cells stimulated by catecholamines, providing insight into potential mechanisms that may contribute to endothelial dysfunction.

To investigate proteomic alterations of endothelial cells following catecholamines stimulation, we established an in vitro cell model in HPMVECs. Cells were treated with catecholamines at different concentrations (0, 0.5 and 5 μM) before being lysed at 4- and 24-hour time points. Proteins were extracted from whole cell lysates, denatured and digested using a Trypsin/Lys-C mix. The peptide mix was desalted using solid-phase extraction, dried and resuspended before being measured using liquid chromatography-mass spectrometry (LC-MS). Samples were measured using both the Waters Synapt XS and Bruker TimsTOF. Data preprocessing and protein identifications were performed using Progenesis QI for Proteomics (Nonlinear Dynamics) and Peaks (Bioinformatics Solutions). Statistical analysis and visualization were carried out using R Studio.

Proteomic data analysis is still ongoing. Multivariate data analysis (MVA), including principal component analysis (PCA), is being used to visualize proteomic differences between treatment conditions. Differentially expressed proteins will be identified using statistical analysis with p-value correction. Volcano plots will highlight significantly altered proteins within the proteome, while Venn diagrams will be used to compare proteomic changes between treatment groups and across different instruments. This study aims to shed light on specific proteomic alterations in endothelial cells upon catecholamine stimulation. Final results will be presented at the conference.

This study aims to provide insight into the proteomic alterations in endothelial cells stimulated by catecholamines. Using an untargeted discovery proteomic approach, we aim to identify key proteins involved in endothelial dysfunction associated with SHINE. By improving our understanding of the cellular mechanisms involved in this condition, our findings may contribute to strategies for better patient outcomes.

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P09 Explorative analysis of microbial communities in chicken production – a chemometric approach

Ingrid Måge [1]Morten Arendt Rasmussen [2], Paula Teixeira [3], Birgitte Moen [1], Clara Santana Sousa [3], Beatriz Nunes Silva [3], Annette Fagerlund [1]

[1] Nofima AS, Ås, Norway
[2] University of Copenhagen, Copenhagen, Denmark
[3] Universidade Católica Portuguesa, Porto, Portugal

Introduction

This study is part of the EU-funded MICROORC project (www.microorc.eu), which aims to reduce food waste by monitoring and utilizing microbiomes in the food processing chain. One key development in the project is a shelf-life prediction model, to enable dynamic and more accurate shelf-life labelling.

Chicken production in Portugal and Norway serves as a case study in the project. The work is divided in three innovation cycles, and the first cycle has just been finalized. This phase consists of a broad exploration of the microbiome in the chicken factories, and in chicken products during storage. The aim is to identify critical control points in the factories and to hypothesize relationships between factory microbiota and chicken shelf life. The findings will guide data collection in the second phase, to ensure that the collected data is relevant for developing the shelf-life prediction model.

Purpose

The study demonstrates how chemometric tools can transform complex, messy, semi-unstructured, high-dimensional industry data into meaningful insights. Key research questions include:

- What are the differences and similarities between factories?

- How does the microbial communities change along the production line

- How does the microbial communities evolve during a production day?

- Are there relationships between the factory microbiota and food spoilage?

- What are the critical control points in the factory?

Methods

We use a combination of various well-known chemometric tools, including multivariate ANOVA, multiblock, and N-way methods, to address the research questions.

Results and Conclusions

Results demonstrate that the good old chemometric workhorses are highly effective in disentangling complex data when applied sensibly and guided by domain knowledge.

P10 Multivariate comparison of growth curves

Guro Dørum [1]Ingunn Berget [1], Nina Solberg [1], Mona E. Pedersen [1], Ingrid Måge [1]

[1] Nofima AS, Ås, Norway

Introduction

Growth curves are essential tools in biology for tracking changes in population size or biomass over time. Typically, these curves are analysed by fitting models to growth data represented in scatter plots. Biological growth usually follows a sigmoid pattern, characterized by an initial slow growth (lag phase), a rapid increase (exponential or log phase), and a leveling off as they approach mature values (stationary phase and plateau). Commonly used growth models include logistic model, generalized logistic model (Richards model), Baranyi-Roberts model and Gompertz model [1]. See Figure 1 for an example. In scientific research, the aim is often to investigate how various extrinsic factors affect growth, and it is therefore necessary to assess if growth curves are statistically different. In many cases, the comparison is simply done by visual inspection of plotted curves. Additionally, it is common to compare parameters estimated by the models, such as lag time or maximum growth rate, but this is usually done univariately.

Purpose

The aim of this study is to develop a method for statistically comparing growth curves from designed experiments. The method should be able to assess how the experimental factors affect the shapes of the curves, and results should be easy to interpret and communicate to biologists. This will enable scientists to better understand treatment effects through statistical testing in combination with graphical visualisation.

Methods

We apply ANOVA-simultaneous component analysis (ASCA) [2] to a case study where human muscle cells have been grown and continuously measured in an Incucyte® Live-Cell Analysis System, and the cells have been exposed to different treatments according to an experimental design. Cell growth measured at multiple time points serves as the response variables, which is modeled in relation to design factors *Treatment* and *Dose*. We compare two approaches: one where ASCA is applied directly to the raw cell growth data, and the other where ASCA is applied to the parameters derived from growth models fitted to the data.

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P11 TCA Smart Sensor for Process Control in the Cork Industry

Vanessa Fernandes Ferreira [1], António César Ferreira [1, 2]

[1] Cork Supply Portugal, 4535 São Paio de Oleiros, Portugal
[2] Centro de Biotecnologia e Química Fina (CBQF), Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

Introduction: Cork taint in wine, characterized by a “moldy-musty” aroma, is primarily attributed to 2,4,6-trichloroanisole (TCA), a chloroanisole compound with an extremely low sensory threshold [1–3]. Conventional methods for TCA detection, such as ISO 20752:2014 (E) [4] and OIV-MA-AS315-16 [5], are time-consuming, destructive, and limited in scope, as they analyze only a small sample from each production lot. To ensure 100% TCA-free corks, Cork Supply developed an industrial real-time, non-destructive inspection system. The DS100+ [6] was introduced as a solution for individual cork analysis, followed by the DS100Ultra, which extends its capabilities to both final products and raw materials. Integrated with an expert system, the DS100Ultra enables data-driven decision-making and process analytical technology (PAT) for full traceability.

Purpose: The scope of this project includes project is twofold: guarantee process control and achieve predictive maintenance.

Methodology: A key challenge was to transfer the calibration from gas chromatography-mass spectrometry (GC-MS) to the DS100Ultra. Stable calibration was required to achieve accurate correlation. By combining machine learning, deep learning and signal processing techniques, a "three-way analysis" approach was developed. To ensure long-term stability, frequent calibration is performed using stable TCA solutions. The system automatically updates the calibration transfer function based on the sensitivity of the detector at each calibration point. Another critical challenge in analytical instrumentation is contamination and maintenance. To address this, an expert system was developed to analyze empty runs using Fast Fourier Transform (FFT) techniques.

Results: Regarding calibration transfer, the DS100Ultra generates a one-dimensional array, which is converted to a scalar value using external pattern normalization methods. By analyzing different levels of contamination and clean granules on both systems, a deep learning transfer function was successfully established with an error of 17%. Concerning predictive maintenance, FFT system was established, determining whether the equipment is production-ready or requires cleaning, optimizing efficiency and ensuring consistent performance.

Conclusions: This innovative solution represents a significant advancement in process control within the cork industry, providing real-time, non-destructive, and reliable TCA detection while incorporating automated calibration and maintenance for sustained analytical accuracy.

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P12 Development of microfluid LC-MS untargeted metabolomic methods and clinical application in newborn screening

Étienne Ljóni Poisson [1, 2]Freyr Jóhansson [3], Óttar Rolfsson [1, 2], Margrét Þorsteinsdóttir [4, 5]

[1] Center for Systems Biology, University of Iceland, Reykjavík, Iceland
[2] Faculty of Medicine, University of Iceland, Reykjavik, Iceland
[3] Department of Genetics and Molecular Biology, Icelandic National Hospital, Reykjavik, Iceland
[4] Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavík, Iceland
[5] ArcticMass, Reykjavík, Iceland

The use of LC-MS in newborn screening is a well-established field, however, using an untargeted approach to investigating inborn errors of metabolism in newborns remains a relatively unexplored field of research. This is due to the very data-intensive nature of untargeted metabolomics, where a single sample can generate many thousands of features. Feature identification in untargeted metabolomics requires an extensive library specific to the conditions of the analysis. Here, we present our progress in creating an extensive library of metabolite features relevant to newborn screening using dried bloodspot of about 400 metabolites, with tens of thousands of features recorded from our standard library under various conditions: positive and negative mode, acidic and basic mobile phases and different types of columns.

The purpose of our LC-MS method and library development is two-fold: first, use our methods to establish a metabolic baseline of Icelandic newborns by screening a thousand healthy newborns. Once we have established this baseline, we intend on testing our library against samples with known conditions that we expect our method will be able to discriminate, thereby showing our method's ability to diagnose newborns based on their metabolome. Following this, our hope is to investigate further a number of inborn errors of metabolism to find new biomarkers for these diseases. Ultimately, we hope to implement our findings in the regular newborn screening methods of the National Hospital. Finally, we hope to compare our methods to a novel microfluidics method using Waters IonKey column system and assess its performance and possible use in newborn screening panels.

So far, our catalogue of 387 serum metabolites have been run in 5 different methods using both HILIC and RP, four of which were run in both positive and negative mode using a Waters Xevo QTOF LC-MS in data-independent mode using HDMSe to capture a maximum number of features. Our current feature library holds over 23.000 individual features. Our in-house method is able to detect over 75% of our total catalogue, withover 90% of our metabolites captured by at least one of our methods.

Upon acceptance of our application to gain access to dried blood spots from the National Hospital, we will begin our goal of screening 1000 Icelandic newborns. With our extensive library, we should be able to establish valuable information about the average metabolome of newborn babies, which should yield a very valuable standard against which to compare newborn screening.

P13 Development and Validation of a UPLC-QD Method for Quantifying Tazarotene and Tazarotenic Acid in Porcine Skin

Helena Hamzehpour [1]Kristófer H. Hauksson [1], Helgi Jónsson [2], Sveinbjorn Gizurarson [1], Bergthora S. Snorradottir [1]

[1] Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavík, Iceland
[2] Faculty of Medicine, University of Iceland, Reykjavík, Iceland

Tazarotene, a retinoid prodrug, holds significant potential as a transdermal treatment for hand osteoarthritis. To evaluate its effectiveness for transdermal delivery, it is vital to assess its permeability, which demands a precise and reliable method for quantifying both tazarotene and its active metabolite, tazarotenic acid, within biological matrices. This research introduces the development and validation of an ultra-performance liquid chromatography method, coupled with a quadrupole detector array (UPLC-QDa), for the simultaneous quantification of both tazarotene and tazarotenic acid in porcine skin.

In vitro permeation tests were carried out using unjacketed Franz diffusion cells with porcine skin. Optimization of the method was achieved using a Design of Experiment (DoE) approach. A central composite orthogonal design (CCO) was utilized to refine factors such as retention time, peak area, peak resolution, and peak width. Method validation complied with FDA guidelines and incorporated ketoconazole as an internal standard (IS).

The ideal mobile phase was identified using DoE screening, comparing methanol and acetonitrile as organic solvents, in combination with various ammonium acetate concentrations (2.5 mM, 5.0 mM, and 7.5 mM) in the aqueous phase. Acetonitrile led to a significantly shorter retention time than methanol, suggesting that it would provide faster separation. Although the ammonium acetate concentration did not considerably influence retention time or peak asymmetry, the 5 mM concentration provided the best peak asymmetry.

Following this, the remaining factors were optimized using the CCO design. The final method adopted a gradient steepness of 1.6 minutes, 70% organic solvent, a flow rate of 0.5 mL/min, a column temperature of 35°C, and a capillary voltage of 1.2 kV. Since cone voltage did not show a significant effect on any responses, a default cone voltage of 15V was chosen. These conditions achieved a resolution greater than 1.5 between tazarotene and tazarotenic acid, with clear separation from the IS.

The UPLC-QDa method was validated for selectivity, specificity, sensitivity, accuracy, precision, matrix effects, dilution integrity, and stability. No interference peaks from impurities, degradation products, or endogenous components were observed at the retention times of the analytes. The method was linear across the concentration range of 0.4-18750 ng/ml for tazarotene and 13.32-12500 ng/ml for tazarotenic acid, with $r^2 \geq 0.99$. The chromatographic analysis demonstrated accuracy and precision within acceptable limits (<15%), and stability testing confirmed that the analytes remained stable after 8 hours at room temperature, 24 hours at 10°C, and 7 days at -20°C. Thus, the method is suitable for both short- and long-term storage.

This validated method provides a reliable and precise tool for the simultaneous analysis of tazarotene and tazarotenic acid, enabling further investigation into their therapeutic potential in the treatment of hand osteoarthritis. By offering a robust platform for advancing research into transdermal drug delivery for osteoarthritis, this method may also serve as a model for future developments in topical drug therapies.

P14 Development of Spectroscopic Process Analytical Technology for Enhanced Optimization and Control of Chemical Modification in Purification Processes

Emil Rye Andersen [1, 2]

[1] Novo Nordisk, Kalundborg, Denmark
[2] University of Copenhagen, Copenhagen, Denmark

A pivotal aspect of Novo Nordisk's aspirations is the implementation of modern technology in automated, multipurpose production facilities aimed at achieving sustainable production and zero environmental impact. A critical enabler of this vision is the adoption of Process Analytical Technology (PAT) [1]. The global PAT market is rapidly growing [2], and advancements in analytical and computer technologies present opportunities to extend PAT techniques to include chemical modification processes.

The objective of this project is to develop spectroscopic PAT tools for optimizing, monitoring and controlling chemical modification steps. Specifically, we will explore the application of mid-IR, Raman, and UV-VIS spectroscopy combined with chemometrics during protein acylation. Our key scientific investigations include establishing a fundamental understanding of spectroscopic-biomolecular interactions during modifications, conducting a comparative study of the employed spectroscopic techniques while investigating the linearity in the response, e.g. by comparing linear (i.e. PCA, MCR and PLS) and non-linear methods (i.e. neural networks and support-vector machines). This understanding and data modelling will enable the implementation and validation of the selected spectroscopic techniques across various scales (lab, pilot, and full-scale) while assessing the influence of process parameters.

By monitoring chemical reactions and measuring both the backbone content and key impurities during modifications, this project aims to enhance process monitoring and control. This approach could significantly improve product yields while addressing the challenges posed by variable impurity levels and unstable reagents, ultimately supporting Novo Nordisk's goal of sustainable and efficient production.

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P15 Evaluation procedure for pansharpening methodologies in environmental trait map generation

Sara Jessica Heikkinen [1]Zina-Sabrina Duma [1], Tuomas Sihvonen [1], Satu-Pia Reinikainen [1]

[1] LUT University, Lappeenranta, Finland

Pansharpening methods are utilized in remote spectral sensing to enhance the spatial information of multi- or hyperspectral images [1]. A low-spatial-resolution spectral image is fused with a high-spatial-resolution panchromatic image to obtain high-resolution spatial and spectral information on the same image. Even though pansharpening techniques are continuously developed and improved, the methods have not achieved wide usage in scientific environmental monitoring due to concerns about data distortions and statistical models' sensitivity to altered data [2]. This study aims to assess how pansharpening affects environmental indicator maps compared to maps made from non-enhanced spectral data. The study focuses on two remote sensing applications: plant trait maps and water quality maps. To evaluate the performance of the pansharpening algorithms concerning the modeling's end goal, a novel procedure is proposed. The utilized images are either multispectral images from the Landsat 8-9 satellites or hyper-spectral images from the PRISMA satellite. This study includes optimized retrieval models based on various multivariate statistics and machine learning methods.

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P16 Triptolide release from gelatine and silicone membranes

Oddny Björgvinsdottir [1], Bergthora Snorradottir [1], Thorarinn Gudjonsson [1]

[1] University of Iceland, Reykjavik, Iceland

The diterpene triepoxide compound Triptolide, found in the plant Tripterygium wilfordii has been used in traditional Chinese medicine for centuries [1]. The compound has gained interest within the scientific community due to its anti-inflammatory properties, immune modulation, antiproliferative and proapoptotic activities [2]. Triptolide, however, has a narrow therapeutic index and its release must therefore be carefully controlled [3]. In this study, Triptolide formulations were made to ensure slow and accurate release in low doses.

Triptolide was incorporated into two types of matrices, gelatine and silicone. Because of the low water solubility of the compound, Triptolide was first dissolved in dimethyl sulfoxide and then mixed with either gelatin or silicone. The compound was released in PBS and quantified by using ultra-performance liquid chromatography.

To ensure detectable amounts of Triptolide, the gelatin membranes were fabricated and tested in T75 cell culture plates. The gelatine was crosslinked with 2% glutaraldehyde and washed with phosphate-buffered saline, containing glycine, overnight. The silicone membranes were fabricated in petri dishes and tested in Franz-cells. Samples were taken every 24 hours and the amount of Triptolide was quantified by using ultra-performance liquid chromatography.

The results showed a sustained release of Triptolide for over 2 weeks. This controlled release over extended period is important for further development of various anti-inflammatory treatments. It will simplify the administration, enable local delivery, and increase the safety of this very potent drug.

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P17 Multi-omics analysis of breast cancer across different biological matrices

Valdis Gunnarsdottir Thormar [1, 2]Kristrun Yr Holm [2, 3], Yassene Mohammed [4], Yuchen Xiang [5], Zoltan Takats [5], Christoph H Borchers [6], Margret Thorsteinsdottir [3, 7], Sigridur Klara Bodvarsdottir [2]

[1] Faculty of Medicine, University of Iceland, Reykjavík, Iceland
[2] BioMedical Center, University of Iceland, Reykjavík, Iceland
[3] Faculty of Pharmaceutical Sciences, Reykjavík, Iceland
[4] Center for Proteomics and Metabolomics, Leiden University Medical Center , Leiden, Netherlands
[5] Faculty of Medicine, Imperial College London, , United Kingdom
[6] Department Oncology, Faculty of Medicine, McGill University, , Canada
[7] ArcticMass, Reykjavik, Iceland

Breast cancer (BC) is the most prevalent cancer worldwide and the second leading cause of cancer-related deaths in women. Challenges relating to timely detection and the heterogeneous nature of BC complicate treatment strategies and influence patient outcomes. Advanced diagnostic techniques that better characterize the disease enable more personalized treatment approaches, which improves prognosis. In this study, we investigate the correlations between BC subtypes and their metabolomic and lipidomic profiles across different biological matrices within the same study cohort, aiming at a holistic view of the biomolecular changes associated with BC.

Metabolomics and lipidomics data were obtained through untargeted desorption electrospray ionization mass spectrometry imaging (DESI-MSI) on both formalin-fixed paraffin-embedded (FFPE) TMA and fresh frozen (FF) BC tissue samples. FFPE TMAs from 222 BC patients and 32 adjacent normal tissues for diagnostic evaluation. The DESI-MSI analysis has already demonstrated the capability to differentiate between tumor and normal breast tissues through their metabolomics signatures. To refine and enhance these findings, the DESI-MSI data is undergoing re-processing using a revised pipeline designed to detect a broader range of metabolites in the study cohort. Moreover, targeted metabolomics and lipidomics analyses have been performed on plasma samples (n=367) from the same study cohort using liquid chromatography mass spectrometry (LC-MS) and NMR.

Advanced machine learning (ML) techniques for predictive modeling will be applied, integrating metabolomic and lipidomic profiles with various BC-related parameters to explore underlying associations in the data. Both univariate and multivariate methods are applied, as each provides distinct information about the data structure. Unsupervised algorithms like PCA, t-SNE, and UMAP will be used to visualize and elucidate data patterns, whilst supervised algorithms such as Gradient Descent, SVM, and Random Forest, including ensemble learning techniques, will be deployed to build robust classifiers. Feature selection methods will be implemented to help identify biomarkers and regulate model complexity. To ensure model reliability and generalizability, rigorous cross-validation and data partitioning into training, testing, and validation sets are conducted. Partitioning and cross-validation allow for the robust evaluation of how well the models perform on new, unseen data.

Through comparative analysis, this study aims to reveal associations between metabolites, lipids, and BC-related parameters across different biological matrices, thereby facilitating the discovery of BC-specific biomarkers.

P18 Synthesis and Antibacterial Assessment of CRAMP-18 Conjugated Chitosan via Click Chemistry

Sankar Rathinam [1]Kasper K Sørensen [2], Martha Á Hjálmarsdóttir [3], Mikkel B. Thygesen [4], Már Másson [1]

[1] Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Ice, Reykjavík, Iceland
[2] Department of Chemistry, Faculty of Science, University of Copenhagen., Copenhagen, Denmark
[3] Department of Biomedical Science, Faculty of Medicine, University of Iceland, Reykjavík, Iceland
[4] Department of Chemistry, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

Synthesis and Antibacterial Assessment of CRAMP-18 Conjugated Chitosan via Click Chemistry

Sankar Rathinam[1,2], Kasper K. Sørensen[2], Martha Á. Hjálmarsdóttir[3], Mikkel B. Thygesen[2], and Már Másson[1]

[1] *Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland,*

[2] *Department of Chemistry, Faculty of Science, University of Copenhagen.*

[3] *Department of Biomedical Science, Faculty of Medicine, University of Iceland,*

Introduction:

Antimicrobial peptides (AMPs), vital components of organisms' innate immune systems, combat diverse microbial infections, including bacteria, fungi, viruses, and parasites, by disrupting microbial membranes and essential processes [1,2]. AMPs offer a promising alternative to traditional antibiotics due to their broad-spectrum activity [3]. CRAMP (Cathelicidin-Related Antimicrobial Peptide), found in mice immune cells, is a specific AMP [4]. Chitosan, derived from chitin in crustacean exoskeletons and fungal cell walls, is a versatile natural polysaccharide with wide-ranging applications in pharmaceuticals, food, cosmetics, agriculture, and wastewater treatments.

Purpose:

The project aims to synthesize chitotriazolan-peptide conjugates and characterize and investigate antibacterial properties.

Methods:

Our approach utilizes azide transfer reactions and copper-catalyzed azide-alkyne cycloadditions (CuAAC) to attach antimicrobial peptides (CRAMP-18) to chitosan and hydroxypropyl chitosan (HPC).) [5]. Chitosanazide and HPC-azide were prepared with controlled azidation using imidazole sulfonyl azide, followed by a click reaction to obtain the desired product. These derivatives were characterized by IR and NMR spectroscopy. The antibacterial activity of the chitosan-peptide conjugates was evaluated against four bacterial species *S. aureus, E. faecalis, E. coli,* and *P. aeruginosa.*

Results:

The successful grafting of pentynoyl-CRAMP18 onto chitosan and HPC was achieved through the CuAAC reaction. This chemical process facilitated robust bonding between the peptide and the polymers. Proton NMR spectroscopy confirmed the conversion of azide groups to 1,2,3-triazole linkages, enabling assessment of the degree of substitution (DS) in chitosan-peptide conjugates. DS values for chitosan-CRAMP18 (**1**) and HPC-CRAMP18 (**2**) were 0.2 and 0.13, respectively, indicating successful peptide incorporation onto both polymers. Notably, chitosan peptide conjugates **1** and **2** displayed significant efficacy against Gram-negative bacteria, especially *E. coli*.

Conclusion:

We successfully conjugated the CRAMP-18 peptide to chitosan using the CuAAC reaction. This study demonstrates that chitosan-peptide conjugates have potential antibacterial properties.

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P19 HATU Coupling for Fast, Efficient, and Selective N-Acylation of Chitosan: DoE-Optimized Synthesis of N-(2-(N,N,N-Trimethylammoniumyl)acetyl)-chitosan (TAC).

Luca Protti [1]Vivien Nagy [1], Sankar Rathinam [1], Daniel Amani [1], Elvar Örn Viktorsson [1], Már Másson [1]

[1] University of Iceland, School of Health sciences, Reykjavík, Iceland

Introduction: Chitosan is a natural, biodegradable, biocompatible, and non-toxic polysaccharide that displays significant antimicrobial activity, making it a promising option for drug delivery systems in treating bacterial infections. However, its practical applications are often hindered by certain physicochemical limitations [1]. These issues can be addressed through chemical modifications that enhance its properties. One noteworthy chitosan derivative is *N*-(2-(*N,N,N*-Trimethylammoniumyl)acetyl)-chitosan (TAC), which demonstrates improved solubility, increased membrane permeability, and strong antimicrobial effectiveness [2, 3].

Purpose: The Traditional synthesis of TAC involves chitosan mesylate and tert-butyldimethylsilyl (TBDMS) protective groups, followed by N-alkylation with glycidyl trimethylammonium chloride (GTMAC) or betaine aldehyde [4]. This method is resource-intensive and time-consuming. To improve the synthesis, this study explores an alternative approach using a Design of Experiments (DoE) strategy to optimize the coupling of betaine to chitosan through Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium (HATU) coupling, aiming for a high degree of substitution (DS) while increasing efficiency.

Methods: Following preliminary investigations, two full factorial DoE optimizations of the reaction, each involving 12 experiments, were conducted at 60°C using a Mya-4 reaction station with varying equivalent ratios of betaine, HATU, 1-hydroxybenzotriazole (HOBt), and triethylamine (TEA). The products were purified by ion exchange and dialysis, freeze-dried, and analyzed by ^{1}H-NMR.

Results: The reaction optimization was conducted using data analysis with MODDE software. This process identified key reaction parameters and optimal reagent conditions to achieve complete substitution efficiently while significantly reducing the reaction time. The optimized procedure allowed for the quantitative conjugation of betaine, forming the

TAC derivative in just three hours. Moreover, the response surface modeling indicated that HATU was the main factor influencing conversion.

Conclusions: The reaction optimization was performed using the DoE approach, identifying key reaction parameters and optimal reagent conditions to efficiently achieve complete substitution. This strategy resulted in a quicker reaction compared to previous methods, improved predictability of the final DS, and significantly reduced reagent consumption. By optimizing the synthesis of TAC, this study successfully developed a novel, efficient, and resource-saving method. The streamlined process not only enhances the sustainability and scalability of TAC synthesis but also establishes a foundation for future applications in nanomedicine and targeted drug delivery systems.

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P20 Mathematical Equation Describing a Bilinear Relationship Between Molecular Weight and Antimicrobial activity of Chitosan polymers and fitting to 29 Datasets

Már Másson [1]

[1] University of Iceland, Faculty of Pharamceutical Sciences, Reykjavik, Iceland

Introduction

Chitosan and its derivatives are known to inhibit the growth and eliminate pathogenic microorganisms, including bacteria and fungi. While the molecular weight (Mw) of the polymer significantly influences antimicrobial activity, the precise relationship between molecular weight and this activity remains unclear. Our recent study [1] demonstrated that the efficacy of *N*, *N*, *N*-trimethyl chitosan (TMC) against *S. Aureus* rises with Mw up to a critical molecular weight for maximum activity (CMW). Beyond this threshold, further increases in Mw did not impact the activity. Subsequent review [2] suggested that this relationship might be a consistent trend for chitosan and its derivatives, based on data from previous publications.

Purpose:

To investigate if literature data can be fitted to a QSAR equation that describes the relationship between antimicrobial activity and molecular weight.

Method

Drawing inspiration from Kubiniy's bilinear quantitative structure-activity relationship equations [3], we formulated a bilinear equation to elucidate the molecular weight-antimicrobial activity association for chitosan and its derivatives.

To encapsulate the bilinear relationship between molecular weight and activity based on the measured minimum inhibitory concentration (MIC) of the polymer, we introduced Equation 1:

The shape of the graph described by the equation is governed by three constants. The CMW is the critical molecular weight for maximum activity, A_{max} (MIC_{min}) represents antimicrobial activity when Mw>CMW, and Amin (MIC_{max}) the projected activity as Mw approaches zero.

Results

Twenty-nine datasets from studies published between 1984 to 2019, providing MIC values for chitosan and its derivatives relative to MW, were used for the analysis [4]. We used the KaleidaGraph Software for least-squares fitting, resulting in excellent conformity to the equations across datasets (Example shown in Figure 1). For datasets with suitable fits and at least three values on either side of CMW, CMW values ranged from 4 to 10 KD.

Conclusion

All the 29 datasets exhibited a good fit (R2 ≥ 0.5) and half excellent (R2 ≥ 0.95) fit to the equation. The CMW generally ranged from 4 to 10 KD for datasets with an excellent fit to the equation.

Bilinear models could be considered for DoE fitting of biological data as they may provide a better fit then polynomial equations.

Acknowledgement

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P21 Synthesis development and optimization of N,N,N-trimethyl chitosan (TMC) with high degree of trimethylation

Vivien Nagy [1]Már Másson [1], Hélène Liette Lauzon [2]

[1] University of Iceland, Faculty of Pharmaceutical Sciences, Reykjavík, Iceland
[2] Primex, Siglufjordur, Iceland

Chitosan is a marine polysaccharide obtained from crustaceans such as shrimp, crab, and lobster shells. Chitosan is an antimicrobial, non-toxic, biodegradable, and biocompatible polymer. However, it possesses poor aqueous solubility, limiting its applications at physiological pH [1].

With chemical modification resulting in chitosan derivatives, the biological properties can be enhanced. N,N,N-Trimethyl chitosan (TMC) has significant antibacterial activity and enhanced aqueous solubility compared to unmodified chitosan [2]. The aim of the current work was to develop and optimize a one-pot process for the synthesis of TMC with high degree of trimethylation (DTM) which would be suitable for economical production.

The optimization was done through the Design of Experiment (DOE) approach, involving systematic investigation of the effect of different reaction parameters on the DTM. In these designs reaction parameters like molar ratios, solvent system, alkylating agent, reaction steps, base, base addition, temperature, and time were varied throughout several Fractional Factorial and Full Factorial designs. The DTM (%) was estimated using ^1H NMR spectroscopy. The results were analyzed using MODDE (Sartorius) software.

With the help of the DOE approach the significant factors of the TMC synthesis were established (temperature, base addition, reagent). It was found that time is an insignificant factor for the yield and DTM (%) responses, which is beneficial for the economic production of TMC. Most publications on the synthesis of TMC use NaOH or $NaHCO_3$ base, however they can lead to viscosity issues and gas production. To overcome this, several new potential bases were tried, and the use of N,N-diisopropylethylamine (DIPEA) was implemented successfully. The highest obtained

DTM in one step was 72%, which is a significant increase in trimethylation compared to the initial design as well as the currently available published methods.

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P22 Adenine phosphoribosyltransferase deficiency: Metabolic profiling of DHA crystal-induced kidney injury

Vazha Dzhedzheia [1]Viðar Eðvarðsson [2], Runólfur Pálsson [2], Richard Lock [3], Laura Goracci [4], Finnur Freyr Eiríksson [5], Ottar Rolfsson [2, 6], Margret Thorsteinsdottir [1, 5]

[1] Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavik , Iceland
[2] Faculty of Medicine, University of Iceland, Reykjavik , Iceland
[3] Waters Corporation, Wilmslow, United Kingdom
[4] Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy
[5] ArcticMass, Reykjavík, Iceland
[6] Center for Systems Biology, University of Iceland, Reykjavik , Iceland

Adenine phosphoribosyltransferase (APRT) deficiency is a disorder of adenine metabolism characterized by abnormal urinary excretion of the poorly soluble 2,8-dihydroxyadenine (DHA), leading to kidney stone formation, crystal-induced kidney injury, and chronic kidney disease. Although treatment options exist, some patients experience adverse reactions or progress to end-stage kidney disease.

The purpose of this study is to understand the mechanisms of DHA crystal-induced kidney injury by identifying distinct metabolic and lipidomic changes linked to disease progression, characterizing inflammatory and renal injury biomarkers, and correlating metabolic disruptions with clinical outcomes and treatment efficacy.

This study employs an integrative omics approach using UPLC-MS-based metabolomics and lipidomics to analyze plasma and urine samples from APRT-deficient patients, collected as part of an open-label, randomized crossover clinical trial evaluating the effects of allopurinol and febuxostat on DHA plasma levels and urinary excretion. Metabolomic sample preparation will involve protein precipitation with organic solvents using an automated liquid handling system to ensure analytical reproducibility. This method will also be applied to plasma lipid extraction, while urine samples will undergo liquid-liquid extraction techniques for more effective lipid isolation. Data will be processed and interpreted using Lipostar2 and Progenesis QI software to identify significant metabolic and lipid alterations associated with the pathophysiology of APRT deficiency.

By elucidating the molecular mechanisms underlying APRT deficiency, this study advances integrative omics methodologies. The findings will provide insights into dysregulated metabolic pathways associated with DHA crystallization, identify prognostic biomarkers, and propose potential therapeutic targets. Ultimately, this research aims to improve the clinical management of APRT deficiency and related crystalline nephropathies.

P23 Green activation of silicone surfaces and chitosan-based coatings for antimicrobial medical devices

Daniel Amani [1], Guðný Eygló Baldvins [1], Vivien Nagy [1], Freygardur Thorstein [2], Már Másson [1]

[1] University of Iceland, Reykjavík, Iceland
[2] Ossur, Reykjavík, Iceland

Introduction

Silicone is widely used in medical devices due to its mechanical properties and biocompatibility. However, microbial contamination of silicone surfaces and biofilm formation remains a significant challenge, particularly in healthcare settings where nosocomial infections can arise from colonized medical implants and catheters. Traditional surface modification techniques, such as oxidative plasma activation, ozone treatment, and silanization, often involve reactive chemicals, high-energy processes, and specialized equipment, limiting their scalability and environmental sustainability. To address these limitations, we explored a green chemistry approach for silicone surface modification, followed by chitosan-based antimicrobial coating to reduce bacterial adhesion.

Purpose

This study aims to develop an eco-friendly and non-toxic approach for modifying silicone surfaces to improve their antimicrobial properties while preserving their mechanical integrity for medical applications. The proposed method focuses on minimizing the use of hazardous chemicals and establishing a simple, efficient, and scalable surface activation process that does not require complex procedures or specialized equipment.

Method

This study investigated the impact of amine-based agents, including ethanolamine (ETA) and ethylenediamine (EDA), on silicone surface activation in eco-friendly solvents such as water, and isopropanol. The efficacy of the surface modification process was assessed by quantifying amine groups using the ninhydrin assay, while the durability of surface activation was evaluated through fluorescence labeling with FITC and ninhydrin assays over a 10-day period to confirm stability. After activation, glutaraldehyde crosslinking was employed to immobilize chitosan, a natural antimicrobial biopolymer, onto the silicone surface. Mechanical integrity post-modification was assessed via tensile and compression testing using a Texture Analyzer. The antibacterial efficacy of chitosan-coated silicone was determined by colony-forming unit (CFU) counts against Gram-positive (*Enterococcus faecalis, Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria.

Results

The results demonstrated that the efficacy of silicone activation was influenced by both the amine agent used and the reaction medium. The highest activation level in isopropanol reached 303±31 nanomoles/cm², observed after treating silicone with ethanolamine. The activated surfaces showed stable amine group concentrations over a 10-day period, confirmed by fluorescence imaging and ninhydrin assays (Figure 1). Furthermore, chitosan-coated silicone significantly reduced Gram-positive and Gram-negative bacteria colony-forming units (CFUs), with *Enterococcus faecalis* CFUs decreasing from 7.1 to 3.7 Log_{10} CFU/mL.

Conclusion

This study presents a simple and eco-friendly approach for silicone surface activation and antimicrobial coating, providing a practical and scalable solution for medical applications. The proposed method enables effective surface modification while preserving mechanical integrity and minimizing reliance on hazardous chemicals and complex procedures. The incorporation of chitosan coating further enhances the antimicrobial properties of the modified surfaces, making them promising candidates for infection-resistant medical devices.

P24 Plastic recycling via pyrolysis: DoE perspective on experimental work

Joona Johannes Lahtinen [1]

[1] VTT, Espoo, Finland

Sustainability and recycling are increasingly central to policy, industry, and public attitudes. Among these challenges, plastic recycling remains a critical and unresolved issue. Pyrolysis—a thermal decomposition process in the absence of oxygen—has emerged as a promising approach for recycling hard-to-recycle plastics like multi-layer films, which are notoriously difficult to process.

To better understand how pyrolysis can optimize the recycling of these challenging materials, we conducted experiments at various scales, each tailored to specific research questions and objectives. Using Design of Experiments (DoE), we systematically varied key parameters in attempt to evaluate how important certain design variables are in pyrolysis when key response factors such as yield, boiling point distribution and share of certain components are behaving. The studies included techniques such as linear regression analysis, variance analysis and chemometric, including principal component analysis (PCA).

The results demonstrated how some key variables influenced the yield and product quality. For example, a multi-dimensional output: boiling point curve of the pyrolysis oil was modelled by using PCA-R from the CC-design. In another example, a full factorial design was conducted from the large pyrolysis piloting campaign where feedstock type along with the temperature was tested in different levels. Later, regression analysis and ANOVA were fabricated from the results to assess the change of these variables. This presentation illustrates some examples on how one can use these techniques on plastic pyrolysis and integrate statistical approach and data-driven approach to experimental research with appropriate domain knowledge.

P25 A Cross-Plant Transfer Method through Feature and Concentration Space Alignment for Odor Concentration Estimation

Zijan Wang [1]Javier Alonso-Valdesueiro [2], Albert Vidal [1], Agustin Gutierrez-Galvez [2], Santiago Marco [1]

[1] Institute For Bioengineering Of Catalonia, , Spain
[2] University of Barcelona, , Spain

Monitoring odors emissions from wastewater treatment plants (WWTPs) is essential to mitigate the negative impacts on the well-being of neighboring communities. The reference method for odor concentration measurement is Dynamic Olfactometry, standardized by EN13725:2022, which relies on the evaluation of odor bags by human panels. This approach leads to infrequent, sparse, and slow measurements. In addition, the associated cost per sample is high. An emerging alternative is Instrumental Odor Monitoring Systems (IOMS). Our research group has developed a drone-based IOMS that can be remotely controlled to collect field odor data at different points in WWTPs[1].

Unfortunately, when we directly applied the model trained by the data from one plant to a different plant, the results were severely degraded. Different plants exhibit significant heterogeneity in both data distributions and concentration profiles of odor compounds. However, building effective internal regression models for every plant is expensive due to costly label acquisition involving human panels. Our purpose is to explore transfer learning methods to develop a cross-plant model, where a model trained on a source plant can be effectively applied to other target plants with minimum recalibration efforts.

The key idea is to align both the data feature distribution and the concentration label distribution. The workflow is shown in the figure. Molina plant was used as the source domain, and the target plant was the Torredembarra plant, for which data were divided into five days. For feature distribution alignment, we apply the Transfer Component Analysis (TCA) method [2] before PLS modeling to enhance model transferability. Specifically, one day's target data was selected as the calibration transfer data. By applying a projection, minimize the Maximum Mean Discrepancy (MMD), an effective metric to measure distribution differences between the training data and the calibration transfer data in a shared subspace. And then find the best parameters to build the transfer model. The data of the remaining days were used as external validation data. We used the mean concentration value of the calibration transfer data to shift the predicted label space to make it closer to the target label space.

Through five days of cross-external validation, the experimental results demonstrate that the proposed transfer model (bias ratio:1.06, std ratio: 2.65) is more effective than direct transfer (bias ratio: 0.31, std ratio: 5.90), and approaches the performance of the internal model (bias ratio: 0.96, std ratio: 2.82), that is a model directly estimated from Torredembarra.

In conclusion, we developed a cross-plant transfer method based on the alignment of feature data space and concentration label space. By using calibration transfer samples, we have effectively achieved performance close to that of the internal model.

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P26 ODOR CHARACTERIZATION IN WASTEWATER TREATMENT PLANTS USING PORTABLE ION MOBILITY SPECTROMETRY AND MCR-LASSO

Luis Fernández [1, 2]Veronica Villa [3], Beatrice Julia Lotesoriere [3], Javier Alonso-Valdesueiro [2], Agustin Gutierrez-Galvez [2], Laura Capelli [3], Santiago Marco [1, 2]

[1] Institute for Bioengineering of Catalonia, Barcelona, Spain
[2] Universitat de Barcelona, Barcelona, Spain
[3] Politecnico di Milano, Milano, Italy

Introduction: Instrumental Odour Monitoring Systems (IOMS) are gaining attention for their ability to continuously monitor environmental emissions, helping to prevent hazardous compound leaks and incidents of unpleasant odors. While IOMS are frequently based in low-cost gas sensor arrays, here we explore for the first time the use of Ion Mobility Spectrometers (IMS) for odour characterization in Wastewater Treatment Plants (WWTP). IMS are a promising alternative since they offer portability, rapid response, and high sensitivity for detecting volatile organic compounds (VOCs). However, the presence of several odor sources dispersed throughout a wastewater treatment plant complicates odor evaluation. This study investigates the use of blind separation techniques to improve chemical information extraction from IMS and a deeper evaluation of odor episodes in WWTPs.

Materials & Methods: We utilized the GDA2 gas detector from AIRSENSE Analytics, a portable IMS. The instrument detects VOCs by ionizing molecules using a radioactive source. Ions in a sample are separated based on their mobility as they are accelerated by an electric field at atmospheric pressure, producing a mobility spectra that serve as unique fingerprints of the gas composition. These spectra are acquired at 0.3 Hz, allowing the capture of slow odour composition changes. We collected a dataset at the Pinedo WWTP in Spain, comprising 46 locations categorized into two classes: 'water' and 'sludge'. Our goal was to explore the molecular fingerprints of those classes and extract the time concentration profiles of the detected VOCs. To achieve this, we applied Multivariate Curve Resolution - Least Absolute Shrinkage and Selection Operator (MCR-LASSO) to the IMS data.

Results: The MCR-LASSO technique was used to decompose each sample matrix into a product of two matrices: one representing time concentration profiles and the other representing mobility spectral profiles. Due to ion correlation the resulting source mobility spectra were not unimodal, so they could not be assigned to a single VOC. To circumvent this, single peaks were identified across the spectral matrix, and these were grouped by location using k-means clustering. New unimodal synthetic Gaussian spectra were then generated based on the cluster representatives. The final concentration profiles were obtained by applying a Least Squares approach to the original data matrix and the matrix of unimodal spectra. The resulting concentration profiles differed between the two sample classes.

Conclusion: The MCR-LASSO method successfully extracted concentration profile information for ions from IMS data collected from WWTP samples, representing two distinct odor source classes.

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P27 Untargeted metabolomics predicts the plant genotype with higher pharmaceutical potential: an example of Icelandic Huperzia species

Maonian Xu [1], Elín Soffía Ólafsdóttir [1]

[1] University of Iceland, Reykjavik, Iceland

Introduction. The plant family Lycopodiaceae produce unique bioactive alkaloids called *Lycopodium* alkaloids(1). The alkaloid huperzine A (hupA) which is a one of the most potent natural acetylcholinesterase inhibitors with high pharmaceutical potential. Previous studies(2,3) have shown large variations of hupA among Icelandic *Huperzia* specimens, and it is interesting to investigate the alkaloid profiles in relation to plant genotypes.

Purpose. The current study included 96 *H. selago* specimens collected all around Iceland and aimed to characterize the overall variation of their hupA contents and alkaloid profiles.

Methods. HupA contents were determined using high performance liquid chromatography-photodiode array detection (HPLC-PDA) and alkaloid profiles were acquired using ultrahigh performance liquid chromatography-mass spectrometry (UPLC-MS). Multivariate data analysis was used to investigate alkaloid patterns in relation to plant genotypes and polyploidy levels. Plant genotypes were recognized using chloroplast DNA barcoding of the Rbcl region, while polyploidy level was determined using flow cytometry.

Results. DNA barcoding indicated three haplotypes in Icelandic *H. selago*, with high correlation to DNA content/polyploidy levels determined by flow cytometry. Our results reveal genotype-specific patterns of alkaloids as well as quantitative variations of hupA between Icelandic *H. selago* genotypes. Genotype 3 with the highest polyploidy level appears to have the highest HupA contents and unique alkaloid profiles with high pharmaceutical potential.

Conclusions. The study emphasizes the need for genotype-level phytochemical knowledge and has broad implications to plant chemotaxonomy and natural product discovery and exploitation. It highlights the utility of integrating plant barcoding and metabolomics in selecting taxa of high pharmaceutical interest.

P28 Preliminary assessment of miniraman assisted by chemometrics to detect emerging contaminant in wastewater

Marina Cocchi [1], Samuele Pellacani [1], Daniele Tanzilli [1], Lorenzo Strani [1], Caterina Durante [1]Barbara Benedetti [2], Alessandra Biancolillo [3], Guido Perra [1]

[1] Dept. of Chemical and Geological Sciences, Univ. of Modena and Reggio Emilia , Modena, Italy
[2] Department of Chemistry and Industrial Chemistry, University of Genova, Genova, Italy
[3] Department of Physical and Chemical Sciences, University of L'Aquila, Aquila, Italy

The possible use of reclaimed water for irrigation represents a great opportunity for water conservation and fits in the circular economy approach. Urban and industrial wastewater needs several treatments to eliminate contamination from different sources. Still, several pollutants may be persistent after treatment and up to now emerging contaminants (ECs), such as pharmaceuticals and personal care products, are not regulated, despite the growing interest of the scientific community. This study, which is part of a larger project, i.e. EXWASTER (see below), is focused on exploiting the potential use of portable Raman (lightnovo miniraman) as un untargeted fast and implementable in-situ approach to detect ECs in wastewater.

In the complete project both spot and passive sampling by Polar Organic Chemical Integrative Samplers (POCIS), strategies will be employed. The spectra will be acquired directly on the POCIS sorbent phases as well as on solid phase extraction cartridges (used to preconcentrate the sample) by evaluating different type of sorbent phases.

The preliminary study here presented will deal with optimization of sample presentation, spectra acquisition, pretreatment (finding best chemometrics tools and pipeline for denoising, baseline correction and normalization), information retrieval and multivariate calibration (evaluating PLS regression as well as spectral unmixing and calibration by MCR-ALS) by using a multi-standards mixture of ECs.

The optimized procedure will be as well tested on few samples coming from a wastewater treatment plant both before and after treatment.

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P29 Statistical inversion of PLS models

Tuomas Iiro Petteri Sihvonen [1]Zina-Sabrina Duma [1]

[1] LUT University, Lappeenranta, Finland

Currently, the inversion of PLS models has been utilised mainly to find process parameters for desired product qualities [1]. In addition, we have previously used such an inversion for a data fusion task in hyperspectral imaging [2]. However, both of these use cases only produce a point estimate of the inversion, disregarding any uncertainties in the data or the forward PLS model. Extending the inversion methods to fully probabilistic would give better uncertainty quantification and could open new use cases. For example, adding prior knowledge in a Bayesian sense from experts already utilising PLS modelling in their fields.

We want to show how Bayesian inversion schemes can be used to invert PLS models. The choice of priors and construction of likelihoods is evaluated. The problem is studied using the MCMC method. We also discuss how these techniques relate to point estimates and how they could be used in spectroscopic data analysis.

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P30 Visualizing Temporal Drug Responses in Live Cells Using Label-Free Techniques and Machine Learning

Edvin Forsgren [1]Jonne Rietdijk [2], David Holmberg [2], Martin M. Johansson [2], Jordi Carreras-Puigvert [2], Johan Trygg [1], Gillian Lovell [3], Ola Spjuth [1], Pär Jonsson [4]

[1] Umeå University, Department of Chemistry, Umeå, Sweden
[2] Uppsala University, Department of Pharmaceutical Biosciences, Uppsala, Sweden
[3] Sartorius BioAnalytics, Royston, United Kingdom
[4] Sartorius Corporate Research, Umeå, Sweden

Morphological profiling is a powerful technique for uncovering modes of action (MOA) of drugs. However, most current methods rely on fixed-cell assays, which capture only a single time point and miss the dynamic nature of cellular responses. Live-cell imaging enables the observation of these responses over time, offering the potential for a deeper understanding of compound-specific effects and mechanisms. Despite its promise, analyzing time-series image data remains challenging due to limited analytical tools.

In this study, we present a workflow for analyzing time-series live-cell images. This approach uses several existing deep learning models, including cell segmentation, live/dead classification, and single-cell feature extraction, along with a data-driven model to capture MOA-specific temporal phenotypic effects.

We demonstrate significantly improved MOA classification when utilizing temporal information compared to single time-point analysis. These results are based on a double-blinded cross-validation and were consistent across two different cell lines and six classes of drugs, each with different MOA.

The proposed workflow is modular, with each step designed to remove unwanted and irrelevant information, thereby enriching the remaining data. This modular design also allows for easy updates to the workflow as new technologies become available. We believe this can become a key component in phenotyping for next-generation drug discovery.

P31 Multi-way decomposition followed by reconvolution of fluorescence time decay data

Åsmund Rinnan [1]Anne Bech Risum [1], Jesper Løve Hinrich [1]

[1] University of Copenhagen, , Denmark

As the price of instruments for measuring time resolved emission spectroscopy (TRES) decreases, these instruments become more available, in particular with the development of cheaper LED light sources. We have proposed a new method for analyzing TRES data, and carefully showing the mathematical background for this method, and why it is a mathematical and TRES sound approach. It combines two existing methods: PARAFAC and reconvolution. PARAFAC is a soft modelling curve resolution technique which has been extensively applied to steady state fluorescence data, and reconvolution is the most common method for fitting TRES data. We have decided to compare our proposed method to two well-established methods of analysing these data, namely global reconvolution and tail-fitting. There recently has been suggested another approach, which involves the use of a method named SLICING, which increases the dimensionality of the data by one dimension. The commonality between all these methods is that they follow the assumption that the measured fluorescence signal is a linear combination of the underlying fluorophores. We compare the methods based on a set of measured TRES dataset with a mixture of three fluorophores, as well as two sets of simulated datasets with up to four fluorophores.

P32 Correlation inflation: a chemistry-based object detection algorithm for plastic characterization in hyperspectral images

Mickey Cornelis Jan Lukkien [1]Gerjen Tinnevelt [1], Jeroen Jansen [1], Mahdiyeh Ghaffari [1]

[1] Radboud University, Nijmegen, Netherlands

Hyperspectral (HS) imaging is an increasingly popular analytical technique which combines the benefits of traditional imaging and spectroscopy. In an industrial setting, HS imaging can be employed for monitoring chemical properties of objects passing along a conveyor belt, for example in plastic sorting. However, applications of HS are challenged as HS images contain large volumes of data, which slow down data preprocessing and (training of) chemometric techniques such as object detection and classification. Additionally, the three-dimensional matrix representing a HS image is often difficult to interpret, which makes the labelling of HS datasets a labor-intensive task.

Motivated by these challenges, we developed an object detection algorithm “Correlation Inflation” for HS imaging. The algorithm works by iteratively inflating the object from a starting pixel, using the Pearson correlation coefficient between the starting pixel and new pixels as criterion. Importantly, the algorithm requires only minimal and rapid preprocessing of HS data. Additionally, it requires the compilation of a spectral library representing background pixels, such as the conveyor belt, which is considerably simpler than the labor-intensive task of annotating individual objects within extensive HS image datasets. This approach effectively addresses common challenges associated with object detection in HS imaging, such as computational loads and the limited availability of labeled datasets.

We demonstrate the algorithm's application on plastic sorting, wherein HS imaging is employed to detect and classify plastic items on a conveyor belt. Following detection, individual plastic objects are characterized using a Partial Least Squares Discriminant Analysis (PLSDA) model. We reduce the training time of the PLSDA model with a novel strategy that uses only the most essential pixels for training. These pixels are found with convex hull calculations, which finds the outer points of each class’s data cloud in the PLSDA latent variable space. We show that this strategy is both faster than training on the full dataset whilst maintaining its accuracy, and more accurate when compared to reducing the data with a stratified split. The results from the plastic sorting case study indicate the algorithm's effectiveness and suggest its potential applicability in a variety of other scenarios, highlighting the advantages of HSI in enhancing object detection capabilities.

P33 Fluorescence spectroscopy as a PAT tool for quantification of low-dose solid pharmaceuticals

Rikke C. A. Helstrup [1, 2]Niels Peter Aae Christensen [1], Dan Henrik Sørensen [1], Holger Grohganz [2], Åsmund Rinnan [3]

[1] Novo Nordisk A/S, Måløv, Denmark
[2] University of Copenhagen, Department of Pharmacy, Copenhagen, Denmark
[3] University of Copenhagen, Department of Food Science, Copenhagen, Denmark

High volume continuous manufacturing of pharmaceuticals requires real-time process analytical technology (PAT), but for established process analytical technologies (NIR, IR and Raman spectroscopy) the limit of quantification (LoQ) is approximately 1 % w/w. This complicates the development and high-volume manufacturing of low dose pharmaceuticals.

Some studies have reported the potential of light-induced fluorescence (LIF) spectroscopy for direct analysis of solid pharmaceuticals and that certain physical and physicochemical attributes of the sample may affect the fluorescent signal.

The aim of this study is to further investigate which parameters affect the fluorescent signal and if it is possible to build a robust model for in-line quantification of low-dose solid pharmaceuticals. For this purpose, blends, compacts, and granules consisting of low-dose model fluorescent drugs, Tryptophan and/or Piroxicam in mixture with microcrystalline cellulose, will be produced and analysed by LIF.

Initially, the effects of particle properties, such as particle size distribution (PSD) and apparent density, will be examined through a Design of Experiments approach using different compaction pressures and screen sizes for granulation. Additionally, the sensitivity of the analytical method will be tested using different concentrations of the drugs in a range of 0.1 % w/w to 1 % w/w.

As fluorescence spectroscopy has a low penetration depth and is seemingly affected by the surface of the samples, the particle properties are expected to affect the signal. Initial experiments have already showed promising results in regard to the sensitivity of the method in concentrations down to 0.1 % w/w.

This research will employ chemometrics to analyse the collected spectroscopic data, focusing on multivariate analysis techniques such as principal component analysis (PCA) and partial least squares (PLS) regression.