Study of forced degradation, by HPLC-UV and electrophoresis, of a new synthetic therapeutic elastic protein formulated in isotonic saline solution

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Introduction

The Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique has developed a **synthetic elastic protein (SEP).** The aim of this recombinant protein is to restore elasticity to tissues altered by genetic elastinopathies by virtue of its molecular prosthetic action.

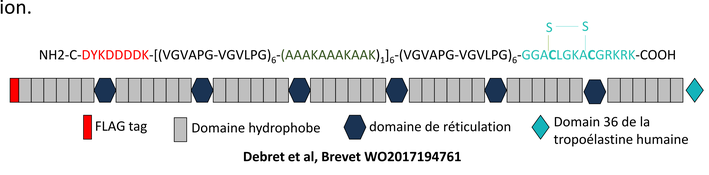


Fig.1: Synthetic elastic protein (SEP)

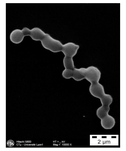


***Fig.2:** Scanning electron microscopy, SEP 1 mg/mL in PBS, stored at 37°C.*

SEP is an unstructured biotherapeutic agent whose action is not linked to its conformation and whose **stability is not yet known**. To date, there is only one validated method for dosing SEP.

Objectives: Carry out the first study of forced degradation of SEP in order to identify the conditions that cause its degradation andverify the stability-indicating nature of the SEP dosage method.

Materials and Methods

Samples	Tested degradation conditions	Exposure time	Neutralisation	Analysis
1 mL SEP **80 µg/mL**	+ 20°C / -80°C	1, 3 ou 5 1 h freezing	Exposure stop	Spectrophotometer
	+ 80°C or + 37°C	Tested exposure durations: 0.5 h - 2 h - 4 h - 8 h - 24 h - 72 h - 168 h	Ice batch 1 min	HPLC Reverse phase Acetonitrile/Water gradient + 0.2% trifluoroacetic acid Coupled with UV detection 210 et 265 nm
	254 nm or 365 nm		Exposure stop	
1 mL SEP **360 µg/mL** (to reach 80 µg/mL after neutralisation)	1 mL HCl pH 1		1 mL equimolar NaOH 1 mL NaCl 0.9%	
	1 mL HCl pH 1, 2, 4 or 6 + 80°C		1 mL equimolar NaOH 1 mL NaCl 0.9% + Ice batch	
	1 mL NaOH pH 13		1 mL equimolar HCl 1 mL NaCl 0.9%	
	1 mL NaOH pH 13, 10 or 8 + 80°C		1 mL equimolar HCl 1 mL NaCl 0.9% + Ice batch	SDS-PAGE & Western Blot
	1 mL H_2O_2 3% + 80°C		2 mL NaCl 0.9% + Ice batch	

Each condition was tested on 2 SEP samples and controlled by a NaCl 0.9% solution

Results

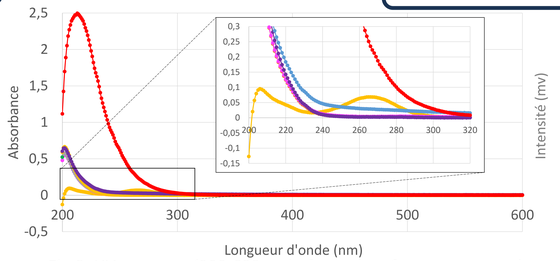


***Fig.3**: UV spectrum (200-600 nm) of samples after exposure to the tested degradation condition.*

Legend fig 3:		
Red: H_2O_2 3%, 80°C	Violet: HCl pH 1, 20°C	Orange: 37°C
Blue: NaOH pH 13, 20°C	Pink: HCl pH 1, 80°C	Green: NaOH pH 13, 80°C

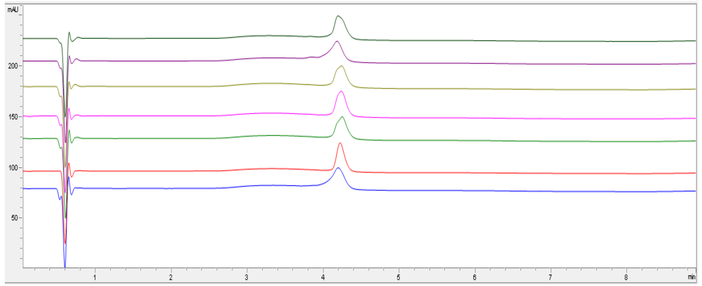
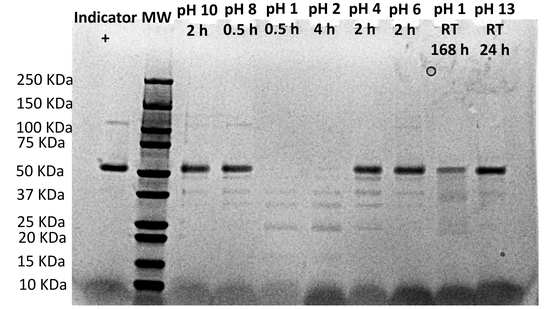


***Fig.4** : Chromatograms of samples after exposure to the tested degradation condition.*

Legend fig 4:	
Dark green: HCl pH4, 80°C	Green: NaOH pH 10, 80°C
Violet: HCl pH2, 80°C	Red: Contrôle SEP avant dégradation
Yellow: HCl pH8, 80°C	Blue: HCl pH1, 20°C



Legend fig 5.
Indicator +: non degraded SEP
MW: Molecular Weight marker
RT: Room temperature

***Fig.5:** SDS-PAGE gel migration and Coumassie blue staining of samples after exposure to pH-degradation conditions*

Conclusions

The present study has enabled the identification of the **circumstances** that lead to **SEP degradation**.

However, the HPLC method used for measuring SEP is unable to distinguish it from its degradation products and, as a result, **cannot be considered a stability indicator method**.

In order to **identify** degradation products by **mass spectrometry**, it is necessary to develop our assay method to **increase its resolution**.

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