International Journal of Pharmaceutics, 16 (1983) 181–190
Elsevier

Adsorption of D-Nal(2)^{6}LHRH, a decapeptide, onto glass and other surfaces

Shabbir T. Anik and Jiin-Yu Hwang

Institute of Pharmaceutical Sciences, Syntex Research, Palo Alto, CA (U.S.A.)

(Received September 30th, 1982)
(Accepted February 18th, 1983)

Summary

Adsorption patters of aqueous solutions of D-Nal(2)^{6}LHRH onto glass, plastic, tubing, syringes and filters were characterized. Effects of ionic species, inert proteins and amino acids on the extent of adsorption onto glass surfaces were also studied. Among the different additives, the phosphate ion at 0.1 M concentration and the acetate ion at 0.16 M concentration, both at pH 5, were the most effective in preventing adsorption onto glass. Siliconization of the glass surface did not inhibit adsorption suggesting that adsorption was not solely due to ionic amine–silanol binding. Adsorption to filters and syringes varied depending on the brand of filters and syringes used, whereas adsorption onto plastic bottles and tygon tubing was minimal.

Introduction

The adsorption of proteins and peptides onto glass and plastic surfaces is well documented (Petty and Cunningham, 1974; Mizutani and Mizutani, 1978a; Bitar et al., 1978; Christensen et al., 1978; Ogino et al., 1979). This phenomenon is particularly significant at low concentrations of peptide/protein in aqueous solution. In the case of peptide drugs such as insulin, secretin, etc., adsorption of the drug to containers, syringes and infusion apparatus can result in significant losses and hence lower dosage to the patient (Kraegen et al., 1975; Petty and Cunningham, 1974; Bitar et al., 1978). The adsorption of peptides/proteins to glass has been ascribed to ionic amine–silanol bonding (Messing, 1975; Mizutani and Mizutani, 1978a) while the nature of peptide/protein loss to other surfaces such as plastic is variable. In the past, the most common method of preventing adsorption has been to use carrier

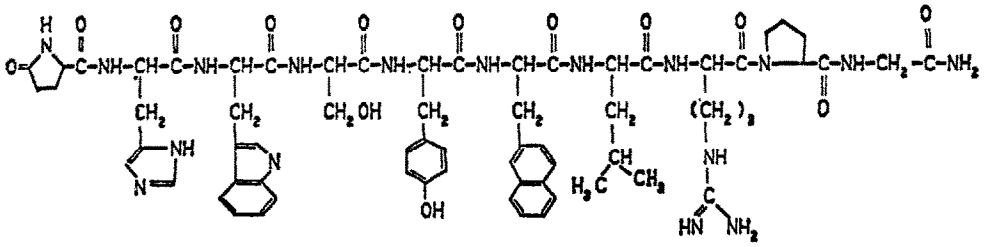

Fig. 1. D-Nal(2)^{6}LHRH (I).

proteins such as albumin or gelatin (Petty and Cunningham, 1974; Kraegen et al., 1975). Surfactants such as sodium lauryl sulphate (Mizutani and Mizutani, 1978b) and amino acid buffers (Mizutani and Mizutani, 1975) have also been suggested. These additives presumably prevent adsorption by competitive binding to the silanol sites on the glass surface. Siliconization and silanization of glassware are also recommended procedures for minimizing adsorption and are useful in laboratory experimentation but are not practical for pharmaceutical application.

D-Nal(2)^{6}LHRH (I) (Fig. 1) (Nestor et al., 1982), a decapeptide, is a derivative of the naturally occurring luteinizing hormone releasing hormone (LHRH) where the 6th amino acid in LHRH has been replaced by 3(2-naphthyl)-D-alanine or Nal(2). It is a highly potent superagonist and has several physiological effects in both males and females at very low doses. The decapeptide has two basic amino acids, histidine and arginine, which are positively charged at acidic pH. Hence, adsorption of the peptide onto glass through ionic amine–silanol interactions may be significant at low concentrations. In order to ensure accurate dosing in bioassay and clinical testing, it was necessary to characterize the adsorption patterns of I onto various surfaces, particularly glass, and determine the effects of additives on the extent of adsorption.

Materials and Methods

Materials

The experimental materials used were D-Nal(2)^{6}LHRH acetate salt [1], D-Nal(2)^{6}LHRH-[^{3}H]acetate salt [1], Oxifluor-H_2O complete oxidizer cocktail [2], borosilicate solid glass beads [3] (2 mm in diameter), Prosil-28 (organo silane) [4], sodium carboxymethylcellulose [5] (type 7M8SXF), glycine [6], bovine serum albumin [7], gelatin [8] (type A, 250 Bloom), Dow Corning 360 Medical Fluid (poly dimethyl siloxane) [9],

[1] Institute of Organic Chemistry, Syntex, Palo Alto, CA, U.S.A.
[2] New England Nuclear, Boston, MA 02118, U.S.A.
[3] Propper Mgf., Long Island, NY, U.S.A.
[4] PCR Research Chemicals, Gainesville, FL 32602, U.S.A.
[5] Hercules, Wilmington, DE 19899, U.S.A.
[6] Eastman Kodak, Rochester, NY 14650, U.S.A.
[7] Miles, Elkhart, IN 46515, U.S.A.
[8] Peter Cooper, Oak Creek, WI 53154, U.S.A.
[9] Dow Corning, Midland, MI 48640, U.S.A.

sodium dihydrogen phosphate monohydrate, 100 μl Hamilton syringe [10], 1 ml Stylex syringe [11], 1 ml B-D Glaspak syringe [12], Millex-GS filter [13] (0.22 μm pore), Nuclepore filter [14] (0.2 μm pore), rubber tubing (0.45 cm in diameter), tygon tubing (0.6 cm in diameter), and high density polyethylene plastic bottles [15].

Methods

(a) General. Adsorption was followed by using tritiated I and scintillation counting to measure changes in concentration. Initial experiments were done on glass apparatus which had been silanized. It was found that there was no loss of this decapeptide from solutions in contact with the silanized glass surface. Hence all glassware used for handling solutions of I was silanized with 1% v/v Prosil. Experiments were done at ambient conditions (22 ± 1°C) without stirring. Stirring was shown not to affect the results.

(b) Adsorption onto glass beads. The solid borosilicate glass beads were washed with distilled water to stimulate normal washing conditions. 10 g of beads (2 mm diameter, approximately 10.3 cm^2/g) were added to 10 ml of the test solution in an Erlenmeyer flask. 100 μl of solution was sampled at different times and counted using 10 ml of Oxifluor scintillation fluid. At least two samples were taken at each time point.

(c) Siliconization of glass beads. Clean glass beads were dipped into a 2% solution of Dow Corning 360 medical fluid in ether, then removed and drained. The beads were initially dried at room temperature and then cured by heating for 4 h at 100°C.

(d) Adsorption onto high density polyethylene bottles. 10 ml of the test solution was placed in a high density polyethylene bottle (~ 49 cm^2 surface area/bottle). 100 μl of solution was sampled in duplicate at different time points and counted as described above.

(e) Adsorption onto syringes, tubing and filters. Syringes and tubing were filled with the test solution. After 5 min, the solution was removed and counted. Each experiment was repeated in triplicate. For filters, the amount adsorbed was determined after a single pass of 2 ml of solution through the filter using a syringe assembly.

(f) Measurement of the partition coefficient in silicone oil. An aqueous solution containing 100 μg/ml of I at pH 7 was equilibrated with silicone oil by rotation in a water bath at 25°C. After 8 days, the aqueous phase was sampled and assayed by HPLC. Duplicate measurements were done.

---

[10] Hamilton, Reno, NE 89510, U.S.A.
[11] Pharmaseal Lab., Glendale, CA 91201, U.S.A.
[12] Becton, Dickinson and Co., Rutherford, NJ, U.S.A.
[13] Millipore, Bedord, MA 01730, U.S.A.
[14] Nuclepore, Pleasantown, CA 94506, U.S.A.
[15] IMCO Container, Kansas City, MO 64132, U.S.A.

Results and Discussion

Adsorption of D-Nal(2)^{6}LHRH on borosilicate glass beads

A typical adsorption profile of I from aqueous solution (pH 7, no buffer) onto glass beads is shown in Fig. 2. Adsorption is fairly rapid and equilibrium is reached within 2 h. The equilibrium adsorption values are plotted as a function of the equilibrium concentration in Fig. 3A. The adsorption isotherm resembles a Langmuir process where saturation of the glass surface occurs with an increase in solution concentration. Many systems such as the adsorption of polymers, which definitely do not conform to the Langmuir assumptions, nevertheless display Langmuir adsorption isotherms. The Langmuir isotherm is mathematically expressed by the following equations (Adamson, 1967)

$$\frac{n^s_2}{n^s} = \frac{KC_{eq}}{1 + KC_{eq}} \tag{1a}$$

$$\frac{C_{eq}}{n^s_2} = \frac{1}{n^s \cdot K} + \frac{C_{eq}}{n^s} \tag{1b}$$

where n^s_2 is the moles of solute (or μg with appropriate correction for units) adsorbed/g of adsorbent, n^s is the number of moles of adsorption sites/g of adsorbent, C_{eq} is the equilibrium concentration of the solute and K is a constant. Fig. 3B is a plot of C_{eq}/n^s_2 vs C_{eq} for the adsorption of I onto glass beads.

The data fit the model well, and the parameters of Eqn. 1b were determined from

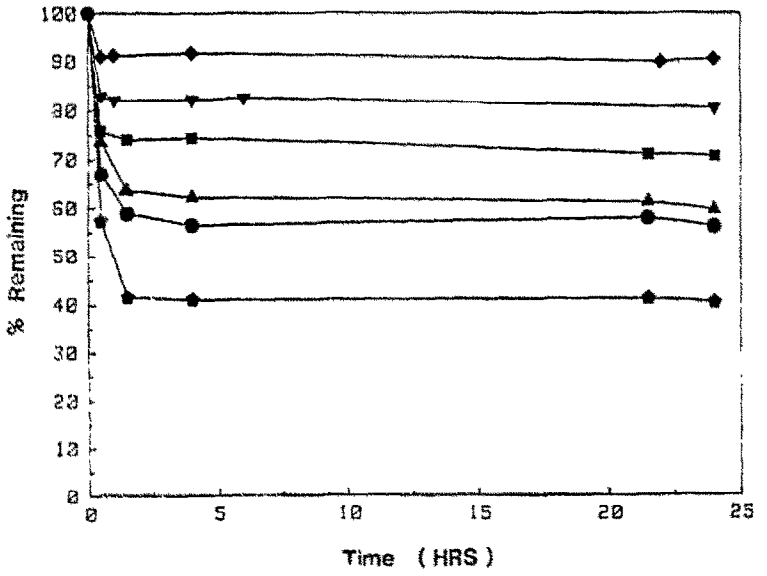

Fig. 2. Adsorption profiles of D-Nal(2)^{6}LHRH from aqueous solution (pH 7, no buffer) as a function of time and concentration. ◆, 1 μg/ml; ●, 5 μg/ml; ▲, 10 μg/ml; ■, 20 μg/ml; ▼ 50 μg/ml; ◆, 100 μg/ml.

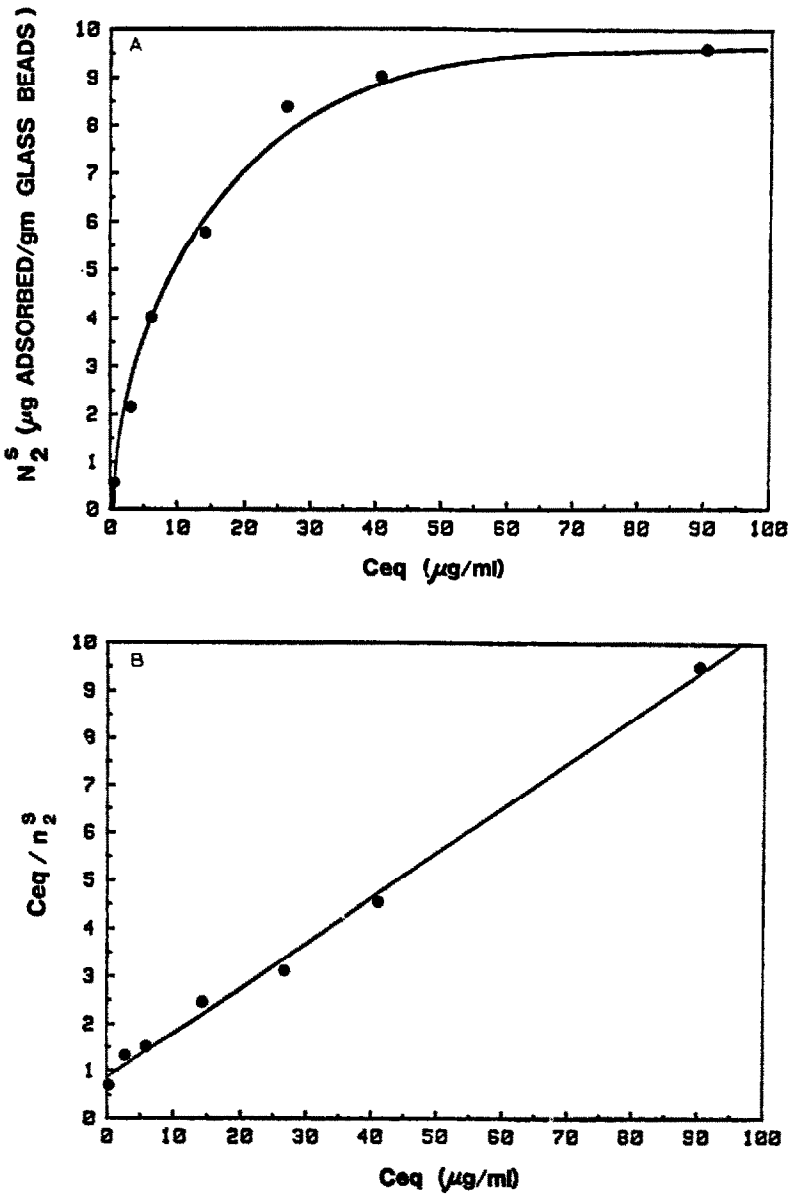

Fig. 3. A: adsorption isotherm of I from aqueous solution. B: Langmuir plot of the adsorption isotherm of I from aqueous solution.

the slope and intercept. Although the physical significance of these parameters is not clear without further evidence supporting the Langmuir mechanism, this approach can be used empirically to compare various adsorption systems.

The effects of different additives on the extent of adsorption are given in Table 1. The additives can be classified into 3 types: (i) ionic compounds; (ii) inert proteins; and (iii) amino acids. The results indicate that the monobasic phosphate ion at a concentration of 0.1 M was very effective in preventing adsorption, particularly at

TABLE I

% ADSORBED AT EQUILIBRIUM OF D-Nal(2)^{6}LHRH ONTO BOROSILICATE SOLID GLASS BEADS (~ 100 cm^2) IN THE PRESENCE OF DIFFERENT ADDITIVES [a]

Additive/initial concentration	20 μg/ml	10 μg/ml	5 μg/ml	1 μg/ml
None (pH = 7)	29	40	43	59
Sodium dihydrogen phosphate				
0.02 M	13	20	35	62
0.05 M	8	15	23	35
0.1 M	4	7	10	18
0.16 M sodium acetate	4	7	12	36
0.2% sodium carboxymethyl cellulose	11	–	16	57
0.1 M glycine	18	–	43	60
0.5% bovine serum albumin	11	–	17	32
0.1% gelatin	10	–	14	19

[a] All solutions at pH 5. All values are ±2%.

drug concentrations of 5–20 μg/ml [16]. The effectiveness, however, decreased with decreasing phosphate ion concentration. Since the mechanism by which the phosphate ion prevents adsorption has not been elucidated, these results may be interpreted in several ways. If the Langmuir model is assumed for this system and the data are plotted according to Eqn. 1b, a linear relationship is obtained for each concentration as shown in Fig. 4. The slope of these lines is a measure of the

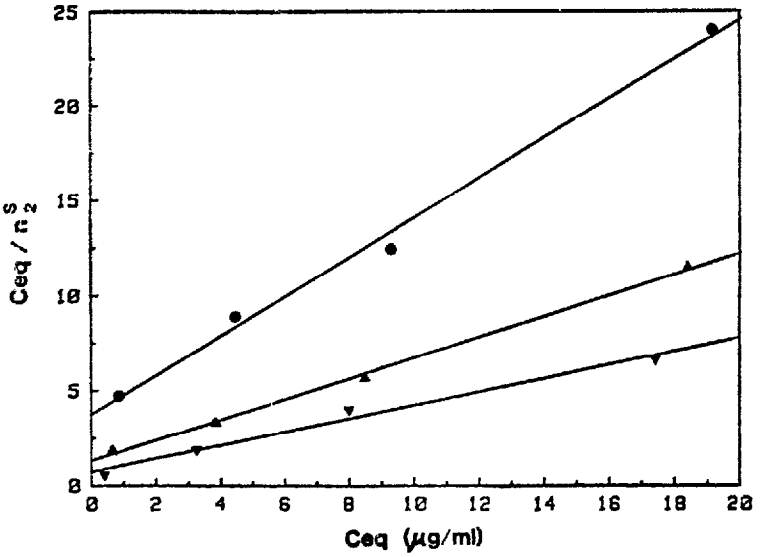

Fig. 4. Langmuir plot of adsorption of I from solutions of different phosphate ion concentrations. ▼, 0.02 M phosphate; ▲, 0.05 M phosphate; ●, 0.1 M phosphate. All solutions at pH 5.

[16] Although it is recognized that a small quantity of the dibasic phosphate ion is present at pH 5, it is assumed to have a negligible effect on the adsorption process.

TABLE 2

LANGMUIR PARAMETERS FOR THE DIFFERENT ADSORBATE SOLUTIONS

Solution	Slope	Intercept	ns (μg/g) (1/slope)	K (ml/μg) (slope/intercept)
Aqueous, pH = 7	0.095 (0.003)	0.86 (0.11)	10.5 (0.32)	0.11 (0.01)
0.02 M phosphate, pH = 5	0.35 (0.03)	0.72 (0.29)	2.8 (0.24)	0.50 (0.21)
0.05 M phosphate, pH = 5	0.55 (0.02)	1.30 (0.20)	1.8 (0.07)	0.42 (0.07)
0.1 M phosphate, pH = 5	1.04 (0.05)	3.74 (0.58)	0.96 (0.05)	0.28 (0.05)
0.16 M acetate, pH = 5	1.16 (0.10)	1.93 (1.1)	0.86 (0.08)	0.60 (0.35)

Numbers in parentheses are standard errors.

available adsorption sites while the ratio of the slope-to-intercept is a measure of the binding affinity of the molecule to the surface. These parameters are listed in Table 2. The most apparent difference in the Langmuir plots of the 3 phosphate concentrations is the increase in slopes (decrease in adsorption sites) with a concomitant increase in phosphate ion concentration. The affinity constant, which decrease with increasing phosphate concentration, however, are not significantly different due to the larger standard errors of the intercept.

Alternatively, a decrease in free-drug concentration in solution due to ion pairing or complexation of the peptide with the phosphate ion could also account for the observed results. Ion pairing has also been implicated in the chromatography of peptides using phosphate buffers as the mobile phase (Hancock et al., 1978). The phosphate ion could also have an effect on the glass surface (such as on the binding sites or effective change on the surface); however, these effects are unknown. In order to determine if the inhibition of adsorption was specific to the phosphate ion, an adsorption isotherm was generated in 0.16 M pH 5 acetate solution (equivalent in ionic strength to 0.1 M phosphate, pH 5). At the higher concentrations of drug, the acetate ion is just as effective in preventing adsorption. Hence the effect of ionic additives is not specific to phosphate and the mechanism of prevention of adsorption may be a combination of the factors mentioned above. In any event, the empirical result is quite clear. The addition of phosphate and potentially other ions such as acetate at appropriate concentrations results in lower adsorption of peptide onto the glass beads.

The inert proteins, gelatin and BSA, were only partially effective in reducing adsorption. Gelatin, however, continued to have an effect even at low concentrations of drug. These proteins have been used successfully for other peptide and protein molecules (Kraegen et al., 1975, Ogino et al., 1979); however, whether protection was due to competition for binding sites on the glass surface or binding of the smaller peptide to the protein is unclear. Differences in mechanism may account for their varying effectiveness with different peptides.

The 0.1 M glycine buffer did not reduce adsorption sufficiently to be of practical value.

Adsorption of siliconized glass beads

Siliconization, although not the most desirable method, has been used for preventing adsorption of drug molecules (Bhargava, 1979). The silicone coating on the glass surface provides a "hydrophobic type' barrier and hence eliminates the silanol–amine interactions which are considered to be the driving force in adsorption of amines and proteins onto glass. The results of the adsorption of I onto silicone-coated glass beads are given in Table 3. At 20 μg/ml, although adsorption on the coated beads is less than on the uncoated beads, it is still significant. The adsorption, however, increases at lower concentrations relative to the uncoated beads. Based on the Langmuir model, this reversal in trend may be explained by the presence of a smaller number of adsorption sites on the coated beads, but a higher affinity of the peptide for the silicone surface compared to the uncoated glass.

Adsorption to the silicone surface suggests that the molecule can also adsorb through hydrophobic interactions between its non-polar residues (naphthylalanine, tryptophan, tyrosine, leucine) and the silicone surface. Measurement of the partition coefficient between the silicone fluid and an aqueous solution gave a value around 7 confirming the affinity of the peptide for the silicone liquid. Adsorption to silicone surfaces has also been observed with several peptide/protein molecules such as secretin, insulin, globulin, etc. (Ogimo, 1979; Mizutani, 1981).

Adsorption onto other surfaces

Based on the above results it became necessary to determine the extent of adsorption of I from an 0.1 M phosphate solution onto a variety of different surfaces and apparatus. The results are listed in Table 4. The plastic surface used here shows negligible adsorption. The results, however, cannot be extrapolated to plastics or even high density polyethylene in general as treatment of finished plastic products as well as resins and additives can vary. Adsorption onto syringes was larger than expected, given the small surface area exposed to the solution. This may be due to the larger surface-to-volume ratio relative to other glass and plastic apparatus. Some loss of drug was observed onto rubber tubing but was negligible on Tygon tubing.

There was a marked difference in the amount of drug lost between the Millex (cellulose ester type) and Nuclepore (polycarbonate) filters. This is probably due to the filamentous structure of Millipore filters as opposed to the pore structure of Nuclepore filters. The former filter provides a much larger surface area resulting in

TABLE 3

% ADSORBED OF D-Nal(2)^{6}LHRH ONTO SILICONE-COATED AND UNCOATED GLASS BEADS[a]

Type of glass beads Initial concentration of solution	20 μg/ml	5 μg/ml	1 μg/ml
Silicone-coated glass beads	17	39	86
Uncoated glass beads	29	43	59

[a] Aqueous solution at pH 7.0 (no buffers added).

TABLE 4

ADSORPTION OF D-Nal(2)^{6}LHRH ONTO VARIOUS SURFACES [a]

Surface	Initial concentration of D-Nal(2)^{6}LHRH (μg/ml)	% Adsorption
High density polyethylene bottle (49 cm^2) [b]	20	2.4
1 ml Stylex plastic syringe 7020D	5	5.5
	20	2.4
1 ml B-D Glaspak syringe	5	12.0
	20	5.7
	50	2.0
	100	0.6
100 μl Gastight Hamilton syringe	50	2.5
Rubber tubing, Amber Latex (0.45 cm diameter, 40 cm length)	20	3.7
Tygon tubing (0.6 cm diameter, 40 cm length)	20	0
Millex-GS filter (25 mm diameter, 0.22 μm)	20	93.2
Nuclepore filter (13 mm diameter, 0.2 μm)	20	4.9

[a] The solution used was an isotonic 0.1 M phosphate buffer at pH 4.4.
[b] Aqueous solution without any additives.

the large amount of drug lost. The possibility of saturating the filter by using larger volumes of solution or higher concentrations was not studied. The above results are a clear indication of the large variations in loss of drug depending on the surface, and the importance of determining this loss for the manufacture, storage and delivery of drug solutions of low concentration.

Acknowledgements

The authors would like to acknowledge the valuable suggestions from Dr. Richard E. Jones and Dr. Allyn Becker in the preparation of this manuscript. Presented at the American Pharmaceutical Association meeting, San Diego, November, 1982.

References

Adamson, A.W., Physical Chemistry of Surfaces, Interscience Publishers, New York, 1967, p. 400.

Bhargava, H.N., Improved recovery of morphine from biological tissues using siliconized glassware. J. Pharm. Sci., 66 (1977) 1044–1045.

Bitar, K.N., Zfass, A.M. and Makhlorf, G.M., Binding of secretin to plastic surfaces. Gastroenterology, 75 (1978) 1080–1082.

Christensen, P., Johansson, A. and Nielsen, V., Quantitation of protein adsorbance to glass and plastics: investigations of a new tube with low adherence. J. Immunol. Meth., 23 (1978) 23–28.

Hancock, W.S., Bishop, C.A., Prestidge, R.L., Harding, D.R.K. and Hearn, M.T.W., Reverse phase, high pressure liquid chromatography of peptides and proteins with ion pairing reagents. Science, 200 (1978) 1168–1170.

Kraegen, E.W., Lazarus, L., Meler, H., Campbell, L. and Chia, Y.O., Carrier solutions for low-level intravenous insulin infusion. Br. Med. J., 23 Aug (1975) 464–466.

Messing, R.A., Adsorption of proteins on glass surfaces and pertinent parameters for the immobilization of enzymes in the pores of inorganic carriers. J. Non-Crystalline Solids, 19 (1975) 277–283.

Mizutani, T. and Mizutani, A., Prevention of adsorption of protein on controlled-pore glass with amino acid buffer. J. Chromatogr., 111 (1975) 214–216.

Mizutani, T. and Mizutani, A., Estimation of adsorption of drugs and proteins on glass surfaces with controlled pore glass as a reference. J. Pharm. Sci. 67 (1978a) 1102–1105.

Mizutani, T. and Mizutani, A., Adsorption of a protein on glass surfaces in detergent solutions. J. Non-Crystalline Solids, 27 (1978b) 437–439.

Mizutani, T., Estimation of protein and drug adsorption onto silicone coated glass surfaces. J. Pharm. Sci., 70 (1981) 493–496.

Nestor Jr., J.J., Ho, T.L., Simpson, R.A., Horner, B.L., Jones, G.H., McRae, G.I. and Vickery, B.H., The synthesis and biological activity of some very hydrophobic analogs of luteinizing hormone releasing hormone. J. Med. Chem., 25 (1982) 795–801.

Ogino, J., Noguchi, K. and Terato, K., Adsorption of secretin on glass surfaces. Chem. Pharm. Bull., 27(12) (1979) 3160–3163.

Petty, C. and Cunningham, N.L., Insulin adsorption by glass infusion bottles, polyvinyl chloride infusion containers and intravenous tubing. Anesthesiology, 40 (4) (1974) 400–404.