Influence of packaging material on the liquid stability of interferon-α2b

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ABSTRACT. Purpose: In this article we studied the effect of the packaging material on the liquid stability of interferon alpha 2b (rhIFN-α2b). **Methods**: The compatibility of this cytokine with type I borosilicate glass ampoules was evaluated by ELISA and RP-HPLC, at 4ºC and after heat sealing. Additionally, the influence of protein concentration (3 and 10 MIU/ml), buffer species (sodium phosphate, sodium citrate and sodium citrate-phosphate) and additives (polysorbate 80 and EDTA Na_2 x $2H_2O$) were studied in samples with and without contact with chlorobutyl stoppers by RP-HPLC. **Results**: The compatibility of this cytokine in sodium phosphate buffer, with type I borosilicate glass ampoules showed a significant adsorption at the lowest concentration. This influence was eliminated with a polysorbate 80/benzyl alcohol-based vehicle. The effect of the heat sealing of ampoules on the stability of rhIFN-α2b showed two degradation peaks when a volume of 1 ml was dispensed. However, with a lower (0.5 ml) volume, the degradation was not detected. On the other hand, samples in contact with chlorobutyl stoppers increased the apparent degradation rate constant in the range of 6.74 ± 0.38 to 46.34 ± 3.11 x 10^3 day^{-1}. This effect significantly decreased in about 1.2- and 1.1-fold when sodium citrate or sodium citrate-phosphate buffers, respectively, were evaluated. Results from the evaluation of EDTA Na_2 x $2H_2O$ or polysorbate 80 showed a similar behavior. These additives reduced the apparent degradation rate constant in the range of 2.01 ± 0.14 to 25.51 ± 3.57 x 10^3 day^{-1}. **Conclusions**: The adsorption of the cytokine to type I borosilicate glass ampoules was eliminated with a polysorbate 80/benzyl alcohol-based vehicle, and the deleterious effect of the heat sealing decreased with a lower (0.5 ml) volume. Experimental data indicated that the contact with chlorobutyl stoppers accelerates the degradation of rhIFN-α2b. However, protein concentration, buffer species and pharmaceutical excipients can modulate this effect.

INTRODUCTION

Interferon alpha 2b (rhIFN-α2b) is a cytokine with a wide use in viral, neoplasic and immunological diseases due to its strongly verified biological properties (1; 2). Many factors can affect the liquid stability of this and other proteins, inducing a great number of physical and chemical degradation pathways (3). One of such factors is the packaging material (3; 4). Several authors have studied the adsorption of proteins to surfaces of untreated glass, siliconized glass, polyester, polypropylene, nylon, silicone rubber and cellulose acetate (3). These studies have demonstrated the instability of proteins at these surfaces, probably due to the adsorption-induced protein denaturation (3).

The general accepted mechanism explaining this effect is based on the high surface tension at the liquid/packaging material interfaces. This tension usually leads proteins to the loss of their tridimensional structure, and increases the probability of adsorption, aggregation, denaturation and other physical and chemical reactions (3).

Surfactants have been frequently used to reduce this effect (5; 6). The role of detergents has been to protect proteins through the competition for the adsorption at different interfaces. Furthermore, interactions with the protein surface have been described (3). In this sense, detergents may cover the hydrophobic sites of proteins reducing the occurrence of aggregation or act as "chaperonins" to catalyze the refolding of partially unfolded molecules (6).

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Most of the biopharmaceutical drugs are prepared in borosilicate glass vials sealed with chlorobutyl stoppers. The effect of these materials on the stability of proteins must also be studied. Earle *et al*. evaluated the increment of the residual moisture due to chlorobutyl stoppers, on the stability of *Haemophilus influenzae* conjugate vaccine (7). However, the gum-stoppers induced degradation has not been clearly studied in protein solutions.

Herein, we evaluated the effect of borosilicate glass ampoules and chlorobutyl stoppers on the stability of rhIFN-α2b in solution.

MATERIALS AND METHODS
Materials
The Center for Genetic Engineering and Biotechnology (CIGB, Havana, Cuba) supplied the rhIFN-α2b with the characteristics previously described for this cytokine (8; 9). All chemicals used were of analytical grade. In particular, the following chemicals were purchased from Merck (Darmstadt, Germany): citric acid, sodium citrate dihydrate, sodium phosphate monobasic dihydrate, sodium phosphate dibasic anhydrous, polysorbate 80 and EDTA Na_2 x $2H_2O$. Acetonitrile was purchased from Caledon (Georgetown, Ont., Canada) and trifluoroacetic acid was acquired from Pierce (Rockford, Illinois, USA). Type I borosilicate glass vials were acquired from Nuova OMPI (Piombino Dese, Italy) and rubber stoppers plus flip-off seals were from Helvoet Pharma (Alken, Belgium).

Methods
Compatibility of type I borosilicate glass ampoules with rhIFN-α2b in solution
rhIFN-α2b was diluted to 1.5, 3, 5, 10 and 20 million international units per milliliter (MIU/ml) in sodium phosphate buffer or in a polysorbate 80/benzyl alcohol-based vehicle, and 0.5 ml was dispensed in borosilicate glass ampoules (Bormioli Rocco, Parma, Italy). Each vial was sealed with parafilm and stored at 4ºC for 24 or 120 h. The compatibility of glass vials with rhIFN-α2b was estimated by determining the concentration of ELISA-quantified rhIFN-α2b present at 24 or 120 h of storage, compared to the initial concentration.

rhIFN-α2b was also diluted to 3 MIU/ml, in 50 mM sodium phosphate buffer, pH 6 (43.8 mM sodium phosphate monobasic dihydrate, 6.2 mM sodium phosphate dibasic anhydrous). The interferon-containing samples of 0.5 or 1 ml were dispensed into borosilicate glass ampoules (Bormioli Rocco, Italy) and analyzed by ELISA and RP-HPLC before and after the heat sealing of ampoules.

Effect of the protein concentration on the liquid stability of rhIFN-α2b in contact with stoppers
rhIFN-α2b was diluted to 3 and 10 MIU/ml in 50 mM sodium phosphate buffer, pH 6. Samples of 1 ml were dispensed in 2R borosilicate glass vials, stored at 37ºC with or without contact with stoppers and analyzed by RP-HPLC at time zero and after 3, 6, 9, 15, 21 and 30 days. Experiments were done in triplicate.

Influence of buffer species on the liquid stability of rhIFN-α2b, in contact with stoppers
PD-10 desalting columns (Amersham Biosciences AB, Upsala, Sweden) were used to change the buffer composition on each sample. Columns were previously equilibrated with 50 mM sodium citrate buffer, pH 6 (6.4 mM citric acid, 43.6 mM sodium citrate dihydrate), 50 mM sodium citrate-phosphate buffer, pH 6 (19 mM citric acid, 31 mM sodium phosphate dibasic anhydrous) or 50 mM sodium phosphate buffer, pH 6. rhIFN-α2b were diluted to 3 MIU/ml, and 1 ml was dispensed in borosilicate vials. Each vial was sealed with a chlorobutyl stopper and 13-mm flip-off aluminum seal, stored at 37ºC with or without contact with stoppers, and analyzed by RP-HPLC at time zero and after 3, 6, 9, 15, 21 and 30 days. Experiments were accomplished in triplicate.

Influence of polysorbate 80 and EDTA Na_2 x $2H_2O$ on the liquid stability of rhIFN-α2b, in contact with stoppers
rhIFN-α2b was diluted to 3 MIU/ml in 50 mM sodium phosphate buffer, pH 6, and then polysorbate 80 or EDTA Na_2 x $2H_2O$ were added to the solution to a final concentration of 5 mM. Samples of 1 ml were dispensed in borosilicate glass vials, stored at 37ºC with or without contact with stoppers and analyzed by RP-HPLC at time zero and after 3, 6, 9, 15, 21 and 30 days. Experiments were done in triplicate.

Analysis of the rhIFN-α2b solutions by Enzyme Linked ImmunoSorbent Assay (ELISA)
This procedure was performed as previously described (10; 11).

Analysis of the rhIFN-α2b solutions by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)
RP-HPLC analysis was performed on a Vydac (Hesperia, Calif., USA) wide pore octyl (C8) column (5 µm; 125 x 4.6 mm). Solvents and gradients were A: 0.1% aqueous trifluoroacetic acid (TFA) and B: 0.05% TFA in acetonitrile (15% to 60% B in 40 min). The flow rate was 0.8 ml/min. Detection was performed at 226-nm with automatic data processing using the Unicorn version 4.10 software (Amersham Biosciences AB, Upsala, Sweden) for data acquisition and analysis. Purity was calculated as the relation between the area of the main peak and the area of contaminant peaks.

Statistical analysis
The statistical significance of the experimental data was determined by the unpaired student-t or ANOVA tests, after a comparison of the homogeneity of variance (Bartlett test) (Sigarroa, 1985). When $P \leq 0.05$, ANOVA test was followed by a Duncan Multiple Range test to determine the specific groups showing significant differences.

RESULTS AND DISCUSSION

Compatibility of type I borosilicate glass ampoules with rhIFN-α2b in solution
Adsorption is one of the physical influences that affect the stability of proteins in solution (3). This degradation route can be induced at different sites such as container/solution and air/solution interfaces. The severity of this process usually depends on the protein concentration and the protein itself, and has been inhibited with surfactants (3; 6).

We evaluated the effect of the glass ampoules on the stability of rhIFN-α2b at different concentrations. In this experiment we found that rhIFN-α2b was adsorbed only at the lowest concentration (1.5 MIU/ml) (Table 1). However, at higher concentrations, differences between results at the initial time and after 24 hours of storage were not significant (Table 1). The use of ELISA to evaluate the adsorption of the active ingredient enhanced the reliability of these results due to the ability of this technique to quantify the correctly folded interferon and recognize when degraded species coexist in the protein solution that has been analyzed (11).

Table 1. Compatibility of borosilicate glass ampoules with rhIFN-α2b diluted in sodium phosphate buffer.

Concentration (x 10^6 IU/ml)	Time (h)	ELISA concentration (µg/ml)	Recovery (%)	α (0.05)
1.5	0	7.61 ± 0.22	-	
	24	7.13 ± 0.16	93.69	0.01
3	0	21.62 ± 2.23	-	
	24	20.71 ± 2.57	95.79	0.07
5	0	30.92 ± 1.37	-	
	24	29.58 ± 0.73	95.67	0.07
10	0	62.71 ± 4.42	-	
	24	60.33 ± 2.98	96.21	0.14
20	0	115.38 ± 2.12	-	
	24	114.91 ± 2.76	99.59	0.55

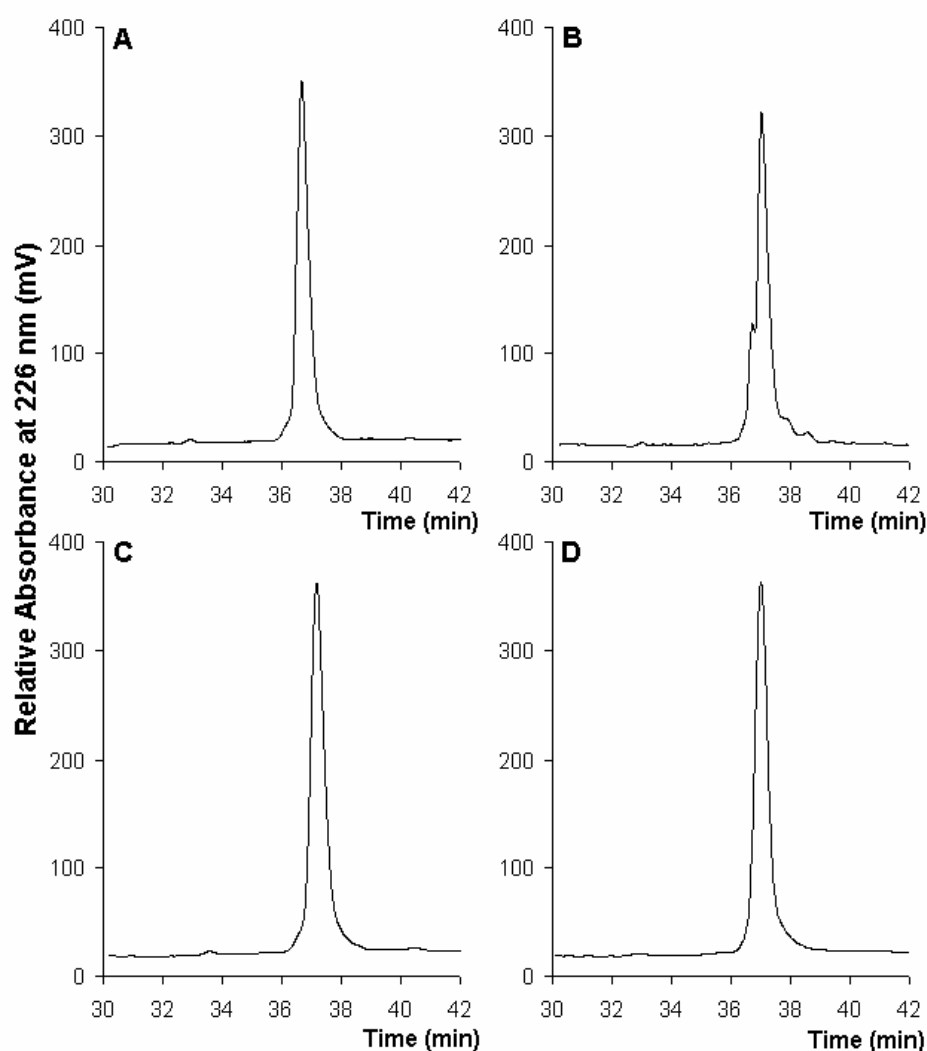Results are expressed as mean (n=3) ± standard deviation (SD).

Figure 1. Effect of the heat sealing of borosilicate ampoules on the RP-HPLC profile of rhIFNα-2b. Chromatographic profiles of 1-ml samples are given in A (before sealing) and B (after sealing). Additionally, chromatographic profiles of 0.5-ml samples are given in C (before sealing) and D (after sealing).

In order to decrease the adsorption of 1.5 MIU/ml rhIFN-α2b to borosilicate glass ampoules, we evaluated the effect of a polysorbate 80/benzyl alcohol-based vehicle, at four concentrations of the cytokine. After this, we did not find statistical differences (α=0.97) between the recovery of rhIFN-α2b in the solution at the initial time and after 24 or 120 hours of storage, even at 1.5 MIU/ml (Data not shown).

The use of surfactants (e.g., polysorbate 80) has seen effective to protect proteins against adsorption (3; 5; 6). The mechanism of protection involves two main pathways (6). One such pathways is based on the specific interaction with the protein surface, covering hydrophobic sites and acting as "artificial chaperonins" to catalyzed the refolding of partially unfolded proteins (6). The second one involves the competition with the protein for the adsorption to various interfaces where chemical or physical degradation may occur (6).

Our results indicated that the stability of rhIFN-α2b at low concentrations can be compromised due to the influence of borosilicate

glass ampoules; however, the use of a detergent-based vehicle may inhibit this process.

Influence of the sealing of type I borosilicate glass ampoules on the liquid stability of rhIFN-α2b

Borosilicate glass ampoules are usually sealed by heating. Although this is a very rapid process, drugs must be very stable to efficiently retain the stability during and after heating. Here, we evaluated the effect of the sealing of ampoules by heating, after dispensing 1 ml of the rhIFN-α2b solution. Results obtained by RP-HPLC showed three degradation by-products which affected the purity of the native rhIFN-α 2b peak (Fig. 1 A, B).

Although the use of the ELISA has proven the ability of this technique to quantify the correctly folded rhIFN-α2b (11), statistical analysis did not show significant differences (p=0.67) before (18.82 ± 0.74 µg/ml) and after (18.61 ± 0.81 µg/ml) sealing despite the chromatographic profile. In fact the three moieties eluting before and after the rhIFN-α2b main peak were only a very low percent of the total protein (less than 5 % each one). Additionally, the early eluting species has demonstrated to retain the same biological activity and immunoidentity than the native rhIFN-α2b (2).

Contrarily, results obtained by RP-HPLC showed one peak without degradation species, when a lower (0.5 ml) volume was dispensed into borosilicate ampoules before the heat sealing (Fig. 1 C, D). It seems that low volumes of rhIFN-α2b

solutions must be dispensed into these vials if the sealing process will be based on heating.

Influence of chorobutyl stoppers on the liquid stability of rhIFN-α2b

Although the stabilization of proteins can be very variable, rhIFN-α2b has shown to be stable at different concentrations as well as in the presence of different buffers and additives (13; 14).

In this study, rhIFN-α2b showed a chromatographic profile characterized by an early eluting species and a moiety eluting just after the rhIFN-α2b main peak (Fig. 2). The first of such degradations could correspond to a Met sulfoxide by-product (2). The exact chemical identity of the later fraction remains under investigation and will be published elsewhere. These degradation products were greater induced in those samples in contact with gum stoppers and the concentration of the protein, the buffer species and the use of additives affected their intensity.

This fact clearly showed the deleterious effect of the gum stoppers on the stability of rhIFN-α2b. Two factors can be considered in order to explain this influence. The possible release of heavy metal ions to the solutions, and other degradation routes induced at the rubber-liquid interface, as explain ahead.
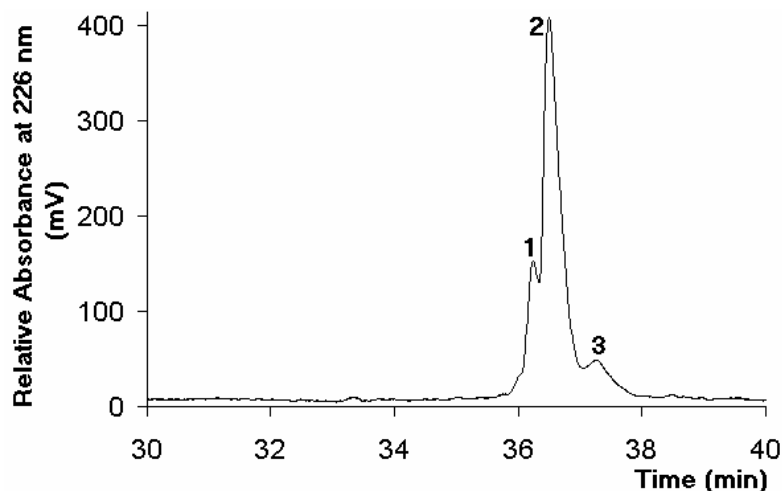

Figure 2. Effect of chlorobutyl stoppers on the RP-HPLC profile of rhIFNα-2b after 30 days of storage at 37 ºC. Peak 1: early eluting species; peak 2: rhIFN-α2b main peak; 3: moiety eluting just after the rhIFN-α2b main peak.

In order to kinetically evaluate the stability of the cytokine, we determined the percent of the area of the main native rhIFN-α2b peak as well as the area under this chromatographic peak. These two parameters offered an idea of the purity and the true quantity of unmodified rhIFN-α2b, respectively. The acceleration of the thermal inactivation resulted in a decline of both parameters with time. However, the decomposition effect was remarkably greater for the area under the chromatographic peak (area). This fact might be explained due to the probable occurrence of degradation routes that affect the area (e.g., precipitation and adsorption), without affecting the purity.

Influence of the concentration of rhIFN-α2b on the liquid stability of this cytokine in contact with chlorobutyl stoppers

Samples at the lowest concentration of the active ingredient (3 MIU/ml) that were stored without contact with stoppers reduced the loss of the purity and area in about 1.82- and 1.28-fold respectively, as compared to those samples that were stored in contact with chorobutyl stoppers (Fig. 3; Table 2). Similarly, 10 MIU/ml rhIFN-α2b stored without contact with gum stoppers decreased the loss of the same parameters in about 1.86- and 1.25-fold, respectively, compared to those solutions in contact with this packaging material (Table 2). Note that protein concentration affected this behavior. Consequently, 3 MIU/ml rhIFN-α2b decreased the RP-HPLC-determined parameters in a lower extension than 10 MIU/ml rhIFN-α2b solutions (Table 2). Kinetic analyses from the comparison between samples at both concentrations showed that diluted solutions that were stored without contact reduced the loss of the purity and area in about 1.43- and 1.22-fold respectively, compared to the concentrated samples at the same storage condition (Table 2).

Similar results were obtained when protein solutions were stored in contact with stoppers. In this case, kinetic analyses indicated that 3 MIU/ml rhIFN-α2b decreased the loss of the purity and area in 1.47- and 1.18-fold, respectively, compared to 10 MIU/ml rhIFN-α2b solutions (Table 2).
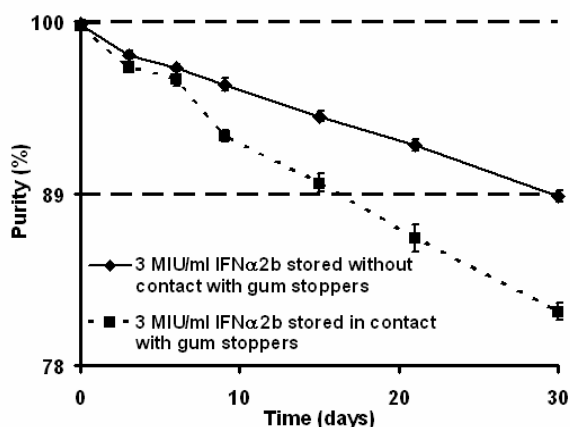

Figure 3. Effect of chlorobutyl stoppers on the RP-HPLC-determined purity of rhIFNα-2b. The cytokine rhIFNα-2b was used at 3 MIU/ml.

It has been suggested that the increase of the protein concentration to higher than 0.02 mg/ml may facilitate the potential aggregation or other degradation routes in these macromolecules (15). This influence can be explained due to the increment on the intermolecular collisions that usually increase the degradation of proteins (16).

The effect of the gum stoppers combined with the high temperature could increase the protein damage due to the adsorption-induced degradation processes (3). This process is usually concentration-dependent, therefore, we might expect a higher interface-induced degradation at higher concentrations of rhIFN-α2b.

Table 2. Kinetic parameters of the influence of chlorobutyl stoppers on the rhIFN-α2b thermal degradation at two concentrations of the cytokine.

Concentrations	In contact with chlorobutyl stoppers	
	Purity[a] (k x 10^3 (day^{-1}))	Area[b] (k x 10^3 (day^{-1}))
3 MIU/ml	6.74 ± 1.22	39.11 ± 2.84
10 MIU/ml	9.92 ± 0.96	46.34 ± 3.11
	Without contact with chlorobutyl stoppers	
	Purity[a] (k x 10^3 (day^{-1}))	Area[b] (k x 10^3 (day^{-1}))
3 MIU/ml	3.71 ± 0.62	30.47 ± 3.61
10 MIU/ml	5.32 ± 0.85	37.12 ± 3.14

[a]Determination of the purity of the native rhIFN-α2b, as determined by RP-HPLC.
[b]Determination of the recovery of area under the peak corresponding to the native rhIFN-α2b, as determined by RP-HPLC.
Results are expressed as mean (n=3) ± standard deviation (SD).

Table 3. Kinetic parameters of the influence of chlorobutyl stoppers on the rhIFN-α2b thermal degradation in the presence of different buffer species.

Buffer species	In contact with chlorobutyl stoppers	
	Purity[a] (k x 10^3 (day^{-1}))	Area[b] (k x 10^3 (day^{-1}))
Sodium citrate	5.52 ± 0.14	29.55 ± 2.41
Sodium citrate-phosphate	6.15 ± 0.31	32.51 ± 3.62
Sodium phosphate	6.74 ± 0.38	39.13 ± 2.95
	Without contact with chlorobutyl stoppers	
	Purity[a] (k x 10^3 (day^{-1}))	Area[b] (k x 10^3 (day^{-1}))
Sodium citrate	2.75 ± 0.23	20.21 ± 2.13
Sodium citrate-phosphate	3.21 ± 0.14	23.15 ± 2.46
Sodium phosphate	3.72 ± 0.29	33.45 ± 2.62

[a]Determination of the purity of the native rhIFN-α2b, as determined by RP-HPLC.
[b]Determination of the recovery of area under the peak corresponding to the native rhIFN-α2b, as determined by RP-HPLC.
Results are expressed as mean (n=3) ± standard deviation (SD).

Influence of the buffer species on the liquid stability of rhIFN-α2b in contact with chlorobutyl stoppers

Buffer species affected the grade of the protein degradation (Table 3). Although the RP-HPLC profile was identical to that described before (Fig. 2), rhIFN-α2b prepared in sodium citrate and sodium citrate-phosphate and stored without contact with chlorobutyl stoppers reduced the loss of the purity in 1.35- and 1.16–fold, respectively, compared to those prepared in sodium phosphate buffer (Table 3). Similar kinetic results were obtained from the analysis of the area. In this case, inhibition of the loss of this parameter in samples prepared in sodium citrate and sodium citrate phosphate buffers was 1.65- and 1.2-fold greater, compared to those prepared in sodium phosphate buffer (Table 3).

Table 4. Kinetic parameters of the influence of chlorobutyl stoppers on the rhIFN-α2b thermal degradation in the presence of EDTA Na$_2$ x 2H$_2$O and polysorbate 80.

Additives	In contact with chlorobutyl stoppers	
	Purity[a] (k x 10^3 (day^{-1}))	Area[b] (k x 10^3 (day^{-1}))
EDTA Na$_2$ x 2H$_2$O	5.01 $\pm$ 0.33	25.51 $\pm$ 3.57
Polysorbate 80	4.24 $\pm$ 0.24	13.37 $\pm$ 2.82
	Without contact with chlorobutyl stoppers	
	Purity[a] (k x 10^3 (day^{-1}))	Area[b] (k x 10^3 (day^{-1}))
EDTA Na$_2$ x 2H$_2$O	2.39 $\pm$ 0.22	18.72 $\pm$ 2.71
Polysorbate 80	2.01 $\pm$ 0.14	8.21 $\pm$ 3.25

[a]Determination of the purity of the native rhIFN-α2b, as determined by RP-HPLC.
[b]Determination of the recovery of area under the peak corresponding to the native rhIFN-α2b, as determined by RP-HPLC.
Results are expressed as mean (n=3) $\pm$ standard deviation (SD).

These results indicated that the stability of rhIFN-α2b is considerably higher in sodium citrate and sodium citrate-phosphate buffers. Differences on the role of these buffer species on the stability of proteins have been previously discussed (17). Chen *et al*. found that citrates decreased the aggregation rate of the keratinocyte growth factor (17). They speculated that this effect was explained due to the presence of negative charges in the buffer ions, which interact with the positive amino acid clusters on the protein. Thus, the protein was stabilized through this charge-charge interaction (17).

Taking into account this speculation we should expect a similar stabilization of other proteins like rhIFN-α2b in the presence of the sodium phosphate buffer due to the negative charges that phosphate ions exhibit. However, our results were largely different (Table 3). The general explanation could be based on the presence of the trace amounts of metal ions on these salts which may accelerate the protein damage (18). In contrast, sodium citrate and sodium citrate-phosphate buffers stabilized rhIFN-α2b on the basis of the combination of the abovementioned mechanism (charge-charge interaction) together with the ability of citrates to act as chelating agents of the trace amounts of metal ions that could be present in protein solutions.
In general, the contact with chlorobutyl stoppers accelerated the protein degradation in all the three evaluated buffers (Table 3).

The impact of the buffer composition on the protection against the stopper-induced degradation was estimated through the comparison of the results that were obtained at both, with and without contact with chlorobutyl stoppers. As a result, sodium citrate-, sodium citrate-phosphate- and sodium phosphate-based samples that were stored in contact with gum stoppers, reduced the purity of rhIFN-α2b in about 2-, 1.91- and 1.81-fold respectively, compared to the same samples stored without contact with this primary pack (Table 3). Similarly, analyses of the area showed a reduction on this parameter in 1.46-, 1.4- and 1.17-fold, respectively, for the same solutions stored in contact with chlorobutyl stoppers, compared to the same samples stored without contact with this packaging material (Table 3). It seems that buffers containing citrates had a higher impact on the inhibition of the stoppers-induced degradation of this protein.

Given the ineffectiveness of citrates to completely inhibit the stoppers-induced degradation of rhIFN-α2b, we can speculate that the release of heavy metal ions from the chlorobutyl gum to aqueous solution is not the main mechanism involved on the deleterious effect of this protein when it is stored in contact with gum stoppers. It seems that other factors like the presence of metal ions in the salts of the buffers may increase the degradation of rhIFN-α2b at both conditions, with and without contact with chlorobutyl stoppers. Therefore, other mechanisms (e.g. adsorption-induced degradation at liquid/stoppers interfaces) might explain the influence of the gum stoppers on the stability of rhIFN-α2b.

Influence of polysorbate 80 and EDTA Na₂ x 2H₂O on the liquid stability of rhIFN-α2b in contact with chlorobutyl stoppers

In order to elucidate the main mechanism that explains the effect of the gum stoppers on the stability of rhIFN-α2b, we evaluated the influence of two additives. One of such additives, EDTA Na₂ x 2H₂O, is a chelating agent that has been frequently used to stabilize proteins because of its ability to bind any harmful metal ion (3). The second one, polysorbate 80, is a nonionic surfactant that drops the surface tension for protein solutions and decreases the forces that drive proteins to aggregation by hydrophobic interactions (6).

In this experiment, EDTA Na₂ x 2H₂O increased the purity and the area of rhIFN-α2b in samples that were stored without contact with gum stoppers in about 2.09- and 1.36-fold compared to the samples stored in contact with this packaging material (Table 4). This fact might indicate that one possible degradation mechanism could be the delivery of metal ions from the gum stoppers to the solution. Nevertheless, we discarded this possibility given the ineffectiveness of EDTA Na₂ x 2H₂O to completely eliminate the stopper-induced degradation of rhIFN-α2b. However, the presence of contaminating metal ions in the buffer salts (as described before) which can accelerate the damage of the protein, and its binding by EDTA Na₂ x 2H₂O, might also explain the effect of this chelator on the preservation of the stability of rhIFN-α2b.

On the other hand, results from experiments with polysorbate 80 indicated that this detergent increased the purity and the area of the cytokine in samples stored without contact with chlorobutyl stoppers in about 2.11- and 1.63-fold, respectively, compared to those stored in contact with this primary pack (Table 4). From these data, it is evident that the non ionic detergent was not absolutely effective on the preservation against the stopper-induced degradation. However, it might be explained due to the probable presence of metal ions in the buffer salts that could accelerate the thermal degradation of rhIFN-α2b. In any case, polysorbate 80 was more effective than EDTA Na₂ x 2H₂O against the stopper-induced degradation of this protein, specially, in the analysis of the area of the main rhIFN-α2b peak, as kinetic evaluation indicated (Table 4). These evidences pointed to that the protein damage induced at the stopper/liquid interfaces could

be the most important mechanism explaining the deleterious effect of chlorobutyl stoppers on the stability of rhIFN-α2b.

CONCLUSIONS

The compatibility of rhIFN-α2b in sodium phosphate buffer with type I borosilicate glass ampoules showed a significant adsorption at the lowest concentration of the active ingredient. However this effect was absolutely eliminated when a polysorbate 80/benzyl alcohol-based vehicle was used instead of the sodium phosphate buffer. On the other hand, the heat sealing of ampoules affected the stability of rhIFN-α2b when a 1-ml volume was dispensed to these vials. In contrast, this effect was not found with a lower (0.5 ml) volume. Additionally, experimental data indicated that the contact with stoppers might accelerate the degradation of rhIFN-α2b, probably due to adsorption-induced destabilization mechanisms. However, this effect can be modulated as a function of the concentration of the protein and the use of different buffer species and excipients.

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