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Evaluation of the effectiveness of different antifoams for an α_1 PI solution

M.P. Flament [a], P. Leterme [a], T. Burnouf [b], A.T. Gayot [a,*]

[a] *Faculté des Sciences Pharmaceutiques et biologiques, Laboratoire de Pharmacotechnie Industrielle, BP 83, rue du Professeur LAGUESSE, 59006 LILE, Cedex, France*
[b] *LFB, 59 rue de Trevise, 59011 Lille, Cedex, France*

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Abstract

Foam appears when α_1 protease inhibitor (α_1 PI) is solubilised and nebulised. The purpose of this study was to select antifoams likely to be associated with an α_1 PI to be nebulised and to reveal the interactions between α_1 PI and the antifoams tested. Antifoam efficacity as regards α_1 PI solution, was evaluated by measuring antifoaming capacity and examining foam stability. The surface tensions of the antifoam solutions and of the α_1 PI solution alone or with antifoams were determined. The influence of the antifoam concentration was evaluated. The compounds tested show high antifoam capacity. Our study revealed different types of foam destabilisation processes: firstly through interactions with α_1 PI in solution, modifying protein diffusion properties at the interface; secondly through a competitive phenomenon at the interface which leads to a total displacement of the protein or to a mixed film containing both protein and surfactant. Span 65 at a 0.025% concentration and cetyl alcohol at a 0.05% concentration associated with tyloxapol at a 0.025% concentration have an antifoaming capacity compatible with the pulmonary administration of α_1 PI. For these compounds, the surface tension study of the different solutions made it possible to reveal the formation of a mixed film containing both protein and antifoam at the interface. © 1997 Elsevier Science B.V.

Keywords: α_1 Protease inhibitor; Antifoams; Antifoaming capacity; Surface tensions; Protein/antifoam interactions

1. Introduction

α_1 Protease inhibitor (α_1 PI), a protein used in pulmonary emphysema treatment and envisaged in that of cystic fibrosis is traditionally administered by the parenteral route. However, only a

* Corresponding author. Tel.: +33 320964048; fax: +33 320959009.

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small proportion of the dose can reach the lungs after intravenous injection. The administration of this protein by the pulmonary route will make it possible to decrease the required doses as it will reach the action site directly.

The α_1 PI concentrate comes in the form of a lyophilized solid that must be reconstituted with water for injection. Foam appears during solubilisation and nebulisation.

Adsorption of the foaming agent at the air/water interface as well as the presence of gas below the liquid surface are necessary for foam formation. This adsorption results in a higher concentration at the interface than inside the liquid (Othmer, 1969). According to Gibbs's equation, adsorption results in a decrease in the surface tension of the solution.

Foaming capacity is influenced by the mechanical properties of the surface film that is formed following adsorption. To have stable foam, cohesion between molecules on the interfacial film must be sufficient to ensure that they remain at the surface.

Foam destruction is connected with a surface activity which corresponds, in the most extreme case, to antifoam adsorption at the air/liquid interface which displaces the foaming agent from this interface and takes its place. This displacement by preferential adsorption creates an interface which does not possess the film elasticity necessary to maintain lasting foams (Othmer, 1969; Derchivian, 1956; Althaus et al., 1974; Flannigan, 1988; Roberts et al., 1976; Ruyssen and Molle, 1965). In the intermediate case, the antifoam does not completely displace the foaming agent. Total or partial adsorption produces surface tension inferior to that obtained with the foaming agent alone.

Antifoam concentration is important: there is an optimal concentration below which efficacity is lower and beyond which foam stabilisation can occur by strengthening cohesion between molecules (Roberts et al., 1976). This concentration has to be determined for each foaming agent/antifoam system.

Because of the presence of foam in the α_1 PI solution, control of droplet size and the quantity nebulised is not possible during nebulisation. This present work is concerned with the choice of antifoams and their concentration likely to be used. The action mechanism of antifoams for which efficiency is satisfactory will be determined by analysing the surface tension measurements of protein and surfactant solutions as well as antifoaming capacity.

2. Materials and method

2.1. Materials

A total of 5 ml of water for injection is added to 100 mg of freeze dried α_1 PI (LFB, Lille, France). The α_1 PI used is a glycoprotein of human origin with a molecular weight of 52 000 Da, made up of a polypeptidic chain with 394 amino acids on which are attached glycanic chains constituting 12% of the molecular weight (Hubbard and Crystal, 1990; Crystal, 1990). α_1 PI shows an isoelectric zone varying from 4.7 to 5.

The antifoams studied, in addition to having antifoam properties in aqueous solution, have to be administrable by the pulmonary route. Those selected from articles already published and their concentrations are as follows: Span 65 (ICI, Essen, Germany), 0.0001–0.1%; Sodium caprylate (Merck, Paris, France), $1.2.10^{-4}$–0.03%; Silbione 70460, Silbione 70414 (Rhône Poulenc, Courbevoie, France), 0.001–0.1%; Sodium oleate (Henkel, Boulogne Billancourt, France), 0.01–0.1%; and Cetyl alcohol (Henkel, Boulogne Billancourt, France), 0.025–0.15%.

A total of 5 ml of antifoam aqueous solution is added to 100 mg of freeze dried α_1 PI. The silbiones used are non-ionic external aqueous phase emulsions containing polydimethylsiloxanic oil. They differ from one to another as regards the content and viscosity of the polydimethylsiloxanic oil.

Cetyl alcohol is used alone or associated with tyloxapol (SIGMA Chimie, St Quentin Fallavier, France), a non ionic surfactant, phenol 4 (1,1,3,3-tetramethylbutyl) polymer with formaldehyde and oxirane, tested at concentrations varying from 0.01 to 0.05%. The tyloxapol facilitates the aqueous dispersion of cetyl alcohol because of its

surfactant properties. It is used, along with the same cetyl alcohol and with the same objective, in 'Surfoxo Neonatal', a preparation for pulmonary administration.

2.2. Method

2.2.1. Antifoaming efficacity evaluation

Antifoam efficacity as regards the α_1 PI solution is judged by evaluating antifoaming capacity and by examining the remaining foam that has to disappear rapidly.

The antifoaming capacity of the selected agents is measured with a device using the principle described in the AF NOR norm NTT 73413 and is determined by comparing the foam of the α_1 PI solution alone to that added to antifoams at the above mentioned concentrations.

The device used consists of a narrow cylindrical glass tube, the bottom of which is sintered-glass (porosity no. 5) through which a compressor emits air into the solution which may lead to foam formation (Fig. 1). A scale graduated in millimeters makes it possible to read the foam height after 2 min contact between air and solution. Actually, a longer time results in a non-homogeneous layer of foam and does not make it possible to read the foam heights. Antifoaming capacity is defined by:

$$\frac{\text{foam heights of}\left(\alpha_1 \text{ PI solution} - \alpha_1 \text{ PI solution with antifoam}\right)}{\text{foam height of } \alpha_1 \text{ PI solution}} \times 100$$

After the addition of each antifoam to the α_1 PI solution, the appearance of the remaining foam is observed, in particular with regard to its fluidity and its transience.

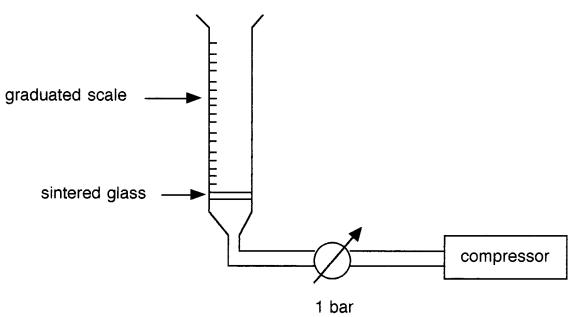

Fig. 1. Device used to evaluate antifoaming capacity.

2.2.2. Surface tension measurement

Destabilization of alphalactalbumine foams by adsorption of tween 20 was studied by Clark et al. (1991) through surface tension measurements of protein and surfactant solutions. In the present work, the surface tensions of the antifoam solutions and of the α_1 PI solution, alone and associated with antifoams, are compared to reveal the action mechanism of antifoams.

The surface tension measurement method consists in measuring the force that has to be exerted on a platine/irridium stirrup piece, which is in contact with the solution surface, to stretch the liquid film.The duration necessary for film breaking varies with the interfacial film characteristics.

Surface tension measurements are made after 30 min, as soon as the solution is obtained, with a LAUDA TD1 tensiometer (Prolabo, Paris, France) whose measuring range is 0–100 mN/m, precision 0.1 mN/m and sensitivity 0.001 mN/m.

3. Results and discussion

The antifoaming capacity increases with the antifoam concentration to reach a maximum and then decreases,except for the Silbiones where a decrease in antifoaming capacity in relation to concentration is not observed (Table 1).

The concentration retained for each antifoam system tested is the one for which the antifoam capacity is higher (Table 2).

All the compounds show high antifoaming capacity, particularly the silbiones, Span 65, cetyl alcohol alone or along with tyloxapol, being in these cases higher than 90%.

The α_1 PI solution presents a very compact foam, hard and very stable. In the presence of sodium caprylate or cetyl alcohol, the foam obtained is also hard, compact and disappears only very slowly. The addition of Span 65 or the cetyl alcohol/tyloxapol mixture leads to the formation of a very liquid foam which disappears quickly. In the presence of sodium oleate, foam behaviour is intermediate between the two just quoted. With

Table 1
Antifoaming capacity of silbione 70414 and Span 65 for α_1 PI solution according to concentration

Antifoam and concentrations (%)	Antifoaming capacity (%)
Silbione 70414	
0.001	40.2
0.01	72.5
0.05	85.7
0.1	93.7
Span 65	
0.01	24
0.0175	61.3
0.025	93.1
0.05	45

the Silbiones, the foam is liquid and very transient; it disappears immediately when the compressor is stopped.

The surface tension of the α_1 PI solution, the α_1 PI/antifoam mixtures and the single antifoam solutions were determined (Table 3).

In all cases, the presence of antifoam in the α_1 PI solution decreases surface tension more than with the α_1 PI solution alone.

All the aqueous solutions of antifoam alone, except for sodium caprylate, have a surface tension lower than that of the α_1 PI solution. These results concord with antifoam activity.

A comparison of the surface tension of the antifoam solutions without α_1 PI to those of the α_1 PI-antifoam associations makes it possible to consider protein antifoam interactions. Three groups can be differentiated:

- The silbione group for which the surface tension of α_1 PI/Silbione association is the same as that of Silbione, indicating a total displacement of α_1 PI by the antifoam.
- Sodium caprylate for which the surface tension of the α_1 PI/sodium caprylate mixture is lower than that of sodium caprylate alone. This latter surface tension is superior to that of water for injection and indicates that sodium caprylate has a lower concentration at the interface than within the solution.

The surface tension of the α_1 PI/sodium caprylate mixture is barely lower than that of the α_1 PI solution alone. The antifoam activity exhibited results from an α_1 PI/sodium caprylate interaction within the solution, modifying protein diffusion properties at the interface, and this decreases adsorption.

- The group containing sodium oleate, Span 65 and cetyl alcohol, alone or along with tyloxapol, for which the surface tension of the α_1 PI solution containing antifoam is higher than that of antifoam alone but lower than that of α_1 PI alone. This shows an interaction between α_1 PI and antifoams at the interface.

To describe this interaction, surface tensions of antifoam solutions and of the α_1 PI antifoam associations, as well as antifoaming capacities were measured for different antifoam concentrations.

Table 2
Antifoaming capacity of the tested antifoamg for a 2% α_1 PI solution.

Antifoam	Antifoaming capacity (%)
Silbione 70460 0.05%	93
Silbione 70414 0.1%	93.7
Span 65 0.025%	93.1
Cetyl alcohol 0.05%	91.7
Cetyl alcohol 0.05%+Tyloxapol 0.025%	90.1
Sodium oleate 0.025%	87.5
Sodium caprylate 1.2 10^{-4}%	83.4

Table 3
Surface tension of the antifoam solutions, alone or with α_1 PI

Solution considered	Surface Tension (mN/m)	
	Without α_1 PI	With α_1 PI
Water for iniection	70	53
Silbione 70460 0.05%	36.7	36.7
Silbione 70414 0.1%	42.9	43
Span 65 0.025%	37.25	49
Cetyl alcohol 0.05%	33.55	49.9
Cetyl alcohol 0.05%+ tyloxapol 0.025%	39.55	47.5
Sodium oleate 0.025%	26.35	35.15
Sodium caprylate $1.2.10^{-4}$%	71.2	51.25

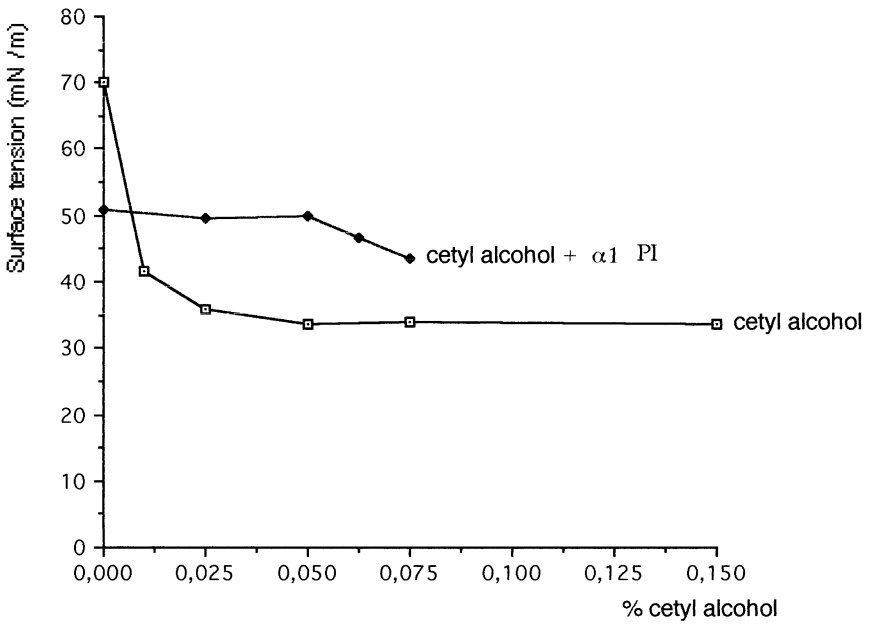

Fig. 2. Surface tension variation of a cetyl alcohol aqueous dispersion at different concentrations, with or without α_1 PI.

For these four agents in aqueous solution, the antifoam solution surface tension curves according to concentration show a gradual adsorption of antifoams up to a maximum.

The results obtained with cetyl alcohol are presented in Fig. 2. It is for a 0.05% concentration of cetyl alcohol dispersed alone in water that its adsorption is maximum. In the case of the α_1 PI cetyl alcohol association, the surface tension is practically constant below this same concentration of 0.05% and then decreases with the cetyl alcohol concentration. The cetyl alcohol begins to adsorb at the interface and penetrates the α_1 PI interfacial film for a concentration of 0.05%. From this same concentration, surface tension of the cetyl alcohol aqueous dispersion alone barely varies with concentration. This concentration is the one for which antifoam capacity towards α_1 PI was found to be maximum as Table 4 results indicate.

Identical behaviour is observed with Span 65 and cetyl alcohol associated with tyloxapol. For the discussion, cetyl alcohol will be taken as a model.

The behaviour of the protein-sodium oleate association is different (Fig. 3). The surface tension variations of the protein/sodium oleate association are parallel to those obtained with sodium oleate alone. The 0.025% concentration corresponds to the bending point of the two curves. It is the concentration at which foam height is minimum (Table 5).

During surface tension measurement of the α_1 PI/sodium oleate solution, film stretching duration is important, indicating that the protein/sodium oleate film is more cohesive than the one obtained with other antifoams. This concords with the stability of the remaining foam.

Table 4
Antifoaming capacity of cetyl alcohol at different concentrations for a 2% α_1 PI solution

Cetyl alcohol concentration (%)	Antifoaming capacity (%)
0.025	66.89
0.050	91.70
0.0625	69.65
0.075	62.75

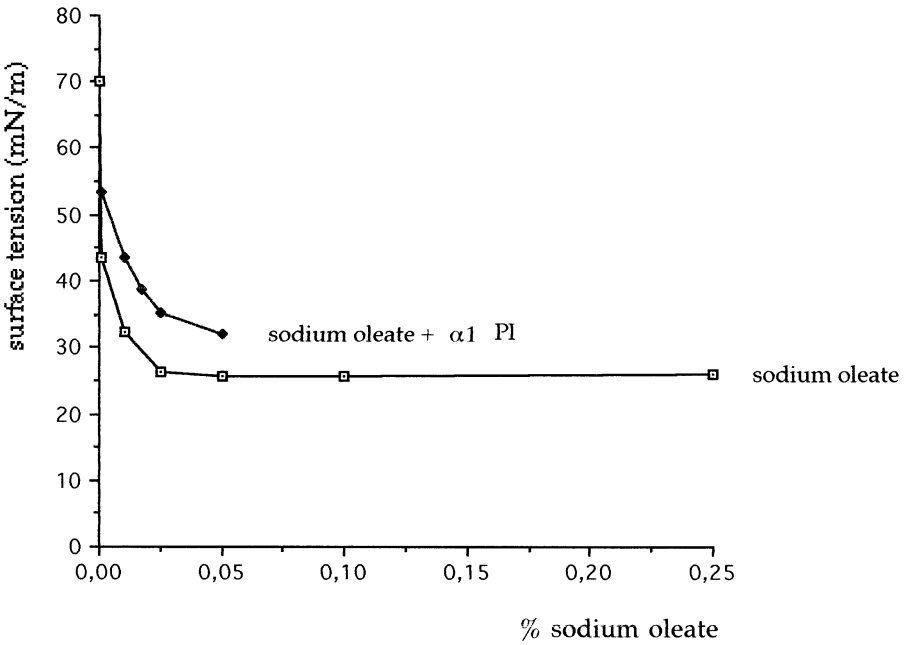

Fig. 3. Surface tension variation of a sodium oleate aqueous solution at different concentrations, with or without α_1 PI.

The comparison of results obtained from agents represented by cetyl alcohol and from sodium oleate shows that their behaviour is different when they are associated with α_1 PI. The first adsorbs at the interface only from a given concentration on, the second as soon as α_1 PI is added. This behaviour should be related to the surfactant properties of these compounds: the surface tension of the sodium oleate solution is lower than that of the other compounds.

When the antifoam capacity is maximum, the agents represented by cetyl alcohol and sodium oleate form a mixed layer at the interface, decreasing the protein/protein interactions at the surface, which in turn increases protein mobility at the surface and decreases film stability. For higher concentrations of sodium oleate or agents represented by cetyl alcohol, stabilising of remaining foam occurs by the strengthening of cohesion between molecules. In the case of sodium oleate, the similitude between the two curves indicates that sodium oleate strongly influences the interfacial film properties for the α_1 PI/sodium oleate association. Sodium oleate greatly penetrates the α_1 PI film and spreads there but it does not completely replace α_1 PI which leads to a surface tension higher than that of sodium oleate alone.

Table 5
Antifoaming capacity of sodium oleate at different concentrations for a 2% α_1 PI solution

Sodium oleate concentration (%)	Antifoaming capacity (%)
0.001	35.02
0.01	40.68
0.0175	76.55
0.025	87.5
0.05	35.02

The characteristics of the interfacial film in the presence of sodium oleate and cetyl alcohol are different.

In the case of sodium oleate, adequate adsorption is necessary to destabilise the film nearly saturated with antifoam and for which subsequent addition leads to a strengthening of cohesion.

In the case of cetyl alcohol, the film is destabilised by cetyl alcohol adsorption which only

appears at a given concentration. Beyond this, cetyl alcohol adsorption is still possible and responsible for the formation of bindings with α_1 PI which stabilise the film.

The antifoam has to be chosen not only from determining antifoam capacity evaluated by measuring height, but also from studying the appearance of this foam. Surface tension measurement, making it possible to estimate film stretching duration, gives us more precisions on foam fugacity.

Sodium caprylate and oleate have therefore not been retained. The former has too low an antifoam capacity, and although the antifoam capacity of sodium oleate is higher, it is associated with a longer film stretching duration. Despite very favorable antifoam properties, silbiones were not retained because an elaborate toxicological study on animals would be necessary for such an application. As regards cetyl alcohol, the appearance of remaining foam is more satisfactory when it is associated with tyloxapol.

We have checked that protein activity is not modified by surfactant addition whatever the agent used and whatever type of interaction takes place.

4. Conclusion

It is difficult to predict the activity of a compound with regard to a foaming agent. Antifoam activity determination can be made through an evaluation of volume, appearance and fugacity of this foam. Surface tension measurements of solutions and comparisons of foaming, antifoam and associations make it possible to reveal interactions.

Our study has made it possible to reveal different types of foam destabilisation processes: through interactions with α_1 PI in solution or through a competitive phenomenon for the interface.

A study of the influence of antifoam addition and technological parameters on aerosol efficiency will be made by using as an antifoam either Span 65 at a 0.025% concentration, or cetyl alcohol at a 0.05% concentration associated with tyloxapol at a 0.025% concentration.

References

Afnor. Norm NTT 73413. Surface agents. Determination of foaming ability, antifoaming capacity and foam stability. Bubbling method.

Althaus, W., Baronnet, M., Voissière, G., Wischniewski, M., 1974. Activité antimousse des diméthylpolysiloxanes à usage gastro-enterologique et mesure de leur activité. Ann. Pharm. Franc. 32, 193–201.

Clark, D.C., Wilde, P.J., Wilson, D.R., 1991. Destabilization of alpha lactalbumin foams by competitive adsorption ofthe surfactant Tween 20. Coll. Surfaces 59, 209–223.

Crystal, R.G., 1990. Alpha 1 antitrypsin deficiency,emphysema and liver disease-Genetic base and strategies for therapy. J. Clin. Invest. 85, 1343–1352.

Dervichian, G., 1956. Les mousses: formation, stabilité, destruction. Bull. Soc. Chim. France 1, 15–22.

Flannigan, W.T., 1988. Silicone antifoams: new perspectives. Speciality Chem. 8, 420–426.

Hubbard, R.C., Crystal, R.G., 1990. Augmentation therapy of α_1 antitrypsine deficiency. Eur. Resp. J. 3, 445–525.

Othmer K., 1969. Foams. In: Encyclopedia of Chemical Technology, 2nd ed., vol. 20, Interscience, New York, pp. 773–791.

Roberts, K., Axberg, C., Österlund, R., 1976. Emulsion foam killers in foams containing fatty and rosin acids. In: Aker, R.J. (Ed.), Foams. Academic Press, London, pp. 39–49.

Ruyssen, R., Molle, L., 1965. Principes de chimie physique a l'usage des pharmaciens et des biologistes, chapitre XIV: les mousses, Masson, Paris, 485.

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