ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2022009

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Co-investigator	
Experiment title	SAXS characterisation of artificial skin samples for application in skincare products
MRF Instrument	**SAXS Xenocs Xeuss**
Access Route	Rapid Access
Science Areas	Biology and Bio-materials
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	Arterra Bioscience S.p.A.

Days requested: 3
Previous GP Number: -
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract

In recent years, cosmetic delivery systems have played an important role for improving the penetration of active molecules and for their release in a controlled way. We propose a series of studies to investigate the permeation of dermal/transdermal delivery systems on synthetic and human skin samples - the penetration of drug molecules within skin, the intermolecular interactions amongst active ingredients, carriers and skin components - using CRS&SEM-EDX (at Unit-Univ Tor Vergata), SAXS (at Unit-CSGI&Univ Florence), and INS measurements on TOSCA. The comparative analyses with the bulk spectra of the isolated components will provide new insight on the diffusion and penetration within skin, hydrogen vibrational dynamics and highlight the effects of intermolecular interactions. The results will be used to optimise the preparation strategies of healthcare products.

Publications

R. Senesi et al., Antioxidants (2021), 10, 643
L. Verdolotti et al., Scientific Reports (2021), 11, 1

ISIS neutron and muon source

IM@IT E-platform: Yes - ISIS Facility

Instruments	**TOSCA**
Access Route	ICRD Access
Science Areas	
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	
Industrial Links	Arterra Bioscience S.p.A.

Days Requested: 2
Previous RB Number: RB2255009
DOI:
Sponsor:
Grant Number:
Finish Date:

Sample record sheet

Principal contact Professor Carla Andreani, University of Rome Tor Vergata, ITALY
MRF Instrument **SAXS Xenocs Xeuss** **Days Requested:** 3
Special requirements:

SAMPLE

Material	artificial Skin	-	-
Formula	collagen, proteins, lipids	-	-
Forms	Solid		
Volume	0.5 cc		
Weight	0.5 g		
Container or substrate	-	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	300 - 300 K	-	-
Pressure Range	0.1 - 1000 mbar	-	-
Magnetic field range	0 - 0 T	-	-
Standard equipment	None	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	Yes	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	-	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed By User	-	-

SCIENTIFIC BACKGROUND

In recent years, cosmetic delivery systems have played an important role for improving the penetration of active molecules and for their release in a controlled way within human skin. The latter has two main layers, the dermis and the epidermis. The dermis is the deeper layer and contains blood vessels, nerves, hair follicles, small muscles, sweat glands. The epidermis is the outside layer and is made up of the skin cells, mainly keratinocytes. Therefore, considering the variety of organic substances (carbohydrates, lipids, proteins and nucleic acids), hydrogen bonding is expected to have a significant effect on the diffusion of active ingredients mediated by organic delivery molecules. In this context, tracking the presence and the interaction of active principles and molecular carriers within synthetic and human skin samples requires a precise characterization of such large systems and complex materials, which is not achievable by, or solely based on, *ab initio* computer modelling.

In recent studies, inelastic neutron spectroscopy (INS) was successfully employed to tackle complex materials such as concrete [1] and extra-virgin olive oils (EVOOs) samples [2]. In the former investigation [1], the degree of interactions between a rubber addition to concrete and the hydrogen-calcium-silicates within the same sample were investigated by monitoring the shifts in characteristic vibrational peaks within polyisoprene. In the second investigation [2], a detailed analysis of the minor polar and most-abundant components within the EVOOs complex materials settled the grounds for future analyses of similar real-life materials. In an INS experiment on cosmetic delivery systems, we aim at quantifying the degree of interaction between series of active ingredients and the gel used as a carrier in the skincare product. However, the atomic-scale information from neutron experiments on such complex systems should be complemented by a characterization at the nano-to-micro-scale. For this reason, this proposal is part of a series of experiments using both ISIS (UK) and ISIS@MACH ITALIA instrumentation.

SAMPLE PREPARATION AND ADDITIONAL CHARACTERIZATIONS

Synthetic skin will be used for this experiment, in the form of human reconstructed epidermis, keratinocytes cultured on a commercially available inert polycarbonate filter (e.g., SkinEthic RHE, Episkin [3]).The experimental procedure consisted of applying the formulation containing the active ingredients, for different exposure times (0-48 hours) on reconstructed skin epidermis. Samples will have a flat geometry, with a thickness of 0.3 mm and a radius of 2.5 cm. Each such disc will be subject to the application of different doses of the active ingredients, and 2-3 discs will be overlapped to maximise the signal from the neutron experiment. We note that, following this pilot investigation on synthetic skin, and provided that the necessary approvals by Ethics committees are obtained, continuation experiments could be run on natural skin as well.

The proponents aim to characterise the samples with preliminary and complementary optical techniques. We have submitted three distinct proposals for open access to the Confocal Raman Microscopy (CRM), Scanning Electron Microscopy (SEM) in combination with Energy Dispersive X-ray Spectroscopy (EDS) - Small Research Facilities (SRFs) – and to the TOSCA neutron spectrometer at the ISIS Neutron and Muon Source. Through the CRM mapping along the section of ex-vivo skin slices we aim to provide a valuable, semi-quantitative method to assess the diffusion of such formulations along the different layers composing the skin. A reconstruction, through several skin layers of the concentration of the active ingredients, will be based on the characteristic vibrational fingerprints (see, *e.g.*, Ref [5,6]) of active ingredients and carriers. Additionally, SEM in combination with EDS will provide complementary information about the morphology of the treated and untreated skin samples and a further way to evaluate the permeation of the formulations through elemental analysis. Finally, INS experiment will have a twofold goal: on one hand to measure and quantify the degree of interaction between series of active ingredients and the gel used as a carrier in the skincare product; on the other hand to record the fingerprint of such active ingredients within skin layers exposed to the abovementioned skincare products.

PROPOSED EXPERIMENT

The aim of this proposal is to use Small Angle X-Ray Scattering (SAXS) to provide insights about the interaction of both active ingredients and carriers with the *stratum corneum*, i.e. the outer layer of epidermis. More specifically, SAXS profiles can provide unique information about the structure of lipids lamellar phases, their packing and the possible inclusion of molecules between them [7].

Therefore, we request access to the SAXS Xenocs Xeuss instrument at the Medium-Range Facility 1 (MRF1) FOURDIM - 4D Imaging–of Complex Systems and Interfaces Facility. The experiment will focus on the permeation of different dermal/transdermal delivery systems on synthetic skin explants treated using a Franz diffusion cell. The latter consists of a cell that holds a chamber for cosmetic application, a membrane or a skin explant (in our case) within which the active ingredients may diffuse, and an acceptor media chamber from which samples may be examined.

Experiments will be performed on a series of skin samples exposed to the skincare product at different conditions and for different times. The latter samples will be composed of a stack of 60-micrometer-thick synthetic and natural skin layers, obtained following the preparation procedures discussed above. The active compounds that will be analysed are antioxidants (*e.g.*, caffeic acid), whitening (*e.g.*, kojic acid) or antiaging agents (*e.g.*, retinoic acid), to be mixed with commercially-available creams.

JUSTIFICATION OF BEAMTIME REQUEST

Considering the number of samples, including the different combinations of active compounds and the commercially available creams, we request 3 days of the SAXS Xenocs Xeuss instrument at the MRF1.

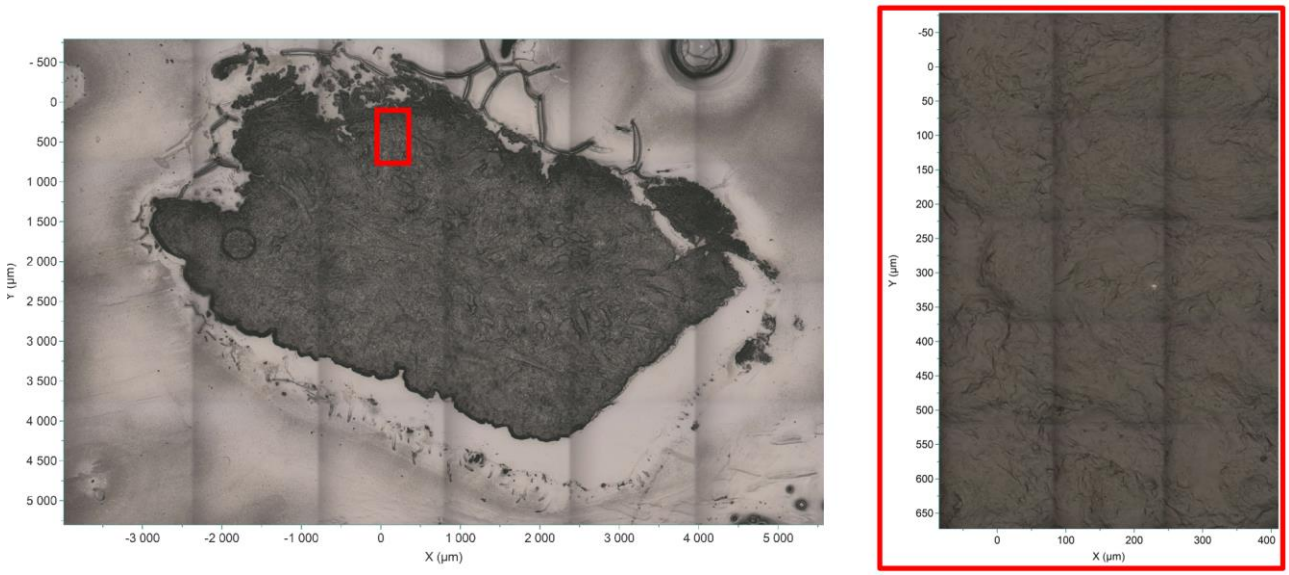

Figure 1 Preliminary images of one of the samples to be investigated, at 5x (left), ranging over 8 mm horizontally and 5 mm vertically, and 50x (right). The image on the right corresponds to the red-rectangle region highlighted on the left image.

References

[1] L. Verdolotti et al., “MWCNT/rGO/natural rubber latex dispersions for innovative, piezo-resistive and cement-based composite sensors”, Scientific Reports (2021), 11, 18975
[2] R. Senesi et al., “Looking for Minor Phenolic Compounds in Extra Virgin Olive Oils Using Neutron and Raman Spectroscopies”, Antioxidants (2021), 10, 643
[3] https://www.episkin.com/SkinEthic-RHE
[4] https://www.isis.stfc.ac.uk/Pages/A-new-partnership-between-ISIS-and-f.aspx
[5] S. Zsikó et al., “Novel In Vitro Investigational Methods for Modeling Skin Permeation: Skin PAMPA, Raman Mapping”, Pharmaceutics (2020), 12, 803
[6] F. Lutz and M. Windbergs, "Applications of Raman spectroscopy in skin research—from skin physiology and diagnosis up to risk assessment and dermal drug delivery", Advanced drug delivery reviews 89 (2015): 91-104
[7] I. Brinkmann et al., “An attempt to clarify the influence of glycerol, propylene glycol, isopropyl myristate and a combination of propylene glycol and isopropyl myristate on human stratum corneum”, Die Pharmazie-An International Journal of Pharmaceutical Sciences (2005), 60(3), 215-220