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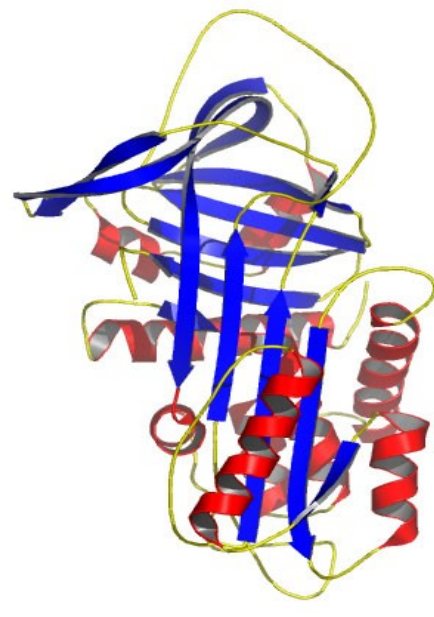
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Rheological properties of cysteine-modified ovalbumin at the air-water interface



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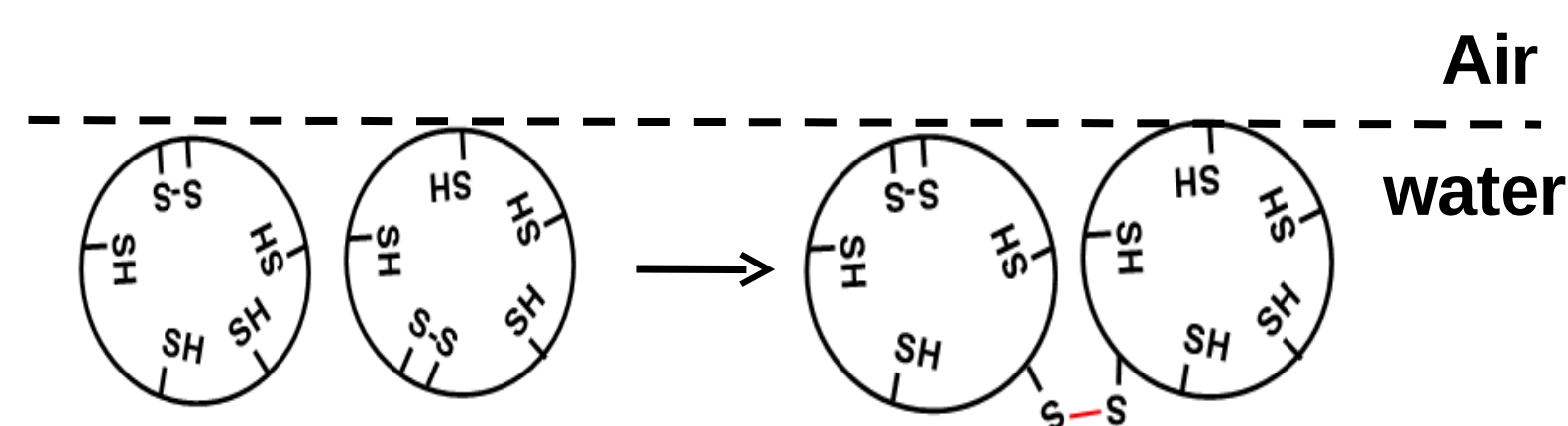
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Introduction

Ovalbumin is a phosphorylated and glycosylated globular protein which contributes to a large part of the functionality of egg-white which it is widely used in the food industry as a foaming agent. However, the molecular bases of its interfacial behaviour are not fully understood.

Objective

To investigate whether the sulfhydryl-disulfide exchange influences the interfacial film rheology, we studied structural and interfacial properties of cysteine-modified ovalbumin.



Egg white
Amino Acids 385
Molecular weight 45 KDa
pHi ≈ 30%
α-helix ≈ 32%
β-sheets 1
-S-S- 4
-SH

Three forms : A3: A2: A1 (Ser⁶⁸ et Ser³⁴⁴)
2: 13 : 85 (%)

0P: 1P : 2P (phosphate groups)

++ glyco forms (Asn²⁹²)
2 N-acétylglucosamines et 4 mannoses

Experimental procedure

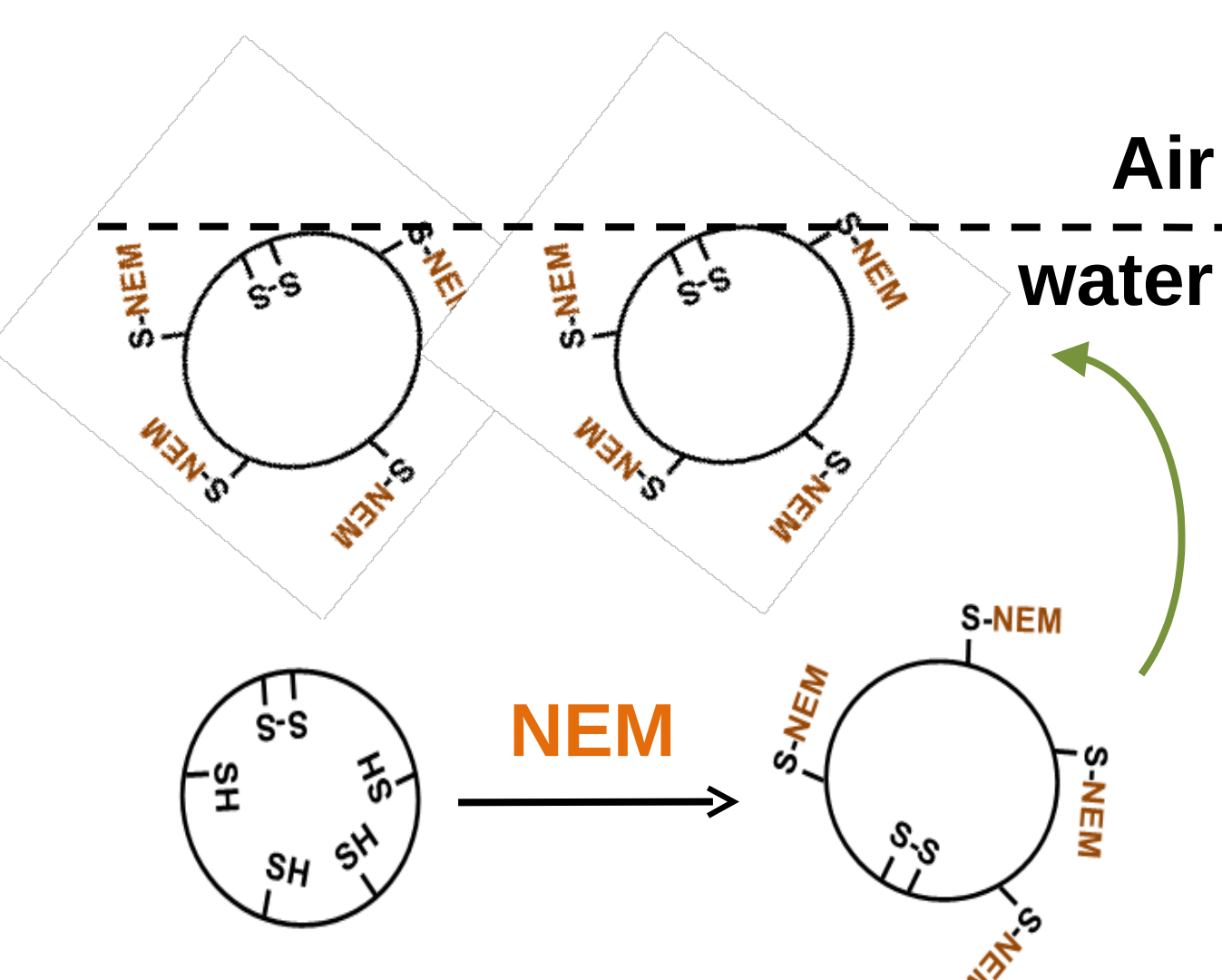
Native Ovalbumin (Native-OVA) was modified by the thiols blocking agent N-ethylmaleimide (NEM).

Native-OVA (0.225 mM) was incubated with a 40-fold molar excess of NEM in 100 mM sodium phosphate buffer (pH 7.2) at 20-21°C for 4 and 24 hours in the dark under stirring.

NEM in excess was removed by dialtrafiltration.

NEM/OVA ratio was estimated and the effect of the binding on the net charge and structure stability of the modified OVA forms was studied.

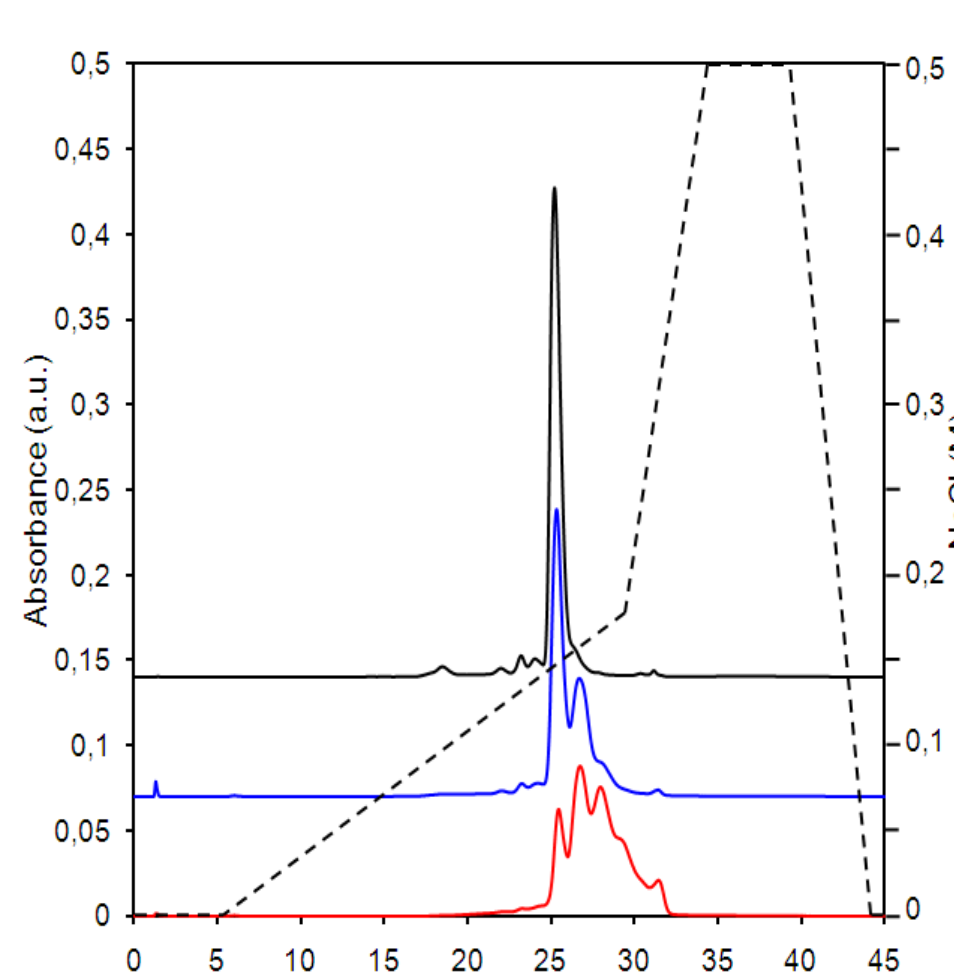
interfacial behavior of different OVA forms was studied at the air-water interface.



Results

Color lines, native-OVA (black), OVA-NEM 4h (Blue), OVA-NEM 24h (Red)

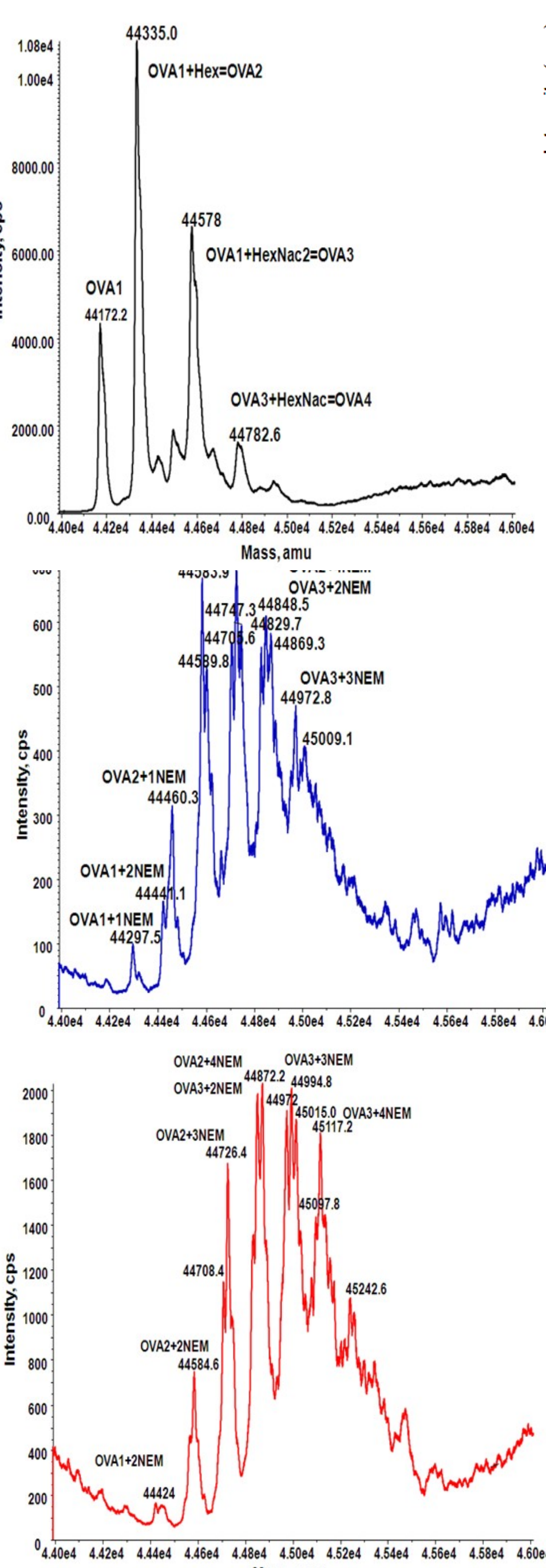
Anion exchange HPLC



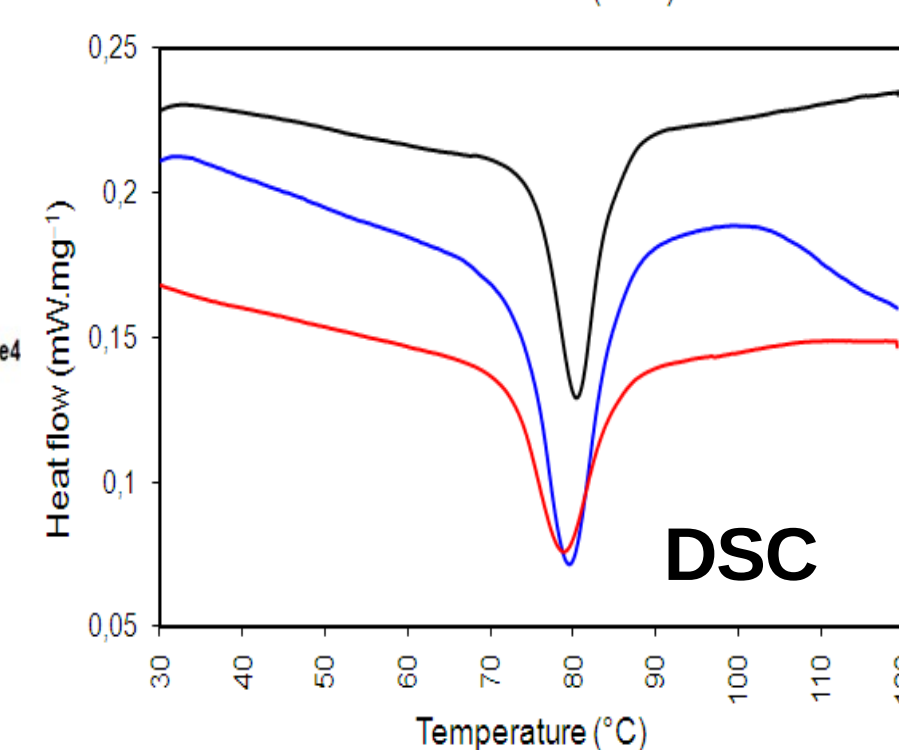
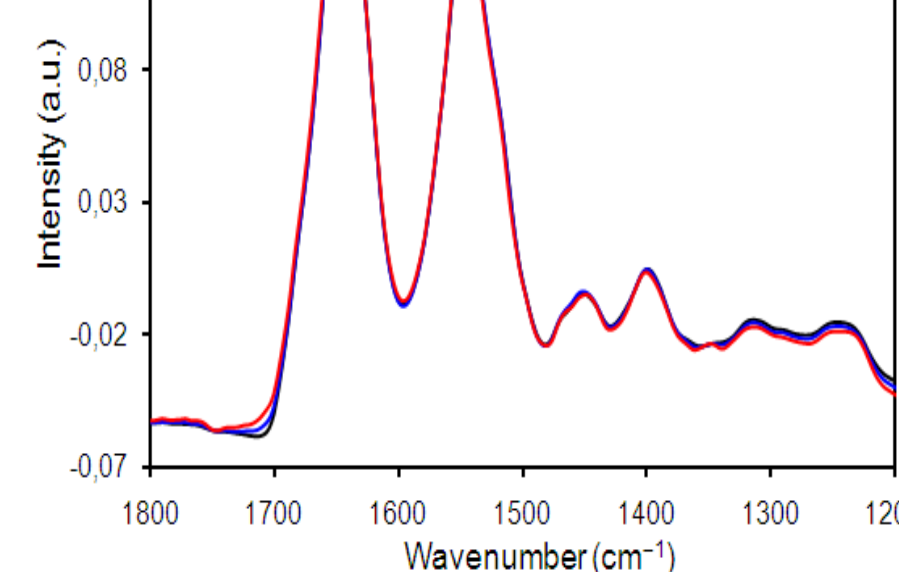
Anion exchange HPLC shows the formation of acidic OVA forms, as probable consequence of NEM hydrolysis after its fixation on thiols OVA groups.

Electrospray-ionization mass spectrometry (ESI-MS) shows the binding of up to four NEM molecules per ovalbumin.

Electrospray-ionization mass spectrometry

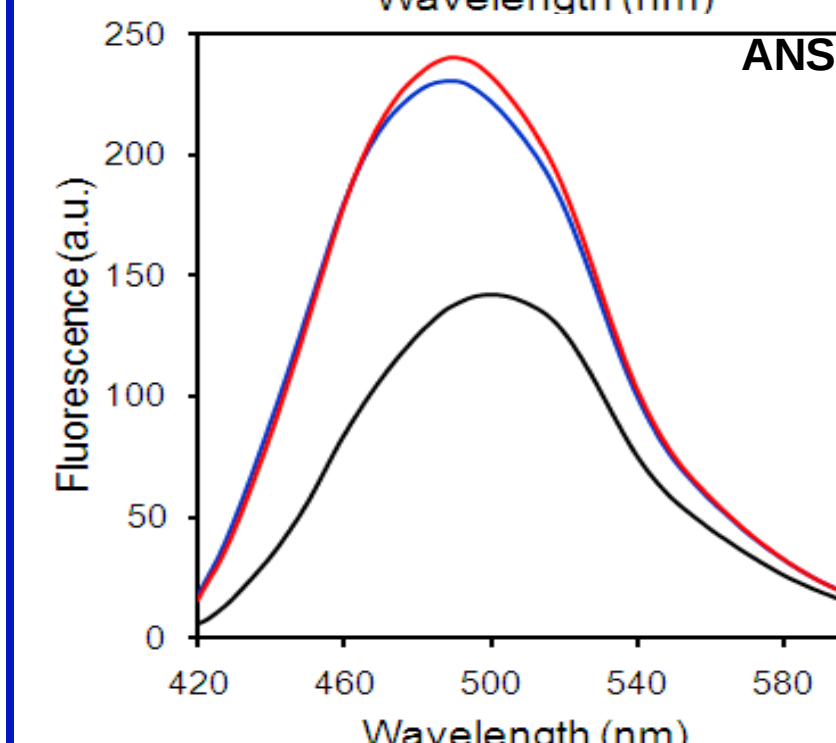
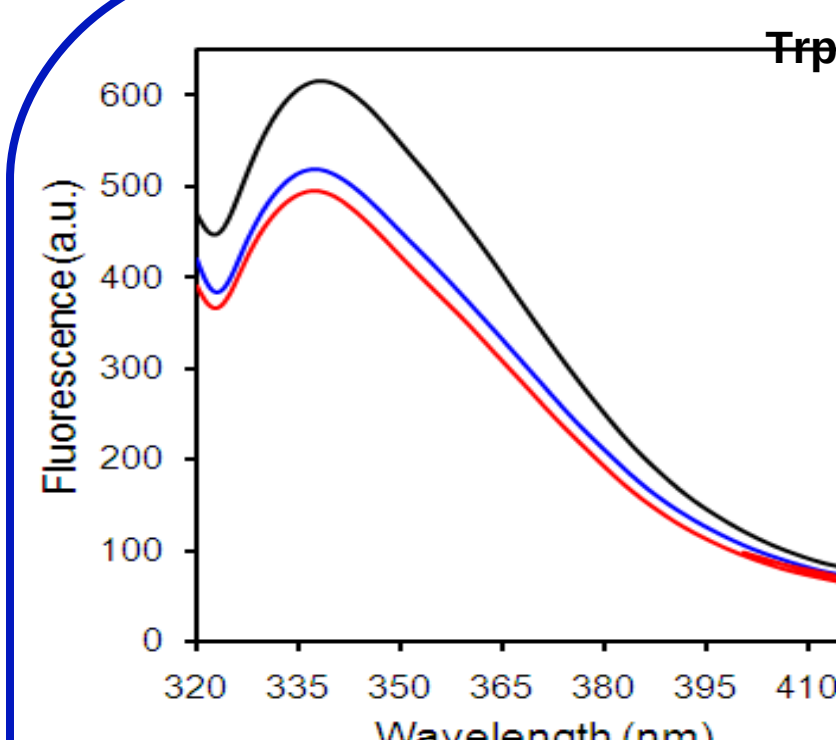


ATR-FTIR



ATR-FTIR does not show any change in secondary structure.

Differential Scanning Calorimetry (DSC) shows a maintained structural stability. The denaturation temperature (T_D) of native-OVA and OVA treated by NEM during 4 and 24 hours slightly decreases (80.3°C, 79.4°C, 78.8°C, respectively).

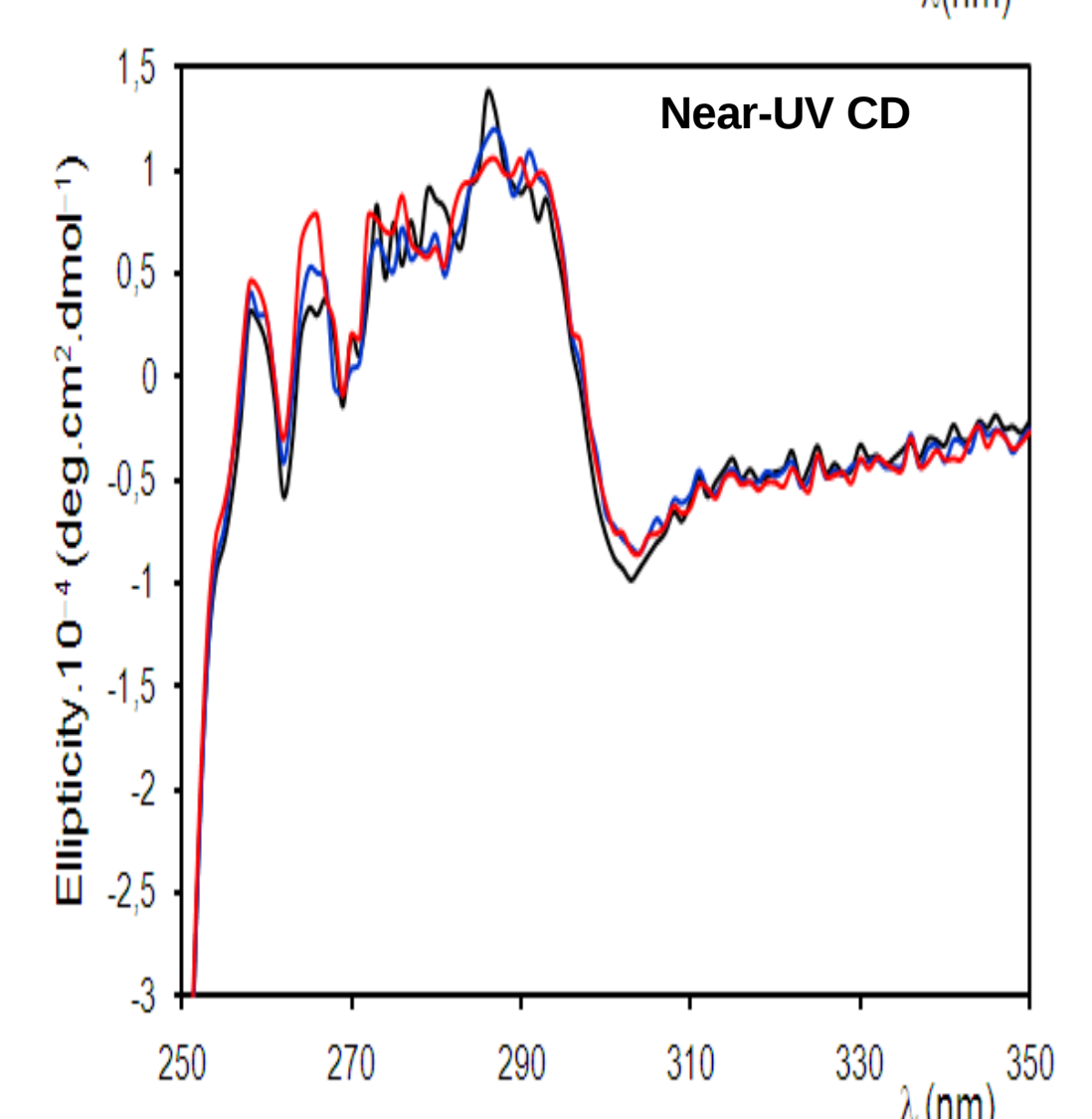
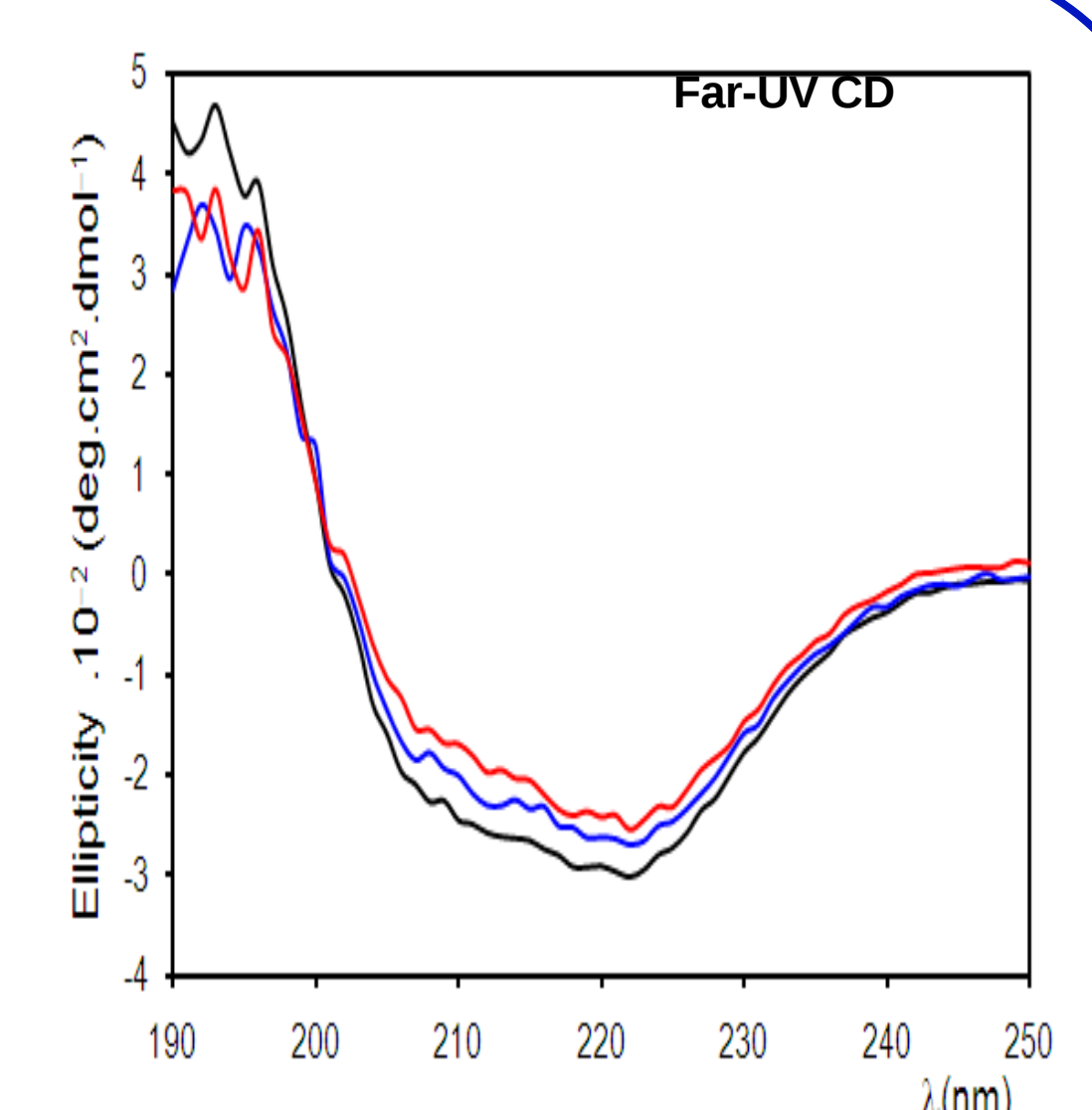


Far-UV CD spectra exhibit negative maxima typical of a protein mainly composed of β-sheets and α-helix with minor modifications at 193, 208 and 222 nm, characteristic of α-helix secondary structure

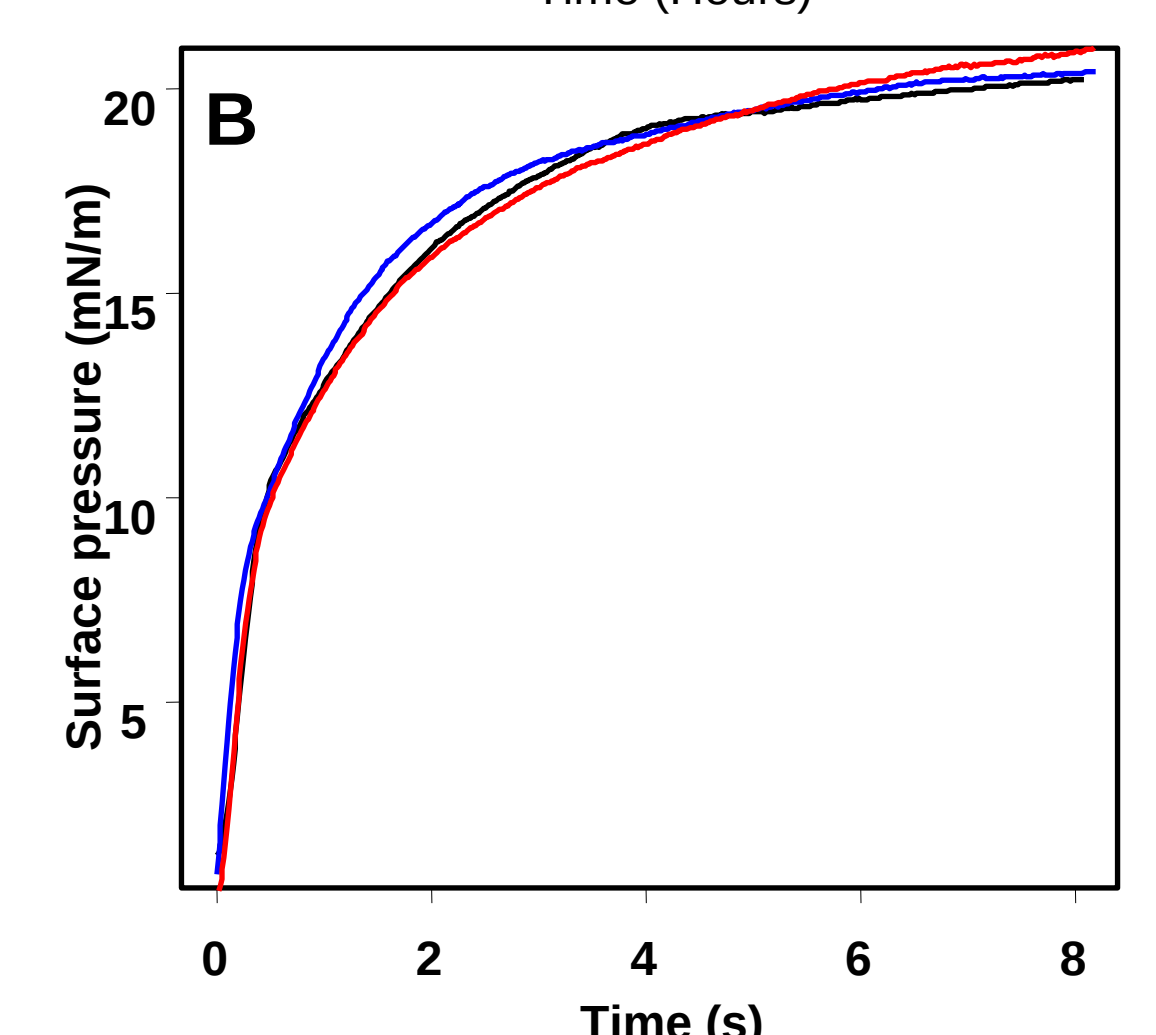
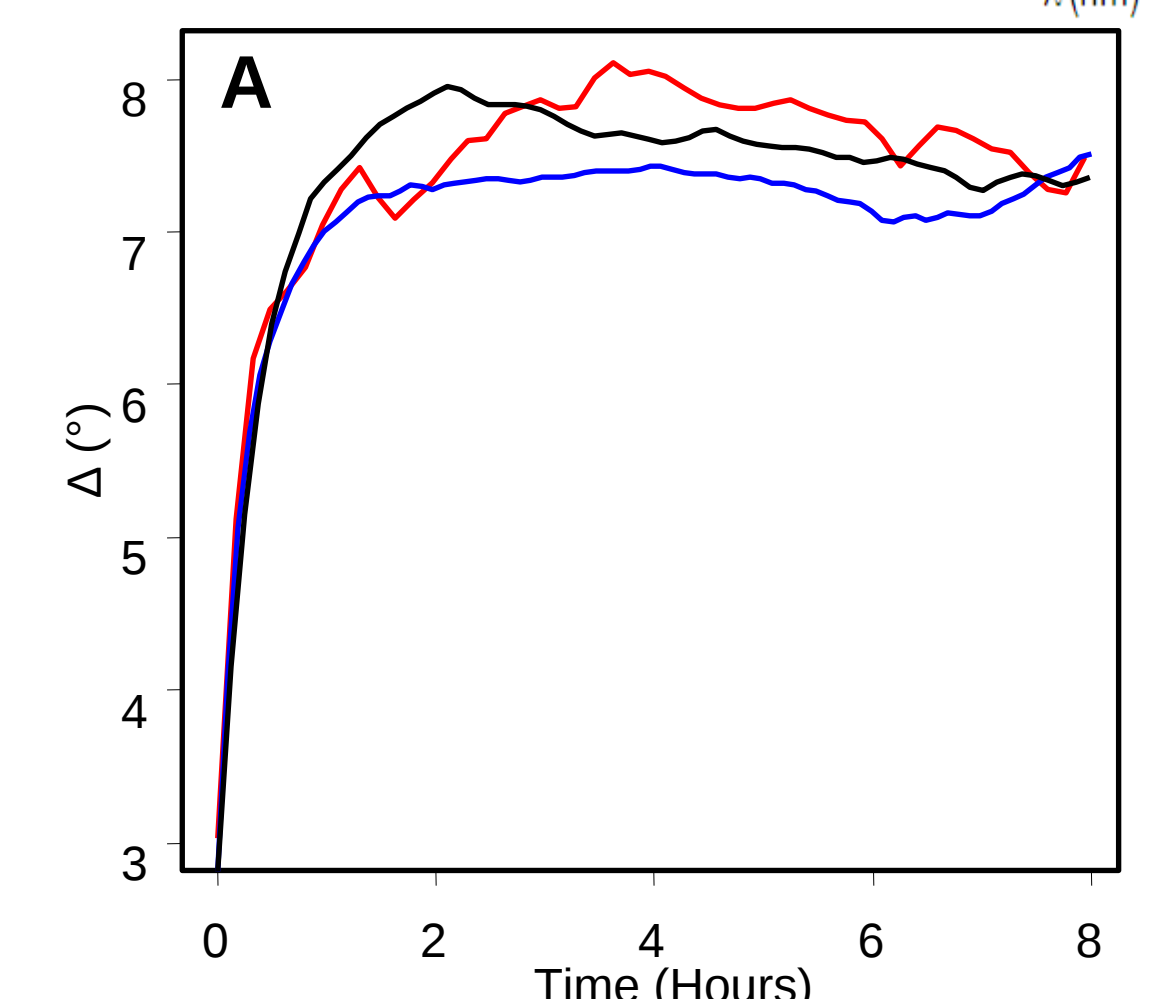
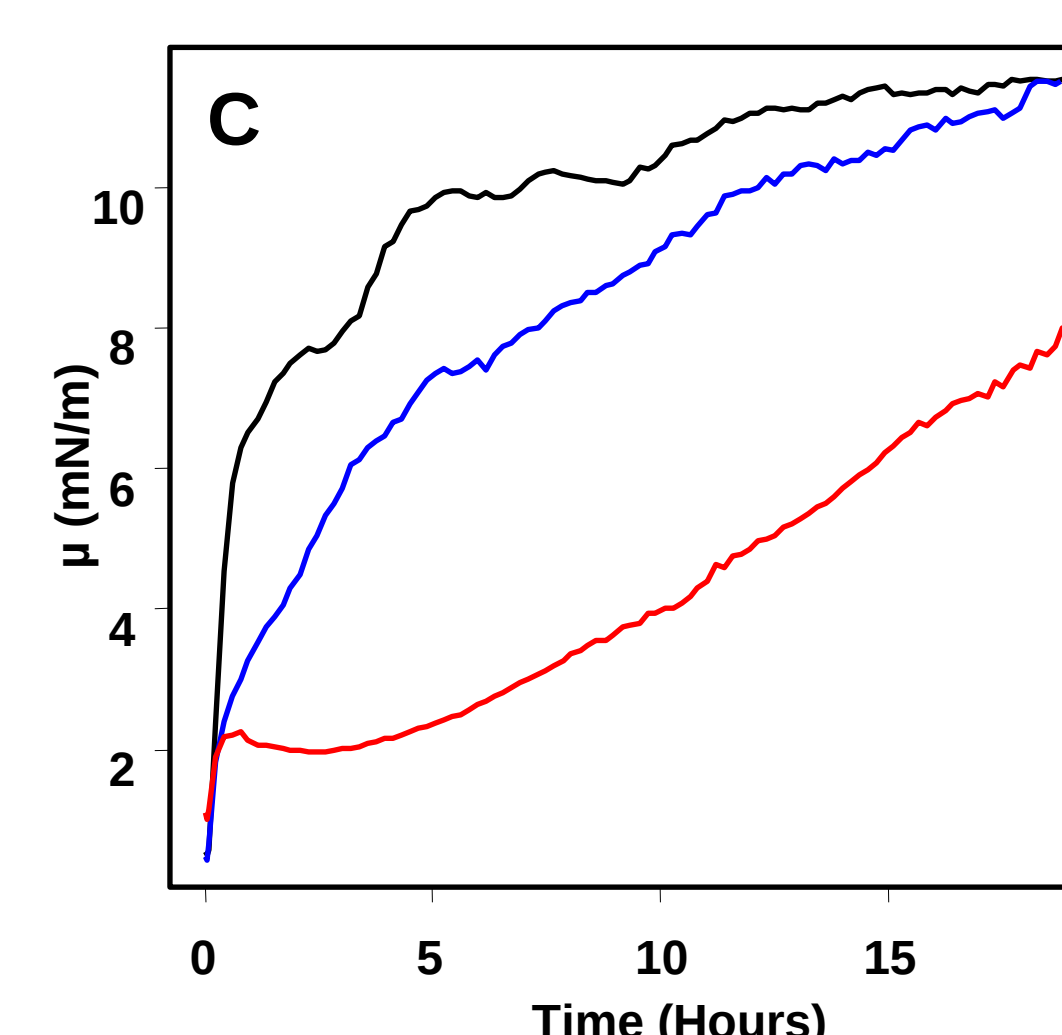
Near-UV CD spectra is characterized by minor modification at 262, 266, 286 and 303 nm attributed to chiral environment of aromatic residues confirming the minor changes in flexibility.

Intrinsic fluorescence (Trp) spectra show a slight blue shift and fluorescence intensity decreases, suggesting that the microenvironment of some tryptophan and/or tyrosine residues changed.

Extrinsic fluorescence (ANS) spectra show a blue shift and fluorescence intensity increases, suggesting the hydrophobicity increases, revealing flexibility differences.



Modification of free thiols does not change adsorption (ellipsometric angle (Δ), A) or surface activity (surface pressure, B) but deeply affect the kinetics of changes in interfacial rheology (Shear elastic constant (μ), C).



Conclusion

Kinetic changes in interfacial rheology can be attributed either to the blocking of thiol groups by the N-ethylmaleimide, or more probably to structural and net charge changes of ovalbumin. Further investigations using other pHs are needed to determine the impact of each factor on the surface rheology of ovalbumin films.