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# Pharmaceutical Perspectives of High Pressure: A Soft Tool for Sterilization of Fragile Drugs

Y. Rigaldie<sup>1,2,4</sup>, G. Lemagnen<sup>1</sup>, A. Largeteau<sup>2</sup>, D. Larrouture<sup>1</sup>, M. Abba<sup>1,4</sup>,  
R. Haller<sup>4</sup>, L. Grislain<sup>1</sup> and G. Demazeau<sup>2</sup>

<sup>1</sup> LTIP (Laboratoire de Technologie Pharmaceutique Industrielle), Faculté de Pharmacie, Université Victor Segalen Bourdeaux 2, 146 Rue Léo Saignat, FR-33076 Bordeaux Cedex, France

<sup>2</sup> IHP (Interface Hautes Pressions), ENSCPB - ICMCB, Avenue Pey-Berland, BP 108, FR-33402 Talence Cedex, France

<sup>3</sup> Bertin Technologies, 10 av. Ampère, Parc d'Activités du Pas du Lac, FR-78180 Montigny-le-Bretonneux, France

<sup>4</sup> Ellipse Pharmaceuticals, 1-2 Allée du Doyen George Brus, FR-33600 Pessac, France

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**Abstract:** In this study, we showed the safety of HHP (High Hydrostatic Pressure) treatment on several fragile biomolecules (molecular weight from 1200 up to 150 000 g/mol : two peptides, insulin, antibodies).

A HHP treatment (10 min at 400 MPa) inactivates totally pure suspensions of *P. aeruginosa* and *C. albicans*. HHP at low temperature induces a stronger inactivation than at room temperature. *S. aureus* needs a longer HHP duration treatment or a multi-step treatment to be totally inactivated. But same tests applied to *B. subtilis* did not lead to a total inactivation; further processes are in progress in order to cause spores of *B. subtilis* to germinate.

## Introduction

Usual methods of sterilisation using temperature or ionising radiation are often inappropriate, especially for fragile drugs and alternative methods (filtration or aseptic processing) are quite expensive. HHP treatment may represent a low cost mild alternative process which transmits few energy [1-6] but modifies the permeability of micro-organisms membranes [7, 8].

This paper deals with the effect of HHP on fragile pharmaceutical compounds and its efficacy on viability of various strains of microorganisms described in Pharmacopoeia. The role of the temperature and of the duration during the HHP process has also been evaluated.

## Materials and Methods

- **High hydrostatic equipment:** We used a high hydrostatic press designed by NFM-FRAMATOME for research with a 3 liters effective volume. This press can reach 800 MPa (8000 bar) and temperature can be specified from -20°C up to 80°C.
- **Samples treated:** Before HHP treatment, every sample has been packaged in polyethylene/polyamide bags already tested under pressure [9]. Then, fragile molecules and bacterial suspensions have been treated in the same conditions but separately.
- **Fragile molecules tested:** Several therapeutic molecules molecular weight ranging from 1200 g/mol up to 150 000 g/mol have been chosen.
- **Pharmacopoeia strains:** For this study, we choose micro-organisms issued from the European Pharmacopoeia : *Pseudomonas aeruginosa* (gram -) : ATCC 9027 ; *Staphylococcus aureus* (gram +) : ATCC 6538 ; *Candida albicans* (yeast) : ATCC 10231 ; Spores of *Aspergillus niger* (mould) : ATCC 16404 ; Spores of *Bacillus subtilis* (gram +): ATCC 9372 (ATCC = American Type Cell Collection)
- **Analytical Devices and Methods**
  - i. Dosage of antibodies has been performed, using an ELISA test.
  - ii. HPLC has been used to dose peptide A, peptide B and insulin.
  - iii. After HHP treatment, surviving colonies of reference samples and treated samples (pure calibrated suspensions of micro-organisms) have been enumerated with a microbiology

analytical method: “plate count method”. The logarithmic reduction was calculated by difference between the references and the samples.

## Results

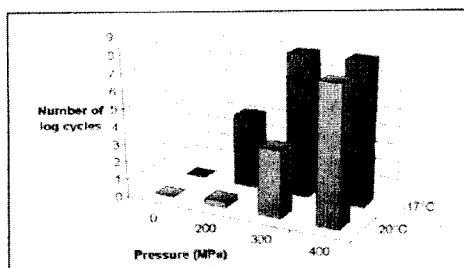
### a – Inocuity of High Hydrostatic Pressure treatment on fragile molecules

- i. *Peptide A and peptide B* have been treated during 10 min at 300, 400, 500 MPa at 20 °C. HPLC dosage results did not show a difference higher than 1% between treated samples and the reference.
- ii. *Insulin* has been treated during 10 min at 400, 500, 600 MPa (20°C) and 600 MPa (–20°C). HPLC assays did not show any difference higher than 1% between treated samples and the reference.
- iii. In the same way, *monoclonal antibodies anti-influenza A* have been treated for 10 min at 400, 500, 600 MPa (20 °C) and for 10 min at 600 MPa (–20°C). An ELISA test showed that at 600 MPa (–20°C) and (20°C) antibodies have lost a fraction of their specificity. But this phenomenon seems to be reversible according to the reference.

### b – Efficacy of High Hydrostatic Pressure treatment of micro-organisms

#### i. *Pseudomonas aeruginosa* : ATCC 9027

**Figure 1** shows values of log reductions obtained after 10 min of HHP treatment at 200, 300, 400 MPa, 20°C (in red) and –17°C (in blue). At 20°C 400 MPa, micro-organisms **were totally inactivated** (the number of log cycles reduction was **7.68**). The association of HHP (300 MPa) with cold temperature (-17°C) has shown a stronger log reduction (**8.05**) than at 20°C (**3.73**), for the same pressure (**Table 1**).



**Figure 1** Inactivation of *P. aeruginosa*

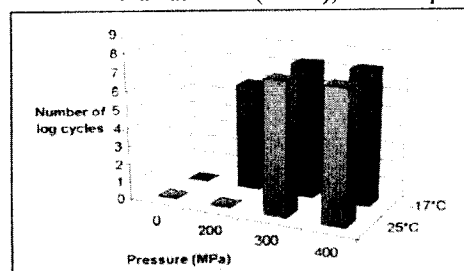
	0 MPa (reference)	200 MPa	300 MPa	400 MPa
20°C	0	0.32	3.73	<b>7.68</b>
-17°C	0	4.12	<b>8.05</b>	<b>8.05</b>

**Table 1** Values of the number of log cycle for each treatment.

#### ii. *Candida albicans* : ATCC 10231

Yeast appeared to be very sensitive to pressure. *C. albicans* was **totally inactivated** by a HHP treatment (10 min at 300 MPa, 25°C or –17°C, **Figure 2 and Table 2**).

Like *P. aeruginosa*, the inactivation by HHP treatment at –17°C (in blue), is higher than at 25°C (in red), at same pressures (**Table 3**).



**Figure 2** Inactivation of *C. albicans*

	0 MPa (reference)	200 MPa	300 MPa	400 MPa
25°C	0	0	<b>7.20</b>	<b>7.20</b>
-17°C	0	5.88	<b>8.05</b>	<b>8.05</b>

**Table 2** Values of the log reduction obtained with the HHP treatment of *C. albicans* at several pressures and temperatures

#### iii. *Staphylococcus aureus* : ATCC 6538

From 200 MPa up to 600 MPa, 10 min HHP treatment could not totally inactivate *S. aureus* (**Figure 3 and Table 3**). The reduction was around 2 log cycles and there is no

significant difference between the results obtained at 25°C (in red) and at -17°C (in blue).

But variations of parameters such as duration or number of cycles allowed us to obtain a total inactivation of *S. aureus* and a log reduction around 8.3 at 500 MPa, 25°C (Table 4, Figure 4).

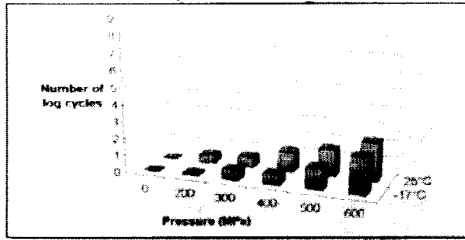


Figure 3 Inactivation of *S. aureus* vs. pressure and temperature

	0 MPa (reference)	200 MPa	300 MPa	400 MPa	500 MPa	600 MPa
25°C	0	0.51	0.63	1.17	1.6	2.26
-17°C	0	0.14	0.60	0.64	1.26	2.11

Table 3 Values of the log reduction obtained with the HHP treatment of *S. aureus* at different pressures and temperatures

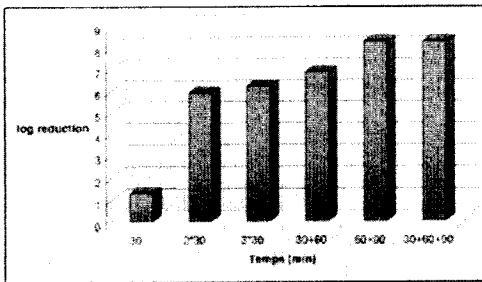


Figure 4 Influence of the duration of the HHP treatment on inactivation *S. aureus* at 500 MPa, 25°C

	30 min	2x30 min	3x30 min	30+60 min	60+90 min	30+60+90 min
25°C	1.27	5.80	6.20	6.86	8.28	8.28

Table 4 Values of the log reduction obtained with several HHP treatments of *S. aureus*.

- iv. Spores of *Aspergillus niger* (ATCC 16404) and spores of *Bacillus subtilis* (ATCC 9372):

Figure 5 and Table 5 show that a HHP treatment (10 min, -17°C, 600 MPa) led to reduce the concentration of spores of *A. niger* to 6.86 log. But spores of *B. subtilis* remain baroresistant at these conditions.

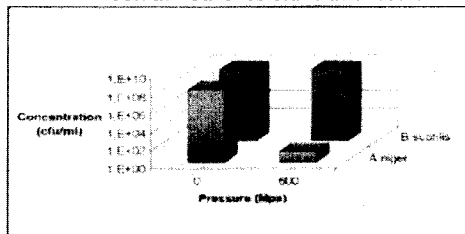


Figure 5 Inactivation of spores of *B. subtilis* and *A. niger* vs. pressure at -17°C, 10 min

	0 MPa (reference)	200 MPa
<i>B. subtilis</i>	0	0
<i>A. niger</i>	0	6.86

Table 5 Values of the log reduction obtained with the HHP treatment of spores of *B. subtilis* and *A. niger* at 600 MPa, -17°C, 10 min

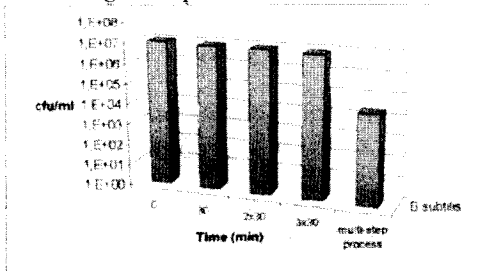


Figure 6 Inactivation of spores of *B. subtilis* at several durations (500 MPa, 25°C)

	0 min	30 min	2x30 min	3x30 min	Multi-step process
<i>B. subtilis</i>	0	0	0	0	3

Table 6 Values of the log reduction obtained with the HHP treatment of spores of *B. subtilis* at 25°C, 500 MPa and several durations of treatment

Spores of *B. subtilis* have been then exposed to several durations of HHP treatment at 500 MPa, 25°C. Only a multi-step process : (30 min at 60 MPa + 30 min at 500 MPa) x 3 decreased the concentration of endospores with a significant value of 3 log (Figure 6, Table 6).

### Discussion - Conclusion

It is well-known that high pressure kill bacteria [10], and that endospores are more resistant than other microorganisms and must be caused to germinate in order to be inactivated [11].

We showed that range of pressure (from 200 up to 600 MPa) and temperature (from -20 up to 25 °C) which allowed us to inactivate yeast cells and vegetative micro-organisms chosen from the European Pharmacopoeia, could be applied to many fragile drugs without altering them. We obtained better results at low temperature (-20°C) than at 25°C for vegetative bacteria (*P. aeruginosa*, + 4.32 log at 300 MPa) and yeast (*C. albicans*, + 5.88 log at 200 MPa).

Results obtained on spores of *B. subtilis* (3 log) are quite encouraging, so further developments are undertaken in order to validate this process as a sterilisation method, especially interesting for decontamination of fragile drugs.

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### References

- [1] HEDEN C.G. : Effects of hydrostatic pressure on microbial systems. Bacteriological Review, 1964, 28 (1), 14-29.
- [2] BALNY C., MASSON P. : Effects of high pressure on proteins. Food Review International, 1993, 9 (4), 611-628.
- [3] MASSON P. : Pressure denaturation of proteins. In: Balny C., Hayashi R., Heremans K., Masson P. : High pressure and biotechnology. Colloque INSERM/John Libbey Eurotext Ltd, 1992, vol. 224, 89-99.
- [4] KUNUGIS S. : Effect of pressure on activity and specificity of some hydrolytic enzymes. In : Balny C., Hayashi R., Heremans K., Masson P. : High pressure and biotechnology. Colloque INSERM/John Libbey Eurotext Ltd, 1992, vol. 224, 129-137.
- [5] HEREMANS K. : Aspects théoriques des effets des hautes pressions sur les biomacromolécules. In : Technologies agroalimentaire, Dossier scientifique de l'IFN n°3, 1993, 3-20.
- [6] MOREAU C. : Semi-continuous high pressure cell for liquid processing. Nottingham University Press. Reading Symposium, 1993.
- [7] MACDONALD A.G. : Effects of high hydrostatic pressure on natural and artificial membranes. In : Balny C., Hayashi R., Heremans K., Masson P. : High pressure and biotechnology. Colloque INSERM/John Libbey Ltd, 1992, vol. 224, 67-75.
- [8] CHONG G., COSSINS A.R. : A differential polarized fluorometric study of the effects of high hydrostatic pressure upon the fluidity of cellular membranes. Biochemistry, 1983, 22 (2), 409-415.
- [9] LAMBERT Y., Application du paramètre pression dans les procédés de l'agro-alimentaire : pascalisation, transport de masse, congélation et mise au point d'une gamme d'emballage, université bordeaux I, 1999, thèse n°2107
- [10] LUDWIG H. and SCHRECK C. The inactivation of vegetative bacteria by pressure, H. P. research in the biosciences and biotech., K. Heremans (Ed.), Leuven Univ. Press, Leuven, Belgium, 1997
- [11] LUDWIG H., BIELER C., HALLBAUER K., SCIGALLA W., Inactivation of microorganisms by hydrostatic pressure, in C Balny, R Hayashi, K Heremans and P Masson (eds), High Pressure biotechnology, colloque INSERM/John Libbey Eurotext Ltd., 1992, 224, 25-32.