International Journal of Pharmaceutics, 100 (1993) 279–284

IJP 03314

Effects on splenic, hepatic, hematological, and growth parameters following high-dose poloxamer 407 administration to rats

Thomas P. Johnston [b], Helen Beris [b], Zjumira G. Wout [b] and John L. Kennedy [a]

[a] *Department of Hematology, University of Illinois Hospitals and Clinics, Chicago, IL 60612 (USA) and* [b] *University of Illinois, Department of Pharmaceutics (M / C 880), College of Pharmacy, 833 South Wood Street, Chicago, IL 60612 (USA)*

(Received 22 October 1992)
(Modified version received 8 April 1993)
(Accepted 14 May 1993)

Key words: Nonionic surfactant; Pluronic® F-127; Poloxamer 407; Splenomegaly

Summary

The nonionic surfactant poloxamer 407, NF (Pluronic® F-127, NF) has previously been shown to produce marked hyperlipidemia in rats at a dose of 1.5 g/kg for greater than 96 h following a single intraperitoneal (i.p.) injection (Wout et al., *J. Parenter. Sci. Technol.*, 46 (1992) 192–200). In an effort to characterize any potential toxicity of the polymeric vehicle to various organ systems in the rat following multiple i.p. injections, a dose of 0.33 g/kg per day (10% w/w solution) or 1.0 g/kg per day (30% w/w solution) of poloxamer 407 was administered once daily for 4 consecutive days. When compared to control (non-injected) animals, rats injected with 0.33 g/kg per day of poloxamer 407 did not show a significant ($p > 0.05$) increase or decrease in spleen, liver, or total body weight. A complete blood count (CBC) with a white blood cell (WBC) differential was performed on blood samples collected on day five from rats injected with poloxamer 407 at both doses. The CBC with WBC differential was conducted to assess any changes in the WBC count, percent lymphocytes (LY), percent monocytes (MO), percent granulocytes (GR), red blood cell (RBC) count, hemoglobin (HGB), percent hematocrit (HCT), and the mean corpuscular volume (MCV) following administration of poloxamer 407. Rats injected i.p. with a dose of 0.33 g/kg per day of poloxamer 407 for 4 days demonstrated a significant ($p < 0.05$) increase in the number of MO when compared to controls. Administration of 1.0 g/kg per day of poloxamer 407 to rats for 4 days demonstrated distinct splenomegaly when compared to non-injected control animals. In addition, a significant ($p < 0.05$) reduction in body weight and significant ($p < 0.05$) decrease in the percent LY, RBCs, HGB, and percent HCT were noted. Lastly, a significant ($p < 0.05$) increase in the number of WBCs and the percent MO was observed in this same group of rats. However, rats administered 1.0 g/kg per day of poloxamer 407 for 4 days were observed to have no detectable changes in the values of the MCV, the percent GR, or liver-to-body weight ratio when compared to control animals. Thus, repetitive i.p. injections of poloxamer 407 to rats at a dose of 1.0 g/kg per day for four days results in splenomegaly and a reduction in total body weight. Splenomegaly in rats administered poloxamer 407 at a dose of 1.0 g/kg per day resulted from red pulp expansion due to infiltration of macrophages which contained phagocytized lipids.

Correspondence to: T.P. Johnston, University of Illinois at Chicago, Department of Pharmaceutics (M/C 880), College of Pharmacy, 833 South Wood Street, Chicago, IL 60612, U.S.A.

The optimal formulation and subsequent delivery of recombinant-derived proteins remains a challenge to pharmaceutical scientists involved with parenteral formulations. Poloxamers (Pluro-

nics®) are a family of block copolymers composed of repeating poly(oxyethylene) and poly(oxypropylene) units and function as relatively inert, nonionic surface active agents. One member of the poloxamer family, specifically poloxamer 407, NF (Pluronic® F-127, NF) has been shown to be inert with regard to lysis of cell membranes (Johnston and Miller, 1985). In particular, poloxamer 407 does not rupture erythrocyte, hepatocyte, myocyte, and lymphocyte membranes when included in in vitro cell cultures or injected in vivo (Johnston and Miller, 1985; Atkinson et al., 1988; Muller, 1991; Johnston et al., 1992; Pec et al., 1992). Moreover, poloxamer 407 at a concentration of 30% (w/w) undergoes reverse thermal gelation at approx. 11°C; that is, the polymeric vehicle changes from a viscous mobile liquid to a semisolid gel (Wang and Johnston, 1991). Poloxamer 407 has found use in various parenteral protein formulations which are currently approved by the Food and Drug Administration since inclusion of the poloxamer has been shown to stabilize various proteins (Wang and Hanson, 1988; Johnston et al., 1992; Pec et al., 1992). It has been reported that poloxamer 407 did not irreversibly denature either a model nonrecombinant protein (urease) or recombinant-derived protein (interleukin-2) when both proteins were incubated in vitro with the nonionic surfactant at temperatures less than or equal to 37°C (Fults and Johnston, 1990; Johnston et al., 1992; Pec et al., 1992). In addition, the biological activity of urease and interleukin-2 was still evident when either protein was homogeneously dispersed in poloxamer 407 and injected intraperitoneally (i.p.) to rats and mice, respectively (Johnston et al., 1992; Pec et al., 1992). Thus, the potential exists for formulating both recombinant and nonrecombinant therapeutic proteins with poloxamer 407 to achieve sustained release of the protein from the semisolid polymer matrix following extravascular administration.

Previous research from our laboratory had also demonstrated that spleens from mice injected daily for 3 days by i.p. injection with an rIL-2/poloxamer formulation containing 33% (w/w) poloxamer 407 (dose of poloxamer ≈ 2.5 g/kg per day) were slightly enlarged (Johnston et al., 1992). Splenic enlargement was hypothesized to occur from inclusion of poloxamer 407 in the IL-2/poloxamer formulation since mice injected i.p. with an equal dose of poloxamer 407 alone also appeared to exhibit splenomegaly (Johnston et al., 1992). Thus, the present study was conducted to determine the effects of poloxamer 407 on overall body weight as well as the weight of the liver and spleen in rats injected i.p. with poloxamer 407 once per day for 4 days at a dose of either 0.33 or 1.0 g/kg per day. In addition, we evaluated whether multiple i.p. injections of poloxamer 407 in rats using these doses altered various hematological parameters. This study was significant in defining any potential toxicity associated with acute, high-dose administration of poloxamer 407 since poloxamer 407 has recently been reported to be used in several obstetric and gynecological surgical procedures in experimental animal models (Leach and Henry, 1990; Steinleitner et al., 1991).

Poloxamer 407, NF was obtained from the BASF company (Parsippany, NJ) and was used as received. All injectable poloxamer 407 formulations were prepared with sterile normal saline (Elkins-Sinn, Inc., Cherry Hill, NJ). Injections were performed using a 1 ml tuberculin syringe fitted with a 19 G, 1.5 inch needle (Becton Dickinson and Co., Rutherford, NJ). The animals used were male, Sprague Dawley rats (300–325 g) obtained from Harlan Sprague Dawley Laboratories (Indianapolis, IN). Male rats were selected so that results from this study could be compared to findings in previous investigations (Johnston et al., 1992; Pec et al., 1992). Blood samples were collected into polypropylene tubes containing EDTA (Microtainer, Becton Dickinson and Co., Rutherford, NJ). Rats were euthanized in a CO_2 chamber and splenectomies and hepatectomies were performed using a scalpel fitted with a no. 20 scalpel blade (American Scientific Products, Chicago, IL). The spleens were placed in preweighed polypropylene tubes purchased from Corning, Inc. (Park Ridge, IL) and weighed on a Mettler AE 240 analytical balance (Hightstown, NJ). Excised livers were weighed using the same balance in tared plastic weigh boats (Fisher Scientific, Chicago, IL). 3 mm thick sections of

splenic and hepatic tissue were placed in 10% neutral buffered formalin obtained from Sigma Scientific Co. (St Louis, MO) and additional similarly sized representative tissue specimens from each organ immediately frozen in liquid nitrogen and stored at −70°C until the time of histological evaluation.

Injectable poloxamer 407 solutions were prepared by weighing 2.5 and 7.5 g of poloxamer 407 in separate tarred glass containers and bringing each container to a final weight of 25.0 g with normal saline to yield a 10 and 30% (w/w) poloxamer 407 solution, respectively. The solutions were then placed on ice overnight to facilitate dissolution of the polymer according to the 'cold method' of incorporation (Schmolka, 1991).

Rats were randomly divided into three groups of five rats each. Each rat in each group was weighed on a 700 series triple beam balance (Ohaus, Inc., Florham Park, NJ) prior to poloxamer administration. All rats in each group were allowed food and water ad libitum during the 4-day study. Rats in groups 1 and 2 were injected i.p. with 1 ml/day for 4 days with either 10 or 30% (w/w) poloxamer 407 solutions which corresponds to a dose of 0.33 or 1.0 g/kg per day, respectively. Group 3 (controls) consisted of rats which did not receive either an injection of poloxamer 407 or a sham-injection of normal saline. It was arbitrarily decided prior to the study that neither normal saline (sham-injected controls) nor injection trauma was likely to result in changes to the parameters evaluated. On the fifth day, 0.5 ml blood samples were collected from each rat in each group using a tail-clipping technique into EDTA containing collection tubes and gently inverted 15 times. A complete blood count (CBC) with a white blood cell differential was immediately performed on all collected blood samples using a JT series, model 682 Coulter counter (Coulter Electronics, Inc., Hialeah, FL).

All rats were euthanized on the fifth day and weighed on the same balance used to obtain whole body weights prior to i.p. injections of poloxamer 407. Splenectomies and hepatectomies were performed on all rats as described above. Additional sections of spleen (0.5–1.0 mm thick) were obtained at the time of killing and were

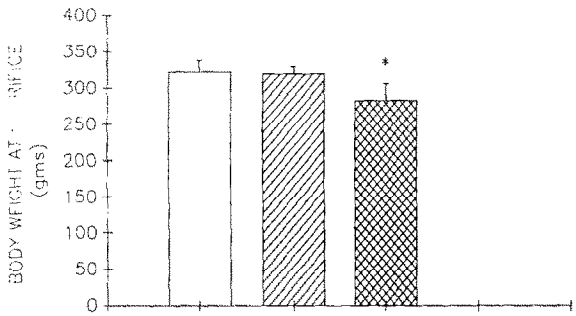

Fig. 1. Effect of repetitive i.p. injections of poloxamer 407 (P-407) on overall body weight at the time of death (day 5). (Empty bars) Non-injected control animals, (hatched bars) 0.33 g/kg per day for 4 days, and (cross-hatched bars) 1.0 g/kg per day for 4 days. * Significant difference ($p < 0.05$) compared to the controls and rats receiving 0.3 g/kg per day of P-407 for 4 days.

immediately fixed in a cacodylate-buffered 2.5% glutaraldehyde-2% paraformaldehyde solution buffered at pH 7.2 for 24 h and then dehydrated in graded ethanol solutions (Karnovsky, 1965). The spleen specimens were then stained with hematoxylin and eosin and examined with a model 410 Reichert-Jung light microscope (Fisher Scientific, Chicago, IL) to assess any changes in morphology. Similarly, liver specimens were also obtained as described above for spleens for light microscopic evaluation. In addition, splenic tissue specimens were stained with the lipid-soluble dye oil Red O. The weight of each excised organ from a given rat was then expressed as a percent of the total body weight at death. The mean value of the percent of body weight that each organ represented for each group of rats was then compared for statistical significance using one-way analysis of variance (ANOVA) coupled with the method of Scheffe'.

The effect of repetitive i.p. injections of poloxamer 407 on overall body weight at the time of killing is shown in Fig. 1. Administration of poloxamer 407 at a dose of 1.0 g/kg per day (30% w/w group) for 4 days resulted in a significant ($p < 0.05$) reduction in the mean value of the total body weight at death compared to non-injected control animals and rats injected with 0.3 g/kg per day of P-407 for 4 days. However, in contrast to total body weight at the time of death,

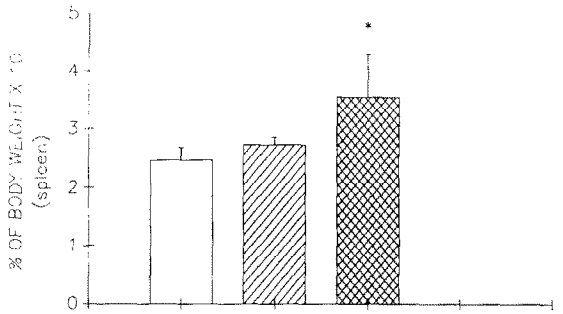

Fig. 2. Effect of repetitive i.p. injections of poloxamer 407 (P-407) on spleen weight at the time of death (day 5) expressed as a percent of the total body weight at death. (Empty bars) Non-injected control animals, (hatched bars) 0.33 g/kg per day for 4 days, and (cross-hatched bars) 1.0 g/kg per day for 4 days. * Significant difference ($p < 0.05$) compared to the controls and rats receiving 0.3 g/kg per day of P-407 for 4 days.

TABLE 1

Pertinent hematological parameters following repetitive intraperitoneal injections of poloxamer 407 (P-407) in rats

Blood parameter	Control	10% P-407 (0.33 g/kg)	30% P-407 (1.0 g/kg)
WBC ($\times 10^3/\mu$l)	8.4±1.0	6.3±0.7	11.4±2.0 [a]
LY (%)	92.0±2.8	91.1±3.3	88.4±1.6 [a]
MO (%)	4.9±1.3	6.2±2.1 [a]	8.1±2.1 [a]
GR (%)	3.0±0.6	2.7±0.4	3.6±0.8
RBC ($\times 10^6/\mu$l)	6.7±0.5	6.3±0.6	3.9±1.0 [a]
(g/dl)	14.2±1.3	14.1±1.5	7.6±2.1 [a]
HCT (%)	39.8±3.3	37.4±3.8	22.9±5.4 [a]
MCV (fl)	58.5±1.0	58.7±0.9	60.0±2.8

All tabulated values are means ± S.D.
[a] Indicates a significant ($p < 0.05$) difference from the mean value for controls using ANOVA.

the mean values of the weights of the excised spleens expressed as a percentage of the total body weight at death were significantly ($p < 0.05$) increased in animals administered poloxamer 407 at a dose of 1.0 g/kg per day for 4 days compared to controls (Fig. 2). While there appeared to be a reduction in the mean values of the weights of the excised livers expressed as a percentage of the terminal body weight as the dose of poloxamer 407 was increased, no statistically significant difference between the three groups was determined (Fig. 3).

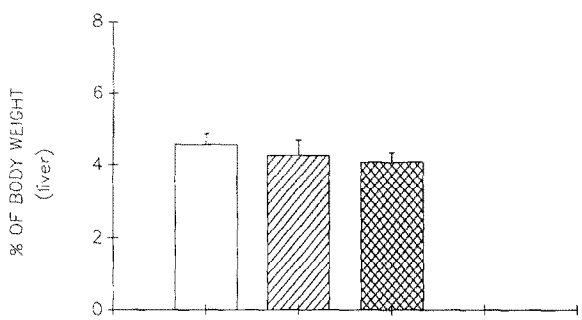

Fig. 3. Effect of repetitive i.p. injections of poloxamer 407 (P-407) on liver weight at the time of death (day 5) expressed as a percent of the total body weight at death. (Empty bars) Non-injected control animals, (hatched bars) 0.33 g/kg per day for 4 days, and (cross-hatched bars) 1.0 g/kg per day for 4 days.

Examination of hepatic tissue obtained and stained post-mortem was unremarkable. However, splenic tissue evaluated by light microscopy displayed red-pulp expansion (micrographs not shown). In addition, oil Red O was incorporated into macrophages contained in the red pulp of splenic tissue indicating phagocytosis of lipids (micrographs not shown). Expansion of splenic red pulp was more noticeable in rats administered poloxamer 407 at a dose of 1.0 g/kg per day for 4 days than for rats administered 0.33 g/kg per day for the same time period.

With the exception of the percent MO observed, i.p. injection of 0.33 g/kg per day of poloxamer 407 (total dose after 4 days = 0.4 g) did not result in a significant increase or decrease in the mean values of the various hematological parameters compared to the same parameters in control (no poloxamer injection) animals as listed in Table 1. However, all the parameters except the percent GR and MCV were significantly ($p < 0.05$) different in rats administered 1.0 g/kg per day of poloxamer 407 for 4 days by i.p. injection when compared to the same hematological parameters for control animals. Of particular note were the number of RBCs, HGB, and percent HCT which were approximately one-half of control values. In contrast, there was a significant ($p < 0.05$) increase in the number of WBCs and

the percent MO following injection of 1.0 g/kg per day of poloxamer 407 to rats for 4 days.

The results of this preliminary study designed to evaluate any potential toxic effects of poloxamer 407 injected i.p. to rats at exaggerated doses (0.33 and 1.0 g/kg per day) for a period of 4 days would indicate that splenomegaly was a dose-dependent phenomenon. In general, splenic pulp may be divided into red and white pulp. The white pulp is composed of compact lymphatic tissue arranged around branches of trabecular arteries (Severin, 1989). The red pulp is composed of vascular sinuses, the cords of Billroth, and terminal branches of the arterial system, the penicilliary arterioles. The organization of the three provides the red pulp with an ideal mechanism for blood filtration (Wolf, 1989). A unique feature of the spleen is that it has an extraordinary capacity to filter blood and behaves as part of the reticuloendothelial system (Severin, 1989).

Splenic enlargement is typically associated with pathology of greatly varied etiology affecting widely disparate organ systems (Severin, 1989; Wolf, 1989). Splenomegaly observed in rats following i.p. administration of poloxamer 407 at a dose of 1.0 g/kg per day for 4 days may potentially be due to either poloxamer-induced proliferation of the lymphocytes contained in the white pulp of the spleen or by phagocytosis of polymer and/or lipids by macrophages in the red pulp of the spleen. Since the lipid-soluble dye was incorporated within macrophages of the red pulp and poloxamer 407 is water-soluble, splenic hypertrophy occurred due to sequestration of lipid-containing macrophages within the red pulp. This histological finding was not surprising when one considers the enormous increase in plasma lipids that occurs following a single injection of poloxamer 407 to rats (Wout et al., 1992).

Splenomegaly due to sequestration of lipid-containing macrophages within the red pulp of the spleen may explain our previous qualitative findings using mice (Johnston et al., 1992). In that study, spleen enlargement was observed in mice that were injected i.p. with either an rIL-2/poloxamer 407 formulation of poloxamer 407 alone for 3 consecutive days (Johnston et al., 1992). It has been reported (Ettinghausen et al., 1991) that injection of an rIL-2 solution alone does not result in increased spleen weight (splenomegaly) in rats. It should be noted that others (Abe et al., 1990) reported severe hepatorenal toxicity following i.p. injection of poloxamer 407 to rabbits and mice. The acute i.p. administered LD_{50} of poloxamer 407 in mice was between 1.7 and 5.0 g/kg body weight (Abe et al., 1990).

The present investigation aimed at identification of hematological parameters that might potentially change following 4 days of repetitive i.p. injection of poloxamer 407 at a dose of either 0.33 or 1.0 g/kg per day to rats would suggest a dose-dependent relationship as well. The increase in the WBC count following administration of 1.0 g/kg per day of poloxamer 407 may suggest an inflammatory reaction such as an acute-phase reactive response due to the severe hyperlipidemia that can be assumed was present in these rats (Okazaki et al., 1990). It is interesting to note that others using a chemically similar nonionic surfactant, namely, Triton WR 1339, observed an increase in the WBC count following i.p. injection of the surfactant to rats (Okazaki et al., 1990). However, the reason for the almost 50% decrease in the RBC count, HGB, and percent HCT following high-dose poloxamer 407 administration in the present study is still uncertain. One possibility is that at the higher dose (1.0 g/kg per day), the polymer may cause hemolysis of red blood cells and subsequently induce the decrease in the RBC counts and HGB values (Okazaki et al., 1990). However, this would appear questionable in view of the fact that poloxamer 407 has been shown to be devoid of cellular membrane lytic effects (Johnston and Miller, 1985; Atkinson et al., 1988; Muller, 1991; Johnston et al., 1992; Pec et al., 1992). Further experimentation is required to elucidate the mechanism of action of poloxamer 407 on blood parameters. In addition, future research will be directed at the reversibility of changes in hematological parameters following cessation of high-dose poloxamer 407 administration.

In conclusion, short-term (4 days), high-dose (1.0 g/kg per day) administration of poloxamer 407 to rats by i.p. injection resulted in a decrease

in total body weight and splenomegaly. Future studies will be directed at the effect of high-dose poloxamer 407 administration on food and water consumption.

Acknowledgements

The authors would like to acknowledge the financial support of the following agencies, all of which supported, in part, the present research. The authors are grateful to the American Heart Association-National Center, the Illinois Cancer Center, and the American Association of Colleges of Pharmacy. T.P.J. was awarded a Young Investigator award from the American Association of Colleges of Pharmacy.

References

Abe, T., Sasaki, M., Nakaiima, H., Ogita, M., Naitou, H., Nagase, A., Taguchi, K. and Miyazaki, S., Evaluation of Pluronic F-127 as a base for the gradual release of anticancer drugs. *Gan. To Kaoaku Ryoko*, 17 (1990) 1546–1550.

Atkinson, T.P., Bullock, J.O., Smith, T.F., Mullins, R.E. and Hunter, R.L., Ion transport mediated by copolymers composed of polyoxyethylene and polyoxy-propylene. *Am. J. Physiol.*, 254 (1988) C20–C26.

Ettinghausen, S.E., Lipford, E.H.J., Mule, J.J. and Rosenberg, S.A., Systemic administration of recombinant interleukin 2 stimulates in vivo lymphoid cell proliferation in tissues. *J. Immunol.*, 135 (1991) 1488–1492.

Fults, K.A. and Johnston, T.P., Sustained release of urease from a poloxamer gel matrix. *J. Parenter. Sci. Technol.*, 44 (1990) 58–65.

Johnston, T.P. and Miller, S.C., Toxicological evaluation of poloxamers for intramuscular use. *J. Parenter. Sci. Technol.*, 39 (1985) 83–88.

Johnston, T.P., Punjabi, M.A. and Froelich, C.J., Sustained delivery of interleukin-2 from a poloxamer 407 gel matrix following intraperitoneal injection in mice. *Pharm. Res.*, 9 (1992) 421–430.

Karnovsky, M.J., A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.*, 27 (1965) 137A.

Leach, R.E. and Henry, R.L., Reduction of postoperative adhesions in the rat uterine horn model with poloxamer 407. *Am. J. Obstet. Gynecol.*, 162 (1990) 1317–1319.

Muller, R.H., Toxicity studies in hepatocyte cultures. In Muller, R.H. (Ed.), *Colloidal Carriers for Controlled Drug Delivery and Targeting*, CRC Press, Boca Raton, FL, 1991, pp. 207–210.

Okazaki, M., Suzuki, M. and Oguchi, K., Changes in coagulative and fibrinolytic activities in Triton WR 1339-induced hyperlipidemia in rats. *Jap. J. Pharmacol.*, 52 (1990) 353–361.

Pec, E.A., Wout, Z.G. and Johnston, T.P., Biological activity of urease formulated in poloxamer 407 following intraperitoneal injection in the rat. *J. Pharm. Sci.*, 81 (1992) 626–630.

Schmolka, I.R., Poloxamers in the pharmaceutical industry. In Tarcha, P.J. (Ed.), *Polymers for Controlled Drug Delivery*, CRC Press, Boca Raton, FL, 1991, pp. 189–214.

Severin, C.M., Anatomy of the spleen. In Pochedly, C., Sills, R. and Schartz, A.D. (Eds), *Disorders of the Spleen*, Dekker, New York, 1989, pp. 1–18.

Steinleitner, A., Lambert, H., Kazensky, C. and Cantor, B., Poloxamer 407 as an intraperitoneal barrier material for the prevention of postsurgical adhesion formation and reformation in rodent models for reproductive surgery. *Obstet. Gynecol.*, 77 (1991) 48–52.

Wang, P. and Johnston, T.P., The kinetics of the sol-to-gel transition for poloxamer polyols. *J. Appl. Polym. Sci.*, 43 (1991) 283–292.

Wang, P. and Hanson, M., Parenteral formulation of protein and peptides: stability and stabilizers. *J. Parenter. Sci. Technol., Techn. Rep. No. 10*, 42 (1988) S1-S23.

Wolf, B.C. and Neiman, R.S., *Disorders of the Spleen*, W.B. Saunders, Philadelphia, PA, 1989, pp. 1–211.

Wout, Z.G., Pec, E.A., Maggiore, J.A., Williams, R.H., Palicharla, P. and Johnston, T.P., Poloxamer 407-mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. *J. Parenter. Sci. Technol.*, 46 (1992) 192–200.