

COST Action CA24111

Event/Meeting Name, Place
Date



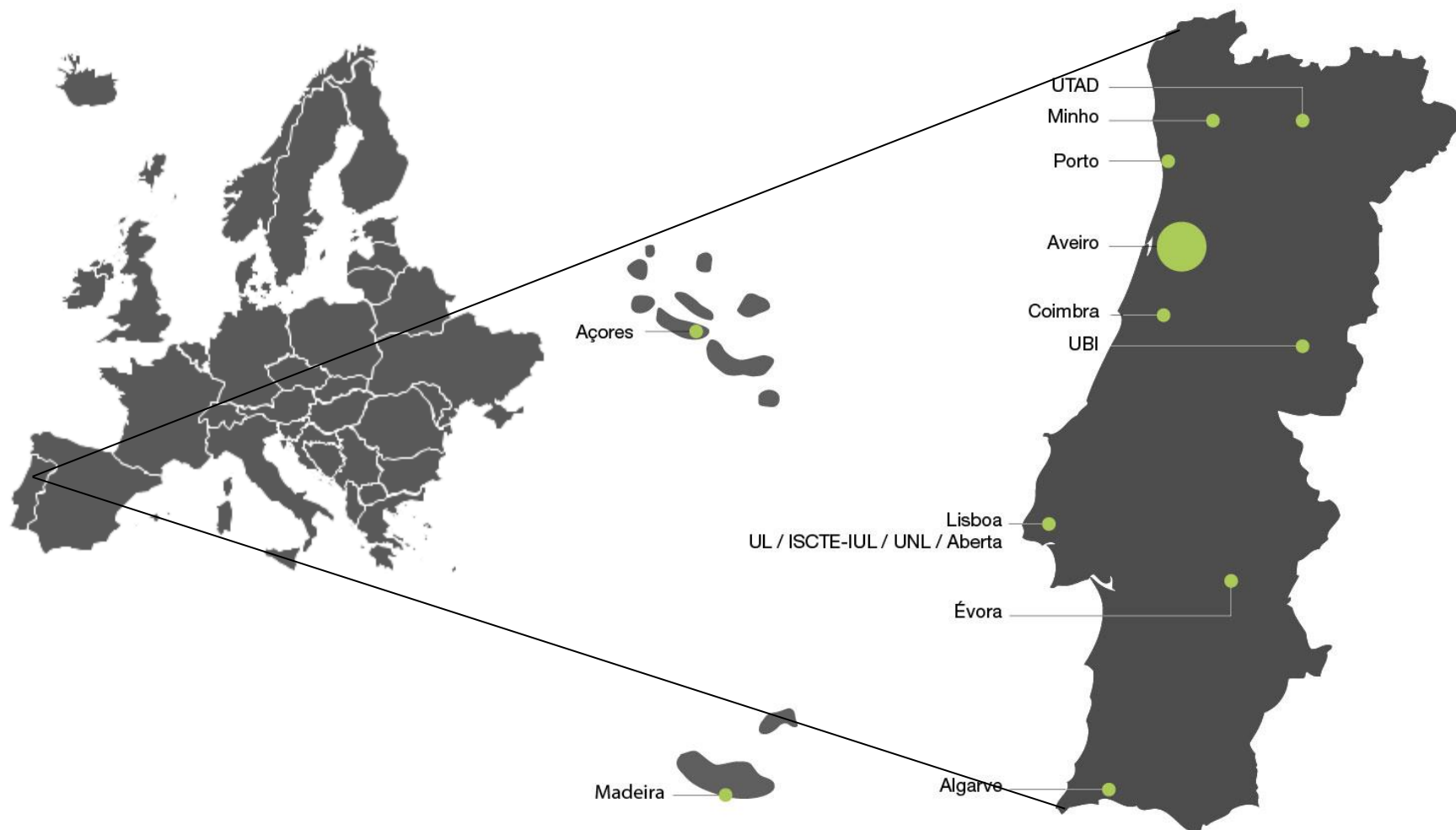
ENFiber

European Network for Valorising Food
Processing Waste into Sustainable Fibers

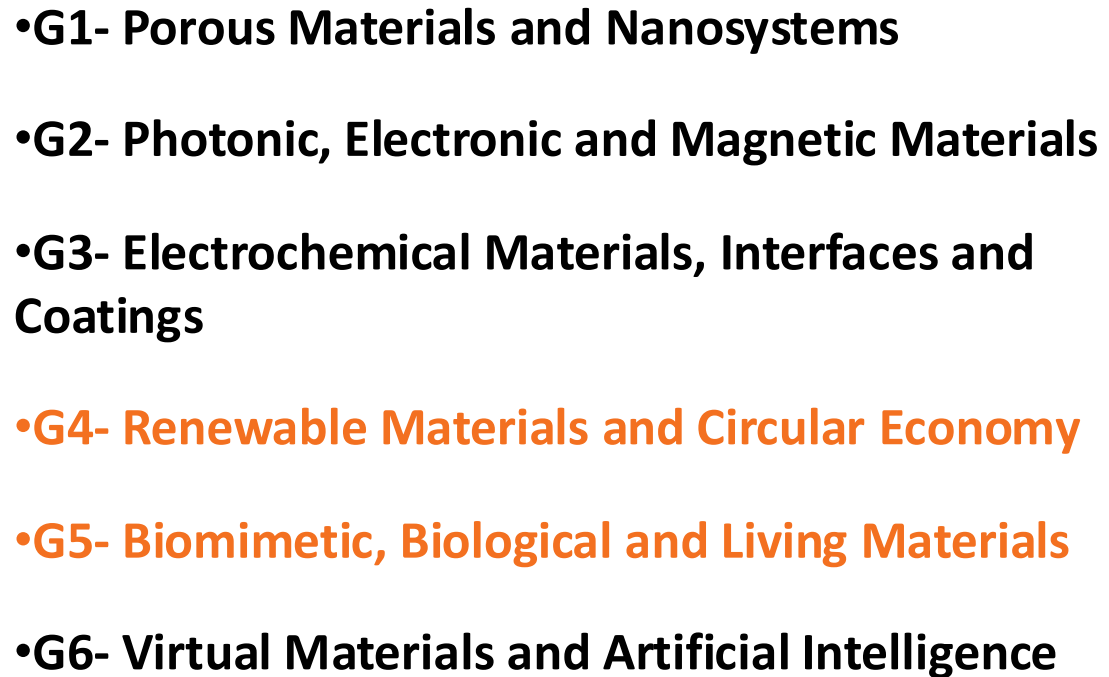
New biomaterials from structural proteins

João A. P. Coutinho, Sónia P. Ventura, Mara G. Freire
CICECO – Aveiro Institute of Materials, University of
Aveiro, Portugal











Aveiro weather

● 16:20 · 1h 28m ago

MEO
BEACHCAM



20h ago

9h ago

7m ago

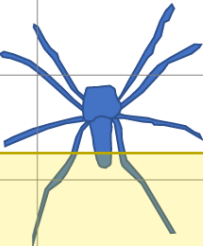
24h ▾

1x ▾

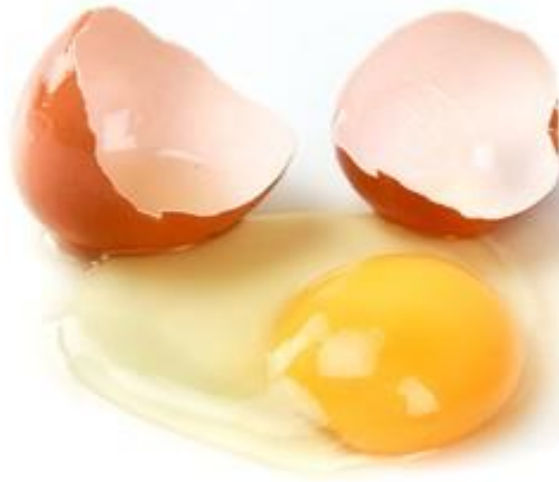
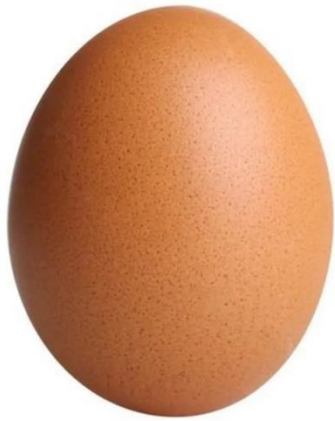


[Dados meteorológicos](#) • [Feedback](#)

Structural Proteins

Structural Protein	In Nature	Repeat Sequence
Silkworm silk [<i>Bombyx mori</i>]	 Cocoon	(heavy chain) $[GAGAGS GAAS(GAGAGS)_{1-11} GAGAGYGAGVGAGYGAGYGAGAGAGY]_n$ GTGSSGFGPYVANGGYSGY EYAWSS ESDFGTGS
Silkworm silk [<i>Antheraea spp.</i>]		$(A)_{13} GSGAGG(SAVR)GGGYGWGDGGYGSDS$ $(A)_{13} SGAGG(SA)(GGY)_{2-3}GSDS$ $(A)_{12} GSGAGGRGDGGYGS GSS$ $(A)_{12} RRAGHDRAADS$
Spider silk [<i>Nephila clavipes</i>]	 Dragline and web frame	(Major Spidroin 1) $(A)_{6-7} GGAG[QGGYGGLG(G/S/N)QGAG]_{1-2}RGGLGGQGAG$
Collagen	 Extracellular matrix in connective tissues	$[Gly-Pro-Hyp]_n$
Elastin		$[VPGVG]_n$
Resilin [<i>Drosophila melanogaster</i>]	Soft rubbery/elastic organs and tissue, such as wings and joints, of insects and other arthropods	(exon I reported as pro-resilin) $[GGRPSDSYGAPGGGN]$ (exon III) $[GYSGGRPGGQDLG]$
Keratin	 Hair, wool, and horn feathers	$MKQLEDK$ $VEELLSK$ $NYHLENE$ $VARLKKL$
Reflectin [<i>Doryteuthis opalescens</i>]	 Iridophores and leucophores of certain cephalopods	$GM PG$ $GM NP$ $GM YG$ $GM PS$ $GM PG$ $GM YP$

What's in the eggs



Purification of IgY from egg yolk



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IgY Technology: A Purification Platform using Ionic-Liquid-Based Aqueous Biphasic Systems

Fact Sheet

Reporting

Results

Objective

With the emergence of antibiotic-resistant pathogens the development of antigen-specific antibodies for use in passive immunotherapy is, nowadays, a major concern in human society. Despite the most focused mammal antibodies, antibodies obtained from egg yolk of immunized hens, immunoglobulin Y (IgY), are an alternative option that can be obtained in higher titres by non-stressful and non-invasive methods. This large amount of available antibodies opens the door for a new kind of cheaper biopharmaceuticals. However, the production cost of high-quality IgY for large-scale applications remains higher than other drug therapies due to the lack of an efficient purification method. The search of new purification platforms is thus a vital demand to which liquid-liquid extraction using aqueous biphasic systems (ABS) could be the answer. Besides the conventional polymer-based systems, highly viscous and with a limited polarity/affinity range, a recent type of ABS composed of ionic liquids (ILs) may be employed. ILs are usually classified as "green solvents" due to their negligible vapour pressure. Yet, the major advantage of IL-based ABS relies on the possibility of tailoring their phases' polarities aiming at extracting a target biomolecule. A proper manipulation of the system constituents and respective composition allows the pre-concentration, complete extraction, or purification of the most diverse biomolecules.

This research project addresses the development of a new technique for the extraction and purification of IgY from egg yolk using IL-based ABS. The proposed plan contemplates the optimization of purification systems at the laboratory scale and their use in countercurrent chromatography to achieve a simple, cost-effective and scalable process. The success of this project and its scalability to an industrial level certainly will allow the production of cheaper antibodies with a long-term impact in human healthcare.

Fields of science

[natural sciences](#) > [biological sciences](#) > [biochemistry](#) > [biomolecules](#)

[natural sciences](#) > [biological sciences](#) > [zoology](#) > [mammalogy](#)

[medical and health sciences](#) > [basic medicine](#) > [immunology](#) > [immunotherapy](#)

Programme(s)

[FP7-IDEAS-ERC - Specific programme: "Ideas" implementing the Seventh Framework Programme of the European Community for research, technological development and demonstration activities \(2007 to 2013\)](#)

Project Information

IgYPurTech

Grant agreement ID: 337753

Closed project

Start date
1 February 2014

End date
31 January 2019

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Total cost
€ 1 386 020,00

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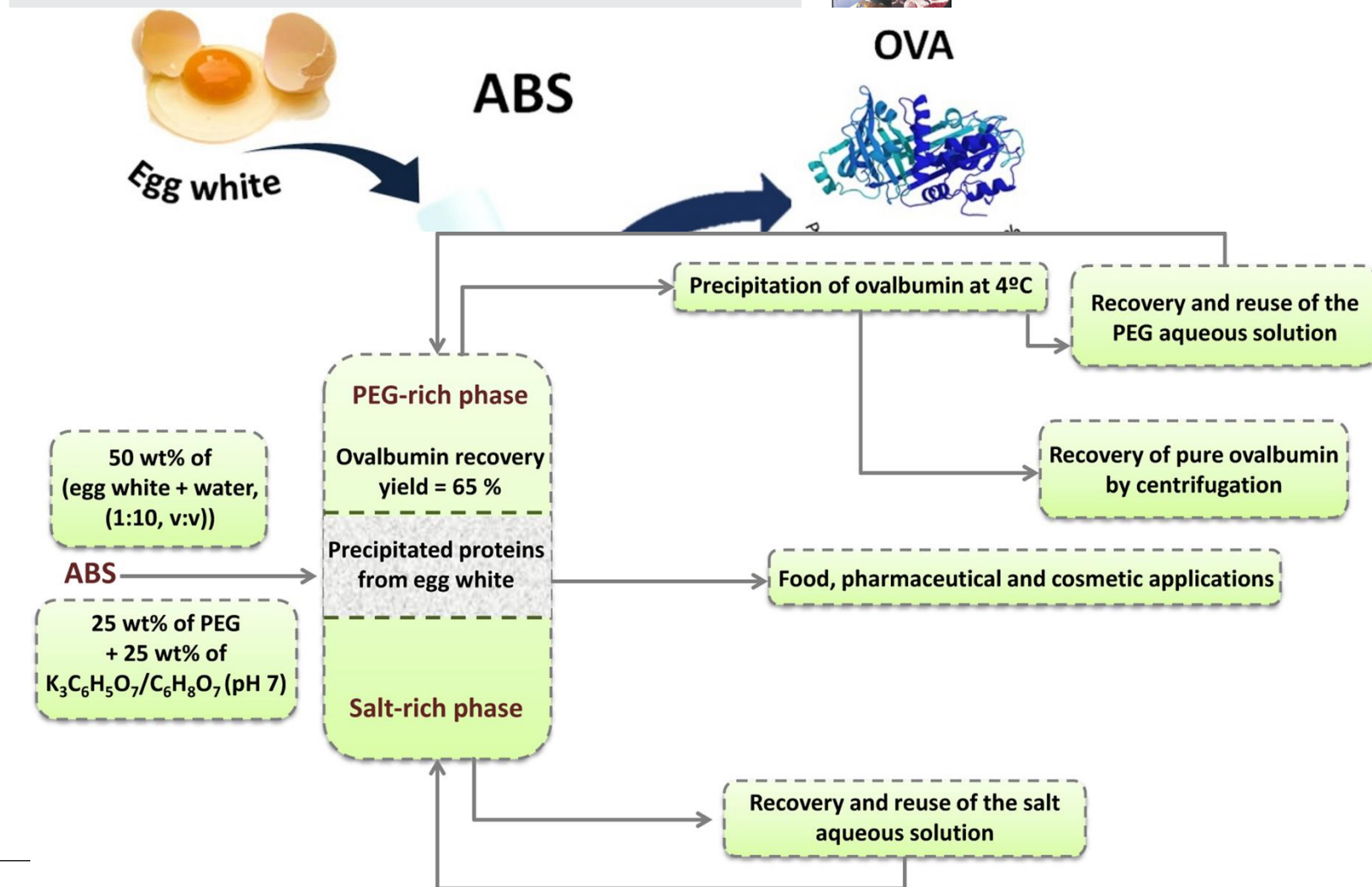
Coordinated by
UNIVERSIDADE DE AVEIRO
 Portugal



Purification of ovalbumin

Process Biochemistry 51 (2016) 781–791

Contents lists available at [ScienceDirect](#)



Fibrillation of egg white



ARTICLE

<https://doi.org/10.1038/s43246-020-0035-0>

OPEN

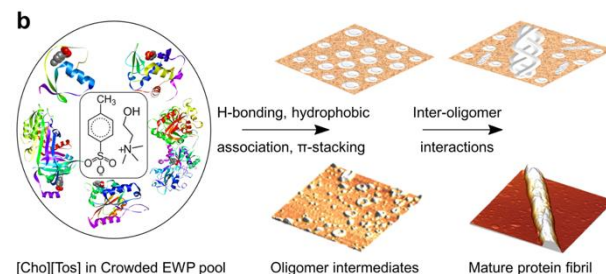
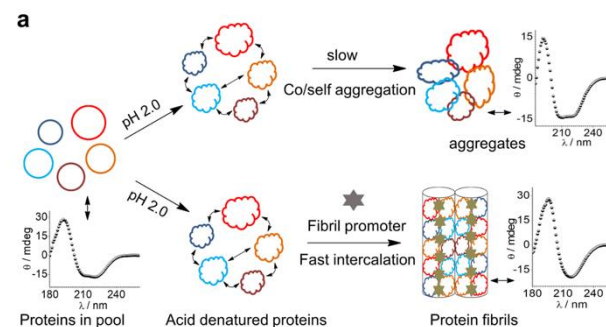
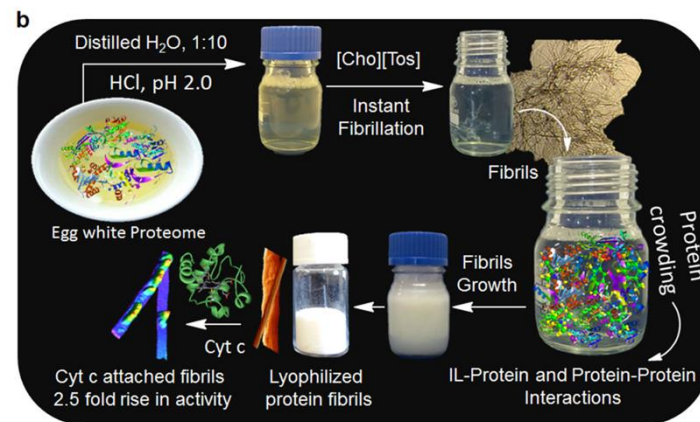


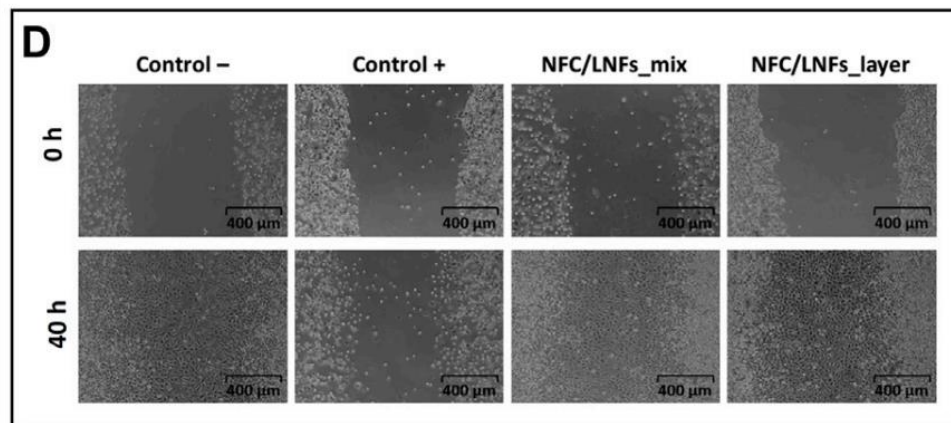
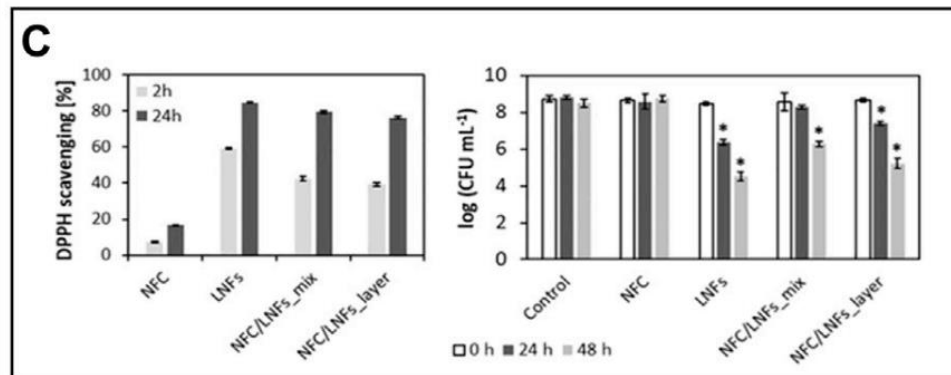
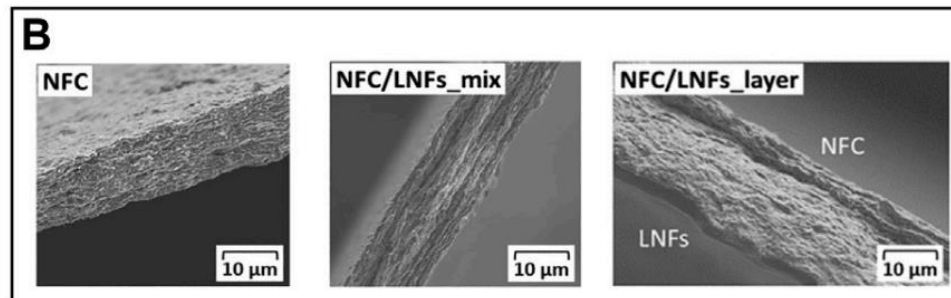
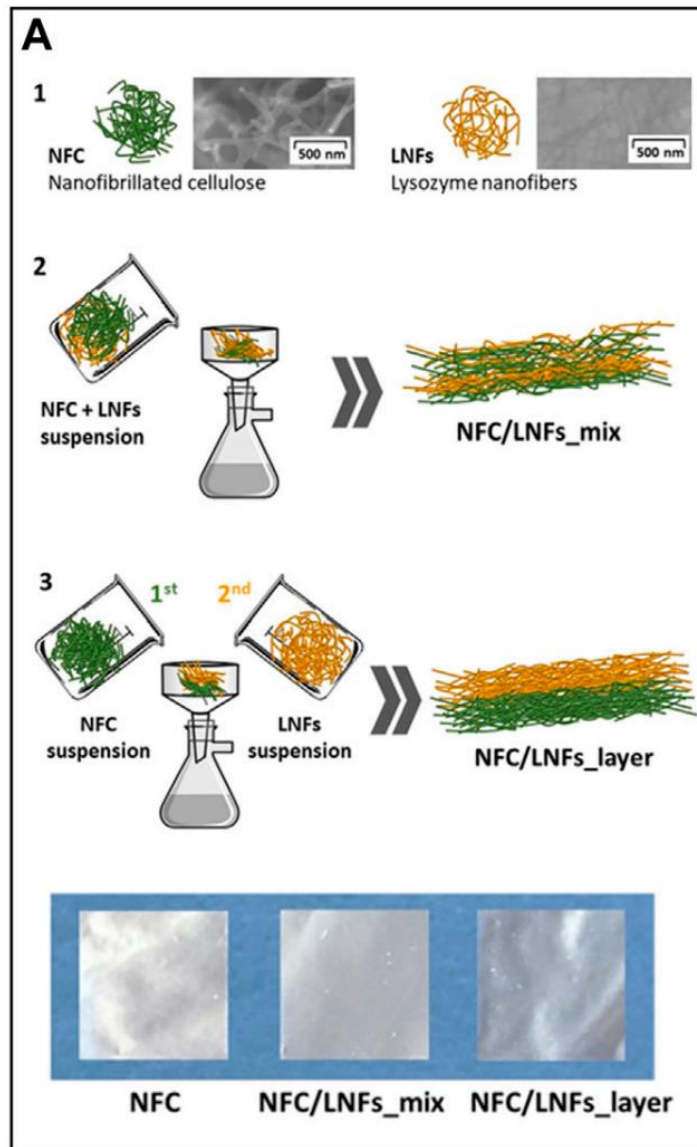
Instantaneous fibrillation of egg white proteome with ionic liquid and macromolecular crowding

Pankaj Bharmoria^{1,5}, Dibyendu Mondal^{2,5}, Matheus M. Pereira¹, Márcia C. Neves¹, Mafalda R. Almeida¹, Maria C. Gomes¹, João F. Mano¹, Igor Bdikin³, Rute A. S. Ferreira⁴, João A. P. Coutinho¹✉ & Mara G. Freire¹✉

a

Fibrillating Agents	<chem>HOCH2CH2N+(CH3)3Cl-</chem>	<chem>HOCH2CH2N+(CH3)3O3P(=O)(O-)-</chem>	<chem>c1ccc(cc1)OCH2CH2N+(CH3)3Cl-</chem>	<chem>HOCH2CH2N+(CH3)3O3P(=O)(O-)-c1ccc(cc1)</chem>
EWP Fibrillation	X	X	X	✓

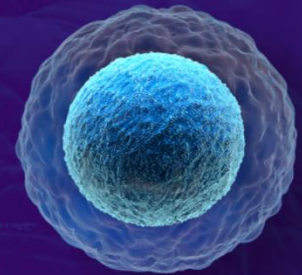




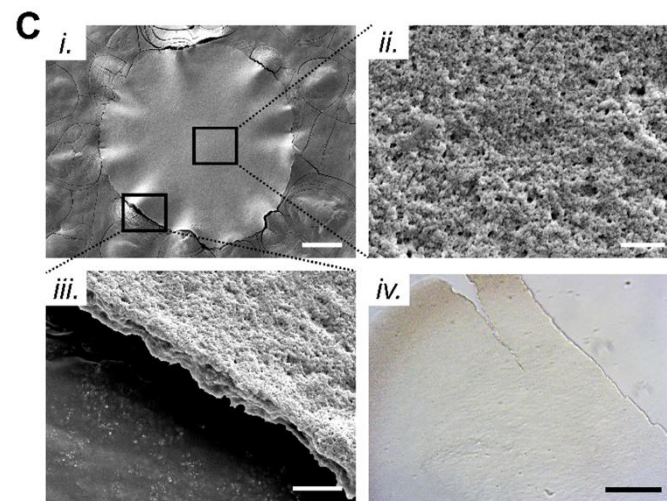
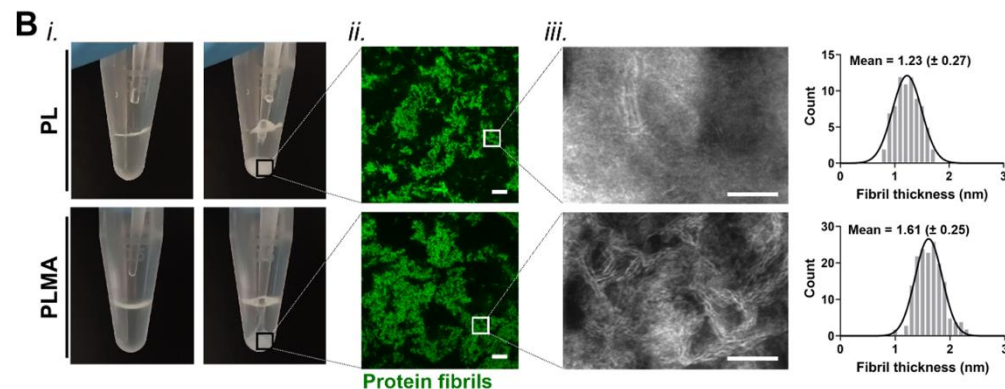
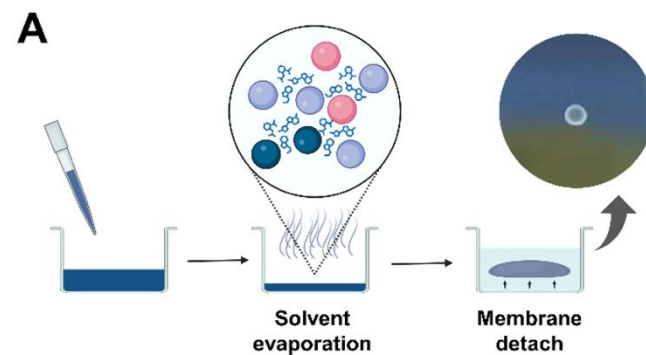
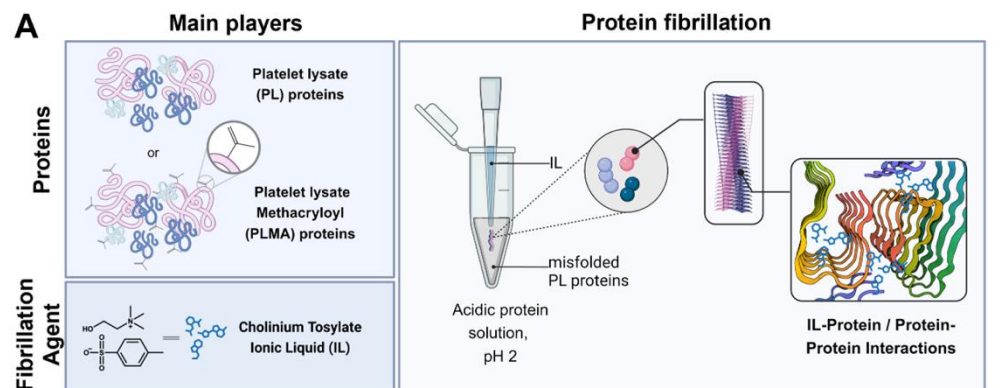
metati§ue

ADVANCING
3D CELL
CULTURE

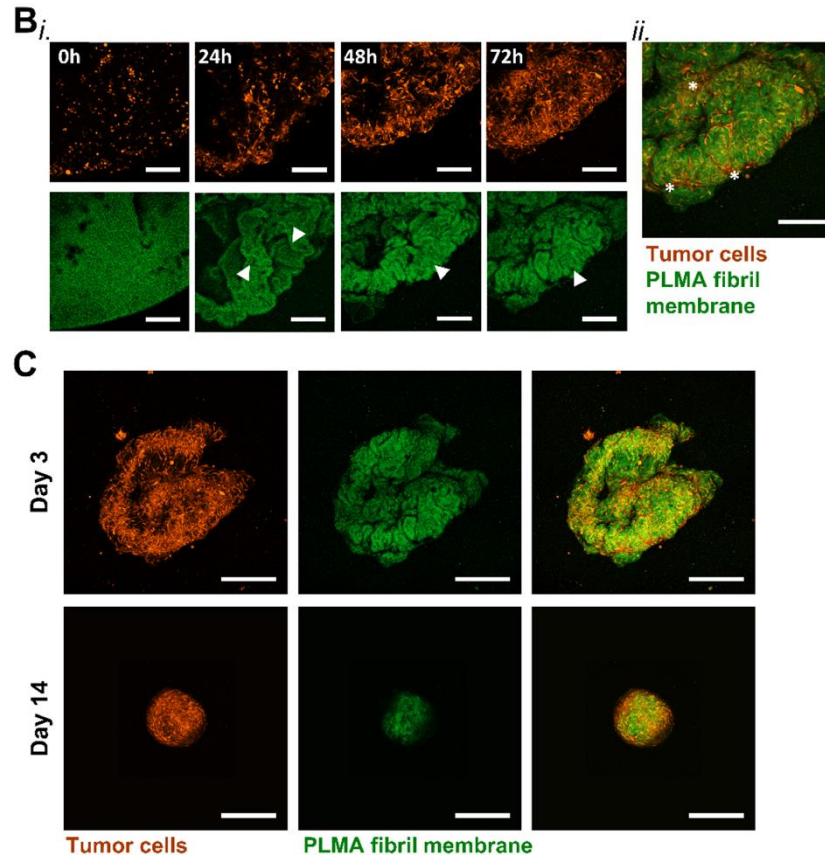
Human-based products
for Cell Culture and
Tissue Engineering



Fibrillation of human platelet lysates



Cell self-aggregation in HPL fibril membranes



Cell-membrane interaction as a driving force for cell self-aggregate formation.

This is the **first time** that a fully **human protein** fibril-based supramolecular free-standing **membrane** is reported as a **functional platform for cellular self-organization**

Purification of IgY from egg yolk



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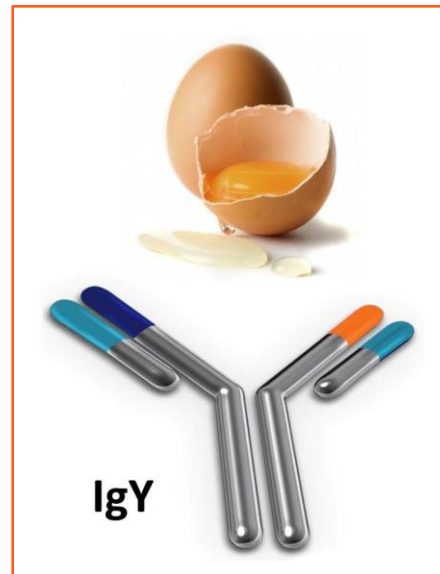
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Coordinated by
UNIVERSIDADE DE AVEIRO
 Portugal



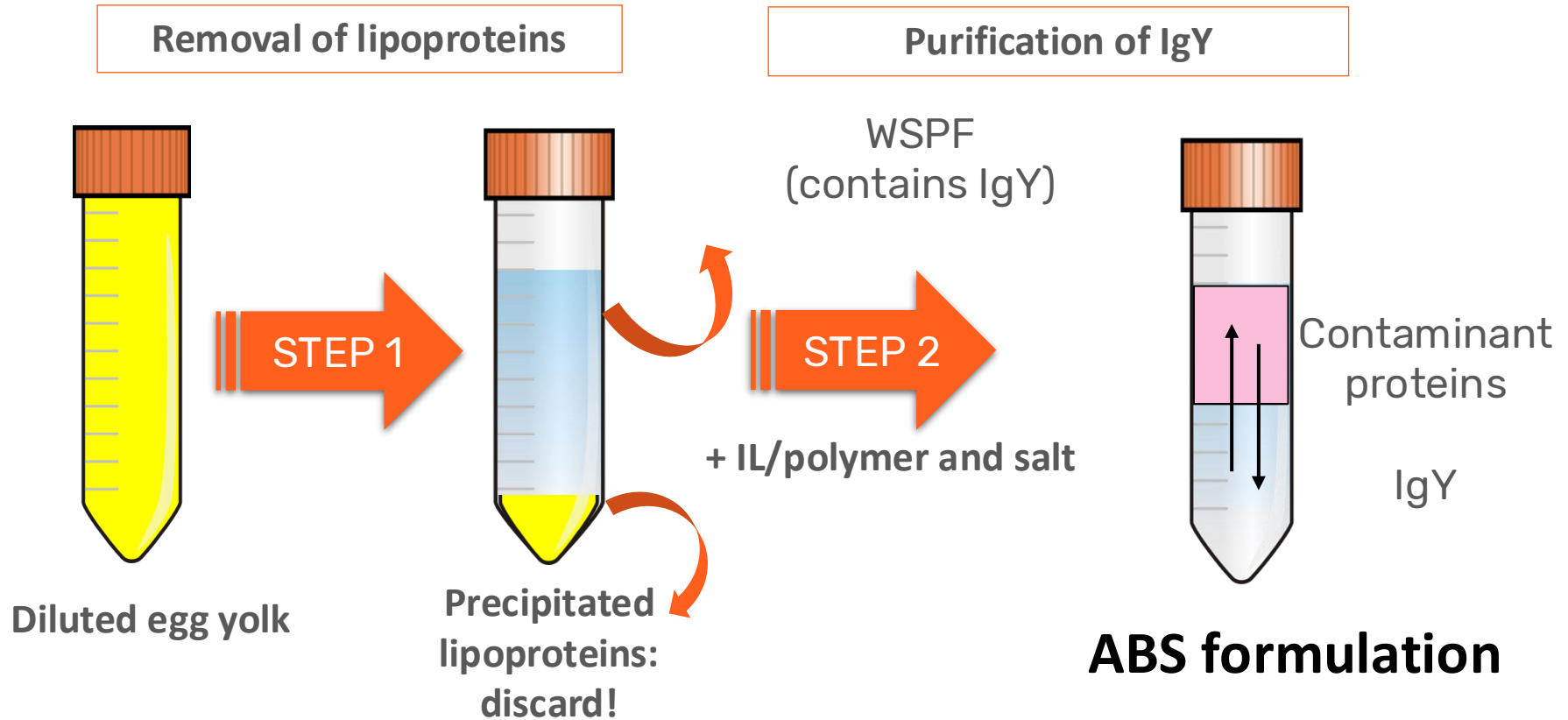
Purification of IgY from egg yolk

- Antibodies obtained from egg yolk of immunized hens, immunoglobulin Y (IgY), are an alternative option to IgG that can be obtained in higher amounts, at a lower-cost and from a renewable matrix.
- The production of high-quality IgY remained a challenge due to the lack of cost-efficient purification strategies.

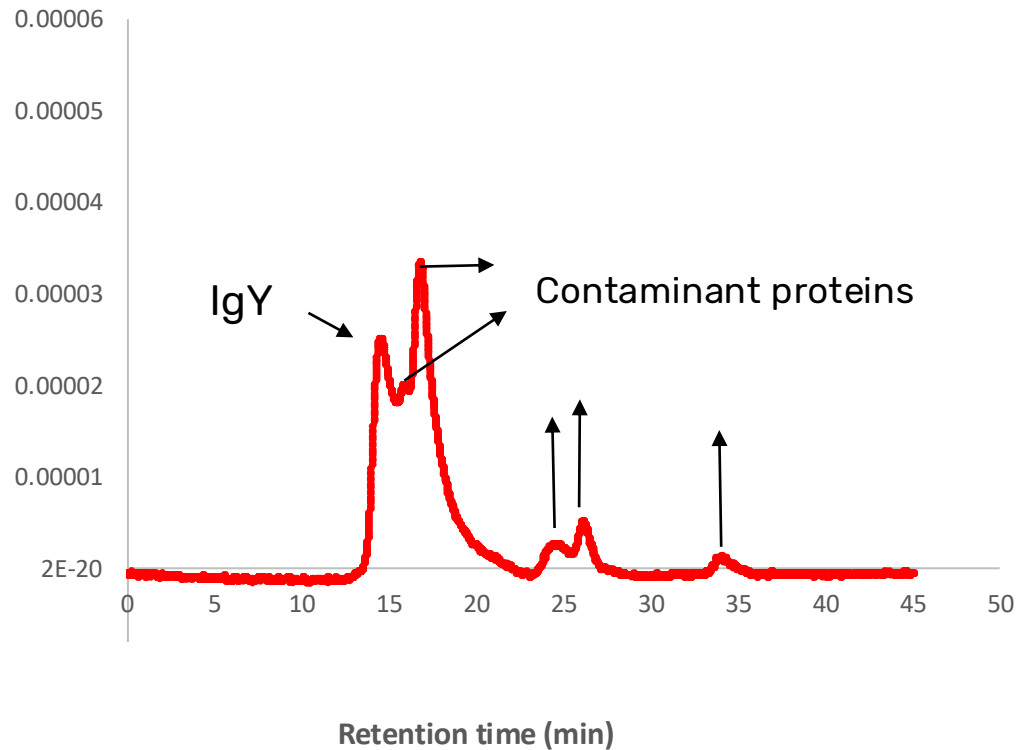


Purification of IgY from egg yolk

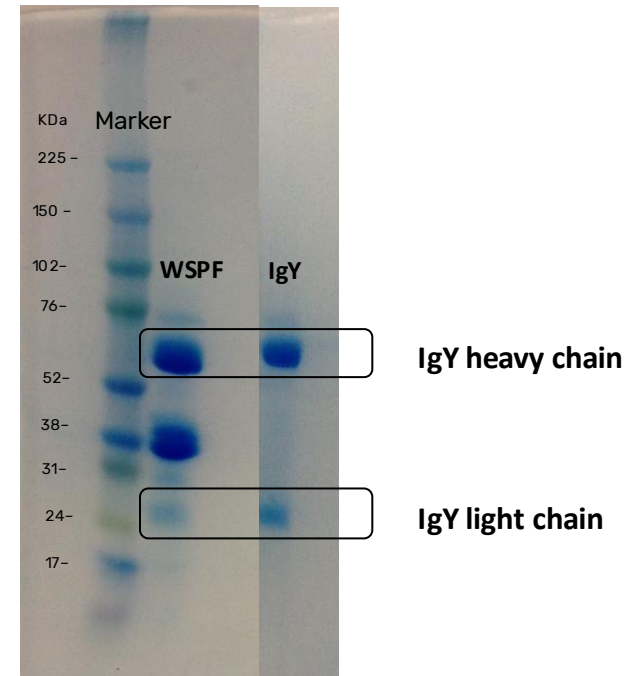
Egg yolk is a complex matrix: Two-step approach



Purification of IgY from egg yolk



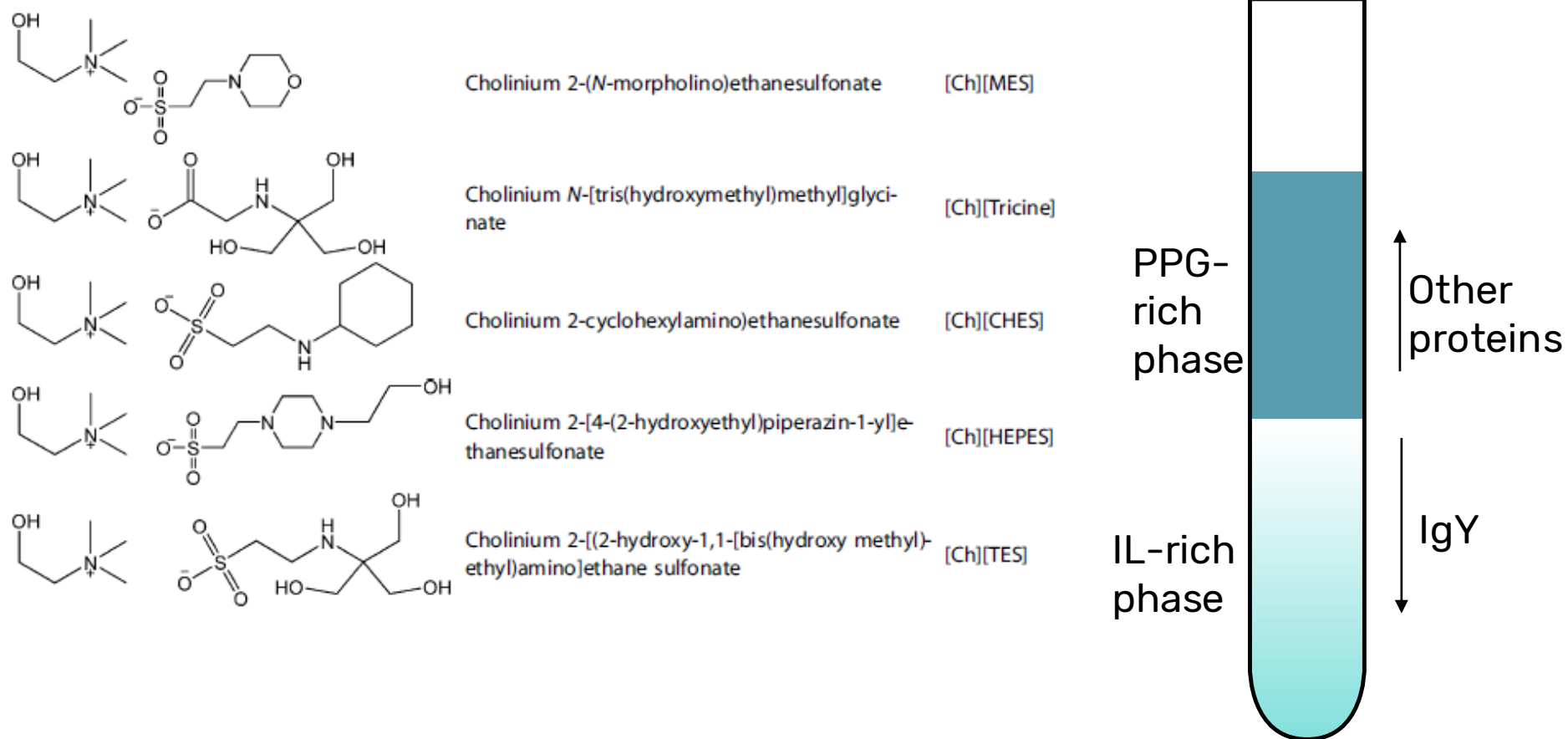
SE-HPLC Chromatogram of the WSPF of egg yolk



SDS-Page results

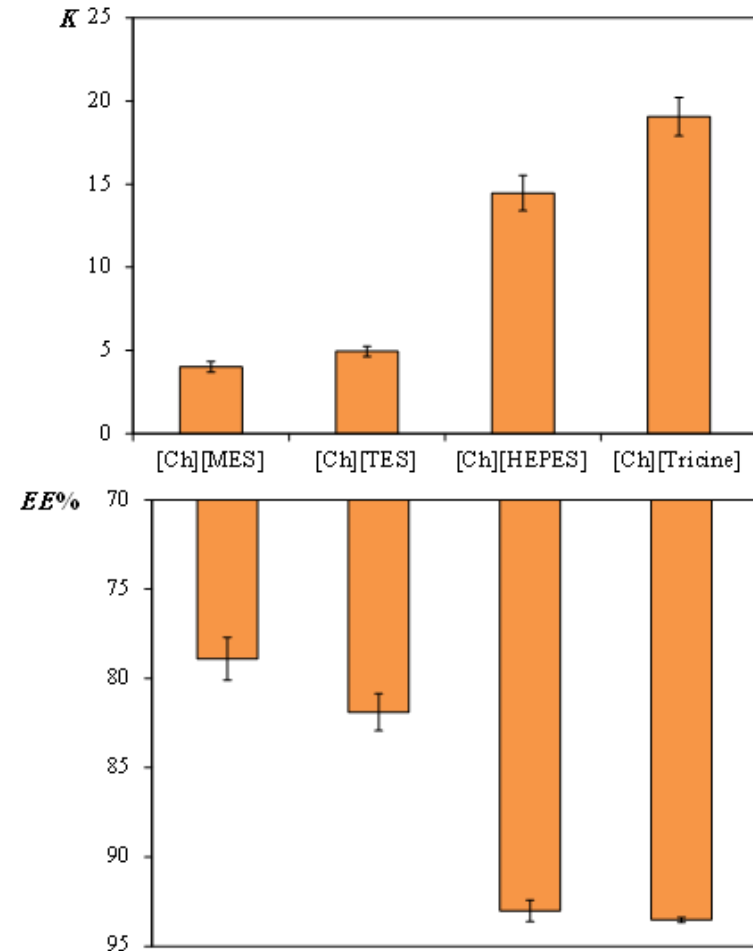
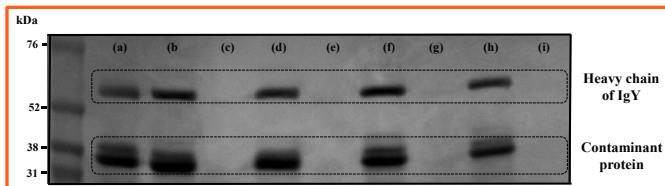
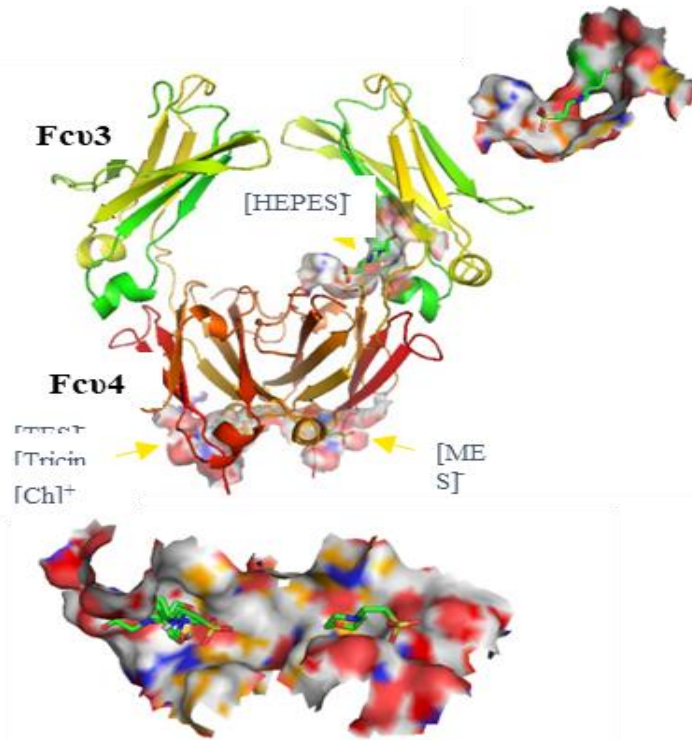
Purification of IgY from egg yolk

Extraction of IgY using ABS composed of GB-based ILs and PPG 400



IL + PPG 400 + WSPF from egg yolk

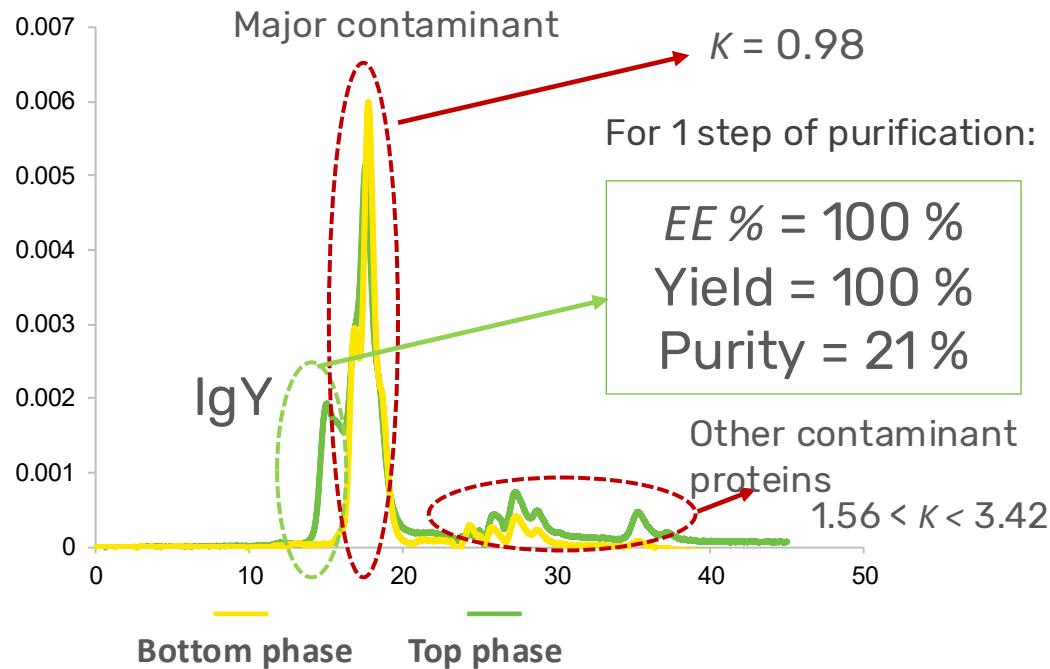
Purification of IgY from egg yolk



Selectivity towards IgY identified

Purification of IgY from egg yolk

After the investigation of several phase-forming components, compositions and pH...



Designed IL-based ABS display high selectivity for IgY

Purification of IgY from egg yolk

Centrifugal Partition Chromatography (CPC)



Liquid-liquid chromatography technique

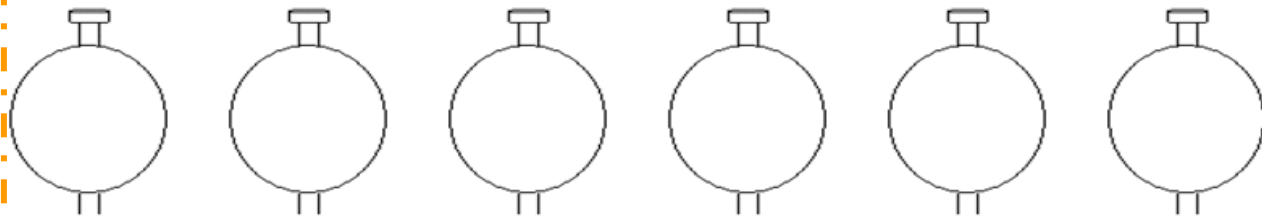
Individual solutes are isolated based on different partition coefficients (selectivity)

$$K_D = \frac{[A]_{\text{stat}}}{[A]_{\text{mob}}}$$

■ $K_D > 1$

● $K_D = 1$

▲ $K_D < 1$



>99% Pure IgY!

RYA a start-up producing IgY antibodies PATH

RYA purification
technologies

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FOR A BRIGHTER FUTURE

Immunoglobulin Y (IgY) therapeutics to tackle infectious diseases

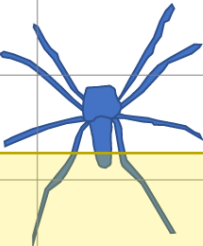
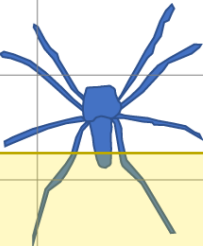
Biopharmaceuticals and proteins' purification and stabilization

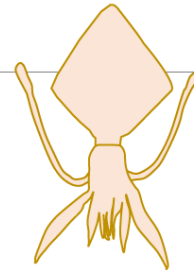
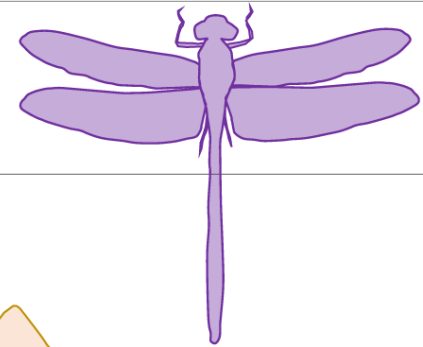
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Reflectin [<i>Doryteuthis opalescens</i>]	 Iridophores and leucophores of certain cephalopods	$GM PG$ $GM NP$ $GM YG$ $GM PS$ $GM PG$ $GM YP$



Collagen from eggshell membrane



Collagen from eggshell membrane

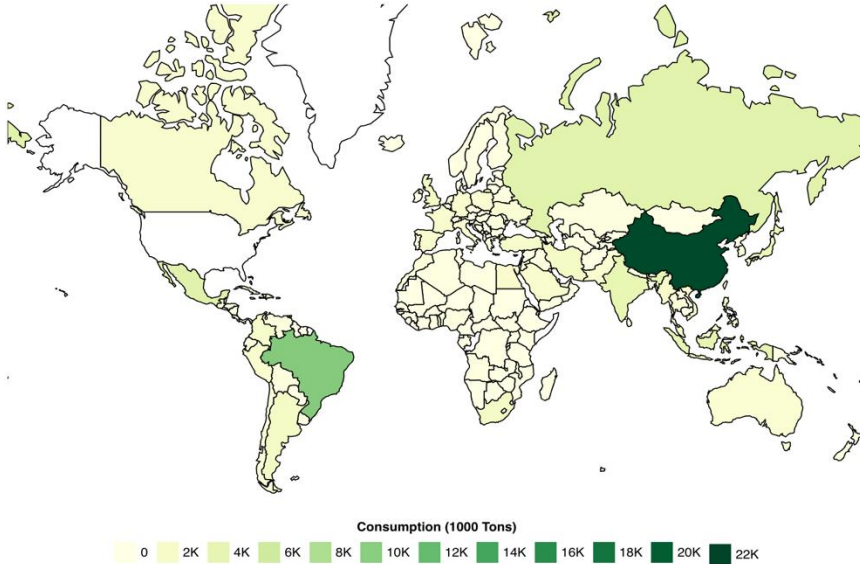


Out of the eggs: Chickens



Why chicken feathers?

Chicken consumption by country 2023



According to the latest statistics:

Consumption (ktons)

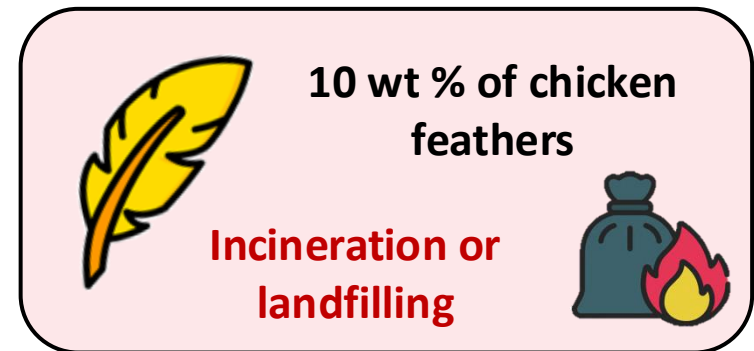
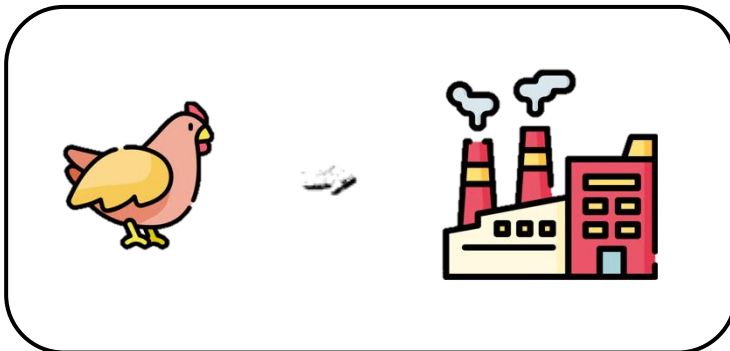
China = **22000**

Spain = **1500**

...

Portugal = **400**

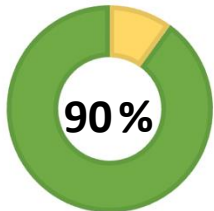
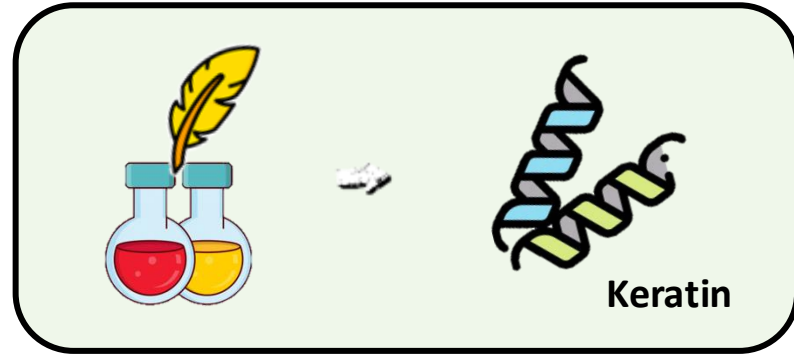
THE PROBLEM



Chicken feather waste valorisation

CHICKEN FEATHER COMPOSITION

Water	
Ash	
Fat	
Protein	



Keratin represents 90 % of the total weight of chicken feathers

Keratin is also found in:





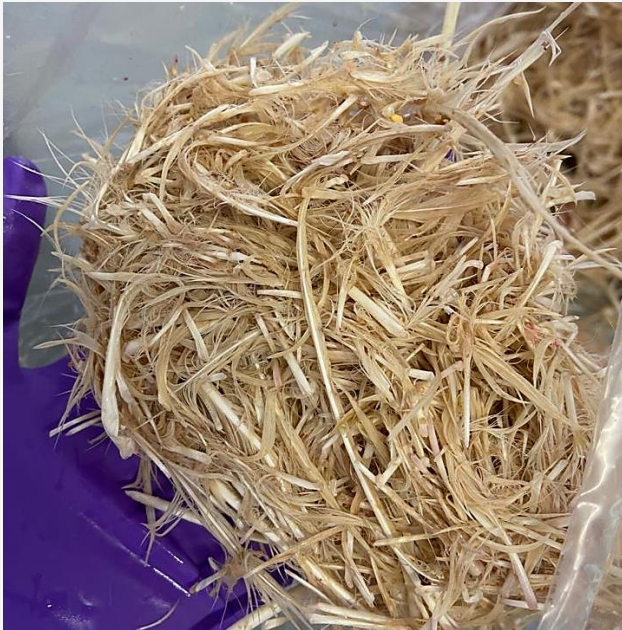
- Keratin is the **third most abundant biopolymer in the environment**, after cellulose and chitin;

- Due to its antioxidant and anti-inflammatory properties, keratin has applications in the **biomedical field** and tissue engineering.

- The potential production of keratin from chicken feathers is **2.5 times higher than from wool** (main source investigated in the literature);

Keratin recovery process

Chicken feathers pre-treatment



PGM recovery from catalytic converters



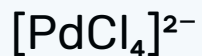
Leaching with:

- $\text{Al}(\text{NO}_3)_3 + \text{AlCl}_3$
- 37% HCl + 1% H_2O_2

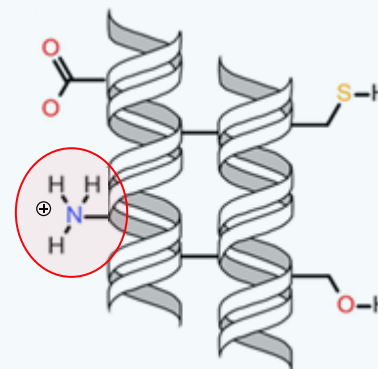


In HCl

Metals



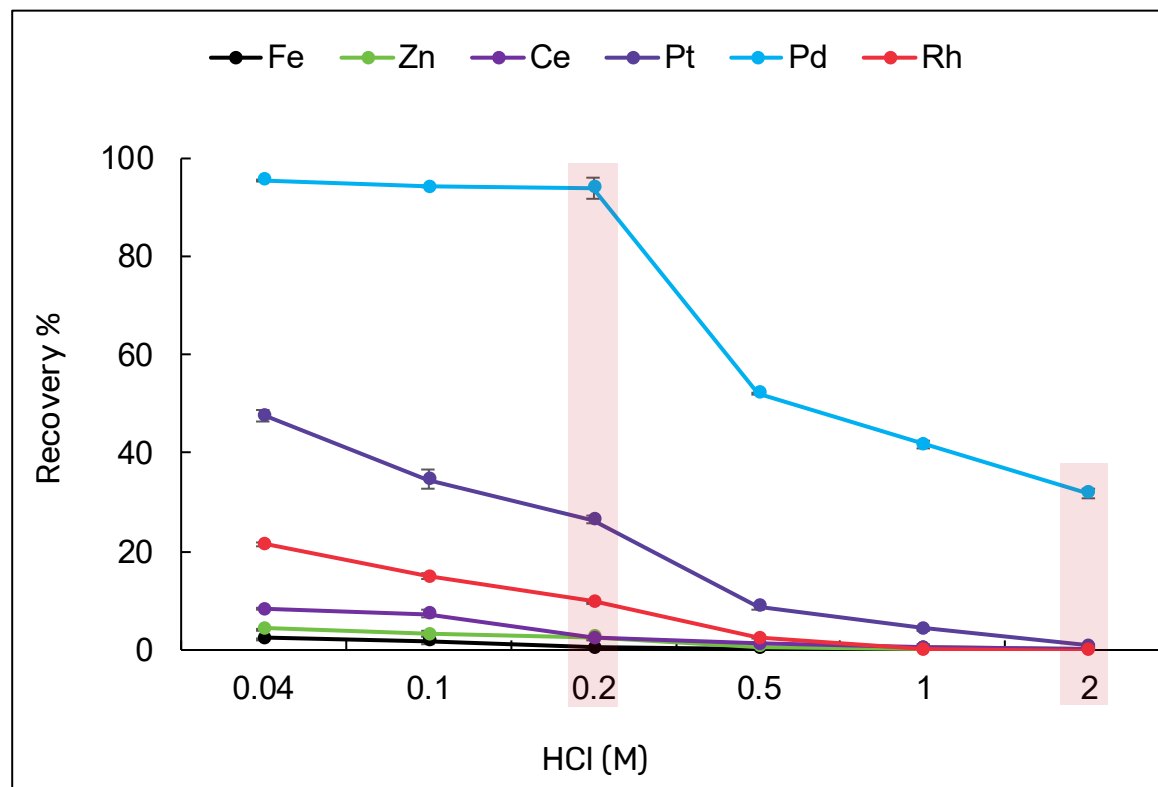
Keratin



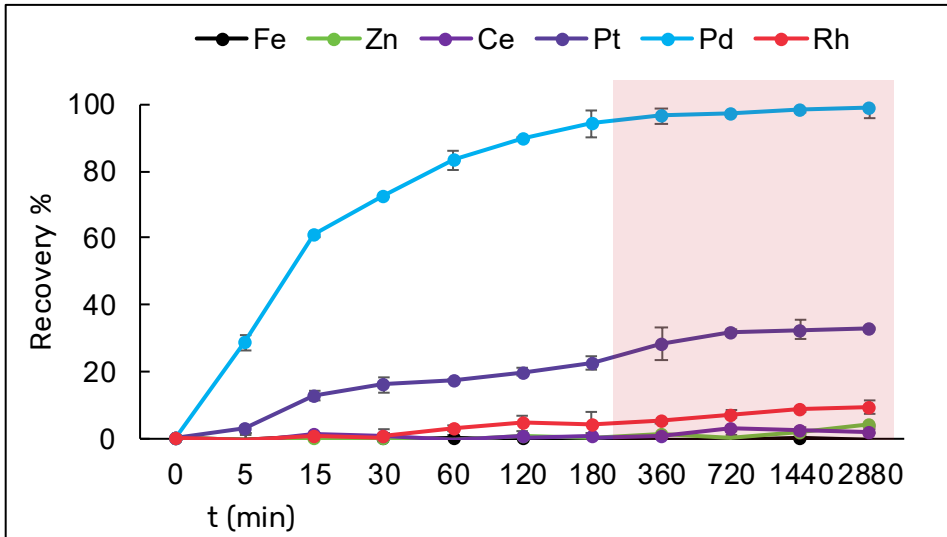
Effect of HCl concentration

Experimental condition:

HCl concentration: 0.04, 0.1, 0.2, 0.5, 1 and 2 M



Adsorption kinetics



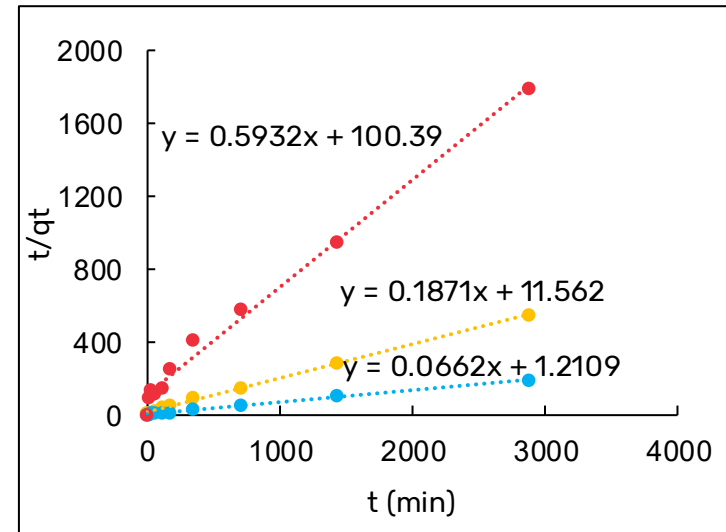
Tested models:

Pseudo-first and Pseudo-second order models

✓ **Pseudo-second-order**
$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

Rate-limiting step of PGMs adsorption by CF is through chemisorption

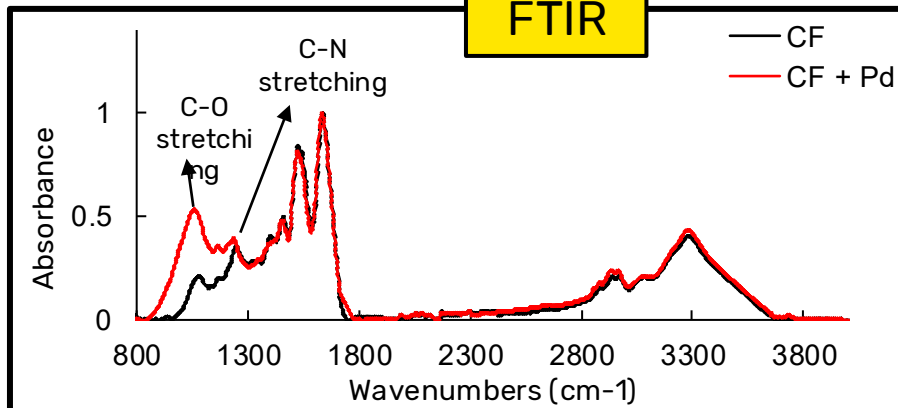
Metal	$q_{e, \text{exp}} \text{ (mg.g}^{-1}\text{)}$	Pseudo -second order model		
		$q_{e, \text{cal}} \text{ (mg.g}^{-1}\text{)}$	R^2	$k_2 \text{ (g.mg}^{-1}\text{.min}^{-1}\text{)}$
Pd	14.67 ± 0.45	15.106	0.999	0.004
Pt	5.23 ± 0.22	5.345	0.999	0.003
Rh	1.61 ± 0.07	1.686	0.991	0.004



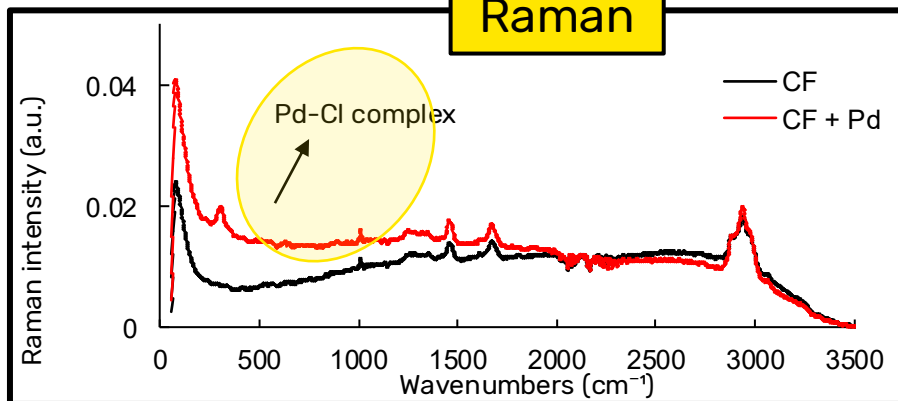
Adsorption mechanism

Feathers before and after Pd adsorption:

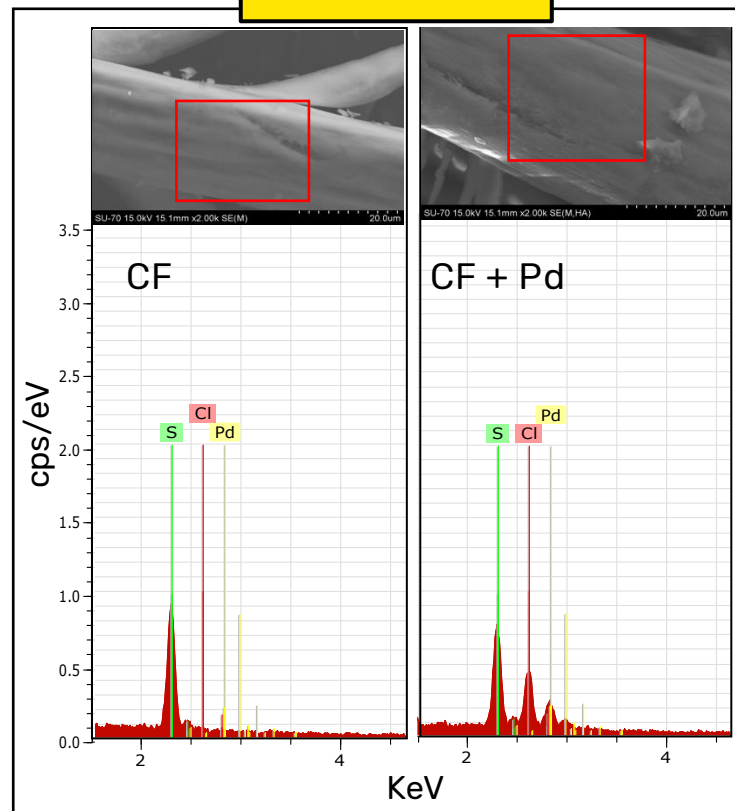
FTIR



Raman



SEM-EDS



Before Pd adsorption

After Pd adsorption

Element

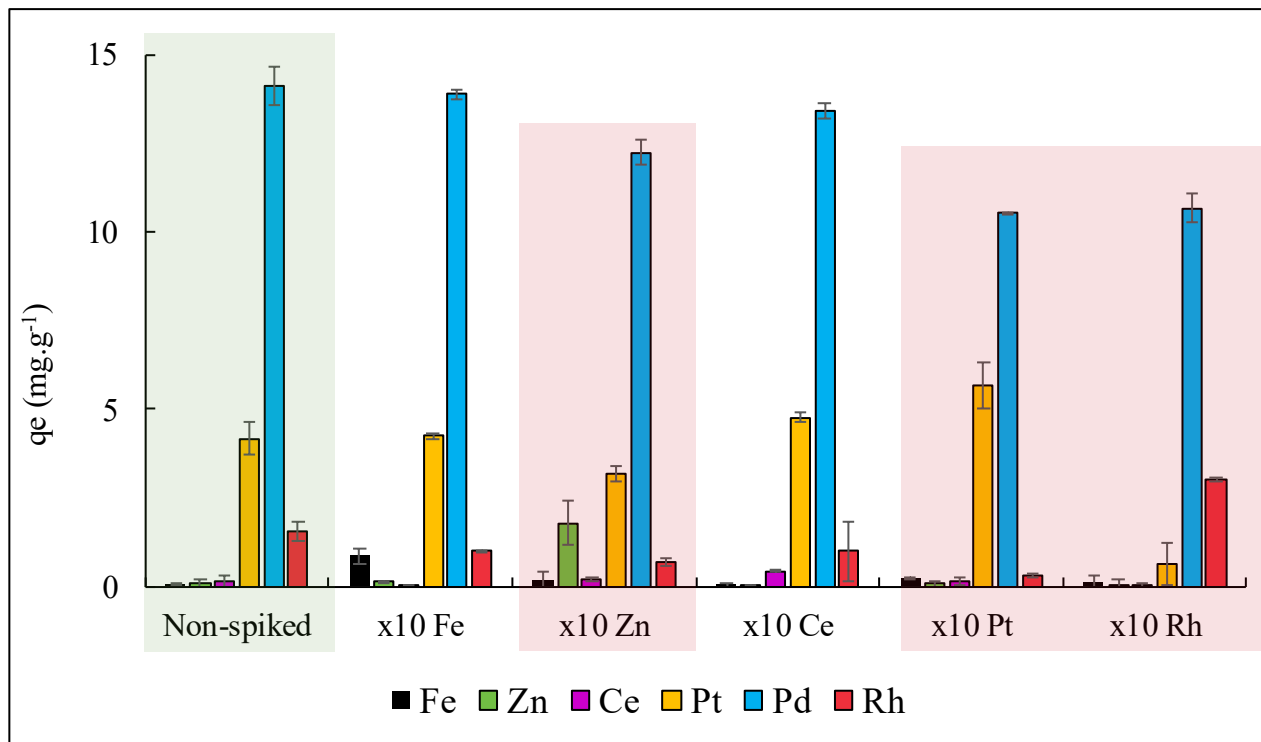
[norm. at.%]

Cl	0.04	1.32
Pd	0.01	0.39

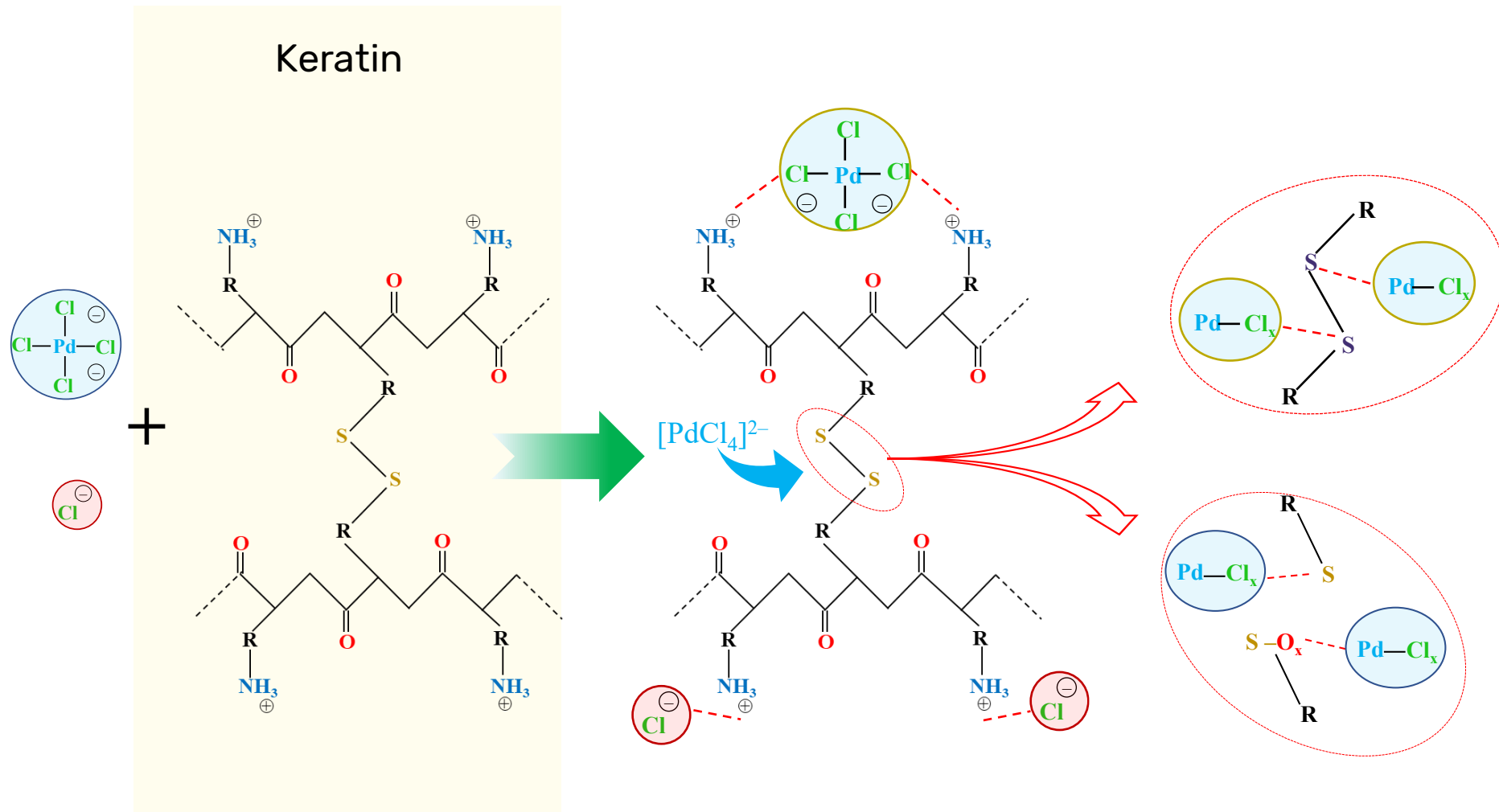
Competitive metal ions effect

Experimental condition:

Concentration of each one of competitive metal increased to 150 mg/L, while maintaining others at 15 mg/L.



Suggested adsorption mechanism

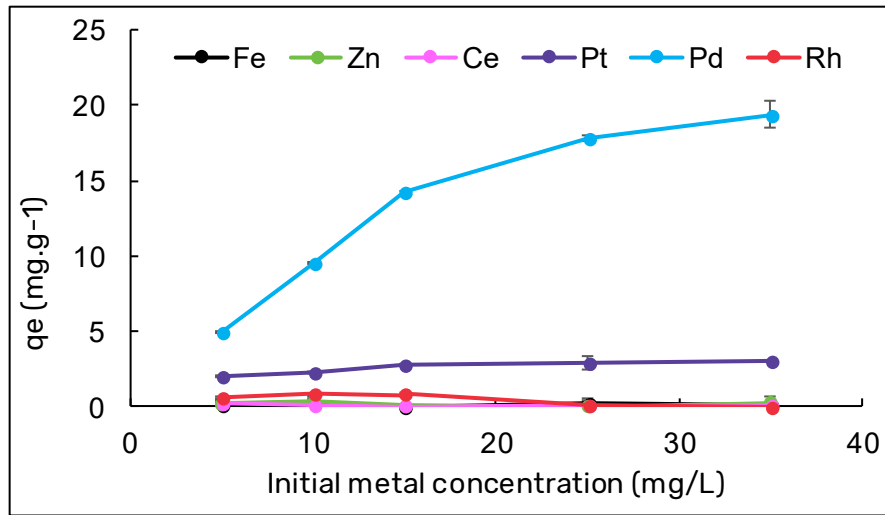


Initial metal concentration effect

Experimental condition: Test performed with solutions containing 5, 10, 15, 25 and 35 mg/L of each metal

Tested models:

Langmuir, Freundlich, Sips and Temkin models

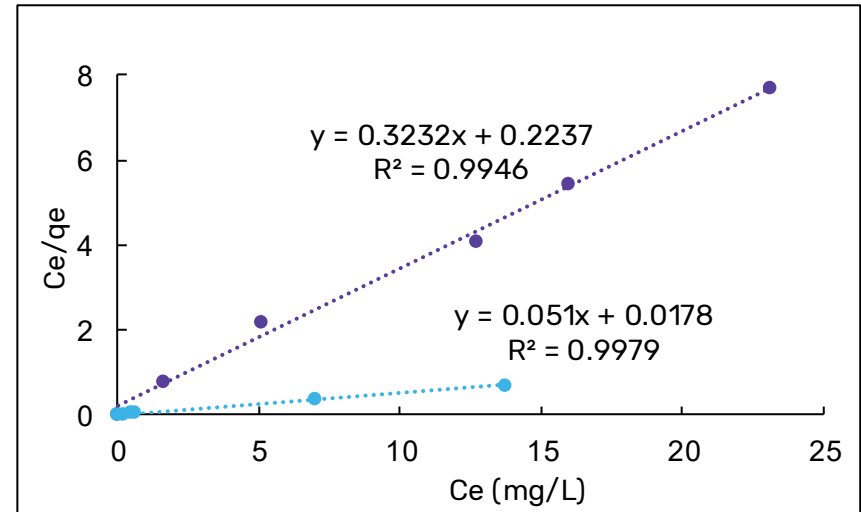


✓ The Langmuir isotherm model

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{b \cdot q_{max}}$$

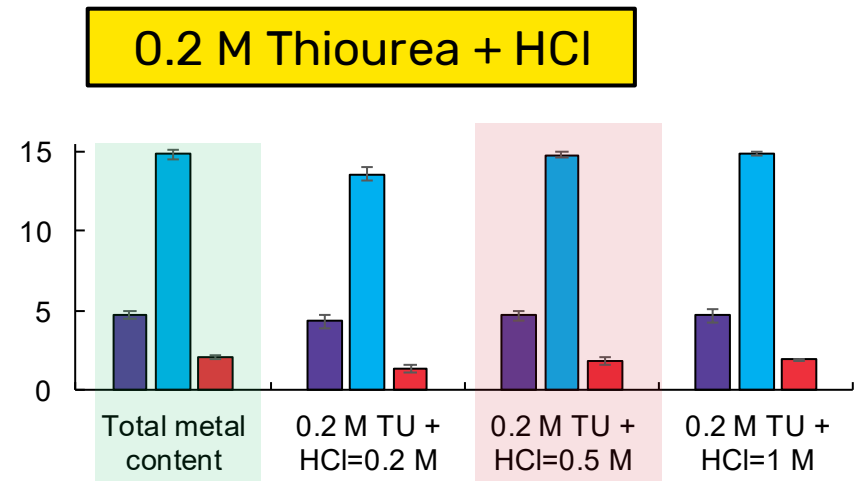
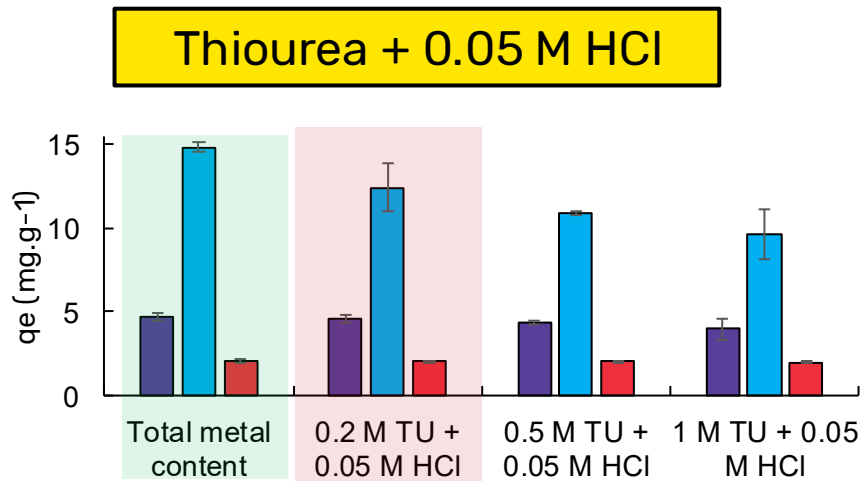
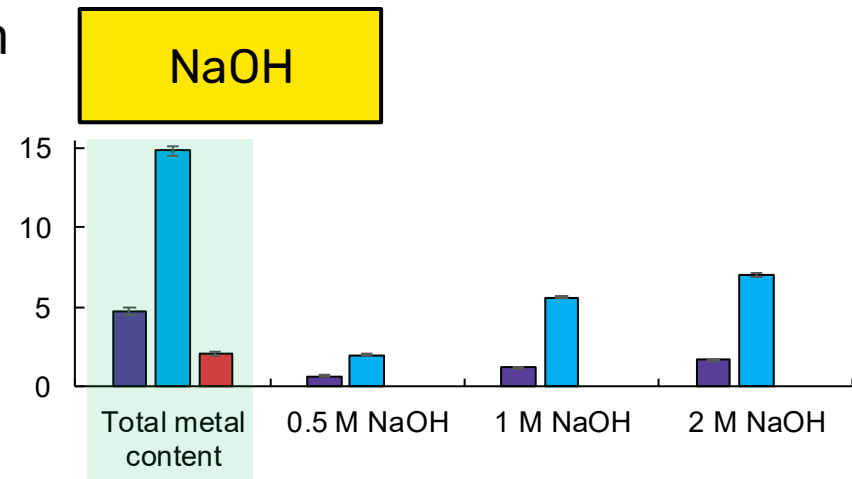
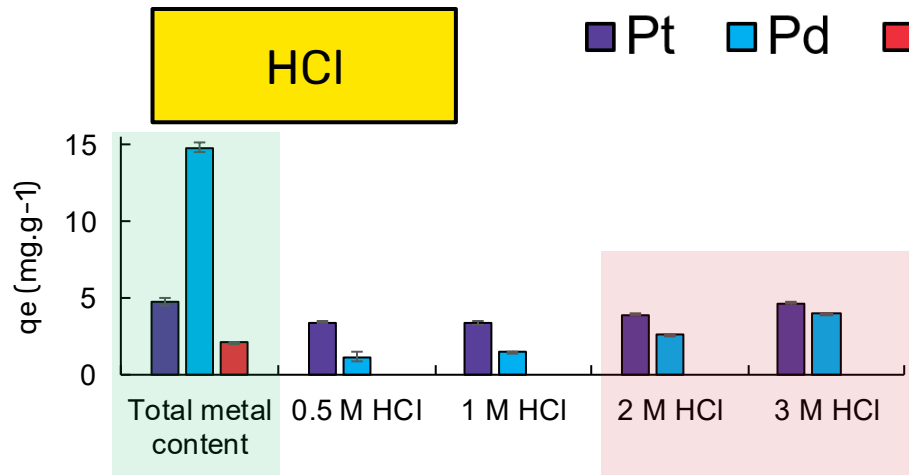
Monolayer adsorption on a homogeneous surface

	R ²	q _{max} (mg.g ⁻¹)	b (L.mg ⁻¹)
Pd	0.9979	19.607	2.865
Pt	0.9946	3.094	1.444



Desorption studies

Metal containing feathers from the optimized adsorption tests were subjected to:





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Research Paper

Waste-to-waste valorization: Sustainable palladium recovery from real spent catalytic converter leachates using chicken feathers

Amir Nobahar^a, Flavia N. Braga^{a,b}, Filipe H.B. Sosa^a, Nicolas Schaeffer^a, João A.P. Coutinho^a, Helena Passos^{b,*}

^a CICECO – Aveiro Institute of Materials, Depart

^b LSRE-LCM, ALiCE, Faculty of Engineering, Uni

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Engineered Biopolymer Architectures for Selective Metal Ions Extraction

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¹CICECO–Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal | ²LSRE-LCM, ALiCE, Faculty of Engineering, University of Porto, Porto, Portugal

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Received: 22 December 2025 | Revised: 19 March 2026 | Accepted: 23 March 2026

Keywords: bio-based adsorbents | biopolymers | chemical functionalization | hydrometallurgy | metal ion adsorption

Chicken feathers dissolution



Organic solvents



- Low protein solubility;
- Poor recovery yield;
- Harsh conditions, leading to amino acid degradation;
- Dialysis is commonly needed to recover keratin



Ionic Liquids (ILs)



- When properly designed, ILs allow complete biomass dissolution;
- Coagulant solvents, such as water or ethanol, ILs can be used for protein recovery;
- Possibility to recovery and reuse.

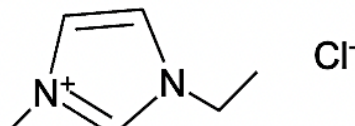
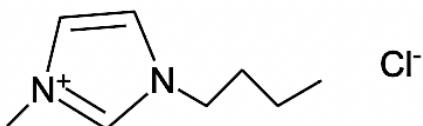
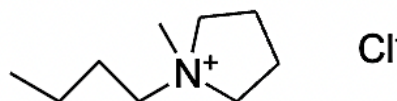
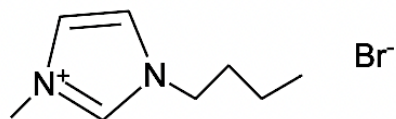
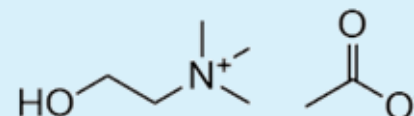
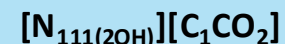
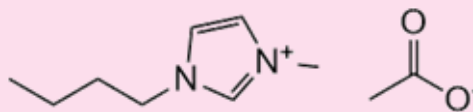
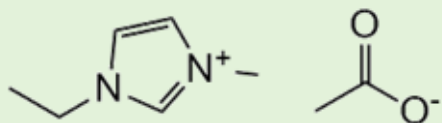
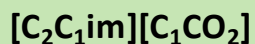


Chicken feathers pre-treatment





Chicken feathers dissolution



100 °C



4 h

$[C_1CO_2]$ -based ILs with 20 wt% water were the best solvents

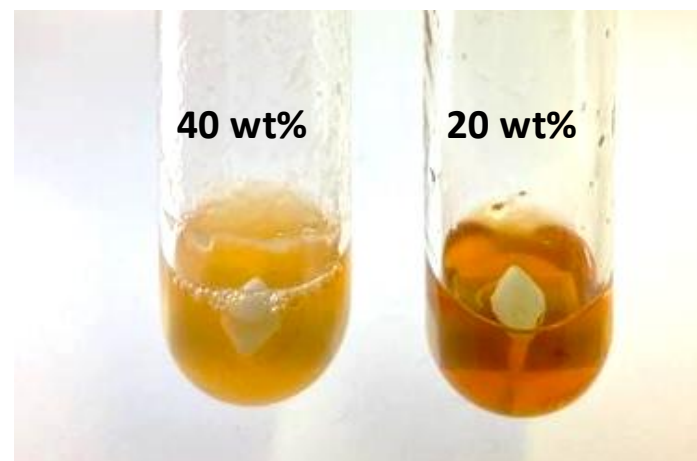


Chicken feathers dissolution

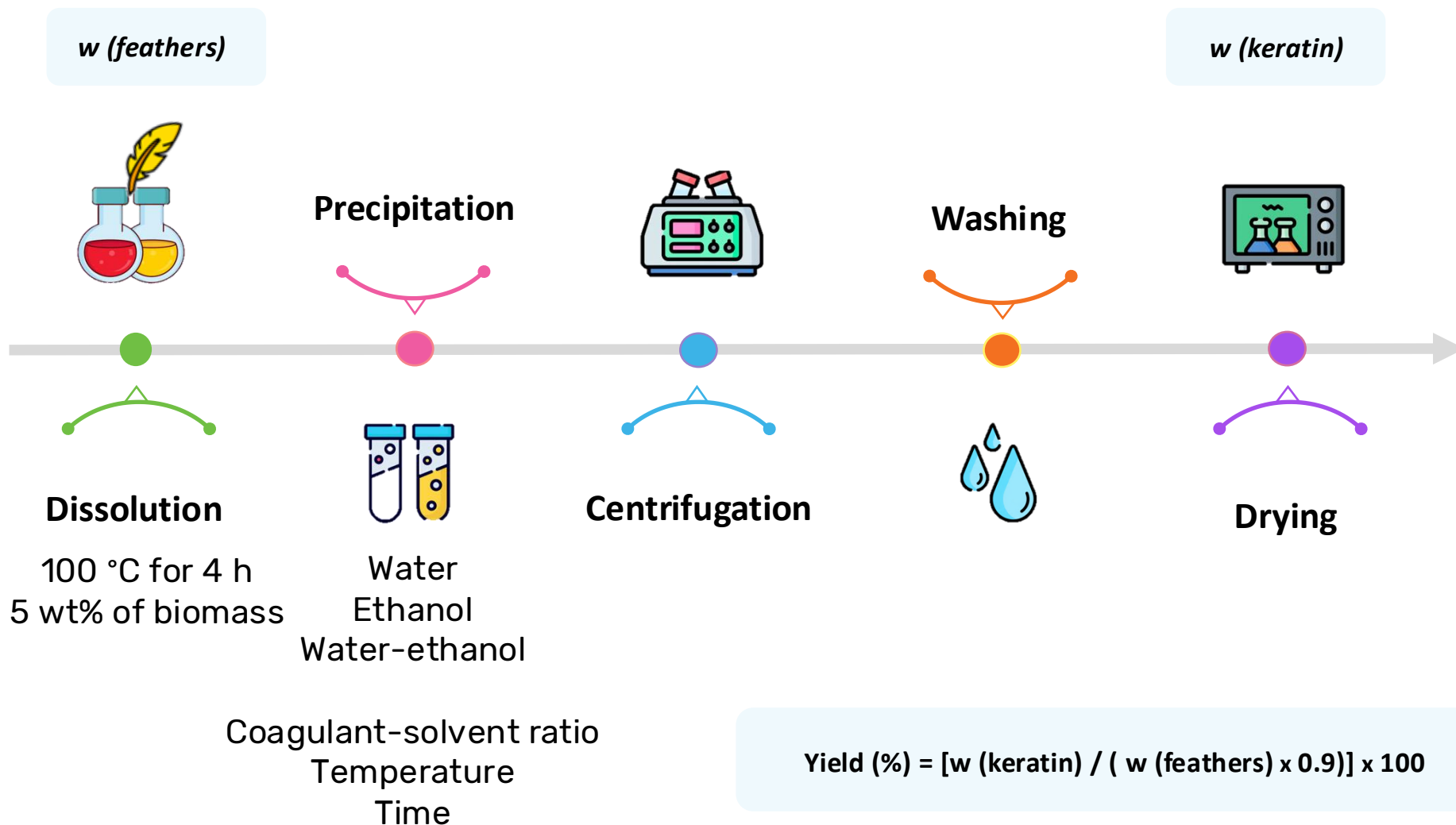
Water at 20 wt% promotes the viscosity reduction, accelerating the dissolution



Increasing water concentration from 20 wt% to 40 wt%, it starts to compete with the hydrogen bonds responsible for dissolution



Keratin recovery process



Optimisation of keratin production

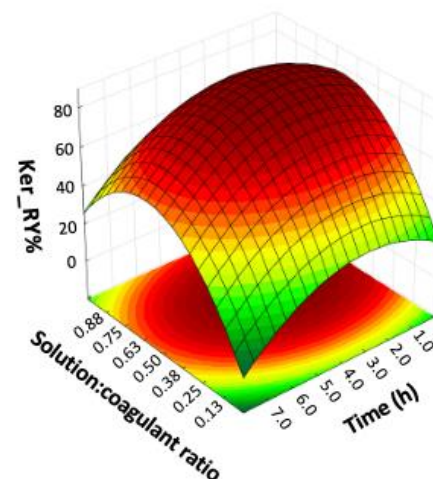
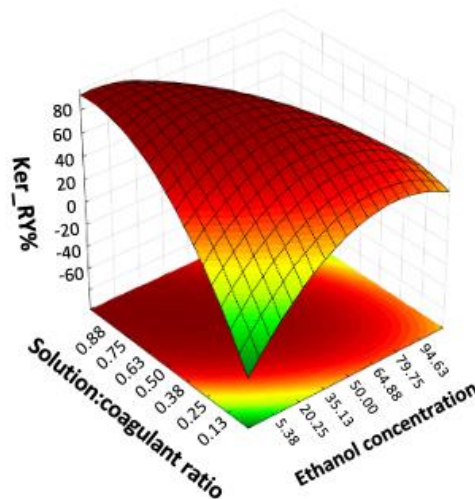
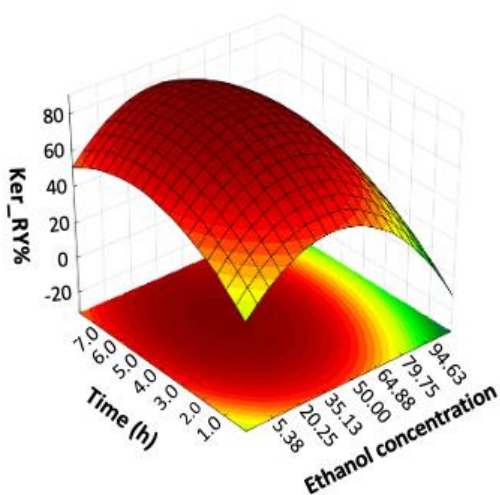


Solvent: Cholinium acetate (80 %) + H₂O (20 %)

Dissolution conditions: 100 °C, 4 h, feathers: solvent ratio = 1:20 w/w

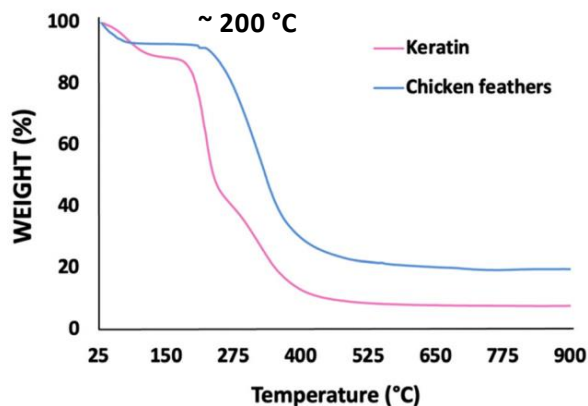


Under the best precipitation conditions (20.25 wt % ethanol in water, 1 : 1.45 w/w, 5 °C, and 5 h), **93 wt % of keratin was recovered.**

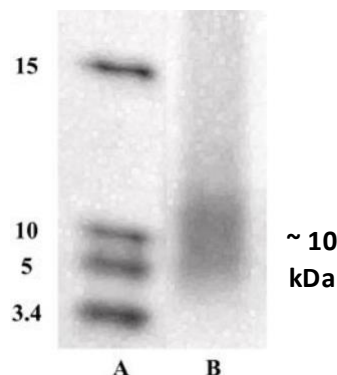


Keratin characterisation

TGA

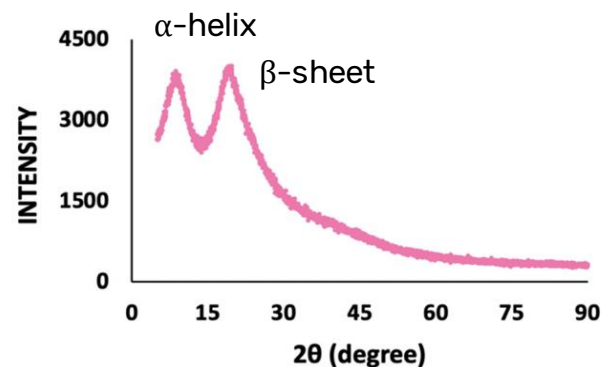


SDS-PAGE

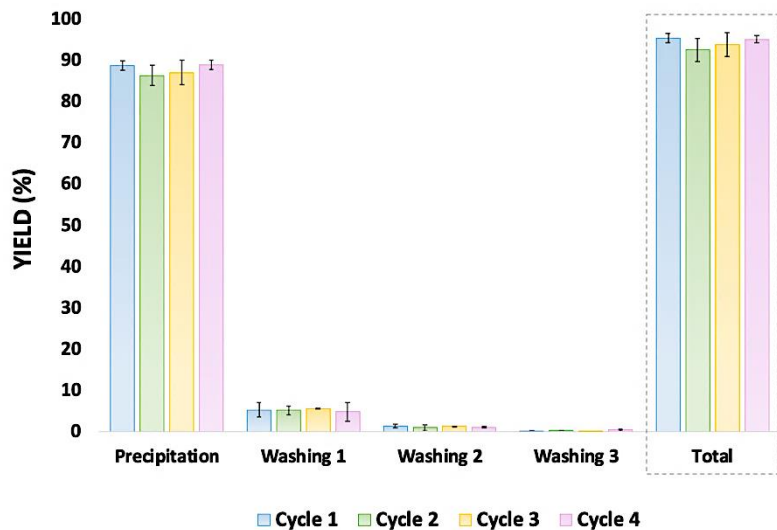
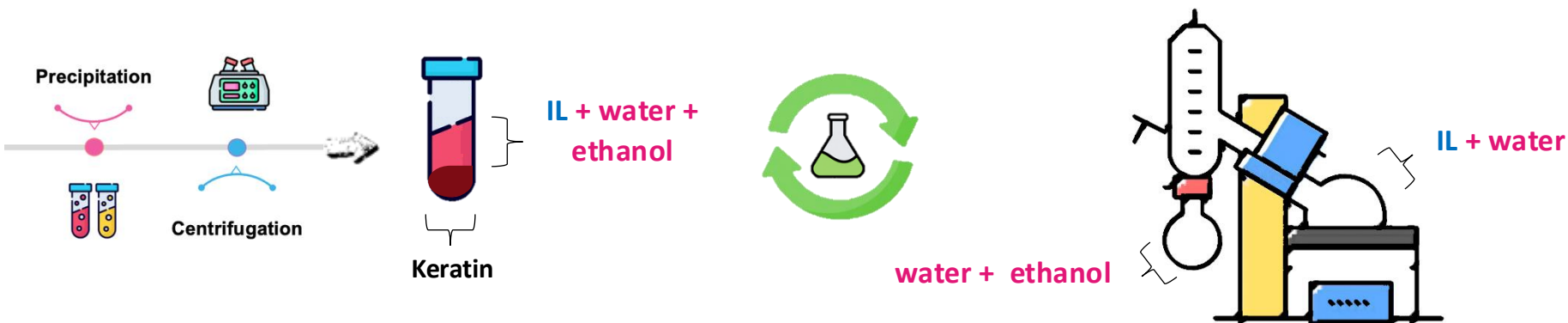


*A= protein marker, B = keratin.

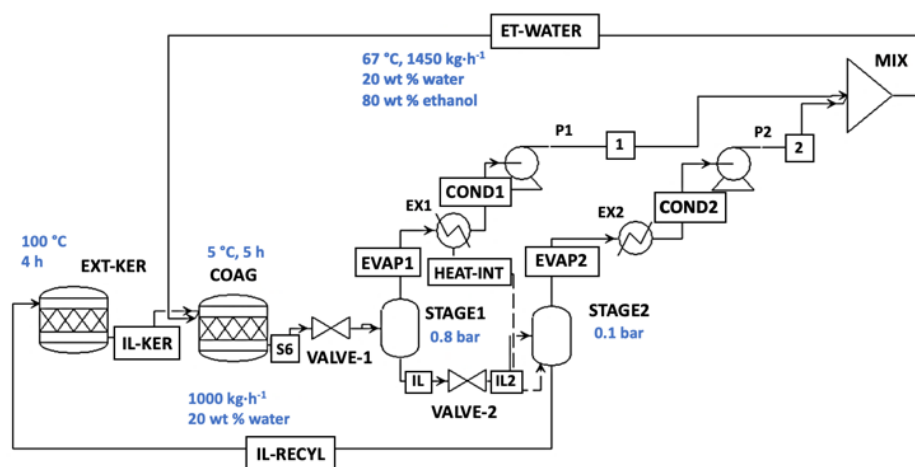
X-ray diffraction



IL recovery and reuse



- 95 wt % of IL was recovered with 4 washing cycles;
- The reused IL does not have an impact on keratin integrity and recovery yield.



Biorefinery process simulation

- The minimum selling price obtained for keratin is 22 \$·kg⁻¹; (personal care, biomedical applications)
- Low CO₂ emission (4kg_{CO2}/kg_{ker})
- The IL recovery has the highest impact on the process costs.

* calculated considering the total productivity of 350 tons of keratin·year⁻¹, the total operating costs and annual plant depreciation.

Green Chemistry



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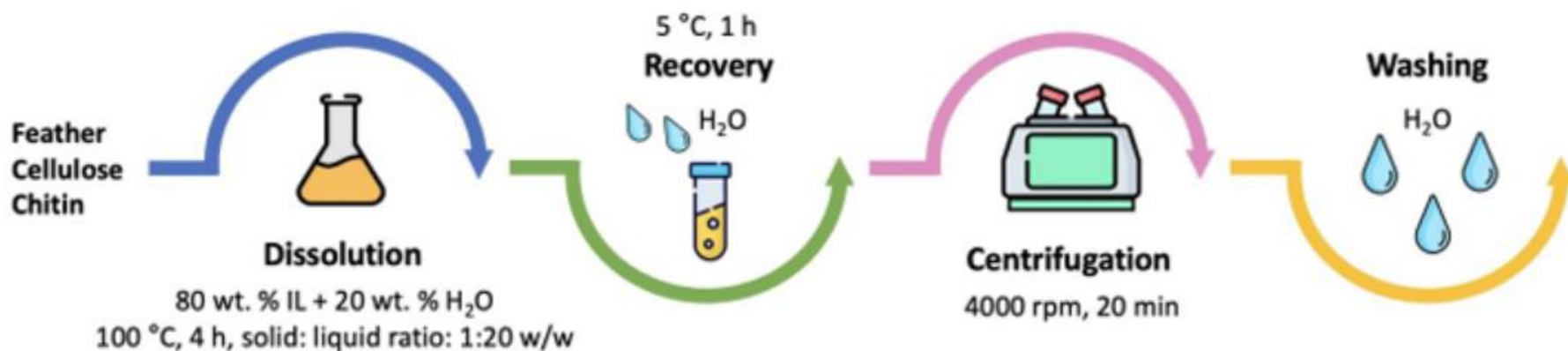
Cite this: *Green Chem.*, 2023, 25, 3995

Sustainable keratin recovery process using a bio-based ionic liquid aqueous solution and its techno-economic assessment†

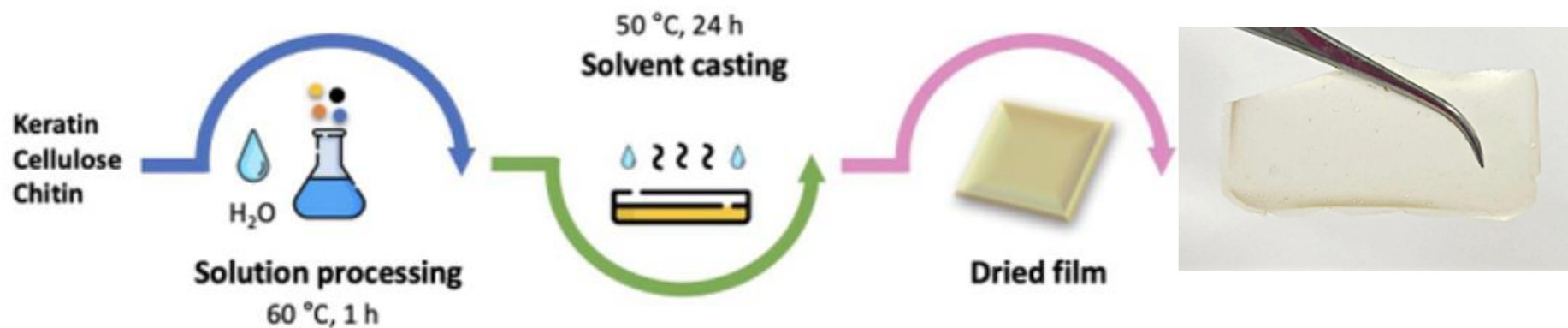
Cariny Polesca, ^{a,b} Amir Al Ghatta, ^b Helena Passos, ^a João A. P. Coutinho, ^a Jason P. Hallett ^{*b} and Mara G. Freire ^{*a}

Keratin film processing

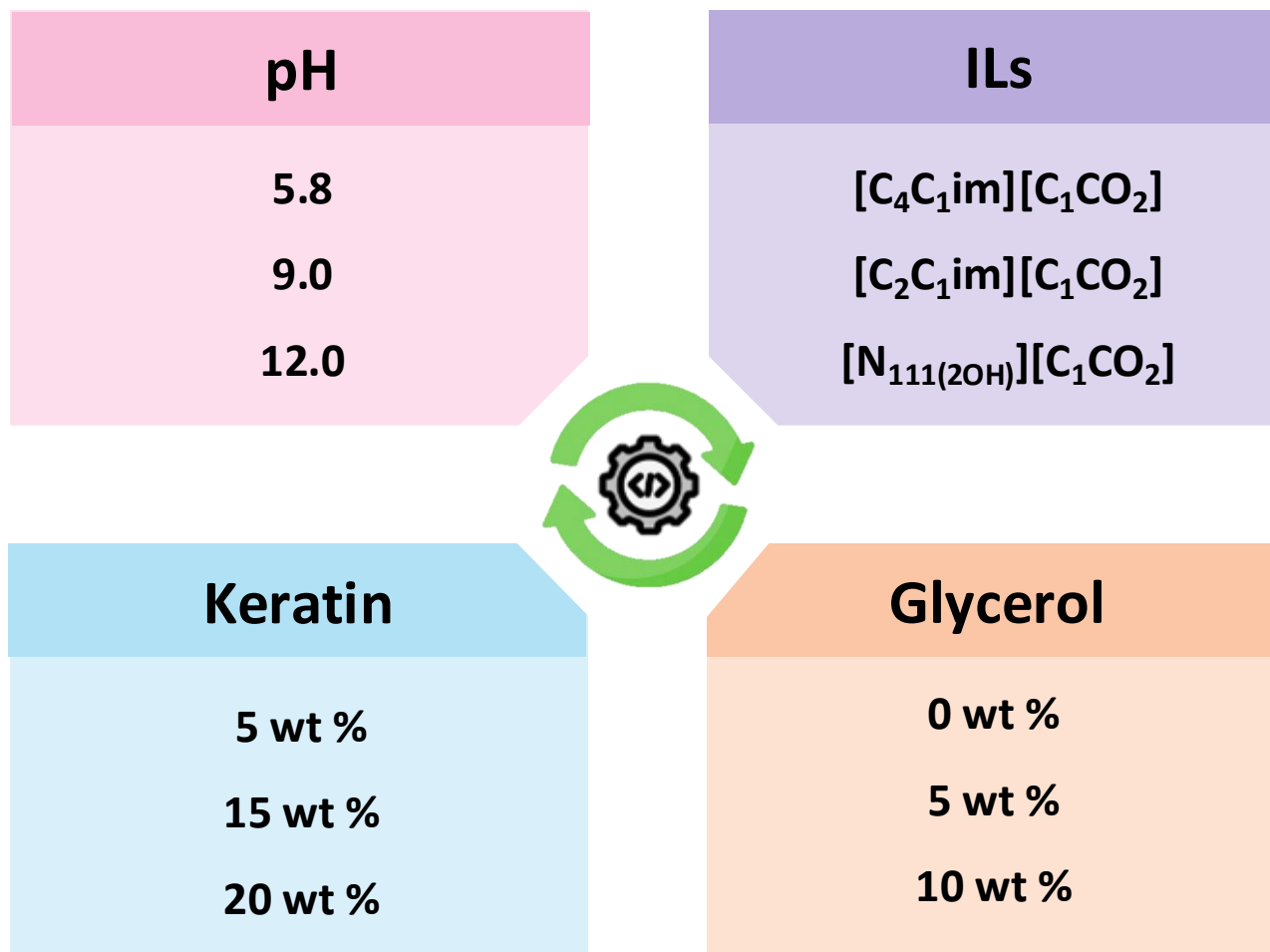
(i) Biopolymer dissolution



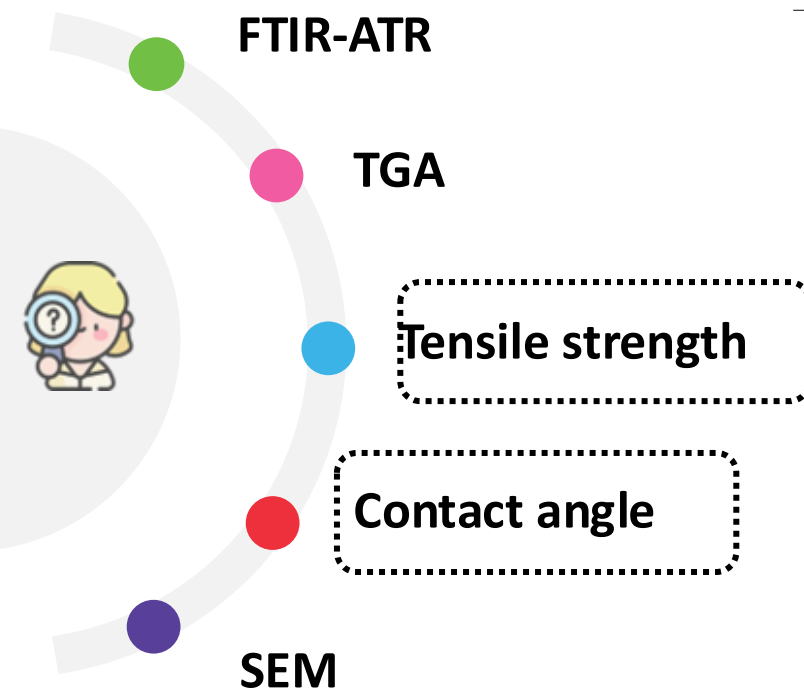
(ii) Bio-based film processing



Keratin film processing conditions



Keratin film characterisation



[41]

Samples	Contact angle (°)	Tensile strength (MPa)
KER-5	23 ± 2	0.53 ± 0.04 14 ± 2 14 ± 2
KER-15*	55 ± 1	
KER-20	62 ± 1	
KER-pH6.4*	55 ± 1	14 ± 2
KER-pH9	86 ± 2	10 ± 1
KER-pH12	84 ± 1	11 ± 1
KER-gly0*	55 ± 1	14 ± 2 36 ± 1 50 ± 1
KER-gly5	49 ± 2	
KER-gly10	47 ± 5	
KER-C4*	55 ± 1	14 ± 2
KER-C2	58 ± 5	12 ± 1
KER-N111	60 ± 5	11 ± 1



Keratin blends

ILs: $[N_{111}(2OH)] [C_1CO_2]$ and $[C_2C_1im] [C_1CO_2]$

Biopolymer concentration: 15 wt %

Glycerol: 10 wt % (in relation to the biopolymer)

pH: without modification



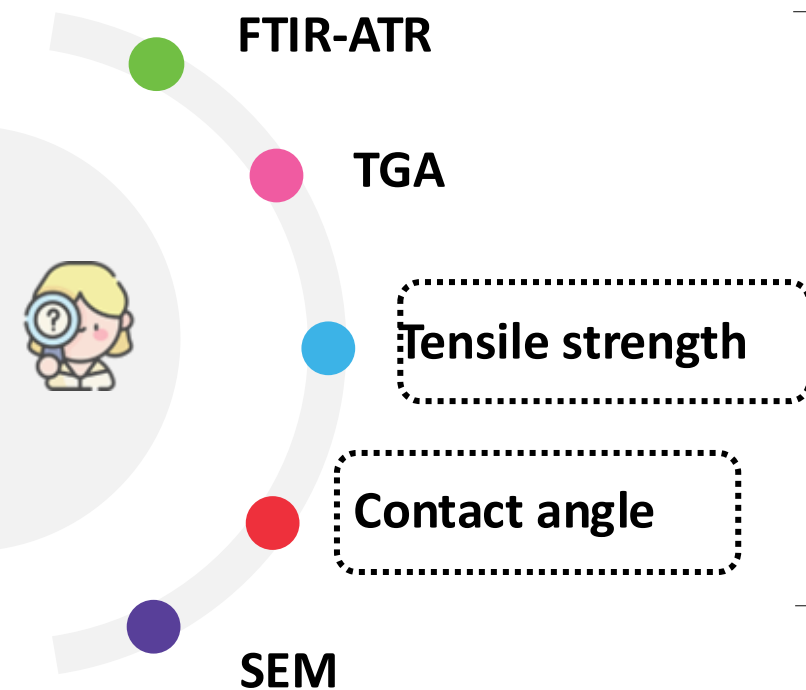
Keratin-chitin 75:25

Keratin-chitin 50:50

Keratin-cellulose 75:25

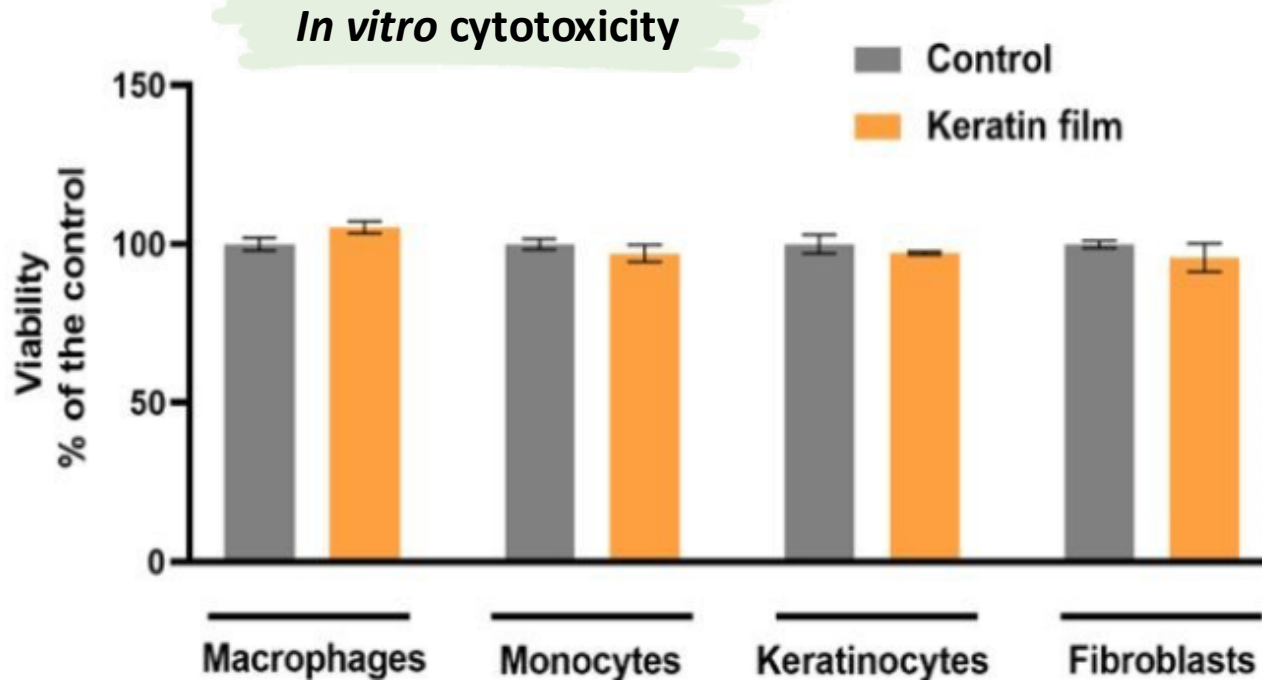
Keratin-cellulose 50:50

Keratin blends characterisation



Samples	Contact angle (°)	Tensile strength (MPa)
KER-C2	58 ± 5	12 ± 1
KER-N111	60 ± 5	11 ± 1
KER-CELL-C2 75:25	54 ± 2	21 ± 1
KER-CELL-C2 50:50	63 ± 3	30 ± 3
KER-CHI-C2 75:25	63 ± 1	53 ± 2
KER-CHI-C2 50:50	47 ± 2	66 ± 1
KER-CELL-N111 75:25	54 ± 3	23 ± 1
KER-CELL-N111 50:50	62 ± 2	26 ± 3
KER-CHI-N111 75:25	59 ± 2	34 ± 2
KER-CHI-N111 50:50	48 ± 2	41 ± 5

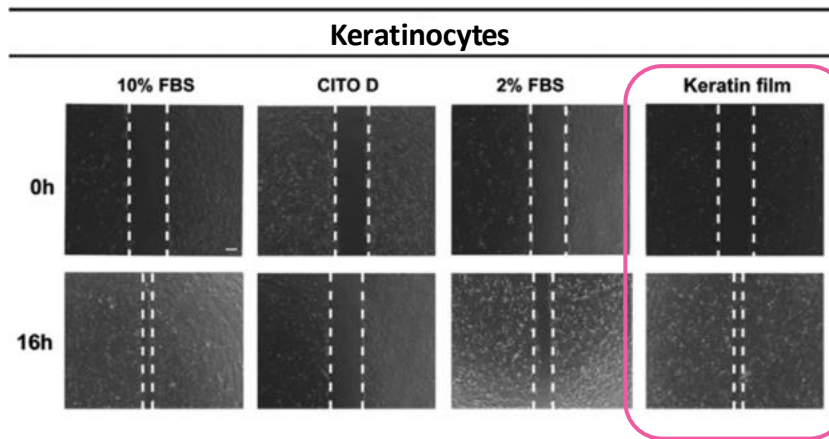
Keratin film applications



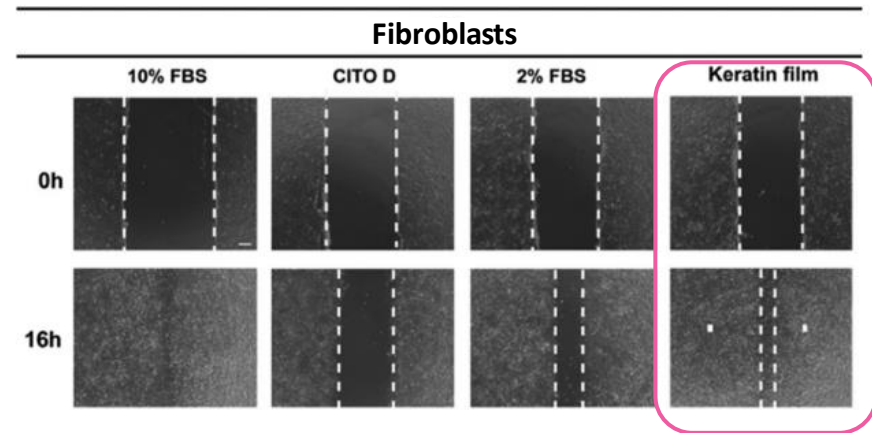
The keratin film presents no cytotoxicity towards the investigated cell lines

Keratin film applications

In vitro wound healing



principal cells of the epidermis



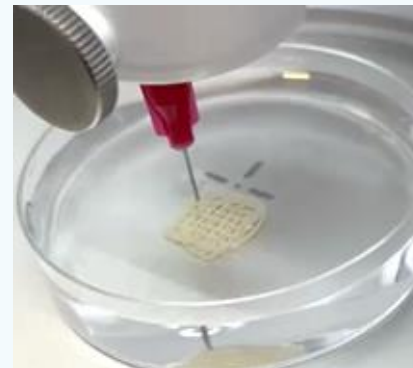
responsible cells for making the extracellular matrix

The keratin films improve the proliferation of the cells, **accelerating wound healing at 16 h.**

This mechanism is associated with some amino acids in keratin (e.g., arginine, glycine and leucine), which promote cellular attachment, migration and proliferation during the healing process.

Keratin film applications

3D printing

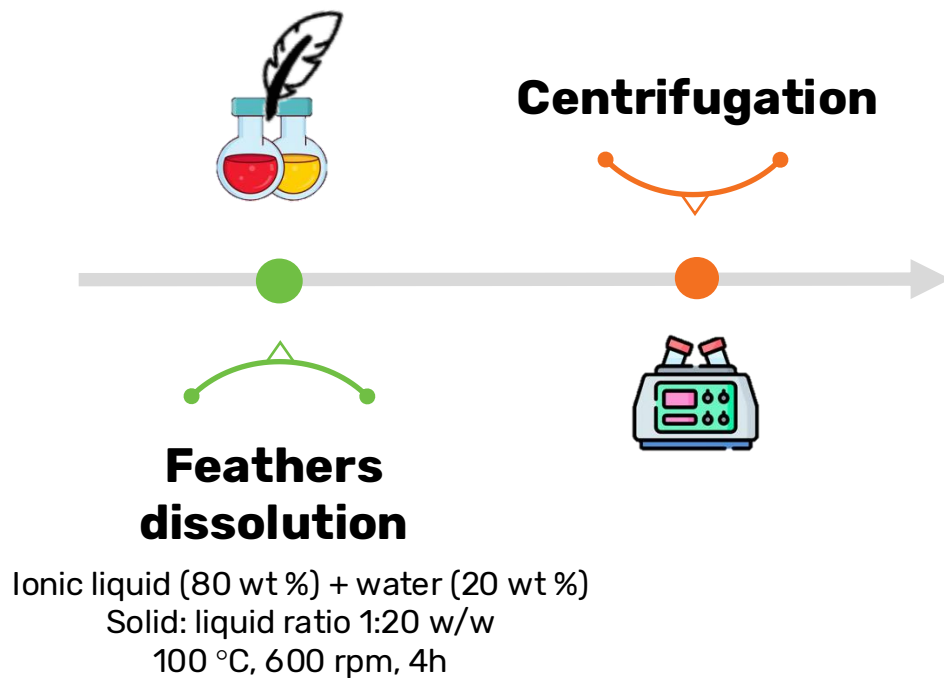


Prof. João
Mano

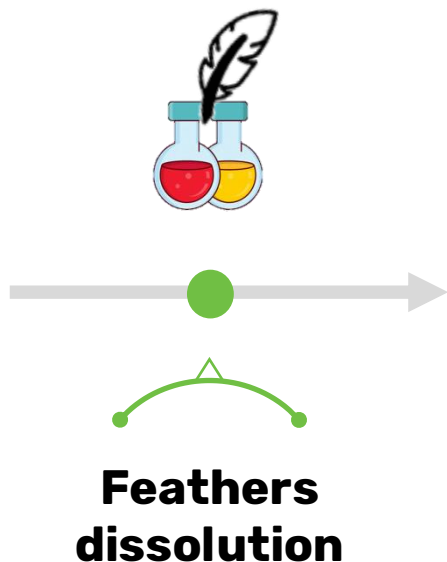


Dr. Rita Almeida

Chicken feathers dissolution



Chicken feathers dissolution



⇒ Solutions:

- (1) White feathers dissolved in $[N_{111}(2OH)][C_1CO_2]$
- (2) Brown feathers dissolved in $[N_{111}(2OH)][C_1CO_2]$
- (3) White feathers dissolved in $[C_2C_1im][C_1CO_2]$
- (4) Brown feathers dissolved in $[C_2C_1im][C_1CO_2]$



Keratin



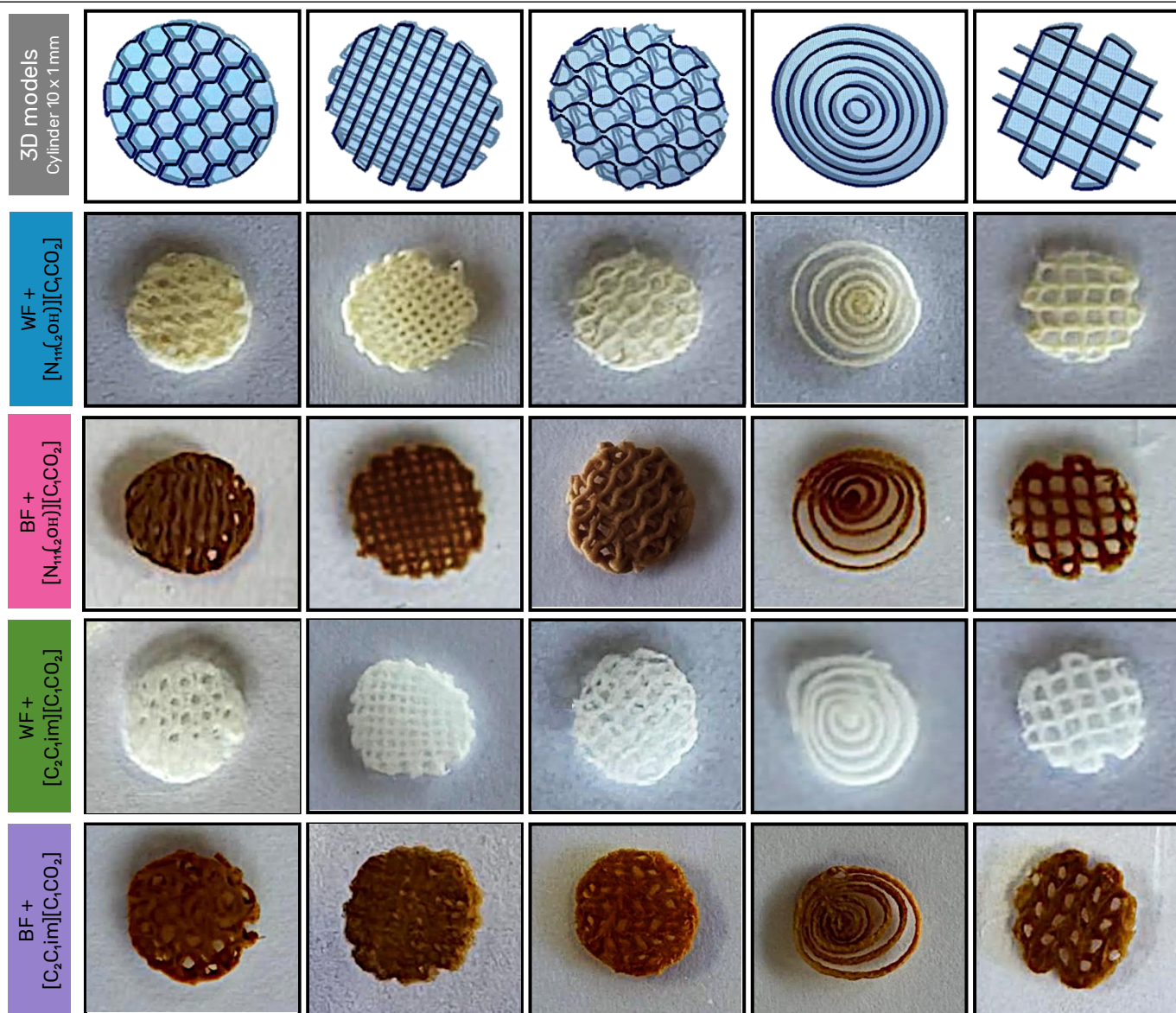
**Keratin +
melanin**

- Reticulation bath: agarose + calcium chloride + carbonate-bicarbonate buffer
- The samples were kept in the reticulation bath for 2 days, and then kept in PBS.



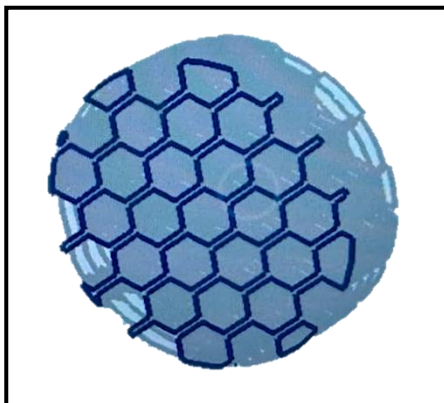
Printing conditions: $P = 35 \text{ kPa}$, Infill density = 25 %, Speed = 8 mm/s

Printability evaluation

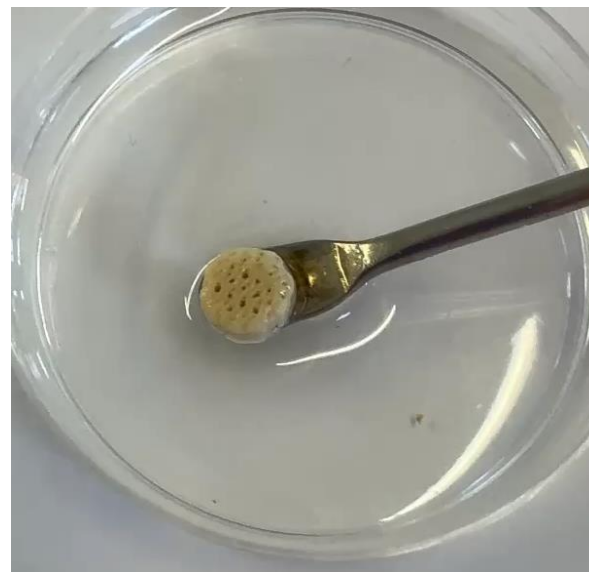
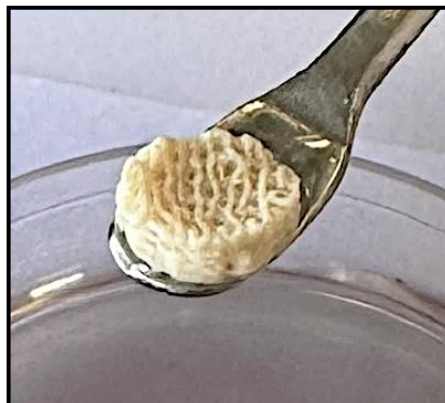


Printability evaluation

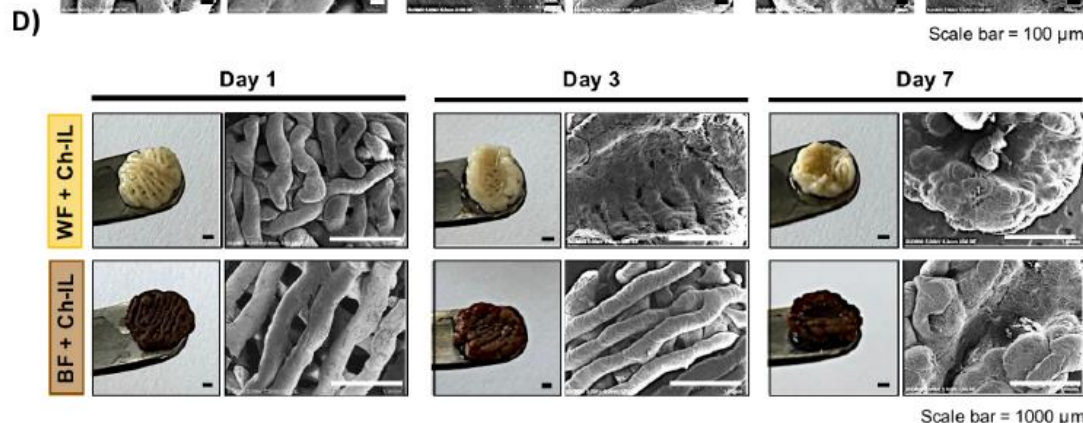
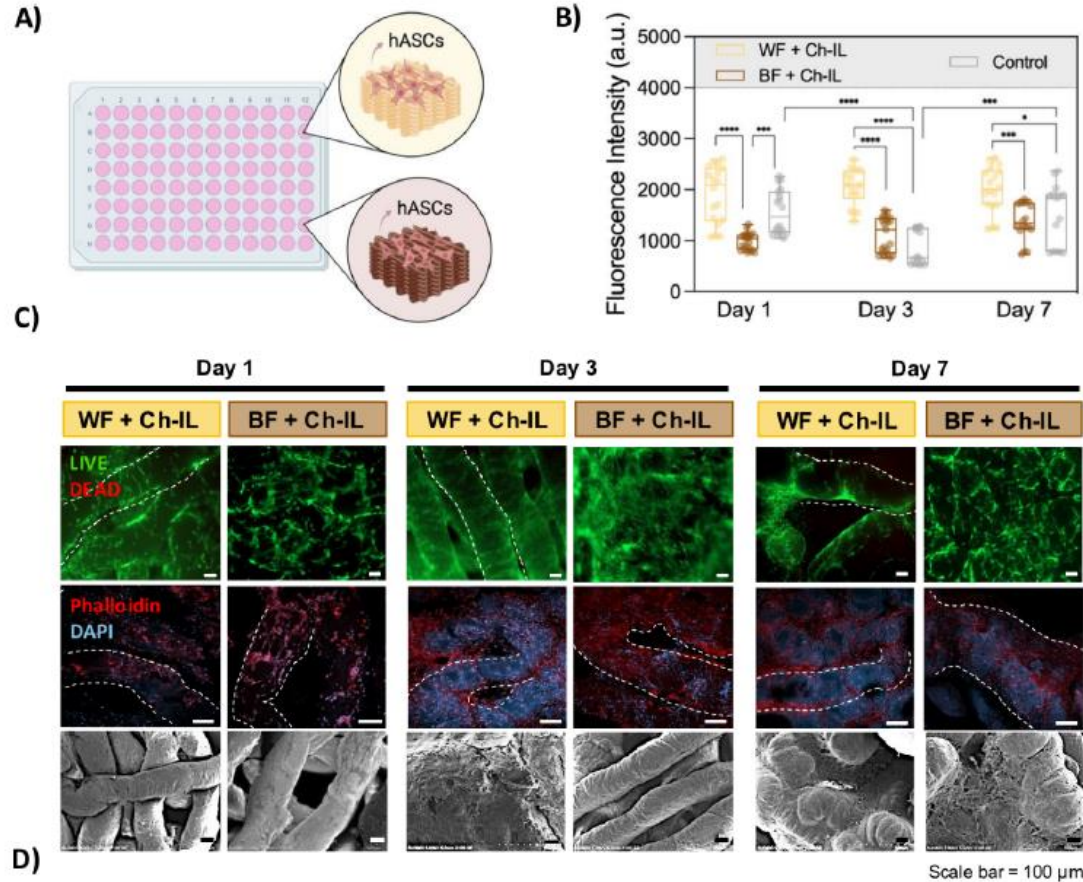
3D model
Cylinder 10 x 3 mm



WF +
 $[N_{111}(2OH)][C_1CO_2]$



Cell growth on keratinaceous scaffold



Engineered from Waste: Ionic Liquid Processing of Keratin for 3D Printing Biomedical Scaffolds

Cariny Polesca,[†] Rita Sobreiro-Almeida,^{*,†} Helena Passos, João A. P. Coutinho, Jason P. Hallett, João F. Mano,^{*} and Mara G. Freire^{*}



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Metrics & More

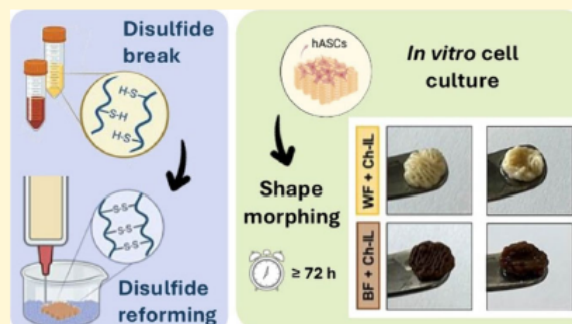


Article Recommendations



Supporting Information

ABSTRACT: Keratin holds significant potential for biomedical applications due to its superior cytocompatibility and ability to promote cellular migration and differentiation. However, despite these advantages, keratin processing is difficult due to its limited solubility in water and most common organic solvents. Herein, a sustainable and cost-effective approach was used for chicken feather dissolution (a keratin-rich waste) using acetate-based ionic liquids. This method simplifies the development of three-dimensional (3D) structures via directly embedded 3D printing keratin dissolved in ionic liquids (ILs). Using a carbonate-bicarbonate agarose microparticle support bath, we successfully promoted disulfide exchange and direct cross-linking of printed structures with diverse patterns and geometries, exhibiting excellent structural integrity. A comprehensive analysis of the rheological, mechanical, and biological properties was conducted, highlighting their potential biomedical applications. Interestingly, the scaffolds exhibited a dynamic shape-change over time, mediated by cellular traction forces, demonstrating their potential for 4D printing toward innovative bioapplications.



Sustainable Recovery of Keratin from Chicken Feather Waste and Its Processing for Biomedical Applications

Cariny Polesca, Helena Passos, Jason P. Hallett, João A. P. Coutinho,* and Mara G. Freire*



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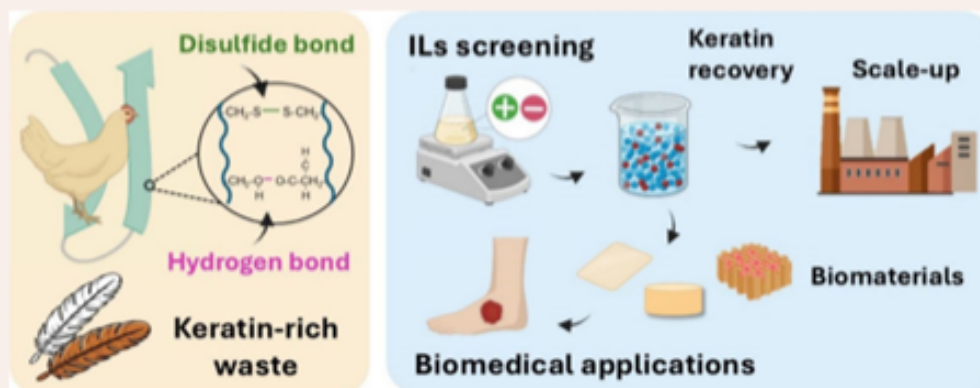
Metrics & More



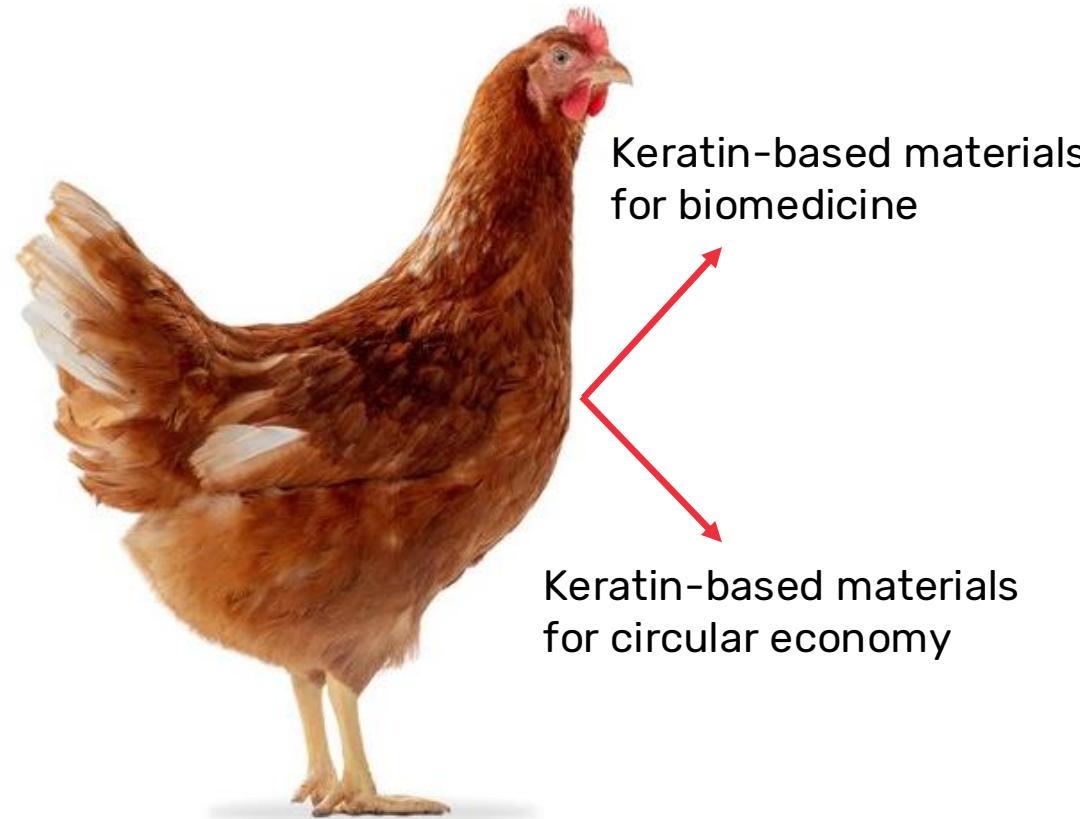
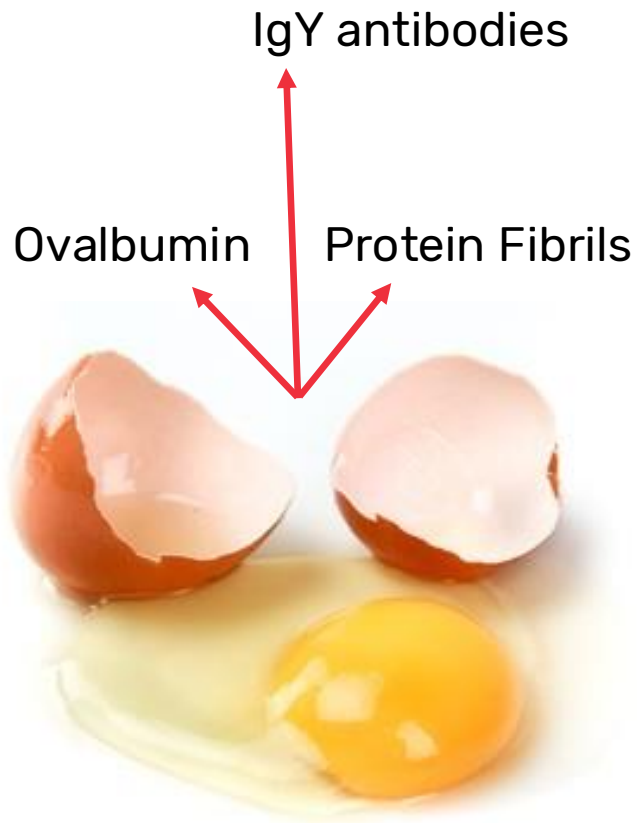
Article Recommendations



Supporting Information



Graphical summary



Acknowledgments



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