PHARMASEL-PROSPERITY NEWSLETTER

PharmaSEL Prosperity

A bimonthly newsletter

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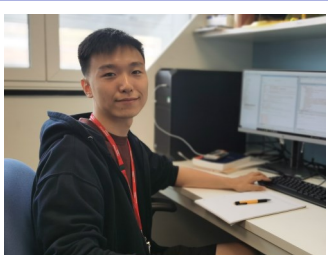
In this issue:

- *Dr Chrysoula D. Kappatou and Mr Lingfeng Gui* share their experiences from their visit to Lilly.
- *Research from the Williams group* on the usage of chromatographic methods to for the evaluation of permeability and stability of oral peptide formulations.
- *Dr Mingxia Guo*, a Research Associate working in Work Package 2, shares her background, research interests, and role within WP2.
- A glance at a *prominent publication* on the latest achievements in prediction of phase behaviour and pH-dependent solubility for common APIs.
- A highlight of our most *recent publications*.

Visit to Lilly: Dr Chrysoula D. Kappatou and Mr Lingfeng (Griffin) Gui



Dr Chrysoula D. Kappatou

Spending the time at Lilly was a unique experience! It gave me the chance to interact with highly-skilled professionals, get familiar with the facilities and labs at Lilly and gain a strong insight on the engineering work within a modern pharmaceutical industry. It was also great as a dissemination activity for my research and how it can be potentially used to address some of the current challenges in the field.

My personal highlight was getting to meet and been able to disseminate my work to some very talented and inspiring people. Of course, the warm hospitality and the excellent food could not be left unmentioned here. To get out of your comfort zone, to be able to talk about your research with different public, to learn and interact and communicate with people with long industrial experience.

In general, I believe it is very important for a researcher to be able to bridge theoretical knowledge with real-world applications. Often standing too long in either the theoretical or the application side of a problem can limit your perspective. In my opinion, the shift in the perspective that such a visit can trigger is extremely interesting and motivating and is also one of the main goals of this prosperity partnership.

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Mr Lingfeng (Griffin) Gui

I paid a three-day visit to Lilly from the 25th to the 27th Aug, during which I met the project team of my internship with Lilly and had a fruitful conversation on the progress and future direction of the project. I also had some guided lab tours and got to know processes of how scientists and engineers at Lilly perform various experiments. What's more, I also got many opportunities to talk to other people working on different areas, including one of my Lilly champions for the PhamaSEL project.

I have been computationally studying peptide synthesis but have never seen the industrial experimental set-ups for peptide synthesis. Through the lab tours, I was fascinated by how the iterative synthetic steps of peptides can be controlled and automated in an efficient way. I also got to know the main challenges people face in practice when performing these experiments, which is inspirational to my future work.

I had a conversation with one of my Lilly champions of the PharmaSEL project, Dr Stan Kolis, who provided me with invaluable advice for my work. We caught up on the project on suppressing HCN formation during peptide synthesis and discussed the potential opportunities of doing experiments to validate my computational results. I especially recommend computationalists (as I am) to go to Lilly as this can help us to better understand the real industrial challenges and how computational work can help overcome these challenges and fit into pharmaceutical development. People I've met at Lilly are so friendly and helpful in sharing their scientific ideas and insights.

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Research Feature: Self-interaction chromatography for the evaluation of oral peptide formulations (Mr Maxim Bird)

Aims and Methodologies:

Professor Daryl Williams, Dr Lucia Lombardi, and I are working within WP4 to develop chromatographic methods of determining the permeability and stability of oral peptide formulations within the gastrointestinal tract. My personal focus is working on self-interaction chromatography (SIC) and IMmobilised Enzyme Reactor (IMER) methods to evaluate peptide aggregation propensity and enzyme degradation susceptibility, respectively, under such conditions. The main challenge identified was establishing optimal conditions for the immobilisation of peptides and enzymes due to their instability under various conditions. For example, in the development of an immobilised pepsin reactor, the pepsin will be denatured at conditions above pH 6, therefore a strategy utilising a glutaraldehyde linker reacting with surface amine groups had to be applied.

Sample Case Study:

Table 1: Size Exclusion Chromatography data representing Semaglutide aggregation under GI tract conditions.

% Monomer	Simulated Gastric Fluid		Simulated Intestinal Fluid	
Time (h)	Fasted State	Fed State	Fasted State	Fed State
0	97.2%	92.0%	98.2%	99.5%
24	74.1%	57.6%	62.6%	90.0%

Within the project, Semaglutide has been applied as a reference peptide in initial studies. Its propensity to aggregate in simulated gastrointestinal fluids was established via size exclusion chromatography (SEC) by storing 0.5mg/mL of the sample at 37°C and eluting it through as Agilent AdvanceBio SEC 130Å column, the results of which are given in Table 1. This work was then corroborated via SIC. The semaglutide was immobilised onto an amino-functionalised polymethacrylate resin via a PEG4-linker to maintain flexibility of the species. The sample was then eluted through the column under various mobile phase conditions. The resultant second virial coefficient calculated (negative indicating stronger peptide-peptide interactions, positive indicating stronger solvent-peptide interactions) are outlined in Table 2.

Table 2: Self-interaction Chromatography indicating second virial coefficient of Semaglutide under GI tract conditions.

	Simulated Gastric Fluid		Simulated Intestinal Fluid	
	Fasted State	Fed State	Fasted State	Fed State
B_2	-1.57	-1.95	-2.21	0.55

Relevance to Lilly:

Developing robust and reproducible SIC and IMER methodologies enables the higher-throughput analysis of oral peptide formulations ensuring the maintenance of constant conditions. These methods have the additional benefit of reducing the required amount of sample peptide for analysis, and in the case of IMERs, removes the necessity for separation of the enzyme from the degraded species.

Future Work:

Currently, the plan is to establish the necessity of the PEG4-linker chain for SIC immobilisation by repeating the process in its absence and comparing the results with those previously collected.

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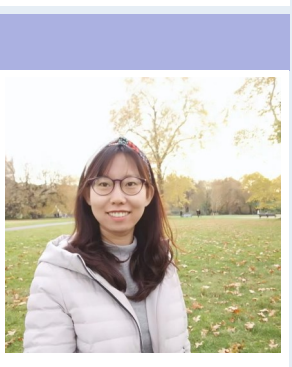
Researcher Spotlight: Dr Mingxia Guo, WP2



Mingxia Guo is currently a Research Associate at Imperial College London, working with Professors Jerry Heng, Amparo Galindo, George Jackson, and Claire Adjiman on Work Package 2 of the Prosperity Partnership. Mingxia completed her BEng in Chemical Engineering at Nanjing University of Technology in 2013, and undertook her masters research in small molecule crystallisation at the National Engineering Research Centre for Industrial Crystallisation Technology, Tianjin University under the supervision of Professor Qiuxiang Yin between 2014 and 2017.

Mingxia then joined Professor Jerry Heng's research group at the Department of Chemical Engineering, Imperial College London under a China Science Council scholarship to undertake research into peptide crystallisation for her PhD, which she successfully defended in May 2022. The research topic of her Ph.D. was the investigation into the crystallization behavior of glycine homopeptides. The research included three parts: firstly, establishing a thermodynamic foundation for the rational design of peptide crystallization processes, and exploring the application of SAFT-γ Mie to biomolecular thermodynamic properties; secondly, investigating the nucleation theory of macromolecules such as peptides; and thirdly, exploring the relationship between solution conformation and peptide crystallization conditions.

Mingxia has co-authored a total of 19 journal papers and presented at a number of international and national conferences. She is an active member in the community, acting as a journal reviewer and was the recipient of an Outstanding Reviewer Award from the journal *Particuology*. This year, she was awarded the Young Scientist Award from The British Association for Crystal Growth (BACG), and won a project under Imperial College London's Julia Higgins funding scheme to design ATR Kinase peptide-based inhibitors as novel chemotherapeutics. Her current research continues on from her PhD topic, and is focused on the investigation of crystallisation mechanisms of peptides, which includes thermodynamic properties, establishment of new cyrstallisation conditions, peptide conformation, and peptide morphologies.

"The Prosperity Partnership gives me a chance to learn from academics and industrial scientists. I really enjoy working with brilliant people together to figure out the tough problems in the world."

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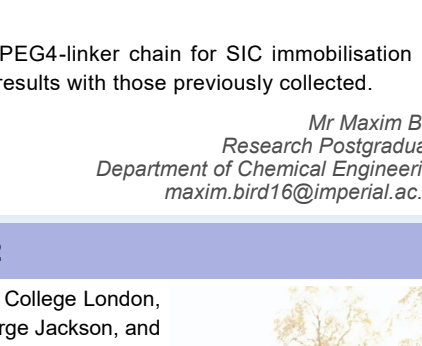
Featured Publication

Phase behaviour and pH-solubility profile prediction of aqueous buffered solutions of ibuprofen and ketoprofen

Malak Wehbe, Andrew J. Haslam, George Jackson, Amparo Galindo

DOI: 10.1016/j.fluid.2022.113504

Abstract



Salt formation is commonly used to increase the solubility of ionisable active pharmaceutical ingredients (APIs) and is monitored via a pH-solubility profile of the API and its salt. Due to the extremely low solubilities of many APIs in water, experiments are difficult to perform, and reliable predictive tools can be especially useful in this context. Here, we use the SAFT-γ Mie group-contribution equation of state to predict the phase diagrams and the pH-dependent solubility of two acidic APIs: ibuprofen and ketoprofen. We consider ibuprofen and ketoprofen in basic buffer solutions of NaOH, KOH, LiOH, RbOH, $Ca(OH)_2$, $Mg(OH)_2$, n-hexylamine, n-octylamine, benzylamine and tert-butylamine, and in acidic buffer solutions of HCl and acetate. A predictive approach is developed in which the unlike interactions involving charged groups are either taken from previous work, calculated using combining rules or, in the case of groups originating from organic groups (e.g., COO^-), taken to be the same as the equivalent interaction involving the neutral (uncharged) group. A new group, NH_3^+, is characterised for the case of amine buffers and salts. Equilibrium constants for the dissociation of the APIs and the formation of salts (pKa and $K_{sp,AvA,BvB}$ values) are also incorporated in the model using experimental values from literature. Predictions of the complete phase diagrams of the APIs in water are presented, including the vapour–liquid, liquid–liquid (oiling out), and solid–liquid (solubility) equilibria. The SAFT-γ Mie approach is shown to provide accurate predictions of the solid–liquid solubility of the compounds, as well as of the presence of liquid–liquid separation. Furthermore, the pH-solubility profiles of the APIs at T = 298.15 K and 310.15 K for the range of buffers and salts considered are predicted in good agreement with the available experimental data.

Publications

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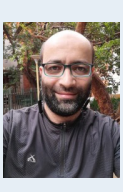

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