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LOSS OF PEPTIDES AND PROTEINS UPON STERILE FILTRATION DUE TO ADSORPTION TO MEMBRANE FILTERS

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ABSTRACT

Sterile filtration through membrane filters is one of the unit operations frequently applied in the pharmaceutical manufacture of peptide or protein solutions. However, proteins tend by nature, to adsorb to filter materials. The adsorption of luteinizing hormone releasing hormone, bovine serum albumin and gamma globulin to five types of filters from three different materials under both static and dynamic conditions was studied. With respect to the filter material, the order of protein affinity that was found is polyvinylidene fluoride < nylon << cellulose nitrate. It was observed that the adsorption process follows time-dependent saturation kinetics. Several recommendations with respect to the filtration process are given which may be helpful in reducing losses of material due to adsorption to filters.

INTRODUCTION

Exposure of peptide or protein solutions to liquid-solid interfaces can lead to physical loss of these compounds by adsorption or to loss of potency due to changes in conformational structure. The investigation of adsorption of peptides and proteins to glass and polymer surfaces has two main objectives: firstly, desired adsorption e.g. in the case of diagnostics research, and secondly, undesired adsorption e.g. upon storage or processing of protein solutions. In this study we deal with unintended adsorption of peptides and proteins to solid surfaces.

Upon storage, adsorption of drugs to the surface of containers, usually glass, has been shown to be a serious problem [1]. However, already during the unit operations of a production process severe losses may occur,particularly in the case of peptides and proteins[2]. Protein adsorption is a complicated process, governed by a variety of factors. Among them are the hydrophilicity/hydrophobicity of the surfaces; the surface charge densities; the ionic strength, ionic composition and pH of the solutions; adsorption times; and several protein characteristics like their surface-active nature [3-6]. Methods to prevent losses due to adsorption have been described, such as the addition of serum albumin, detergents or large amounts of glycerol [7]. However, whether this approach is generally applicable to pharmaceutical peptide or proteinaceous products, remains questionable.

Sterilization by filtration through 0.2 μm membrane filters is an important step in the pharmaceutical production process. To gain insight into the degree of losses during filtration processes we studied the adsorption loss from solutions containing bovine serum albumin (BSA), luteinizing hormone releasing hormone (LHRH) and mouse immunoglobulin G (IgG) caused by five different filters.

TABLE 1

Name	Material	Supplier	Pore Size (μm)
Durapore	Polyvinylidene difluoride (GVWP)	Millipore	0,2
Ultipore	Nylon	Pall	0,2
Sartorius	Cellulose nitrate	Sartorius	0,2
S&S 1121	Carton-lined cellulose nitrate	Schleicher & Schüll	0,2
S&S 1119	Carton-lined cellulose nitrate	Schleicher & Schüll	0,6

EXPERIMENTAL

Materials

BSA was from Armour and dissolved to a concentration 1 mg/ml in either 20 mM sodium phosphate buffer (pH 8) or water adjusted to pH 5 with hydrochloric acid. LHRH and monoclonal mouse IgG were produced by Organon, Oss, The Netherlands and used as a solution of 1 mg/ml in 10 mM sodium acetate (pH 4) and 20 mM sodium phosphate (pH 8), respectively. The membrane filters used are listed in Table 1. All filters were of 47 mm diameter.

Methods

Four types of experiments were carried out. Static adsorption was studied by a 24 h incubation of filter material from one filter, cut to pieces, with 2.5 ml of protein solution. The loss of material during a filtration step was determined by passing a protein solution either three times through the same filter or five times with replacement of the filter after each filtration. Finally, the influence of the volume of the protein solution was analyzed by determining the relative loss after a single filtration of 10, 25 and 100 ml. Protein concentrations were determined spectrophotometrically at 210 nm (BSA and IgG) or 217 nm (LHRH). All experiments and protein measurements were performed in triplicate.

RESULTS

Measurement of static adsorption revealed vast differences in protein binding capacities for the various types of filters (Fig. 1). Substantial losses for all protein solutions studied were found for the nitrocellulose filters. The high binding capacity is further increased when the filter is provided with a carton-lining. We suppose that a smaller total surface area of the 0.6 μm carton-supported cellulose nitrate filters accounts for the somewhat lower protein affinity compared to identical filters of 0,2 μm porosity. Little or no protein is bound by the nylon and PVDF filters. The adsorption of BSA from a water solution of pH 5 can be opposed by increasing the ionic strength of the solution and changing the pH of 5, which is about the pI of albumin, to pH 8. Similar observations have been reported previously [6].

For some proteins repeated filtration of a 25 ml solution through one filter results in higher amounts of adsorbed material than were observed in the static

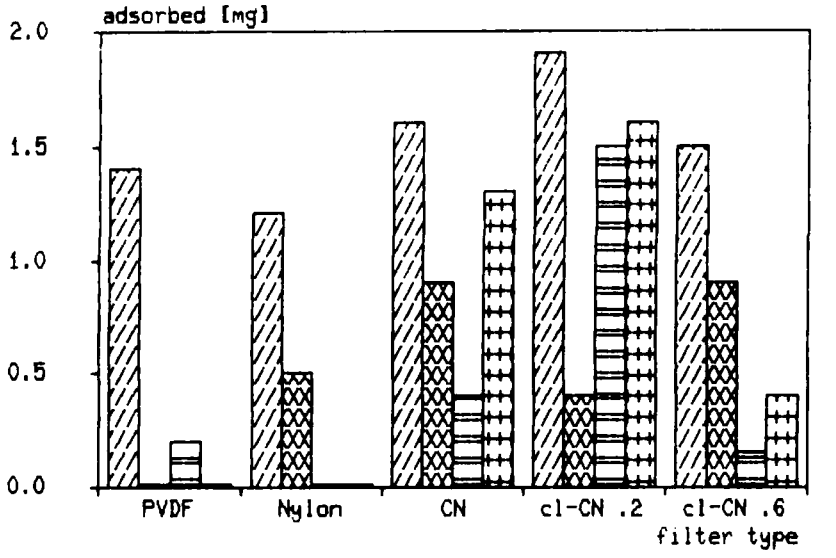

FIGURE 1

Static adsorption to filter material. Loss by adsorption to a single 47 mm filter was measured after incubation for 24 h in 2.5 ml containing 1 mg/ml BSA in water (▨), BSA in buffer (▩), LHRH (▤), or IgG (▦).

experiment (Fig. 2). Thus, the amount of adsorbed protein increases when larger quantities are presented to the filters. In our set-up, no additional adsorption was observed in the second and third filtration steps. This contrasts with static experiments on the adsorption of albumin to glass surfaces. It has been reported that the glass surface could not be saturated due to the fact that BSA deposited in multiple layers [4,8].

Cumulative losses of protein in five subsequent filtrations of 25 ml with replacement of the filter after every step are depicted in Fig. 3. In general, equal amounts are lost in each filtration step, although in some cases somewhat less protein is adsorbed due to the decreased protein concentration of the sample. No

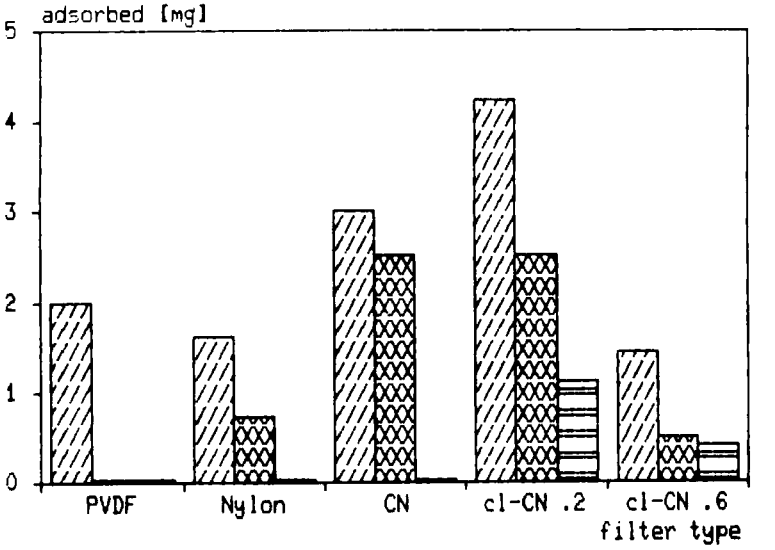

FIGURE 2

Adsorption after repeated filtration over a single filter. Loss by adsorption to a single 47 mm filter was measured after triple filtration of a 25 ml containing 1 mg/ml BSA in water ( ), BSA in buffer ( ), or LHRH ( ).

explanation was found for the increased adsorption of IgG and LHRH in later steps upon filtration over 0.2 μm carton-lined cellulose nitrate filters. This phenomenon was not observed with the 0.6 μm version of this filter type.

The relative amount of protein lost decreases with increasing volume of the protein solution (Fig. 4). It must be stressed, however, that the absolute amounts that were adsorbed were often larger when larger volumes were filtered. This indicates that the filters are not readily saturated.

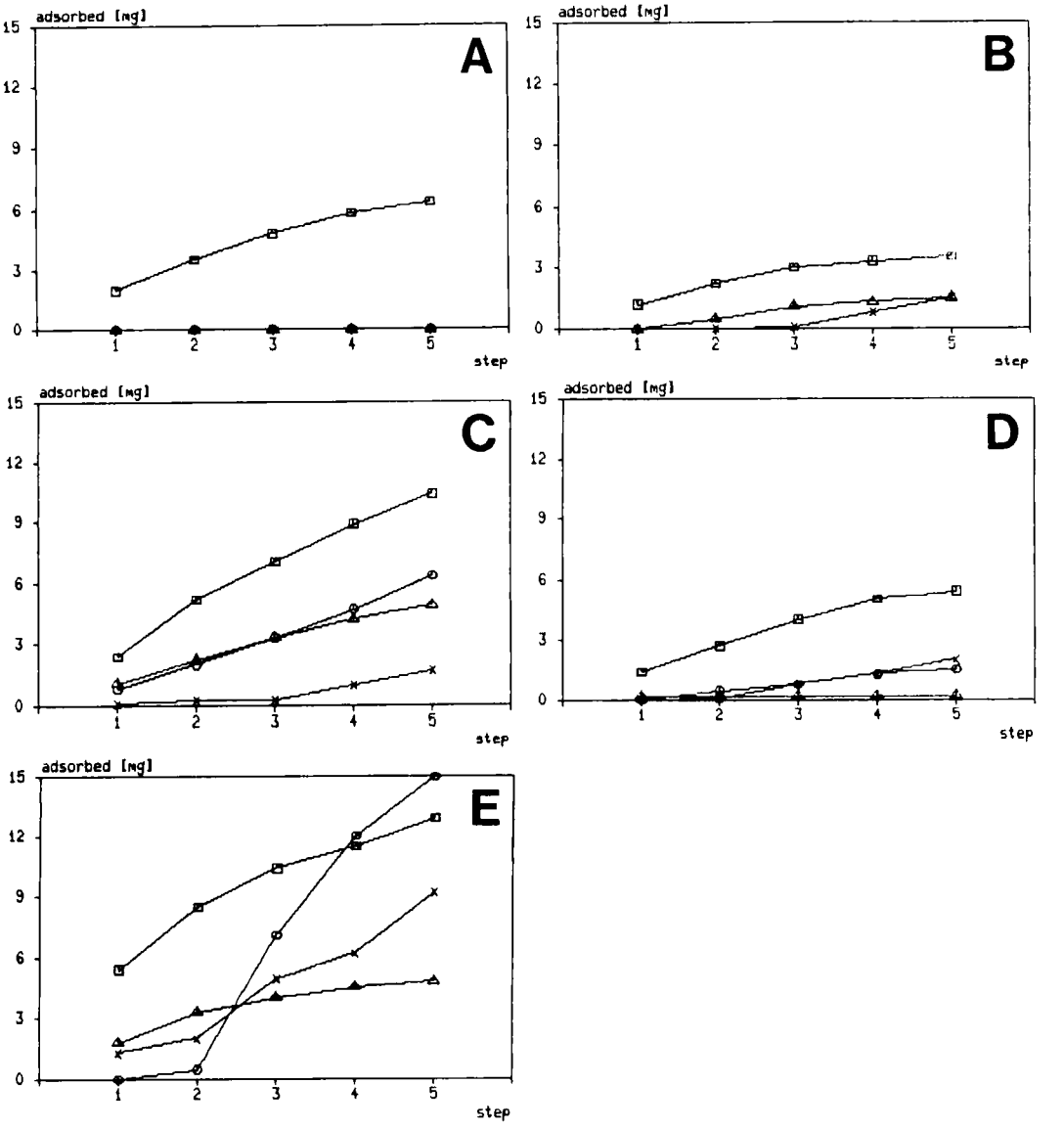

FIGURE 3

Cummulative losses of protein in five subsequent filtrations with replacement of the filter. Volumes of 25 ml containing 1 mg/ml of BSA in water (□), BSA in buffer (△), LHRH (x), or IgG (o) were repeatedly filtered. A, PVDF; B, nylon; C, cellulose nitrate; D, carton-lined cellulose nitrate 0.6 μm; E, carton-lined cellulose nitrate 0.22 μm.

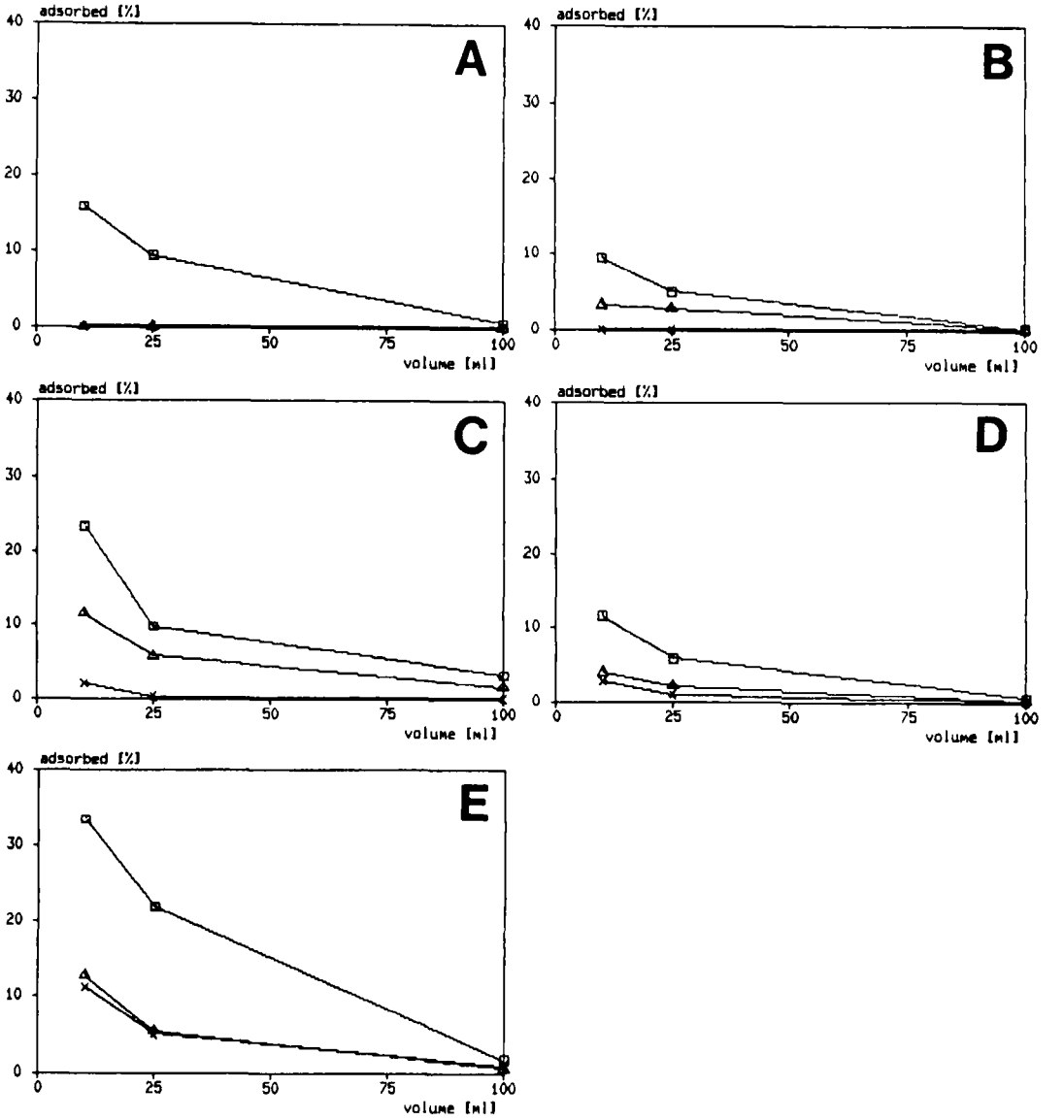

FIGURE 4

Relative loss of protein from 10, 25 or 100 ml of a 1 mg/ml solution. The losses were determined for BSA in water (□), BSA in buffer (Δ), and LHRH (x). A, PVDF; B, nylon; C, cellulose nitrate; D, carton-lined cellulose nitrate 0.6 μm; E, carton-lined cellulose nitrate 0.22 μm.

DISCUSSION

Substantial differences in protein affinity were observed for the five filters used in our experiments. All cellulose filters exhibit large adsorption capacities both for the peptide (LHRH) and the proteins (BSA and IgG) tested. The somewhat lower loss when using a 0,6 μm cellulose nitrate filter compared with a 0,22 μm filter may be caused by a larger surface area of the latter. The nylon and PVDF filters show much lower protein affinity and especially the one based on PVDF seems to be the most suitable for sterile filtration of proteins and peptides. This is confirmed by conclusions that were drawn from other experiments [2,9]. However, due to the multitude of factors that can be involved in the adsorption process [4-6], no general rule can be given for the choice of filter for a specific product. We have shown for instance that the difference between a buffered and non-buffered solution of BSA was considerable. Therefore, it is recommended to test several types of filters before processing a new peptide or protein.

The flattening of the curves in Fig. 3 and the large amounts of material adsorbed in the static experiment (Fig. 1), indicate that the kinetics of the adsorption process are of a time-dependent saturation type. The largest quantities are lost from the first few milliliters from the bulk that are filtered. Therefore, when filtering small volumes, in order to limit the relative reduction in concentration, the first few milliliters of the filtrate should be discarded. Fig. 4 shows that for large volumes this effect is less significant, despite the fact that in absolute sence larger quantities may be lost.

Other recommendations that follow from our experiments are that the number of filtrations should be kept minimal, the area of the filter should be as small

as is practicable, and the time of contact with the filter should be as short as possible.

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