



Helix Aspersa

CHARACTERIZATION OF EXTRACT FROM *HELIX SPP.*

Roma IZSLT 11th April 2019

Prof. Claudio Trapella; Department of Chemical and Pharmaceutical Sciences
University of Ferrara ITALY.

Discovery and history

FERNANDO BASCUNAN:
Chilean snail breeder



20 years ago, he discovers that his employees who collected snails for the French market had very hydrated and soft hands, besides they did not show cuts wounds and the scars were healed more quickly and without getting infected.



Discovery and history



DIPARTIMENTO DI CHIMICA FARMACEUTICA

UNIVERSITA' DEGLI STUDI DI PAVIA

Segreteria amministrativa

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EVALUATION OF THE EFFECTIVENESS OF ANTI-WRINKLE COSMETIC PRODUCTS

Report number: S/10/1850

Test product: **Elicina**

Anti-wrinkle effect study from
University of Pavia, Italy

t zero



t 2 mesi



Applications

Anti-wrinkle, anti-acne, scars, skin pots and wound healing.



Applications

Why the snail mucus presents all this effects?



In *Helix aspersa*, as for other gastropods, locomotion occurs through the "sliding" on rough surfaces, and the richness of the mucus in mucopolysaccharides is justified in the first instance by the mechanical functions necessary for locomotion and defence (ability to remain strongly clinging to vertical surfaces hidden in the shell). But the constant sliding of the snail on hard surfaces justifies the contemporary restructuring function of the slime necessary to repair the tissues of the snail itself, confirmed by analytical studies concerning the composition (allantoin, amino acids, collagen and elastin) and clinical studies concerning the functionality restructuring the skin and antibiotics against the main skin pathogens

Extraction

The mechanical extraction has been achieved using a patented BEATRIX[®] cruelty free extractor machine.



The extraction has been done by chemical and thermal stimulus that allow us to obtain a pure and controlled product with a 98% snail survival.

Characterization

The chemical characterization of the snail mucus will be achieved as follow:

1. Lyophilisation to calculate the per cent of dry substances present, followed by a IR analysis and a Bradford assay to quantify and qualify the protein part.
1. HPLC analysis to quantify the glycolic acid and allantoin, colorimetric assay for the mucopolysaccharides (GAGs) determination.
1. Microbiological analysis for the total bacteria and fungi.

Chemical Characterization

The Chemical Composition of Snail Gelatin

By A. P. WILLIAMS

The British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N. 7

(Received 7 July 1959)

SNAIL GELATIN

Table 1. *Amino acid composition and related analytical data for snail gelatin*

	Amino acid		Amino acid N as % of total N	No. of residues of amino acid per 1000 total residues of amino acids
	(g./100 g. of dry, ash-free sample)	(moles/10 ⁶ g.)		
Alanine	5.69	63.8	5.82	72.3
Glycine	21.24	283.1	24.95	321.0
Valine	2.22	18.97	1.75	21.5
Leucine	3.02	20.74	2.07	23.5
Isoleucine	1.40	10.64	0.97	12.1
Proline	10.57	91.8	8.34	104.1
Phenylalanine	1.45	8.75	0.78	9.9
Tyrosine	1.41	7.78	0.71	8.8
Serine*	5.70	54.2	4.92	61.4
Threonine*	2.90	24.4	2.2	27.7
Cystine	0.00	0.00	0.00	0.00
Methionine†	0.16	1.07	0.13	1.2
Arginine	7.88	45.6	16.43	50.9
Histidine	0.36	2.33	0.65	2.6
Lysine	1.07	7.30	1.33	8.3
Aspartic acid	7.84	58.9	5.37	66.8
Glutamic acid‡	12.85	87.4	7.89	99.1
Hydroxyproline	11.50	87.7	7.95	99.5
Hydroxylysine	1.14	7.2	1.29	8.2
Amide N§	0.69	49.2	4.46	—
Total	99.09	930.9		

Allantoin

Glycolic acid

First evidence in literature for the Infrared characterization of pulmonate mucus

J. Moll. Stud. (2000), 66, 363–371

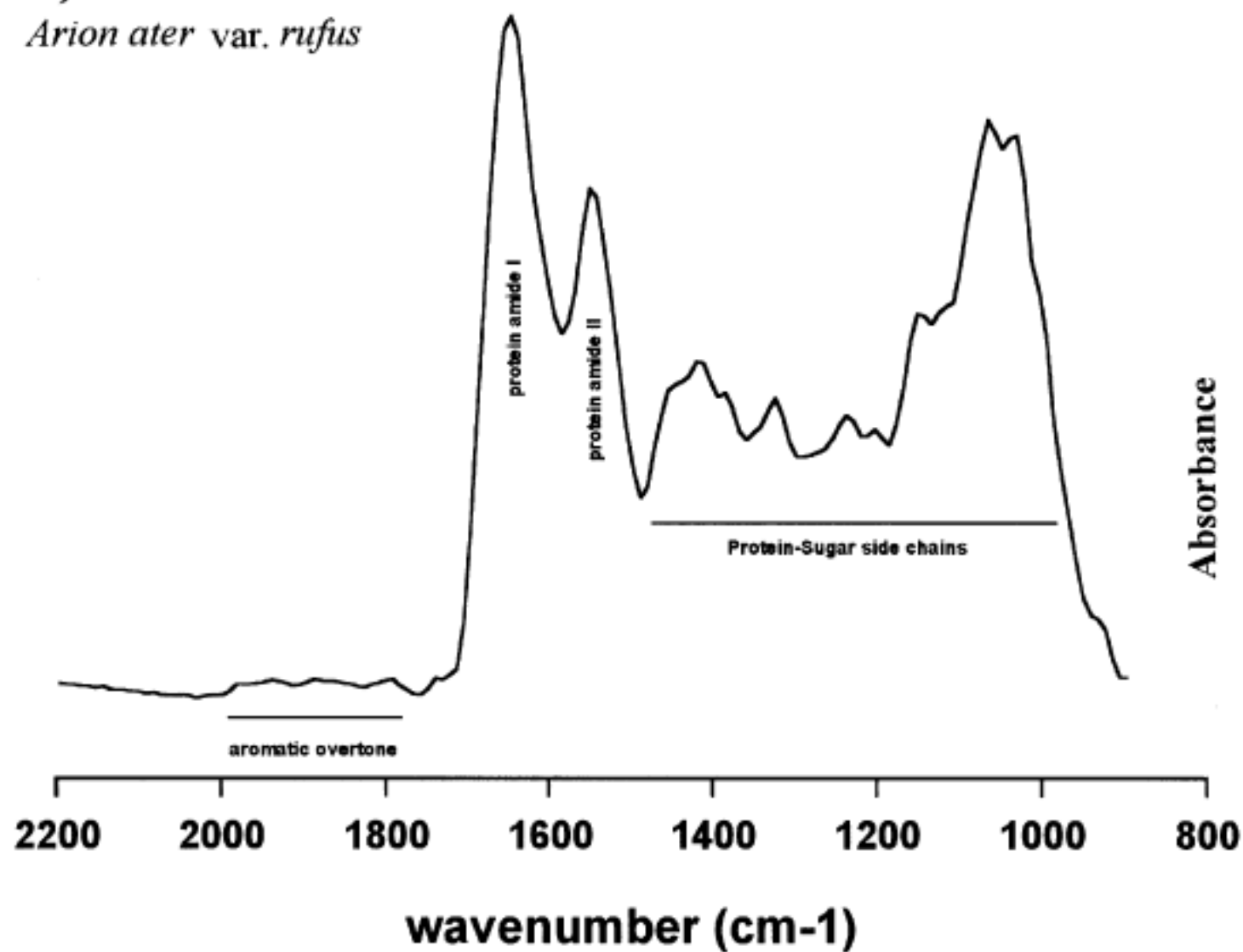
© The Malacological Society of London 2000

ANALYSIS OF PULMONATE MUCUS BY INFRARED SPECTROSCOPY

D.R. SKINGSLEY*, A. J. WHITE, and A. WESTON

Staffordshire University, School of Sciences, Biology Division, College Road, Stoke-on-Trent, ST4 2DE

(Received 23 July 1999; accepted 24 November 1999)

a)*Arion ater* var. *rufus*

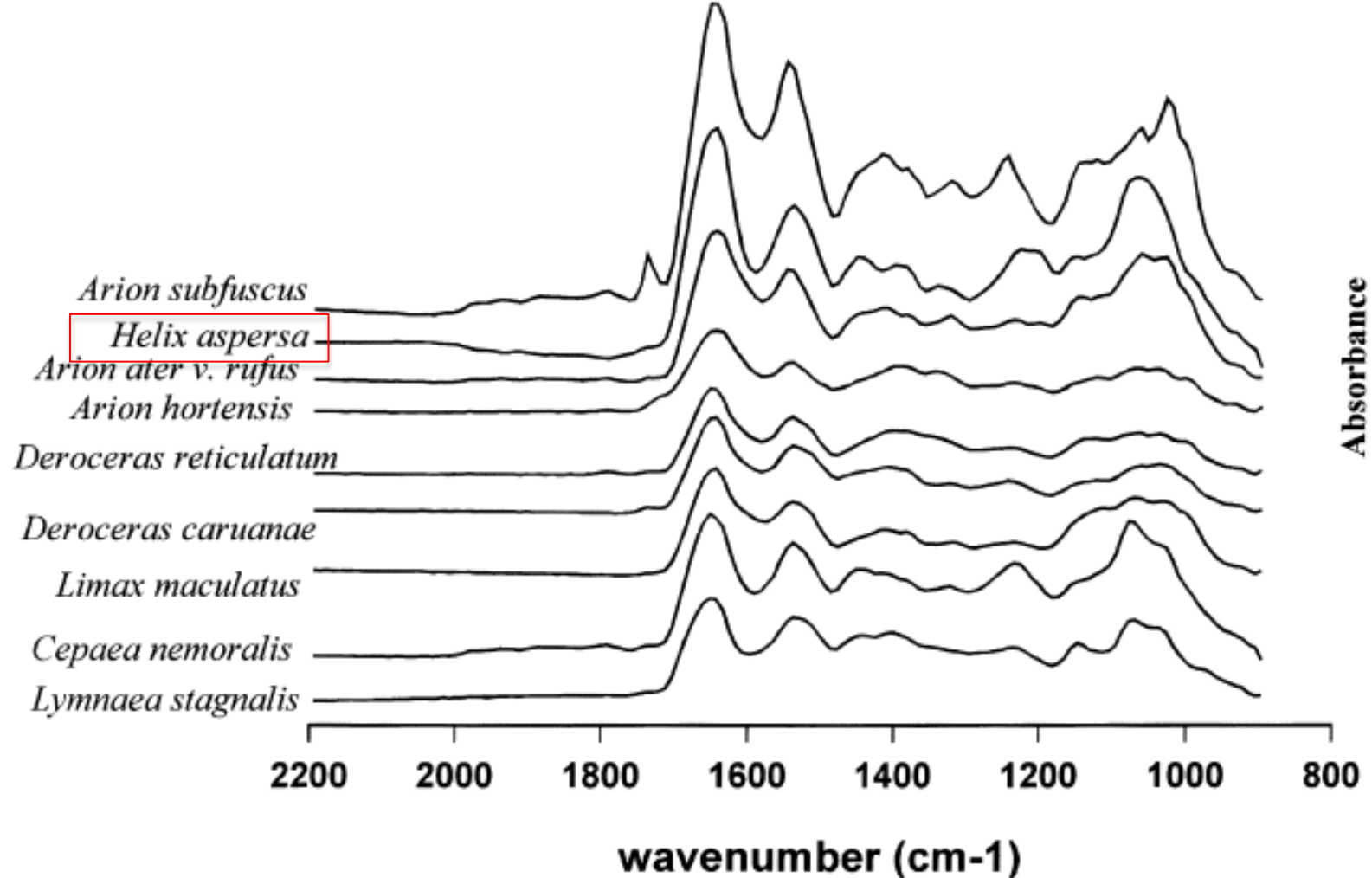


Figure 2. Spectral analysis of the pedal mucus of a variety of gastropods (*Arion ater* var. *rufus*, *Arion subfuscus*, *Arion hortensis*, *Deroceras reticulatum*, *Deroceras caruanae*, *Limax maculatus*, *Helix aspersa*, *Cepaea nemoralis*, *Lymnaea stagnalis*) showing the spectral profile between 900 cm^{-1} and 2200 cm^{-1} . *Arion ater* var. *rufus* is included in the figure for comparison. All spectra show the presence of Amide I and Amide II bands (about 1640 cm^{-1} and 1545 cm^{-1} respectively). The bands between 929 cm^{-1} and 1445 cm^{-1} are the most variable within this section of the spectra. The majority of spectra do not exhibit strong benzene overtones (1800 cm^{-1} and 2000 cm^{-1}) with the exception of *Cepaea nemoralis* and *Arion subfuscus*. The latter also has a strong band in the ester/acetyl banding at 1738 cm^{-1} which is weakly mirrored in *Arion hortensis* and *Deroceras caruanae*.

Chemical Characterization

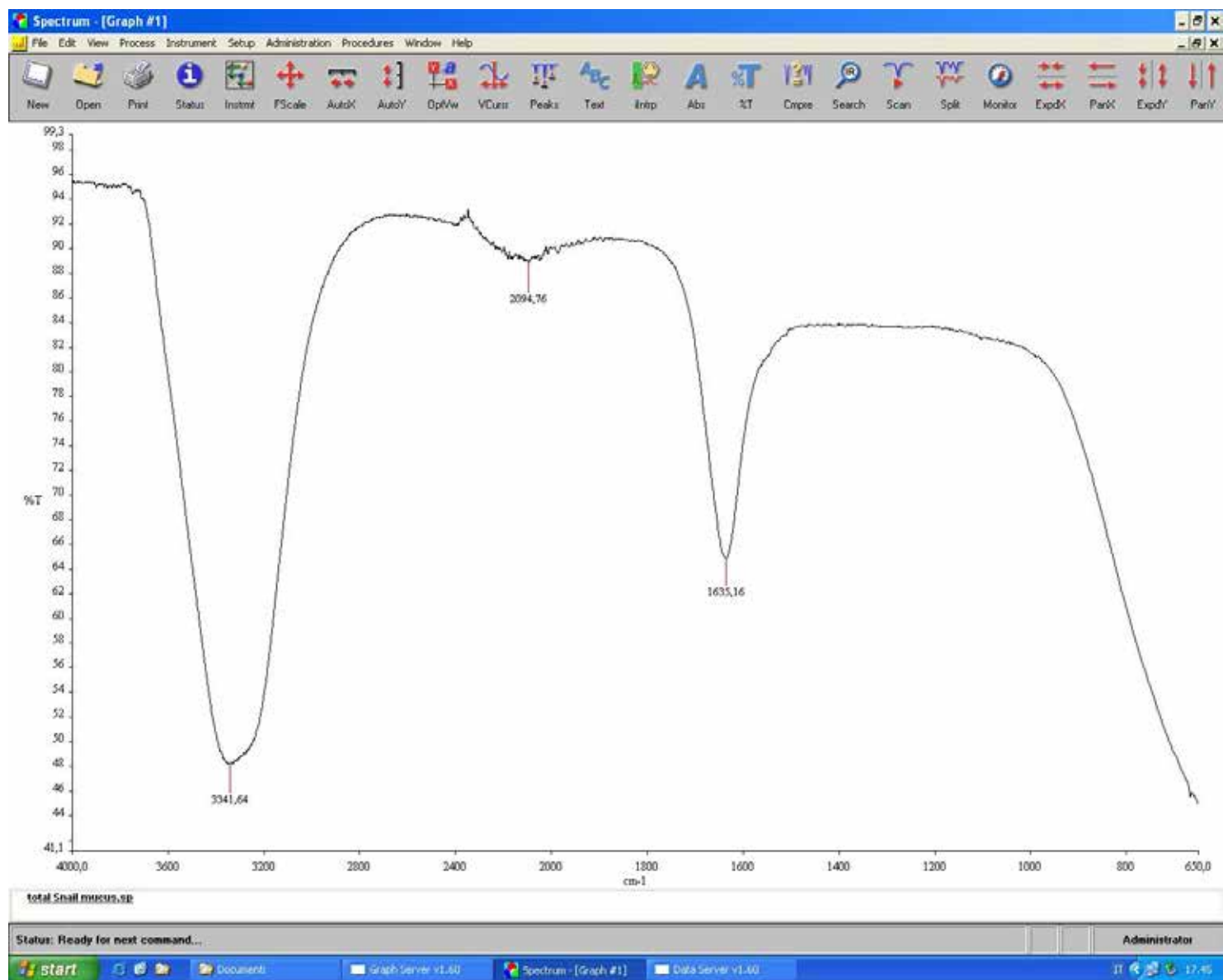
Infrared spectroscopy



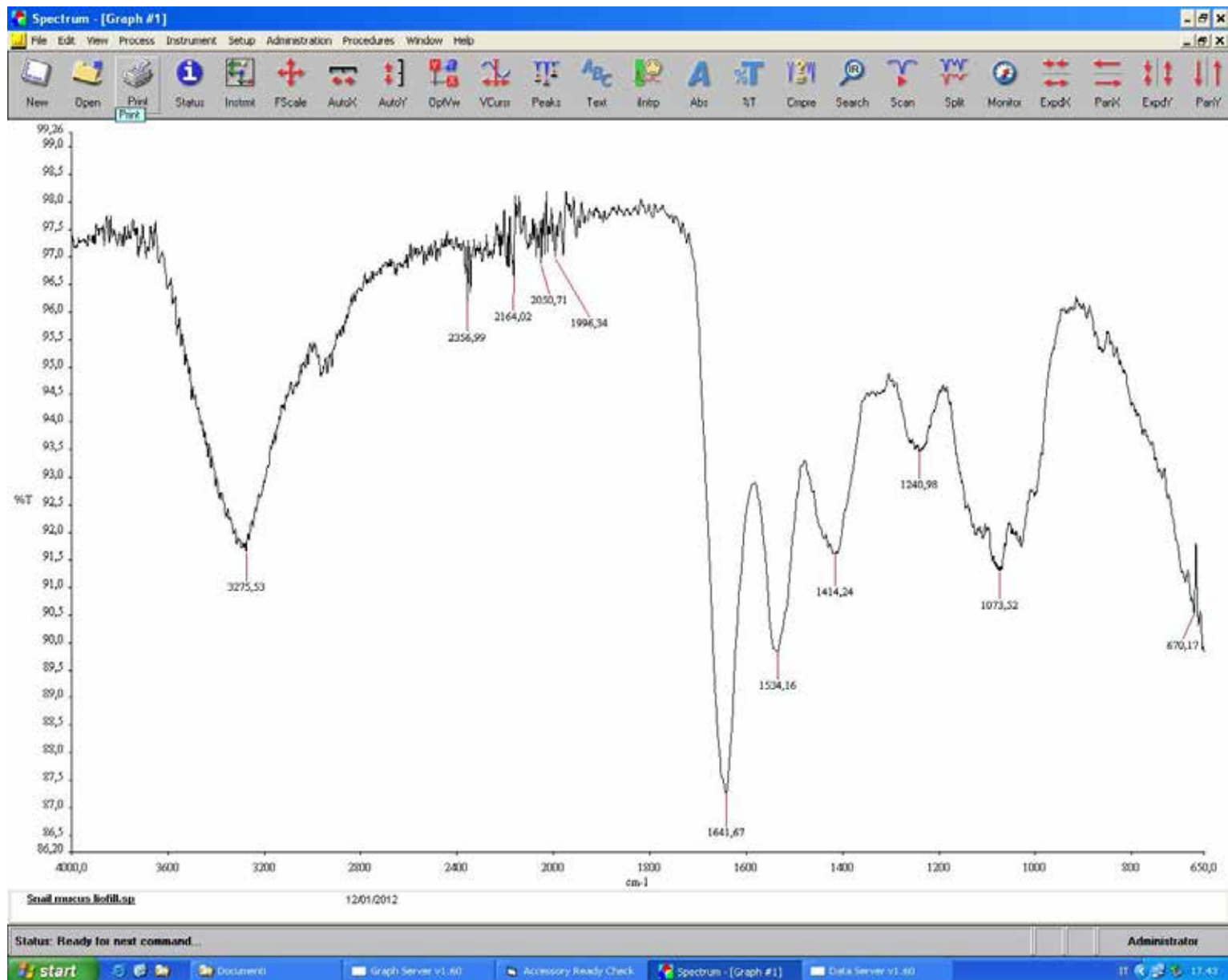
Due to the high chemical complexity of the snail mucus the IR spectroscopy is the key technique to better understand the presence of proteins and their integrity.

Proteins are the most important compounds to explain the biological properties of the mucus.

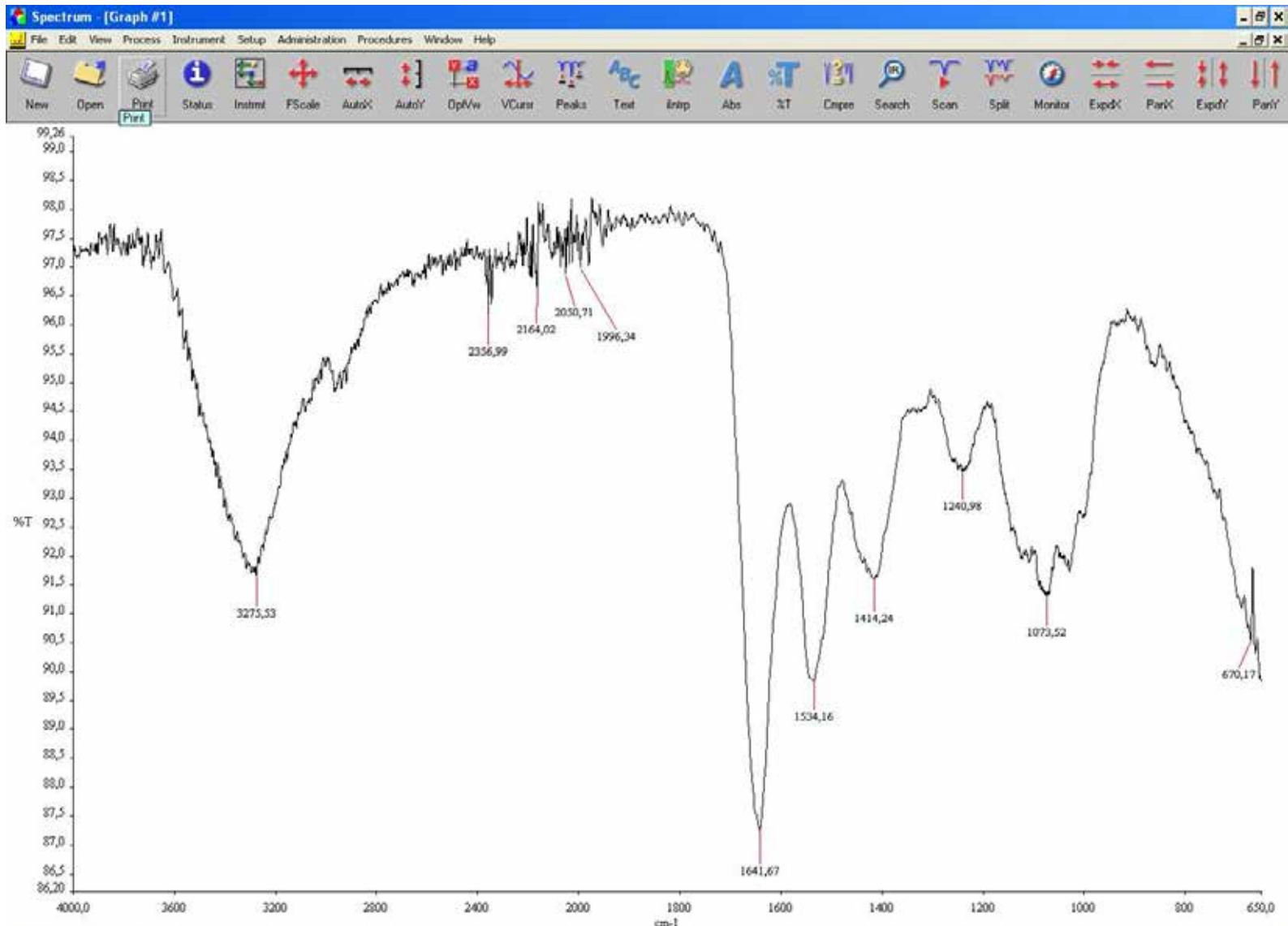
IR analysis of crude *HELIX ASPERSA* extract



IR analysis after lyophilisation of the extract



Differences between species: HELIX ASPERSA MULLER

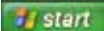


Small muscles Biofil.sp

12/01/2012

Status: Ready for next command...

Administrator



Document

Graph Server v1.60

Accessory Ready Check

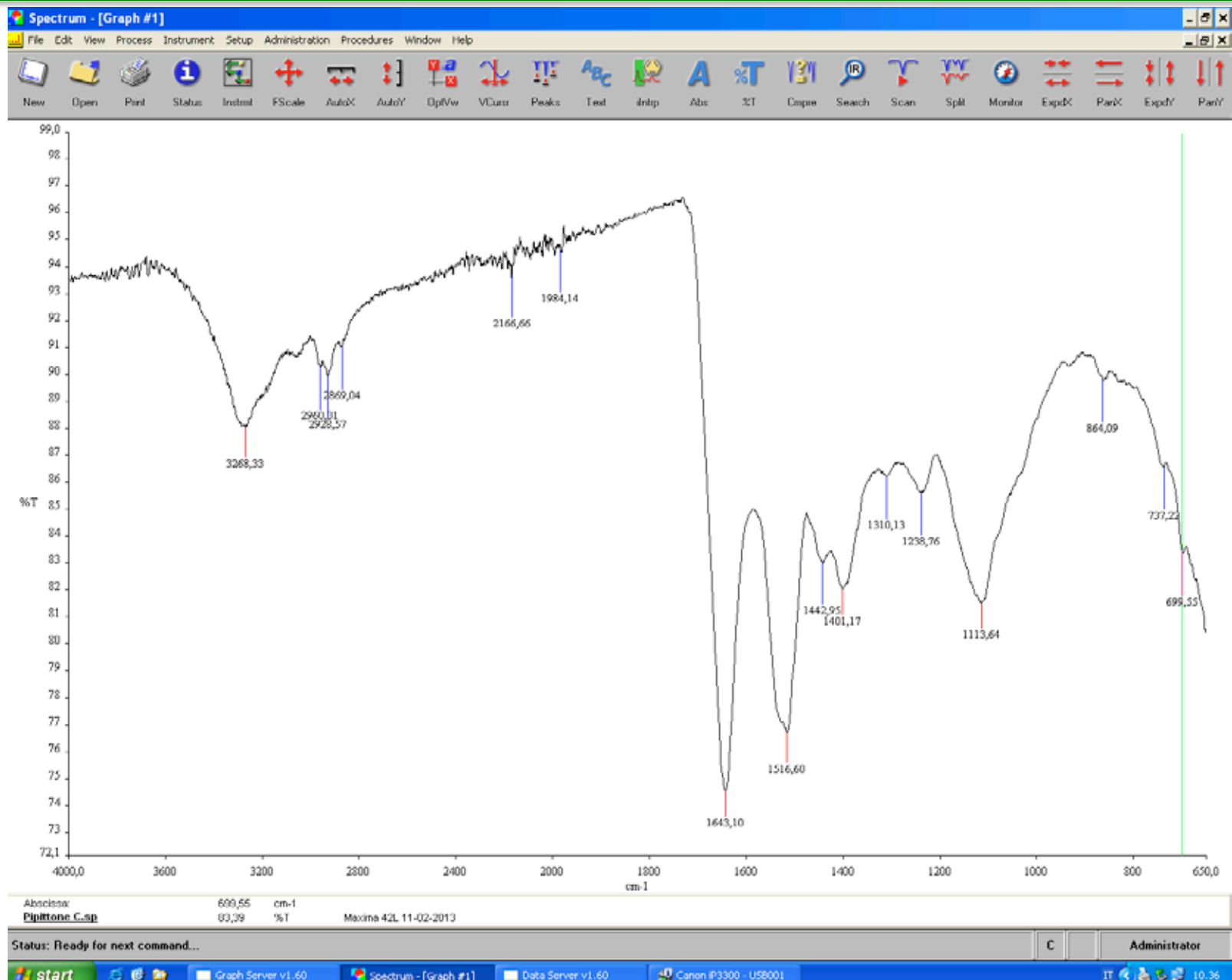
Spectrum - [Graph #1]

Data Server v1.60

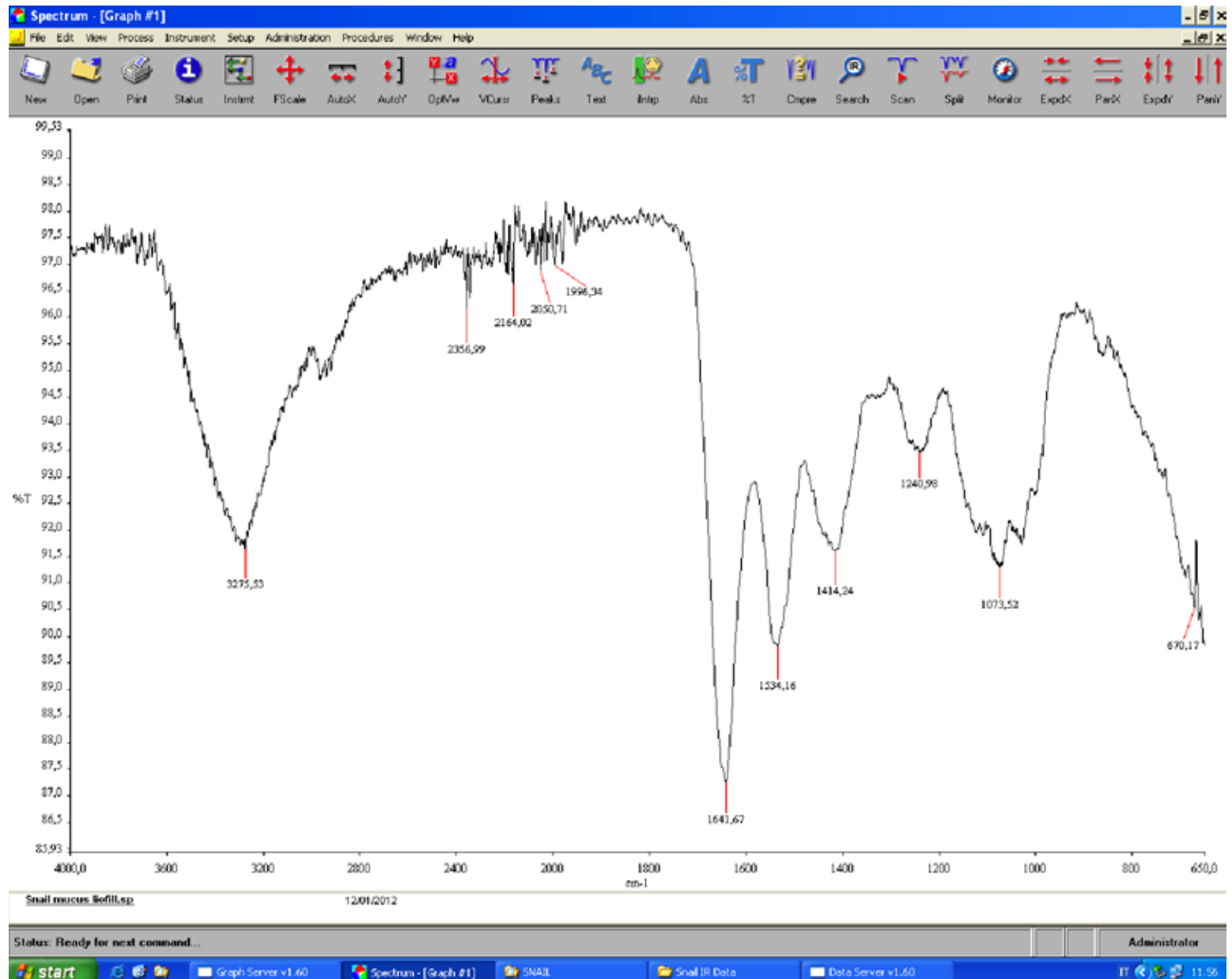
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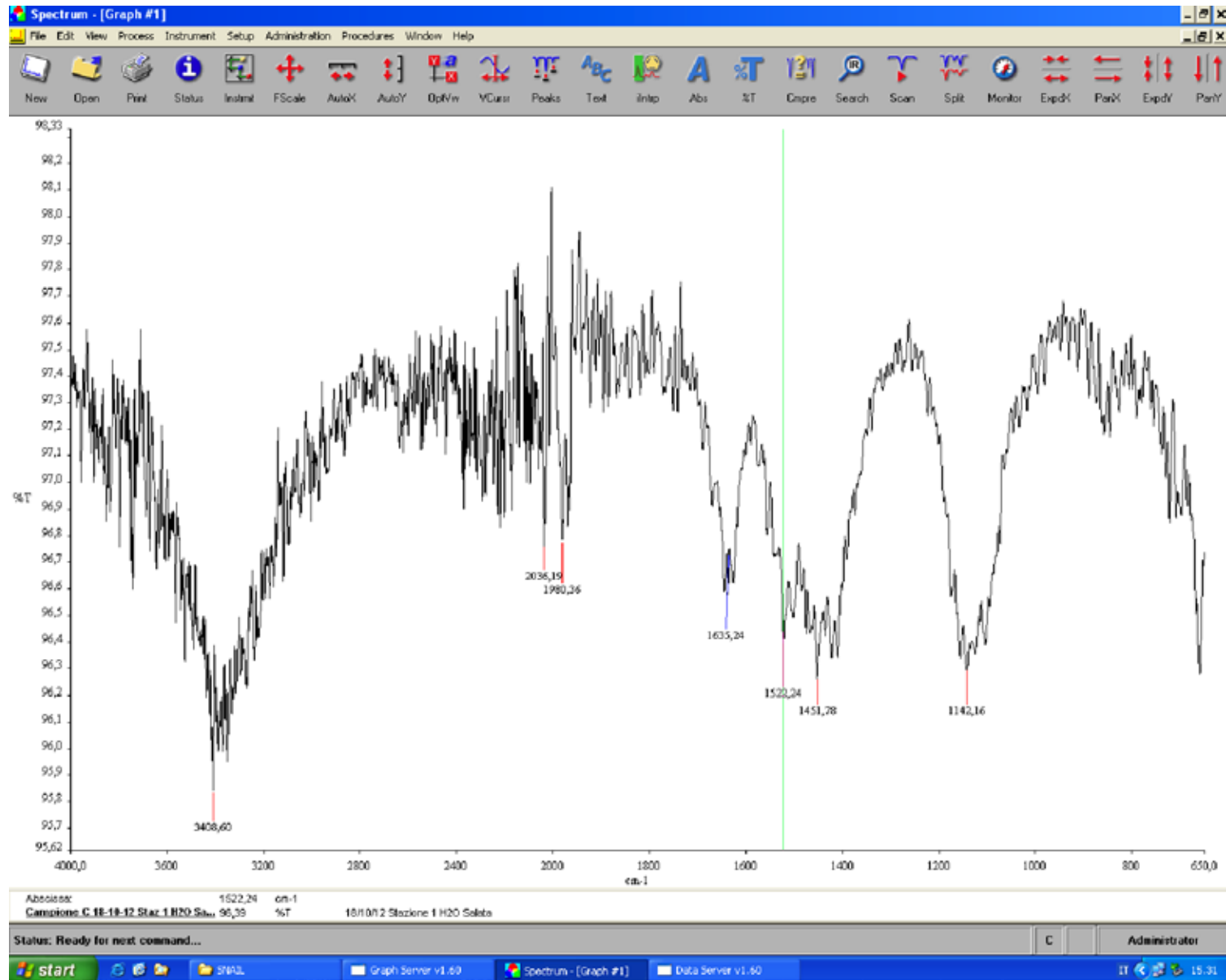
HELIX ASPERSA MAXIMA



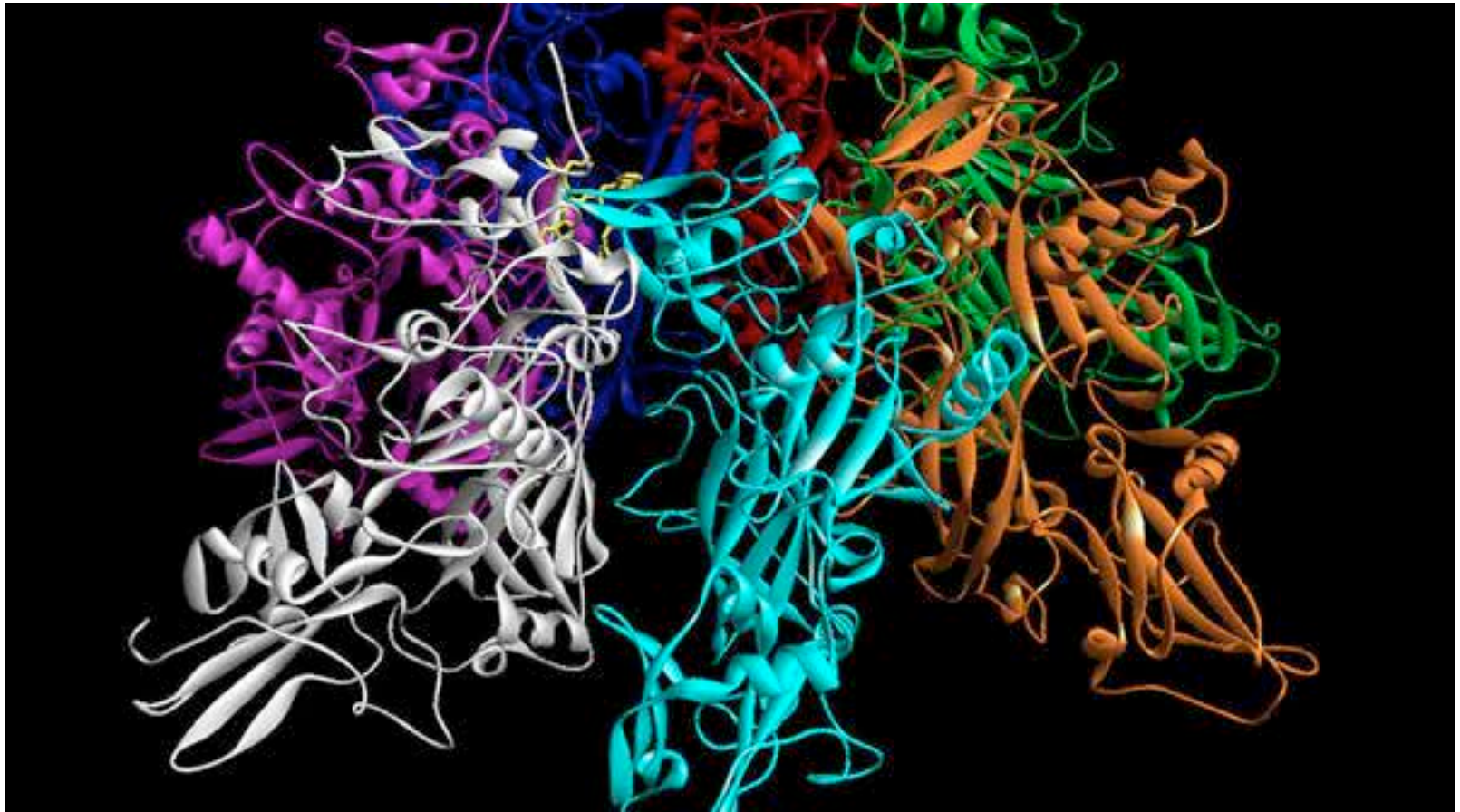
HELIX POMATIA



Low quality IR spectra of snail mucus proteins

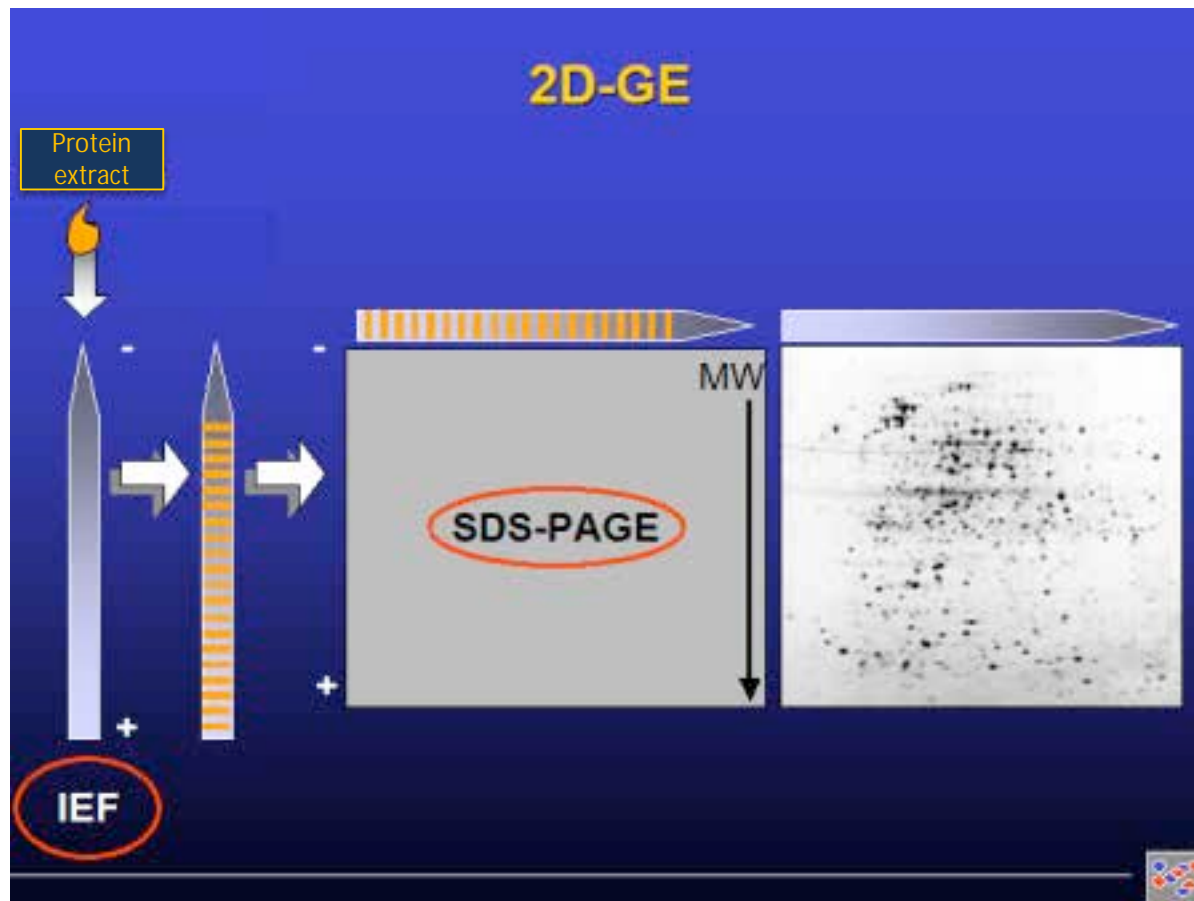


PROTEINS ANALYSIS



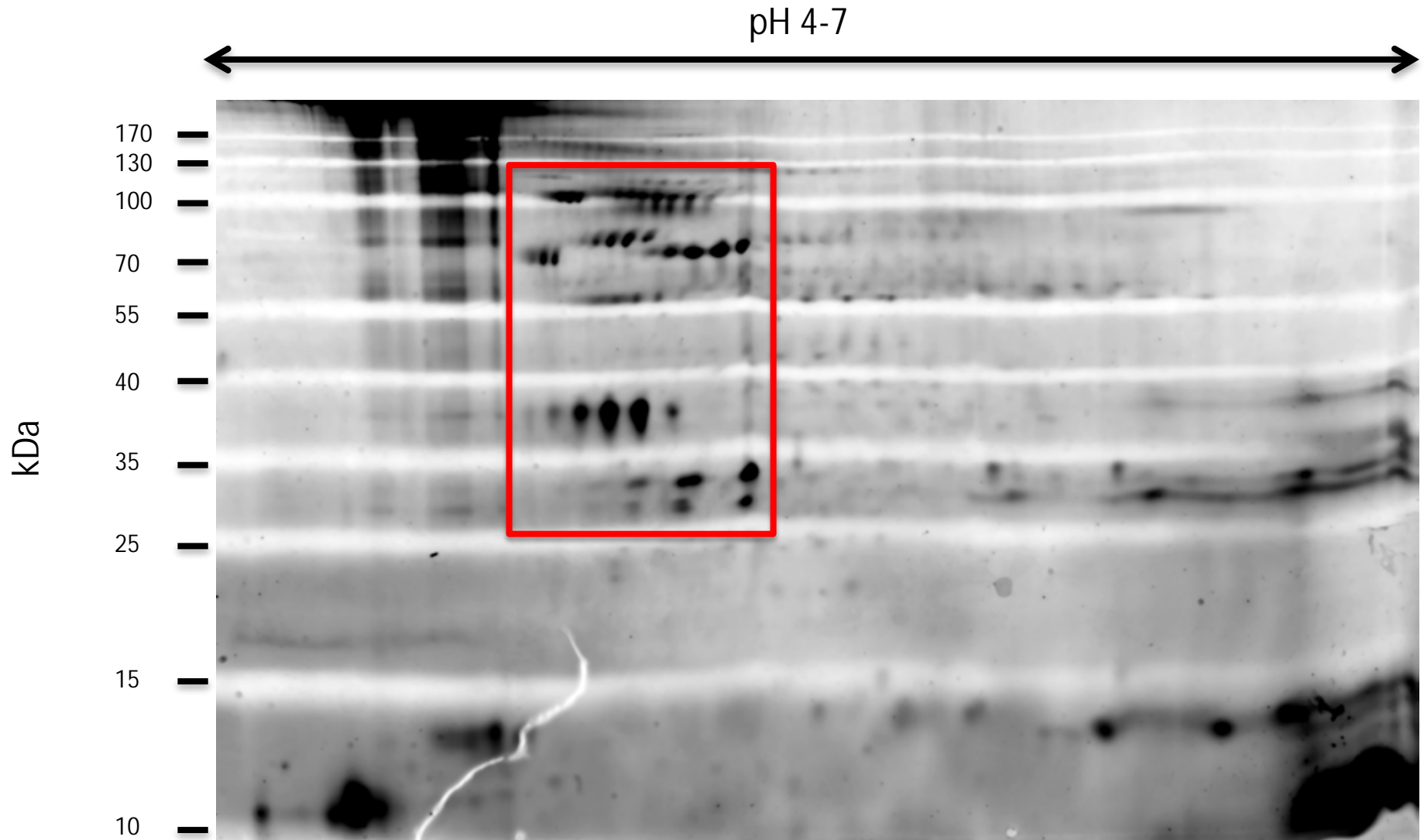
PROTEINS ANALYSIS

The proteins analysis will allow us to understand if there are protein substances that give reason of the biological properties.

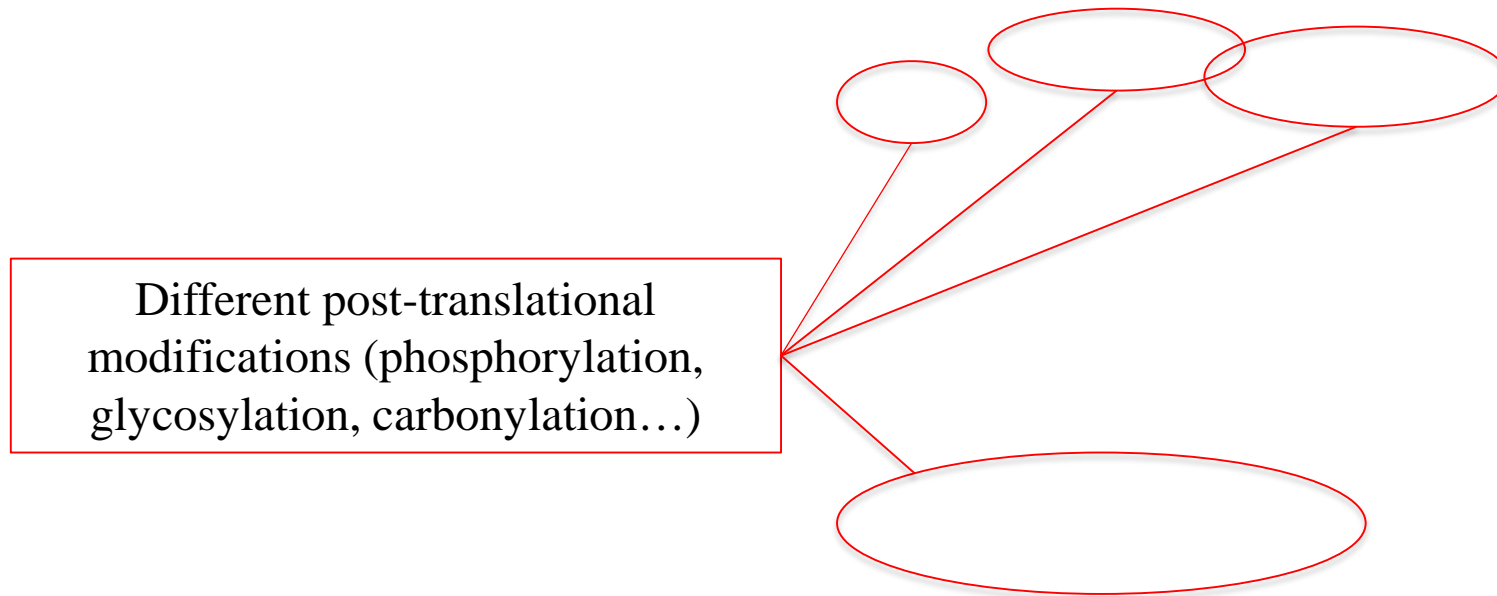


PROTEIN ANALYSIS: RESULTS

Snail mucus protein extract 2D-GE



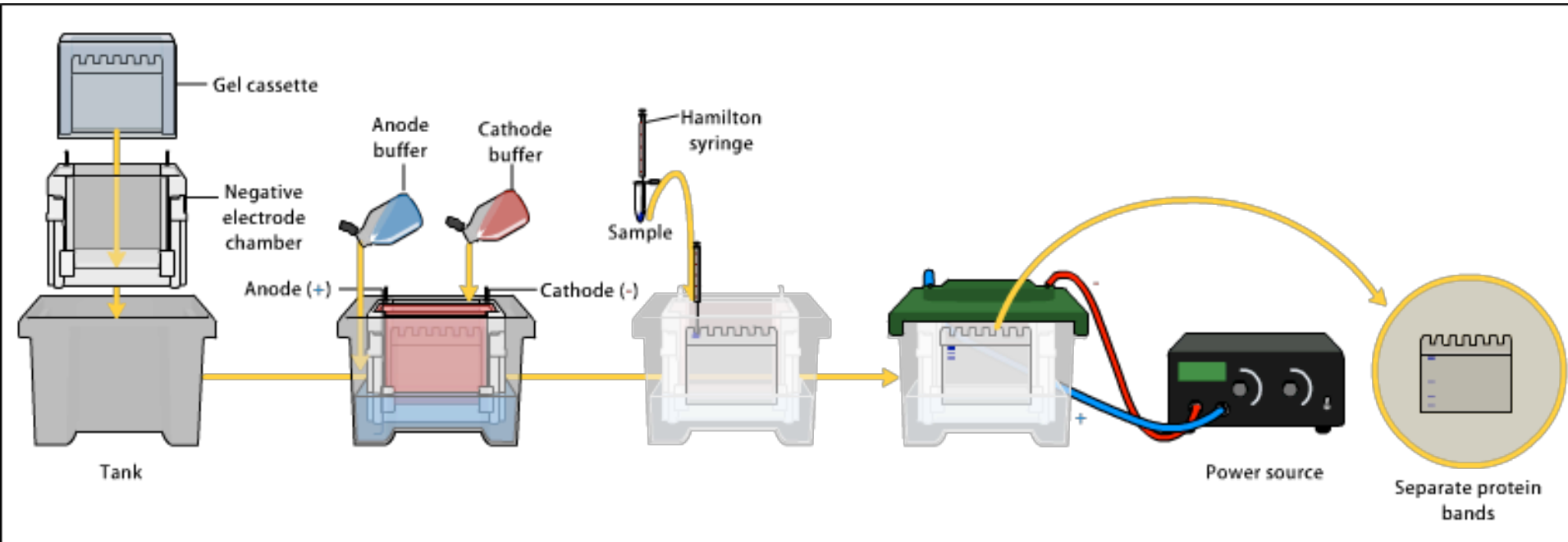
Snail mucus proteins of interest:





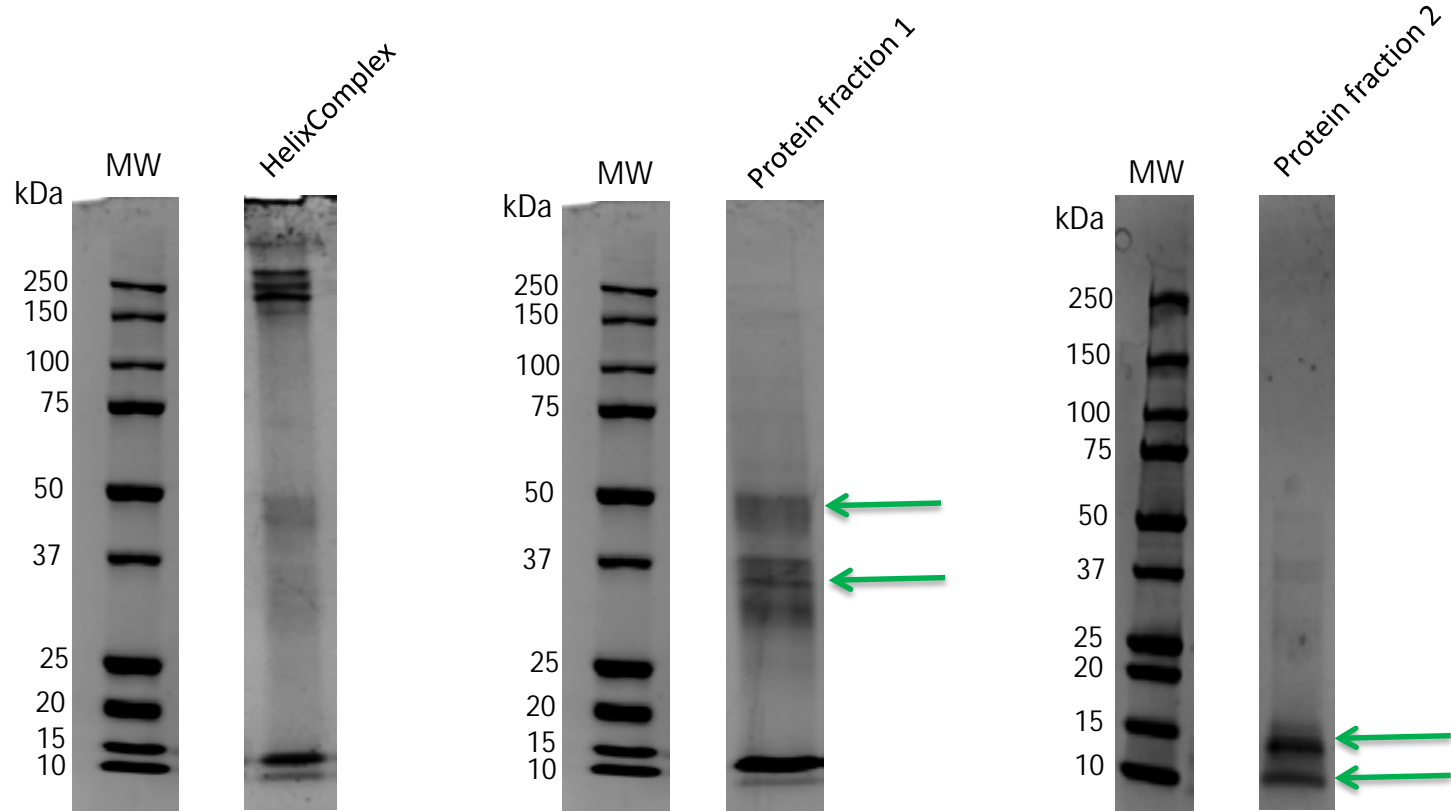
Capillary electrophoresis:

SDS-PAGE



SDS-PAGE of HelixComplex® shows characteristic bands at different molecular weight.

With ultracentrifugation and concentration of the HelixComplex® it is possible to separate specific protein fractions for subsequent analyses.



BIOLOGICAL PROPERTIES

Ø **Regenerative**

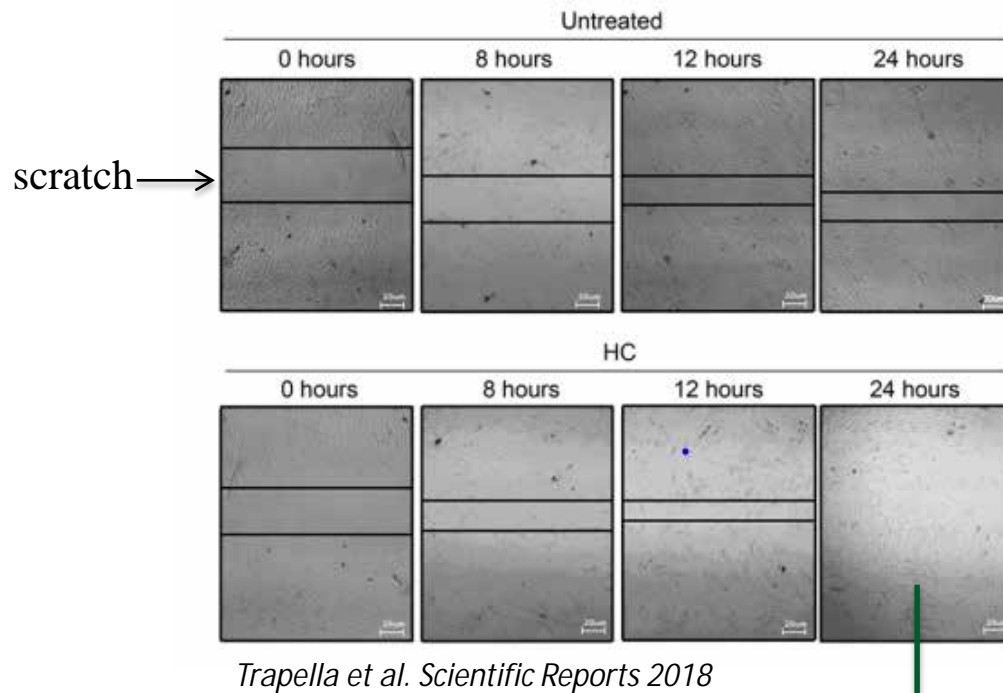
Ø **Antimicrobial**

Ø **Mucoadhesive**

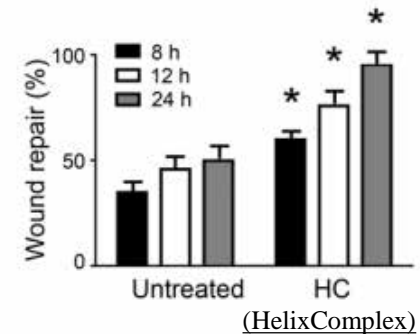
Ø **Mucolytic**

Ø **Anti-Maillard**

Ø Regenerative property:



Scratch-wound healing assay on monolayers of fibroblast cell line: exposure to HelixComplex® for 24 hours after scratch.



After treatment with HelixComplex®, the monolayer is completely repaired.
HelixComplex® promotes cellular migration and wound healing.

SCIENTIFIC REPORTS




OPEN

HelixComplex snail mucus exhibits pro-survival, proliferative and pro-migration effects on mammalian fibroblasts

Received: 22 May 2018

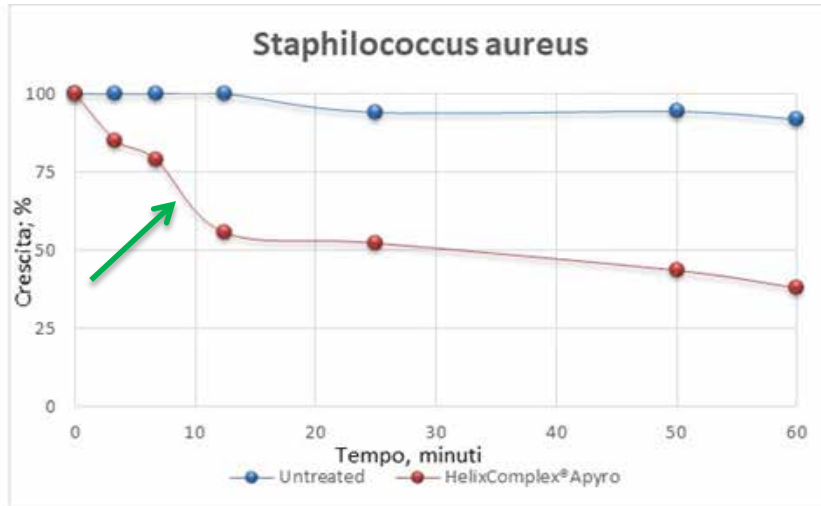
Accepted: 7 November 2018

Published online: 05 December 2018

Claudio Trapella¹, Roberta Rizzo², Stefania Gallo³, Andrea Alogna¹, Daria Bortolotti², Fabio Casciano ³, Giorgio Zauli³, Paola Secchiero³ & Rebecca Voltan³

Ø Antimicrobial properties:

- ANTIBACTERIAL effect of HelixComplex® (HC) on Gram positives (*Staphylococcus aureus*) and Gram negatives (*Pseudomonas aeruginosa*) bacteria

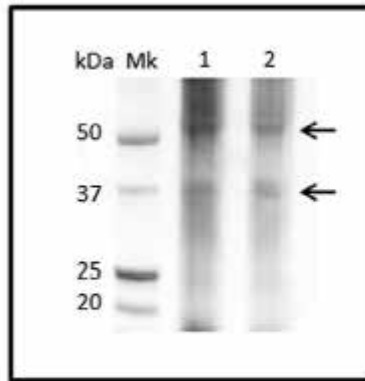


HelixComplex®-treated bacteria exhibit a stronger decrease in growth compared to untreated bacteria, already observed after 15 minutes of treatment.

Identification of protein responsible for antimicrobial activity

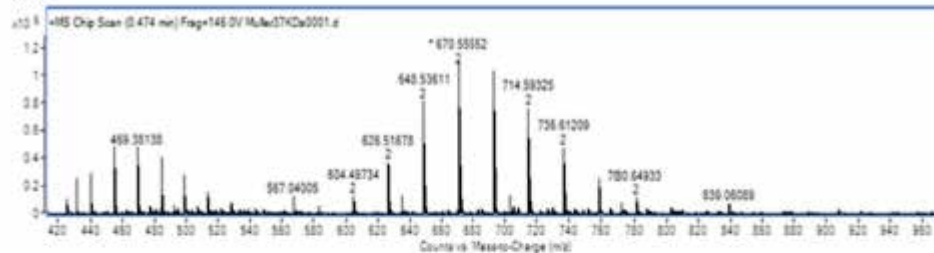
Bortolotti et al.
Br J Biomed Sci;
2016

(a)



- 50-kDa protein band is composed by several proteins.
- Protein band in the range of 40–30 kDa is composed by a single protein of 41 kDa

(b)



(c)

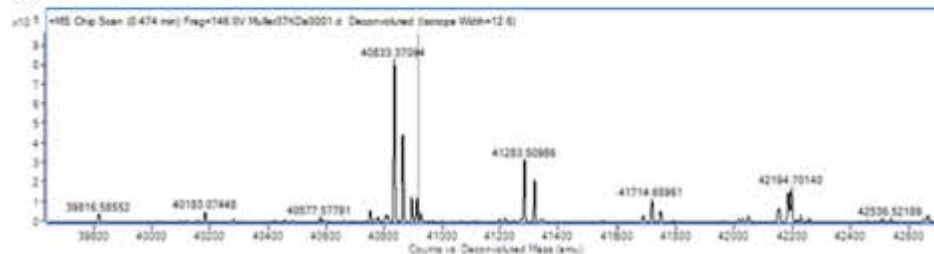


Figure 1. Analysis of protein content in lyophilised *H. aspersa* mucus. (a) SDS–PAGE and Coomassie Blue R250 staining of two samples (1, 2) of lyophilised mucus. Two sets of proteins, one above 50 kDa and one between the range of 40–30 kDa are shown. Mk: standard protein marker (Precision Plus Protein Prestained Standards, Biorad). (b) Mass spectra of multicharge protein between 40 and 30 kDa. (c) Deconvolution analysis of the multicharge spectra.



British Journal of Biomedical Science

ISSN: 0967-4845 (Print) (Online) Journal homepage: <http://www.tandfonline.com/loi/tbbs20>

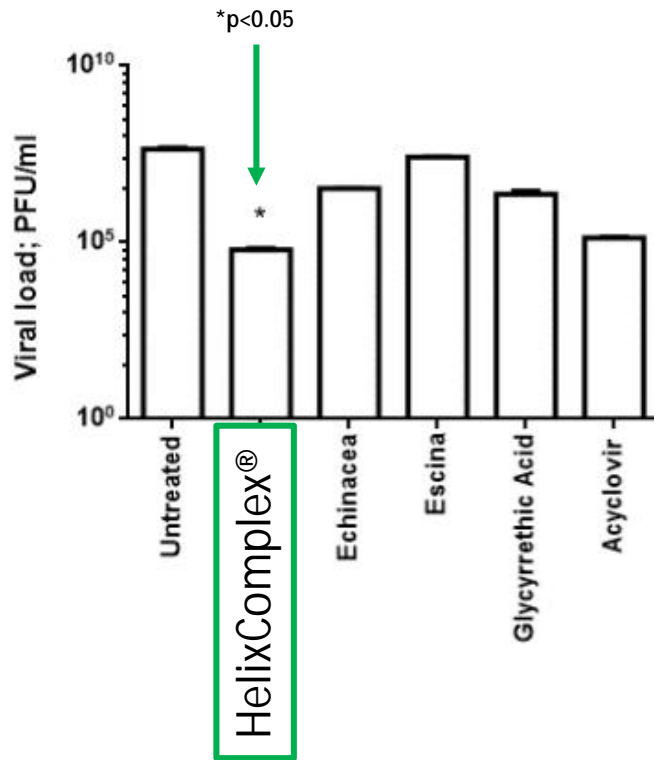
Letter to the Editor: Antimicrobial properties of mucus from the brown garden snail *Helix aspersa*

Daria Bortolotti, Claudio Trapella, Tatiana Bernardi & Roberta Rizzo

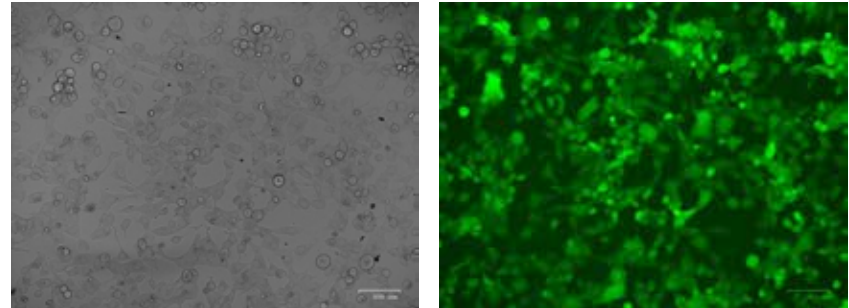
To cite this article: Daria Bortolotti, Claudio Trapella, Tatiana Bernardi & Roberta Rizzo (2016) Letter to the Editor: Antimicrobial properties of mucus from the brown garden snail *Helix aspersa*, British Journal of Biomedical Science, 73:1, 49-50

To link to this article: <http://dx.doi.org/10.1080/09674845.2016.1155377>

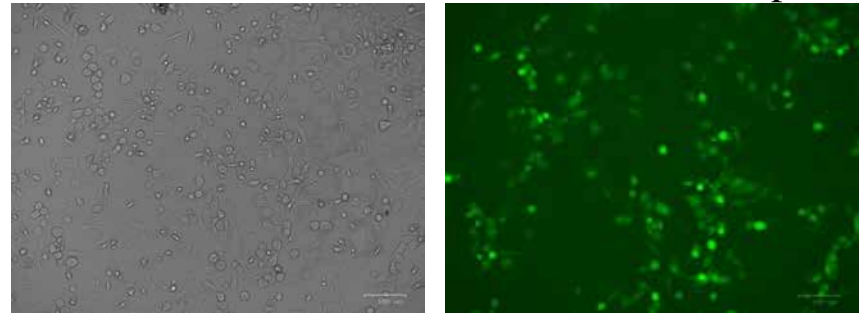
- ANTIVIRAL effect of HelixComplex® (HC) on Herpes Simplex type 1 virus



Untreated HSV-1 infected cells



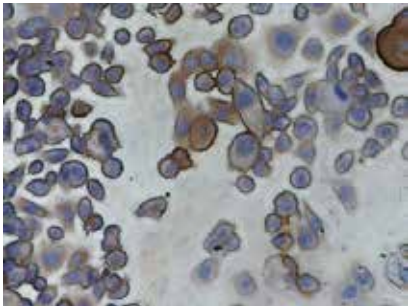
HSV-1 infected cells treated with HelixComplex®



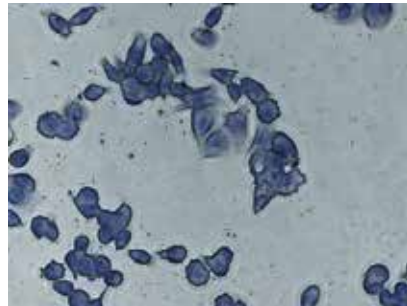
Decrease of viral load in infected cells treated with HelixComplex®

Ø Mucoadhesive properties:

Snail extract is rich in mupolysaccharides with hygroscopic activity that helps the formation of a **protective film** on mucosal cells.

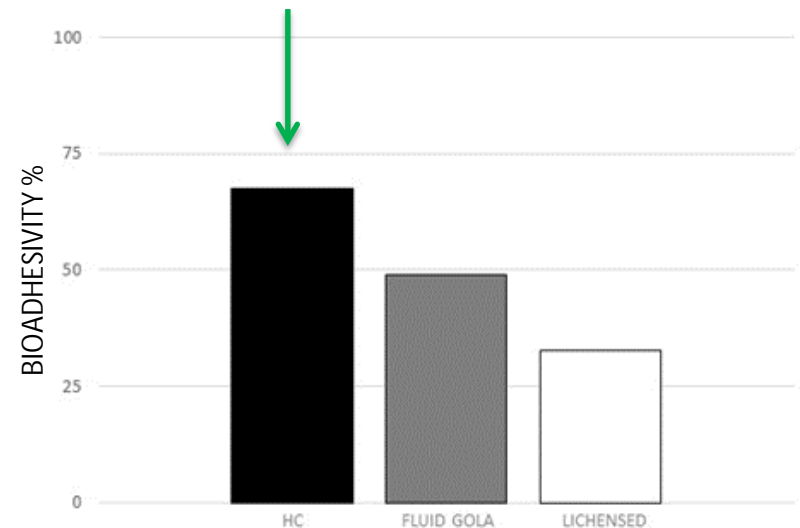


Untreated



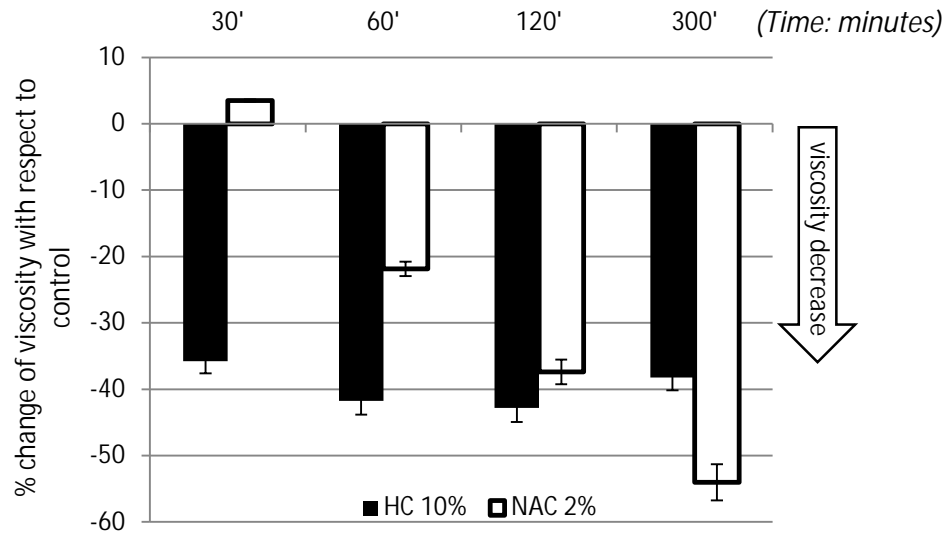
HelixComplex® 10%

HelixComplex® prevents adhesion of carbohydrate-binding proteins (brown rim) on the cell surface.



Bioadhesivity of HelixComplex® on bronchial and gastric epithelium (A549 and AGS cells), in comparison to other commercial medical devices.

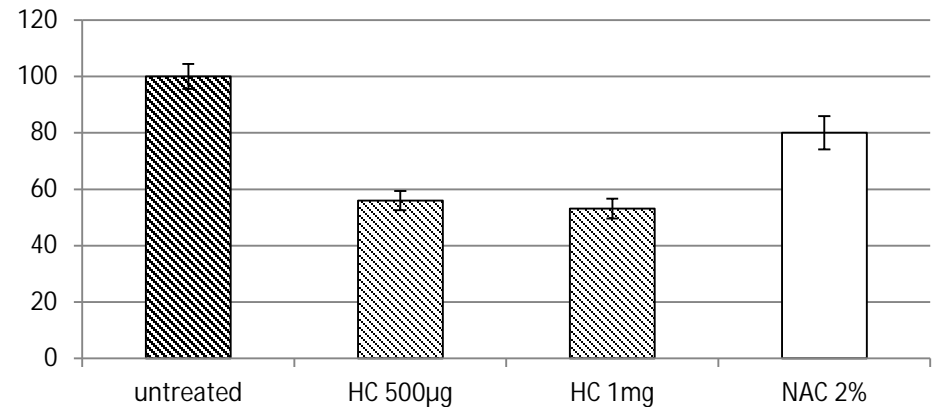
Ø Mucolytic properties:



HelixComplex® decreases the viscosity of mucin solution at high levels after 30 minutes of incubation. N-acetylcystein (positive control) reaches the same level after 120 minutes of incubation.

HelixComplex® exhibits disruptive properties on pre-cast *P. aeruginosa* biofilm after 24 hours of incubation.

% of *P. aeruginosa* biofilm after 24 h of treatment



Ø Anti-Maillard properties:

reducing sugars



amino groups of proteins, lipids
and DNA

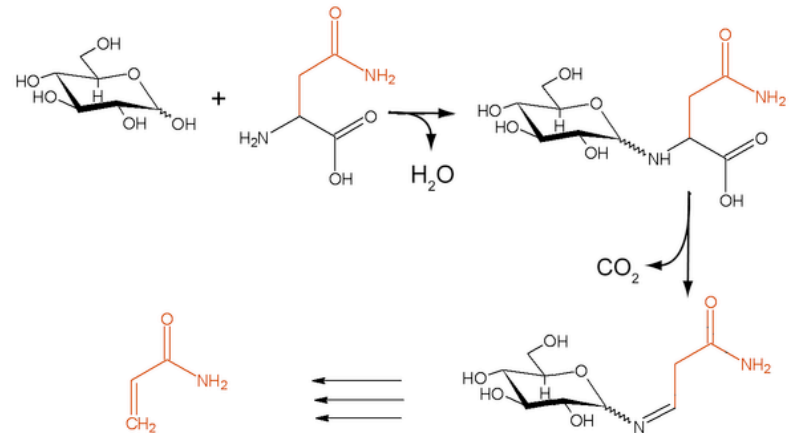
Maillard
reaction

Advanced glycation
end products (AGEs)

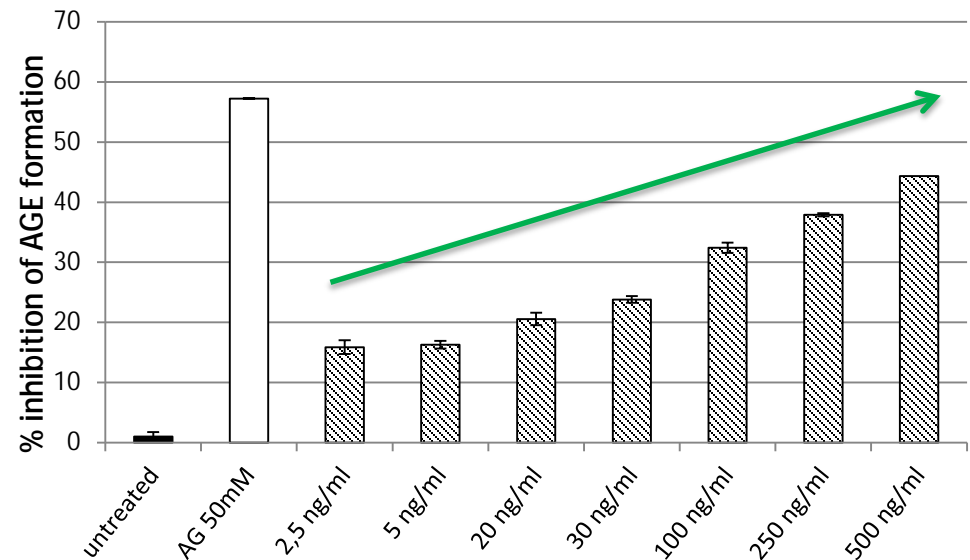
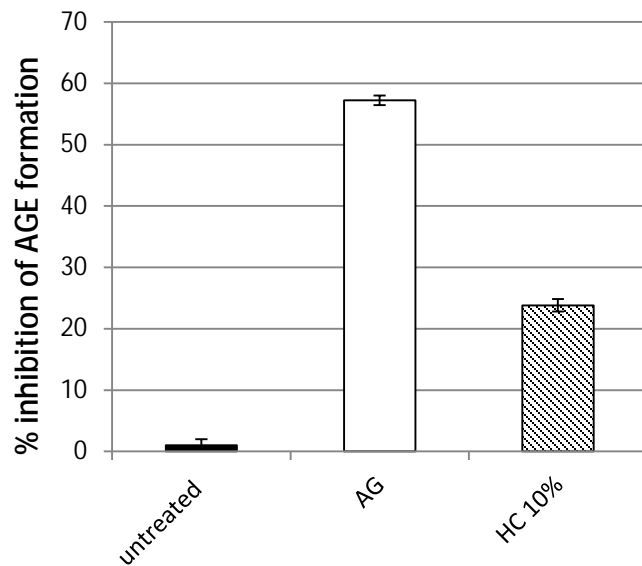


Browning, fluorescence, and cross-linking.

Accumulation of AGEs plays an important role
in aging and age-related diseases.



In vitro model for assessment of anti-glycation activity of HelixComplex[®] and protein extracts from HelixComplex[®] : **BSA-Glucose Assay**
After 7 days of incubation, fluorescent intensity was measured.



AG= aminoguanidine, positive control

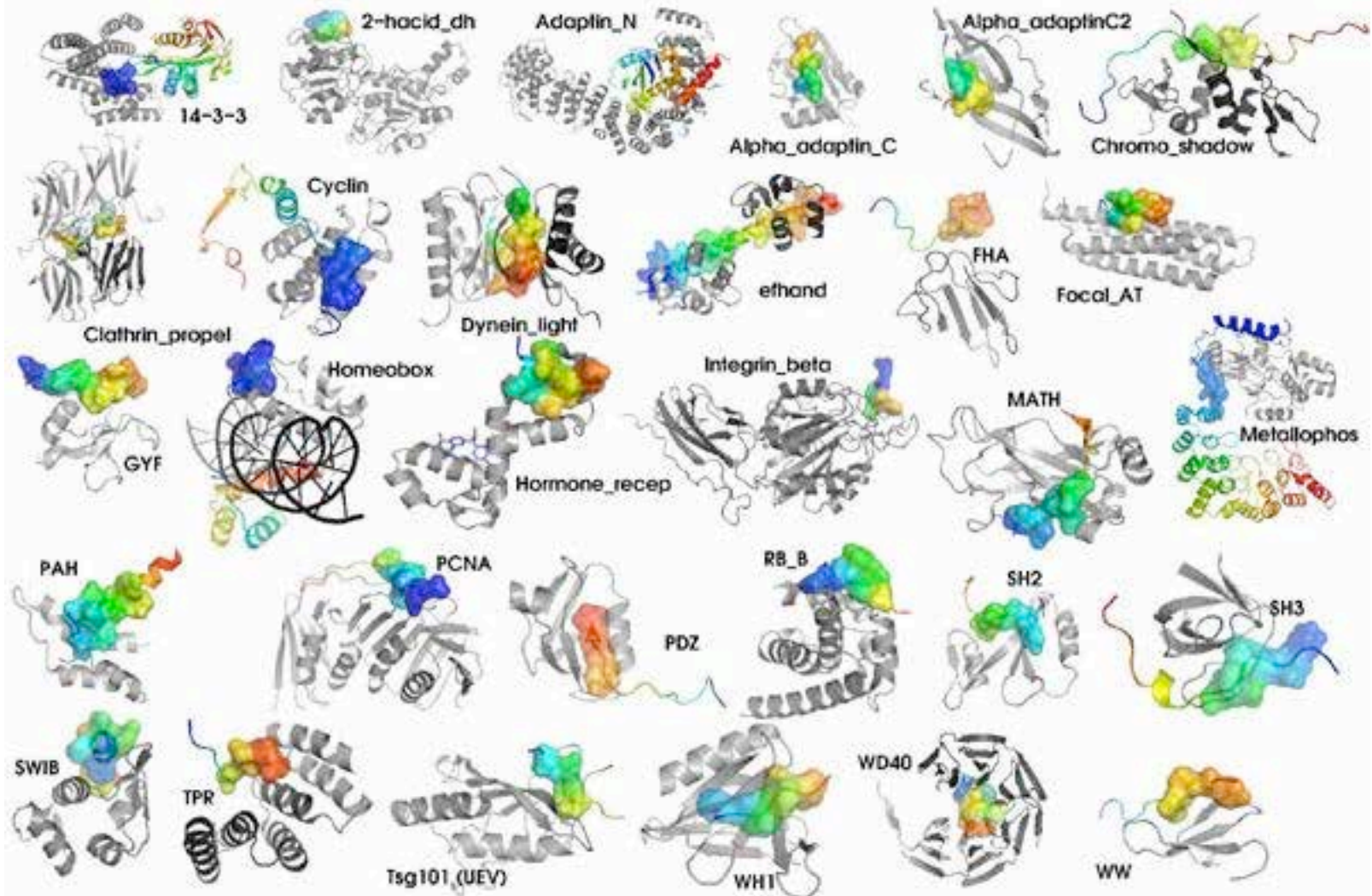
- HelixComplex[®] inhibits AGEs formation
- Dose-dependent inhibition of AGEs formation by protein extracts of HelixComplex[®]

FUTURE WORKS: PROTEIN ANALYSIS



AGILENT ESI-Q-TOF 6520 HPLC/MS

PROTEINS ANALYSIS



Ongoing experiments on HelixComplex® activity:

- Protection against pollutants and UV rays.
- Intestinal uptake of microelements and functional substances

Potential use in:

- Respiratory diseases (COPD, CF...)
- Skin spots
- Food Supplementation (nutraceutical)



**THANKS FOR YOUR
KIND ATTENTION**