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INTRODUCTION

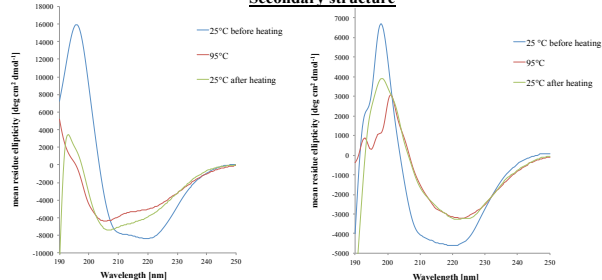
The use of conventional aqueous electrolyte on the realisation of biosensor can give some life-time issues related to the volatility of water, limited temperature range and the very narrow pH range for protein stability.[1] Ionic Liquids (ILs) are low melting point salts (<100°C) that represent a new class of non-aqueous solvents composed entirely of ions, which have been extensively used in biocatalysis. [2]

It was shown that certain proteins are soluble, stable and remain active for up to 18 months when dissolved in hydrated ILs. [3] In fact, the addition of small amounts of water to ILs strongly influences the protein solubility while the main properties of the selected Ionic liquid are retained. In the present study, the structural and the catalytic properties of LOx were investigated using Circular Dichroism and colorimetric assay, respectively.

The final aim of this study is the realisation an electrochemical biosensor for the sensing of lactic acid in sweat. [4]

RESULTS – Circular Dichroism

Temperature Scan of LOx in PBS and Choline dhp
Secondary structure



Choline dhp

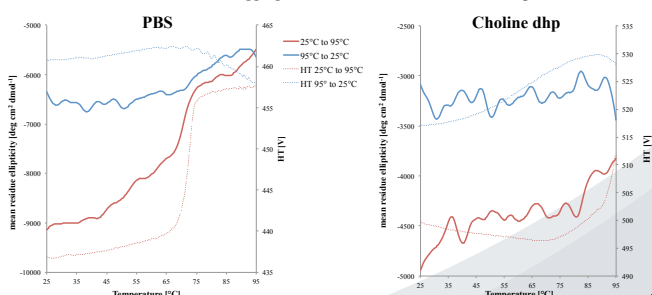
	(1) 25 °C	(2) 95 °C	(3) 25 °C
α -helix	11	4	8
β -sheet	34	28	37
Turns	24	24	25
Unordered	30	32	30
NRMDS	0.087	0.067	0.116

PBS

	(1) 25 °C	(2) 95 °C	(3) 25 °C
α -helix	28	12	18
β -sheet	23	30	30
Turns	20	24	28
Unordered	30	32	23
NRMDS	0.011	0.042	0.039

DichroWeb

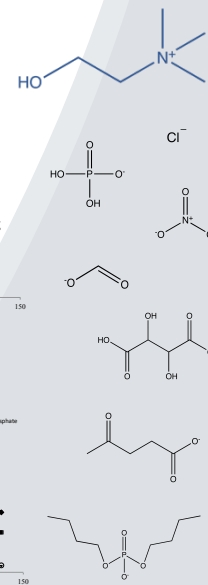
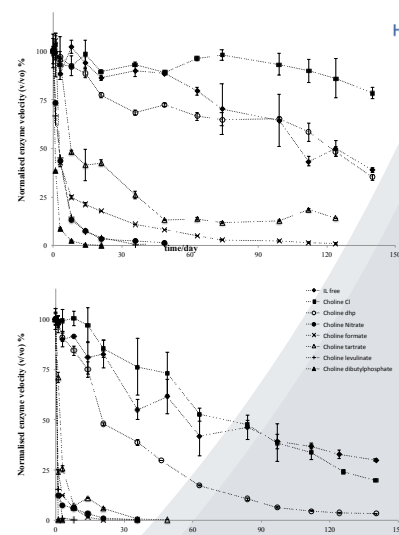
Thermal denaturation and aggregation of LOx in PBS and Choline dhp at 222 nm



RESULTS – Residual Activity

Colorimetric enzyme activity assay

ILs/LOx stock solutions were stored at 5 °C and 37 °C



CONCLUSION and FUTURE WORK

In this work we studied the structural conformation and residual activity of LOx enzyme when dissolved in conventional PBS and hydrated choline ionic liquids. Circular Dichroism showed that hydrated choline dhp is capable to retain the secondary structure and enhance the heat induced denaturation of the enzyme. Moreover, residual activity experiments revealed that the bio-functionality of the enzyme is strongly influenced by the nature of the anion of ILs. Future work will focus on the study of the kinetic constants and the integration of choline dhp in to a biocompatible polymeric matrix, such as chitosan and Gelatin A.

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