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International Journal of Pharmaceutics 114 (1995) 177–184

international journal of pharmaceutics

# Inhibition of granulocyte colony stimulating factor (G-CSF) adsorption to polyvinyl chloride using a nonionic surfactant

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Received 28 July 1993; modified version received 17 June 1994; accepted 21 July 1994

**Abstract**

The effect of poloxamer 407 (Pluronic® F-127), a nonionic surfactant, on the adsorption of granulocyte colony stimulating factor (G-CSF) to polyvinyl chloride (PVC) was assessed. G-CSF, at concentrations less than 15 μg/ml, was combined with human serum albumin (HSA), poloxamer 407, and HSA plus poloxamer 407 in dextrose 5% in water (D5W) and the percent of G-CSF bound to PVC compared with G-CSF in D5W alone. The concentrations of poloxamer 407 evaluated for their effect on G-CSF adsorption to PVC were 0.005, 0.05, and 0.5% (w/w), whereas solutions which contained HSA with or without poloxamer 407 employed HSA at a concentration of 2 mg/ml only. A concentration of 0.05% w/w poloxamer 407 in a solution of G-CSF in D5W provided a significant ($p < 0.05$) decrease in the percent of G-CSF bound to PVC compared to a solution of G-CSF alone. Poloxamer 407 alone at a concentration of 0.05% w/w was just as effective as HSA alone (2 mg/ml) at inhibiting adsorption of G-CSF to PVC. However, inhibition of G-CSF surface adsorption to PVC was not dependent on the concentration of poloxamer 407 when the surfactant was combined with HSA. Incorporation of poloxamer 407 at each of the three concentrations selected in a G-CSF/HSA solution provided no additional reduction in G-CSF adsorption to PVC when compared to a G-CSF solution which contained only HSA at a concentration of 2 mg/ml. Over the G-CSF concentration range of 0.3–300 ng/ml, the amount of G-CSF adsorbed per square centimeter of PVC was significantly greater at 45°C compared to G-CSF solutions evaluated at 22°C. Poloxamer 407 at a concentration of 0.05% w/w may show promise as a solvent additive with which to minimize G-CSF adsorption to PVC.

*Keywords:* Recombinant granulocyte colony stimulating factor; G-CSF; Poloxamer 407; Protein adsorption; Polyvinyl chloride; Temperature effect

## 1. Introduction

With an ever-increasing number of protein drugs being approved by the Food and Drug Administration for use in humans, there exists a need to optimize formulation strategies designed to prevent protein drugs from undergoing aggregation, precipitation, adsorption, and denaturation with subsequent loss in biological activity. To date, most protein drugs have been formulated as a lyophilized powder for reconstitution at the

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*SSDI* 0378-5173(94)00236-3

time of administration. Alternatively, solvent additives are often incorporated into protein drug solutions.

Since adsorption of protein drugs to glass and polymeric materials may negate the intended therapeutic effect, this process of physical instability of a protein drug formulation must be inhibited. One method suggested to decrease surface adsorption of protein drugs to glass and polymeric surfaces is to add a surface-active agent in low concentration. Preferably, the surface-active agent should be nonionic and nondenaturing. We have shown previously that poloxamer 407 (Pluronic® F-127), a block copolymer comprised of polyoxypropylene and polyoxyethylene units, does not cause a reduction in the enzymatic activity of both a model enzyme (urease) (Fults and Johnston, 1990) and a model recombinant-derived protein (interleukin-2; rIL-2) (Pec et al., 1992). In addition, this same surfactant was shown to prevent loss in the biological activity of urease and rIL-2 when both proteins in solution were subjected to vigorous solution agitation (Wang and Johnston, 1993a). The secondary structures of both urease and rIL-2 were also preserved when each protein was incubated with poloxamer 407 (Wang and Johnston, 1993a). Lastly, poloxamer 407 was shown to prevent thermally induced, irreversible inactivation of urease and rIL-2 at temperatures less than 50°C (Wang and Johnston, 1993b). Thus, it was reasoned that poloxamer 407 might be useful as a solvent additive to minimize/eliminate protein surface adsorption.

Recombinant-derived granulocyte colony stimulating factor (G-CSF) was selected as a model recombinant protein drug with which to study surface adsorption. It has been reported that the continuous administration of G-CSF as an intravenous (i.v.) infusion may have distinct advantages with regard to reversing drug and/or disease-induced neutropenia compared to intermittent i.v. bolus doses (Lieschke et al., 1990). Since recombinant G-CSF administered as an i.v. infusion might potentially be delivered from a polyvinyl chloride (PVC) infusion bag, we investigated whether incorporation of poloxamer 407 into a G-CSF solution would inhibit surface adsorption of the protein. One manufacturer of G-CSF states that at G-CSF concentrations less than 15 $\mu$g/ml, human serum albumin (HSA) should be added to the G-CSF solution to a final concentration of 2 mg/ml. Our aim in this study was to determine whether poloxamer 407 alone or in combination with HSA would offer an advantage in terms of G-CSF adsorption to PVC. In addition, we sought to characterize the adsorption profile of G-CSF binding to PVC and determine whether we could identify a critical concentration of poloxamer 407 which would prevent G-CSF adsorption to PVC. Lastly, we investigated the effect of temperature on G-CSF adsorption to PVC.

## 2. Materials and methods

### 2.1. Materials

Recombinant granulocyte colony stimulating factor (Neupogen,® Amgen Inc., Thousand Oaks, CA) at a concentration of 300 $\mu$g/ml (specific activity = $1.0 \pm 0.6 \times 10^8$ U/mg), human serum albumin (Alpha Therapeutic Corp., Los Angeles, CA) at a concentration of 25 mg/ml, and dextrose 5% in water (D5W) were purchased directly from The University of Illinois Hospital Pharmacy (Chicago, IL) and used as received. Poloxamer 407, NF (conforming to NF XVII specifications) was a gift from BASF (Parsippany, NJ) and used as received. ELISA kits used for the quantitative determination of G-CSF were purchased from Research and Diagnostic Systems (Minneapolis, MN).

### 2.2. Adsorption studies using poloxamer 407

This study evaluated the extent of G-CSF adsorption to PVC in the presence of HSA, poloxamer 407, or both excipients. Solutions were prepared as listed in Table 1. All solutions containing poloxamer 407 (solutions 3–11 in Table 1) were prepared the evening prior to the experiment to facilitate dissolution of the polymer in D5W. All i.v. infusion bags used were 1 l in volume (except i.v. bags used in the G-CSF-tem-

perature study) and were made of polyvinyl chloride (Viaflex® (Baxter Health Care, Round Lake, IL)). On the day of the experiment, HSA and/or G-CSF were added to each bag (except bags 3–5) as indicated in Table 1. Addition of the two proteins to the 1 l i.v. infusion bags was performed using a standard pipetteman. A triangular section of the top edge of each bag was removed using a scissors so that the HSA and G-CSF could be conveniently added to each bag. The volume of G-CSF solution added to each 1 l bag was 30 $\mu$l to yield a final G-CSF concentration of 9000 pg/ml. The cut corner of each bag was then closed and secured so that the bags were neither open to the air nor contained a headspace above the G-CSF solution. All G-CSF/D5W solutions were then gently mixed by inverting the bags in an end-over-end fashion. In addition to the i.v. infusion bags made of PVC, one glass volumetric flask was also evaluated for G-CSF adsorption.

At 1 h following the preparation of each solution listed in Table 1, the solutions were sampled in triplicate. Unlike removal of the G-CSF from the stock solution contained in the original vial of G-CSF (300 $\mu$g/ml), solution samples from each bag were removed using a pipetteman with pipette tips which had previously been washed three times with a portion of the test solution in each bag. The solution samples (20 $\mu$l) removed from each bag were diluted 10-fold directly in the wells of the 96-well microtiter plate contained in the ELISA detection kit. It should be noted that the samples of each solution were added to 180 $\mu$l of D5W already contained in the wells. The assay was then performed and the results expressed as the percent of the original amount of G-CSF placed into each bag which was unbound or soluble. The percent of the original amount of G-CSF placed into each bag that was soluble was first corrected for the diminution in the absorbance reading caused by HSA and poloxamer 407 (Fig. 1 and 2). Previous pilot studies in our laboratory had quantitatively determined the reduction in the absorbance values that a G-CSF/D5W solution (9000 pg/ml) which contained these additives would produce as the concentration of the excipients was incrementally increased (Fig. 1 and 2). Therefore, any additional reduction in the mean absorbance values from the values expected when G-CSF was quantitated in the presence of these excipients was assumed to reflect G-CSF adsorption to PVC. The surface area calculated for each PVC bag was approx. 711 and 248 cm$^2$ for the 1 and 0.25 l i.v. bags, respectively.

Table 1
Composition of test solutions to evaluate surface adsorption of G-CSF to polyvinyl chloride (PVC)

| Solution no. | Surfactant (% w/w) | | Protein (9 ng/ml) | Stabilizer (2 mg/ml) |
|---|---|---|---|---|
| 1 | – | | +G-CSF | – |
| 2 | – | | +G-CSF | +HSA |
| 3 | +0.5% | P-407 | – | – |
| 4 | +0.05% | P-407 | – | – |
| 5 | +0.005% | P-407 | – | – |
| 6 | +0.5% | P-407 | +G-CSF | – |
| 7 | +0.05% | P-407 | +G-CSF | – |
| 8 | +0.005% | P-407 | +G-CSF | – |
| 9 | +0.5% | P-407 | +G-CSF | +HSA |
| 10 | +0.05% | P-407 | +G-CSF | +HSA |
| 11 | +0.005% | P-407 | +G-CSF | +HSA |

HSA, human serum albumin, G-CSF, granulocyte colony stimulating factor, and P-407, poloxamer 407

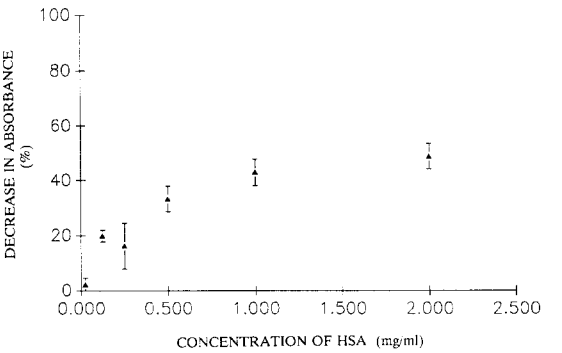


Fig. 1. Effect of HSA on G-CSF quantitation using the enzyme-linked immunosorbent assay. All values represent the mean ± standard deviation of three absorbance readings.

## 2.3. Quantitative determination of G-CSF

The ELISA kit used to measure the amount of G-CSF in an aqueous medium had a calibration curve that ranged from 39.1 to 2500 pg/ml. In brief, the principle of the assay involves the use of a quantitative immunometric, 'sandwich en-

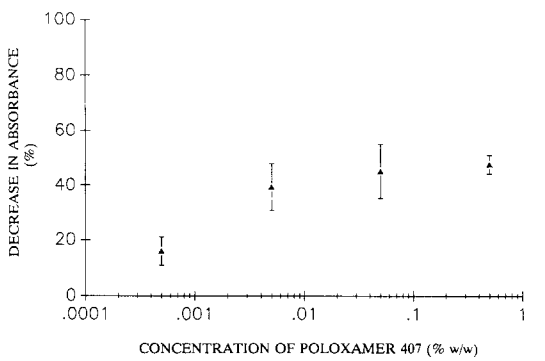


Fig. 2. Effect of poloxamer 407 on G-CSF quantitation using the enzyme-linked immunosorbent assay. All values represent the mean ± standard deviation of three absorbance readings.

zyme technique. A monoclonal antibody specific for G-CSF had been coated onto a microtiter plate provided with the kit. Standards with known amounts of G-CSF and samples were pipetted into the wells, and any G-CSF present was bound by the immobilized antibody. After washing away any unbound sample proteins, an enzyme-linked polyclonal antibody specific for G-CSF was added to the wells and allowed to bind the G-CSF which was bound during the first incubation. Following a wash to remove unbound antibody-enzyme reagent, a substrate solution was added to the wells and the color which developed was proportional to the amount of G-CSF bound in the initial step. The color development was stopped and the intensity of the color was measured. All microtiter plates were read at 450 nm on a 96-well microplate reader (Bio-Rad, Richmond, CA).

### 2.4. Adsorption-temperature study

This study examined the effect of increasing temperature on the adsorption of G-CSF at various concentrations of the protein. The concentrations of G-CSF tested were 0.3, 3.0, 30, 100, and 300 ng/ml. All solutions were prepared the evening prior to the experiment. Into ten, 250 ml i.v. infusion bags made of PVC, G-CSF was added as described above. Half of the bags were then placed in a water bath maintained at 45 ± 0.1°C and half were stored at ambient temperature (22 ± 0.1°C). The next day, prior to sampling the 10 bags, five additional bags were freshly prepared with the above concentrations of G-CSF. After careful mixing of the last set of five bags, all bags were then sampled as described above and the concentration of G-CSF determined in each bag. This protocol was selected based on previous findings in our laboratory which evaluated the kinetics of G-CSF adsorption to PVC (data not shown). In that experiment, storage of G-CSF solution samples in vials made of either glass or plastic (polypropylene) prior to the ELISA procedure resulted in extensive adsorption.

From a knowledge of the concentration of G-CSF detected at each G-CSF concentration evaluated in the five bags prepared immediately prior to the experiment, the amount of G-CSF

Table 2
Statistical comparison of the percent soluble G-CSF for the G-CSF test solutions

| | G-CSF HSA | G-CSF 0.5% P-407 | G-CSF 0.05% P-407 | G-CSF 0.005% P-407 | G-CSF HSA 0.5% P-407 | G-CSF HSA 0.05% P-407 | G-CSF HSA 0.005% P-407 |
|---|---|---|---|---|---|---|---|
| G-CSF | [a] | – | [a] | – | [a] | – | [a] |
| G-CSF HSA | | [a] | – | [a] | – | – | – |
| G-CSF 0.5% P-407 | | | – | – | [a] | – | – |
| G-CSF 0.05% P-407 | | | | [a] | [a] | – | – |
| G-CSF 0.005% P-407 | | | | | [a] | – | [a] |
| G-CSF HSA 0.5% P-407 | | | | | | – | – |
| G-CSF HSA 0.05% P-407 | | | | | | | – |

[a] Significant difference ($p < 0.05$) between test solutions.

which had been adsorbed to each bag at 22 and 45°C was calculated. The amount of G-CSF adsorbed was then normalized for surface area of PVC and the data expressed as the mass of G-CSF adsorbed per $cm^2$ of PVC vs the G-CSF concentrations evaluated.

### 2.5. Statistical analysis

Statistical significance between mean values was determined using Student's *t*-test (Snedecor and Cochran, 1980). A significant difference in the mean values of the percent soluble G-CSF (Fig. 3) between any two solutions sampled 1 h following their preparation is shown in Table 2.

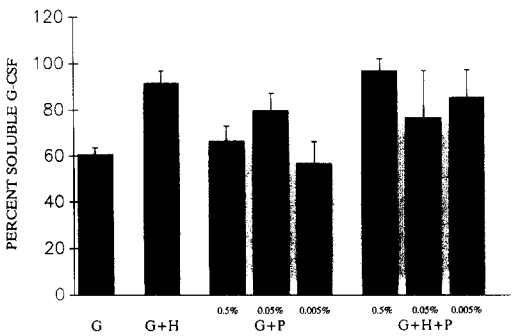


Fig. 3. Effect of solvent additives on the adsorption of granulocyte colony stimulating factor (G-CSF) to polyvinyl chloride (PVC). G, G-CSF (9000 pg/ml); H, human serum albumin (2 mg/ml); P, poloxamer 407. Concentrations shown above groups G+P and G+H+P refer to the concentrations of poloxamer 407 evaluated. All values represent the mean values ± standard deviation.

## 3. Results

### 3.1. G-CSF adsorption study

It can be noted in Table 2 that a concentration of 0.05% w/w poloxamer 407 in a solution of G-CSF in D5W provided a significant ($p < 0.05$) decrease in the percent of G-CSF bound (i.e., increase in the percent soluble G-CSF) to PVC when compared to a solution of G-CSF alone. Poloxamer 407 alone at a concentration of 0.05% w/w was just as effective as HSA alone (2 mg/ml) at inhibiting adsorption of G-CSF to PVC (Fig. 3 and Table 2). However, addition of poloxamer 407 at each concentration evaluated to a G-CSF solution which contained only HSA was no more effective at preventing adsorption of G-CSF to PVC than HSA alone (Fig. 3). That is, inhibition of G-CSF adsorption to PVC was not additive for the two excipients (HSA and poloxamer 407) tested.

### 3.2. Effects of temperature on G-CSF adsorption

Fig. 4 clearly illustrates that adsorption of G-CSF to PVC occurred to a much greater extent at 45°C compared to G-CSF solutions evaluated at 22°C. It should be noted that the data in Fig. 4 were not plotted as the amount of G-CSF adsorbed per $cm^2$ of PVC ($x/a$) vs the equilibrium concentration ($C_{eq}$) as for a standard adsorption isotherm. Instead, the data were plotted as $x/a$ vs the original concentration ($C_0$) of the G-CSF solutions tested. As stated above, this results from our previous kinetic study which demonstrated that storage of the G-CSF solution samples in either glass or polymeric (polypropylene) vials prior to assay resulted in extensive adsorption to the storage container (data not shown).

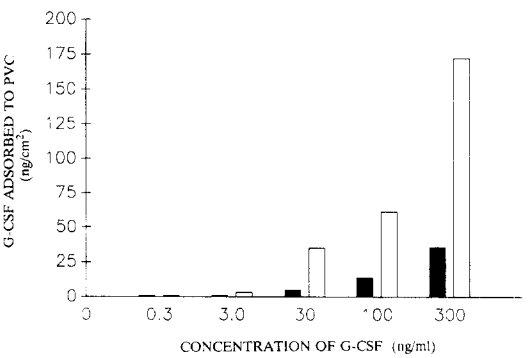


Fig. 4. Effect of temperature on G-CSF adsorption to PVC at various concentrations of G-CSF. (□,■) Mean values of the amount of G-CSF adsorbed per area of PVC at 45 and 22°C, respectively. All data points represent the mean values of duplicate determinations.

## 4. Discussion

Various approaches have been attempted to prevent loss of protein by adsorption to glass and plastic. These include coating polypropylene tubes with bovine serum albumin (Felgner and Wilson, 1976; Beyerman et al., 1981), coating glassware with 1% solutions of polyethylene glycol 20 000 in deionized water followed by drying at 110°C (Kramer et al., 1976), or by adding glycerol and Triton X-100 (Suelter and DeLuca, 1983). Suelter and DeLuca (1983) compared the effectiveness of two approaches to prevent adsorption of proteins to glass and plastic surfaces: modification of solvent and modification of the surface. Using bovine serum albumin, luciferase and mitochondrial creatine kinase as model proteins, these investigators found that modifying the solvent by adding Triton X-100 (0.2 mM final concentration) or glycerol (50% final concentration) was more effective than treating surfaces with proteins such as fibrinogen, globulin, ovalbumin, bovine serum albumin, and cytochrome *c* in reducing protein adsorption to glass and polyethylene. This may be attributed to the ability of the pharmaceutical protein to bind to the proteins coating the surface or to displace the proteins from the coated surface. The type of container used in an experiment is also an important consideration. For plastic containers, glycerol or Triton X-100 at either low or high ionic strength was effective in preventing adsorption of proteins. For glass containers, glycerol at low ionic strength and Triton X-100 at high ionic strength were the preferred solvents. Clearly, the best solvent to use needs to be determined for a specific protein. Our evaluation of G-CSF adsorption to glass demonstrated that 96.1% of the initial amount of G-CSF placed in the volumetric flask had undergone adsorption after an overnight incubation at room temperature. Surfactants such as poloxamer 188 (Pluronic® F-68) or polysorbate have also been used in injectable formulations (Coval, 1979) to prevent denaturation at surfaces, since proteins tend to concentrate at interfaces.

The mechanism by which either HSA or poloxamer 407 inhibited G-CSF adsorption to PVC is unclear. Further experimentation is required to elucidate the exact mechanism by which these additives inhibit G-CSF adsorption to PVC and will be the aim of future work conducted in our laboratory. However, the results presented in Fig. 3 of this study, as well as work by others (Law et al., 1983; Norman et al., 1992), provide suggestive evidence that G-CSF adsorption to PVC was inhibited by HSA due to binding of G-CSF with HSA. Presumably, binding of G-CSF to HSA would decrease the free fraction of G-CSF available for binding to the PVC surface. Alternatively, G-CSF adsorption to PVC could be inhibited in the presence of HSA due to a greater binding affinity of HSA for PVC than G-CSF. However, inhibition of G-CSF adsorption to PVC in the G-CSF/HSA/poloxamer 407 solutions tested in our study (Fig. 3) was not significantly ($p > 0.05$) greater than the inhibition in G-CSF adsorption in the G-CSF/HSA solutions even though the binding affinity of HSA to PVC in the former group should have potentially been negligible. This is supported by the findings of others (Norman et al., 1992) which reported that human serum albumin adsorption to a polymeric material (polystyrene) was negligible when the surface was in contact with poloxamers 235, 237, 238, and 407. Further support for the mechanism of binding of G-CSF with HSA to reduce the free fraction of G-CSF available for adsorption to PVC is provided by the findings of Law et al. (1983). It was demonstrated that an increased loss of thyroid hormone occurred on a filter at albumin concentrations of 2.5–5.0%. These authors (Law et al., 1983) attributed the loss of thyroid hormone on the filter to binding with HSA.

Evidence that poloxamer 407 may also bind with G-CSF and minimize its adsorption to PVC is also provided in Fig. 3. The optimal concentration of poloxamer 407 with which to inhibit G-CSF adsorption to PVC was 0.05% w/w in the group of solutions containing only G-CSF and poloxamer 407. This concentration is below the critical micelle concentration for poloxamer 407 which is 0.095% w/w at 25°C (Rassing and Attwood, 1983). If solubilization of G-CSF into surfactant micelles had been the mechanism by which poloxamer 407 alone inhibited adsorption of G-CSF to PVC, then the greatest inhibition of adsorption

should have occurred with a concentration of 0.5% w/w poloxamer 407. In fact, examination of Fig. 3 for the G-CSF/HSA/poloxamer 407 solutions tested would suggest that HSA has a greater binding affinity for G-CSF than poloxamer 407 since the concentration-dependent inhibition of G-CSF adsorption observed with poloxamer 407 alone is lost when HSA is also present. Again, further experimentation is required to determine the relative binding affinities of HSA and poloxamer 407 to G-CSF.

The present study has also shown that temperature plays an important role in the extent of surface adsorption of G-CSF to PVC. The increased adsorption of G-CSF to PVC at an elevated temperature may have been caused by partial/total unfolding of the tertiary structure of G-CSF. Unfolding of the three-dimensional conformation of G-CSF would presumably expose a greater number of hydrophobic residues typically buried within the interior of the protein to the more hydrophobic PVC surface. This may then result in a greater number of potential binding sites on the protein for the surface of the PVC bag and increase the extent of adsorption. However, further experimentation is required to verify this hypothesis. Increased adsorption of bovine serum albumin, $\beta$-lactoglobulin, and gelatin at an alumina-water interface have been shown to increase with an increase in temperature (Hajra and Chattoraj, 1991a–c). These authors suggested (Hajra and Chattoraj, 1991b) that the increased adsorption of these proteins at 45°C occurred from the hydrophobic effect controlling the adsorption process. Lastly, others (Zalazar et al., 1992) have demonstrated that the rate of adsorption of pure human IgG to polyvinyl chloride was proportionally increased with an increase in temperature from 4 to 37°C.

The present study has demonstrated that the nonionic detergent, poloxamer 407, can be used at a concentration of 0.05% w/w to inhibit adsorption of G-CSF to i.v. infusion bags made of PVC. Poloxamer 407 was just as effective as HSA alone in preventing surface adsorption of this recombinant protein 1 h following its dilution in D5W to a concentration less than 15 $\mu$g/ml. This nonionic surfactant may show promise as a solvent additive with which to minimize surface adsorption of other recombinant-derived protein drugs to glass and various polymeric materials.

## Acknowledgements

The research was supported, in part, by grants from the Parenteral Drug Association Foundation for Pharmaceutical Science and the American Association of Colleges of Pharmacy awarded to T.P.J. In addition, a portion of this work was supported by the Department of Pharmacy Practice, University of Illinois (G.O.U.).

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