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Strategies for the depyrogenation of contaminated immunoglobulin G solutions by histidine-immobilized hollow fiber membrane

C. Legallais[a], F.B. Anspach[b], S.M.A. Bueno[a], K. Haupt[a], M.A. Vijayalakshmi[a,*]

[a]*Génie Biologique, Université de Technologie de Compiègne, Centre de recherches de Royallieu, B.P. 529, 60205 Compiègne Cedex, France*

[b]*Gesellschaft für Biotechnologische Forschung (GBF), Mascheroder Weg 1, 38124 Braunschweig, Germany*

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Abstract

The depyrogenation of different IgG solutions using the histidine-linked hollow fiber membrane developed in our laboratory is presented here. Three strategies for endotoxin (ET) removal were investigated according to the immobilized histidine's ability to bind different immunoglobulins: (1) ET removal from 1 mg/ml non histidine-binding mouse monoclonal IgG_1 (MabCD4) solution was achieved in the presence of acetate buffer (pH 5.0) without any protein loss. (2) For contaminated human IgG, combined adsorption of ET and IgG in the presence of MOPS or Tris buffer was tested, followed by differential elution using increasing salt concentrations. This attempt was not successful since ET were quantitatively found in the IgG elution fraction. (3) Alternatively, it was proposed to adsorb selectively ET in the presence of acetate buffer (pH 5.0) under non binding conditions for human IgG. Human IgG could then be purified if necessary with the same membrane in the presence of MOPS buffer (pH 6.5). With a 1 m^2 histidine-PEVA module under these operating conditions, it is estimated that the depyrogenation of 3 l of 1 mg/ml IgG (human or murine) solution containing 80 EU/ml of ET should be possible.

Keywords: Depyrogenation; Histidine-immobilized hollow fiber membrane; Immunoglobulin G; Histidine; Endotoxins

1. Introduction

Immunoglobulin G (IgG) from human serum or of monoclonal type (obtained from mouse ascite fluids or from hybridoma cell culture supernatant) are of growing interest for therapeutic applications, in vivo diagnostics, etc. When proper preventive measures are not extensively taken, endotoxin (ET) liberated by gram-negative bacteria might contaminate the IgG production. ETs are able to produce fever in humans and hence have to be removed for the safe administration of these highly purified products. In general, therapeutic applications require endotoxins level to be below 5 EU/ml.

Different methods are available for ET removal from biological samples [1] but not all are suitable in the case of contaminated IgG. Ultrafiltration is not helpful since IgG are relatively large proteins (M_r: 160 000) and would be retained along with ET (M_r: 10 000–9 000 000). Adsorption on activated charcoal presents the drawback of high IgG losses [1]. Ion-exchange chromatography on DEAE columns

*Corresponding author.

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studied by Hou and Zaniewski [2] provided fairly interesting results for gamma-globulins although ET level was reduced only to 35% of the initial level. Neidhardt et al. [3] proposed the preparation of ET free murine IgG_1 using a rather long procedure based on the combination of a mixed-mode ion-exchange chromatography resin and of a QAE Sepharose column. In such a case IgG recovery was about 80% and eluate ET concentration was below 0.25 EU/ml. Affinity chromatography methods are also described for ET removal. Ligands such as polymyxin B [4] and polyethylenimine [5] were fixed to insoluble fibers or to macroporous cellulose beads, respectively, and were able to bind ET from blood or plasma. However, both affinity ligands if present in the final plasma or IgG preparations even in trace amounts due to leaching of the affinity matrix may pose serious problems: polymyxin B is neurotoxic and nephrotoxic and polyethylenimine presents low biocompatibility [5].

In this study, we used the histidine-linked hollow fiber membrane (His-PEVA) already developed in our laboratory for the separation of IgG from ET [6]. Immobilized histidine is well known for its properties of binding ET [7,8]. It is also capable of adsorbing different types of IgG under different operating conditions [9,10]. As pseudobiospecific ligand, this amino acid offers a medium affinity to different therapeutic proteins such as IgG and factor VIII [8], requiring mild elution conditions for these proteins. In addition this ligand has high chemical and physical stability and is of low cost, making it an attractive choice for this purpose. Matsumae et al. [11] have already studied the behaviour of histidine towards ET in presence of different proteins in solution. With proteins such as albumin and tumor necrosis factor (TNF), they showed that ET preferentially adsorbed to the agarose-linked histidine.

The hollow fiber module was preferred to soft chromatographic matrices because of its larger throughput, easier scale-up and mechanical stability. The IgG preparation chosen for this study was (a) monoclonal IgG_1 and (b) whole IgG from human serum. Of the various strategies attempted, the one which worked best was selective adsorption of ET to the His-PEVA membrane under optimized conditions.

2. Experimental

2.1. Module

The membrane used in this study was a hollow fiber conventionally used in plasma fractionation in an extracorporeal system. It was composed of a copolymer of ethylene and vinyl alcohol (Kuraray, Japan, Model EVAL 4A). Fiber characteristics were the following: 200 μm I.D., wall thickness 20 μm and a M_r cut-off 600 000. The PEVA hollow fiber cartridges (laboratory minimodule surface area $A=41$ cm^2 and commercial module $A=1$ m^2) were activated with 1,4-butanedioldiglycidyl ether and L-histidine was coupled as described elsewhere [6].

2.2. Tested fluids

Purified IgG from human plasma (164 g/l) was kindly supplied by Institut Mérieux (Lyon, France) or purchased from ICN (Meckenheim, Germany). Mouse anti-human CD4 monoclonal antibodies (MabCD4) of IgG_1 subclass were purified from cell culture supernatant harvested from the hybridoma cell line MAX 16 H 5 at GBF. Protein concentration was adjusted to approximately 1 mg/ml. These solutions were spiked with ET either from *E. coli* DSM 10498 cultivated at GBF or from *E. coli* 055:B5 purchased from Sigma (Saint Quentin Fallavier, France).

2.3. Reagents

The LAL COATEST® Endotoxin kit was purchased from Chromogenix (Mölndal, Sweden) and the Gelification Method kit was from Sepracor (Villeneuve la Garenne, France). The endotoxins content measurements were performed according to the manufacturers instructions.

2.4. Adsorption and elution experiments

2.4.1. Chromatographic experiments with finely cut hollow fibers

For preliminary studies using small amounts of IgG, histidine-linked fibers were finely cut (length of 1 mm) and packed into a chromatographic column.

The experiments concerning the buffer influence on IgG adsorption were carried out as described elsewhere [6].

2.4.2. Open loop experiments with His-PEVA module for IgG adsorption studies

Frontal breakthrough curve and elution were obtained based on the usual chromatographic procedures [12]: after equilibration with an appropriate buffer, the minimodule was fed with 10 ml of IgG solution (1 mg/ml) in a dead end filtration mode (retentate line totally closed) at a flow-rate of 0.20 ml/min. In these experiments, no endotoxins were added to the protein solution. After the rinsing procedures, elution was performed with the same buffer containing 0.4 *M* NaCl. Fractions were collected every 5 min at the filtrate line outlet and the absorbance at 280 nm was measured for each sample.

2.4.3. Closed loop experiments with His-PEVA module

All experiments for IgG/ET adsorption were run in a closed loop circuit, as shown in Fig. 1. The solution was removed from the stirred tank by a peristaltic pump and fed to the filtration module in cross flow mode. The filtrate flow-rate was adjusted through a second peristaltic pump in order to maintain a filtrate flow-rate to inlet flow-rate ratio of 0.85, as described elsewhere [12]. The filtrate flux was approximately 5×10^{-3} cm/min. Both filtrate and retentate lines were returned to the tank. The duration of each adsorption experiment was 1 h. The IgG concentration was monitored by a UV detector located either on the filtrate line (during the adsorption experiment) or on the retentate line (during the elution steps obtained under backflushing conditions).

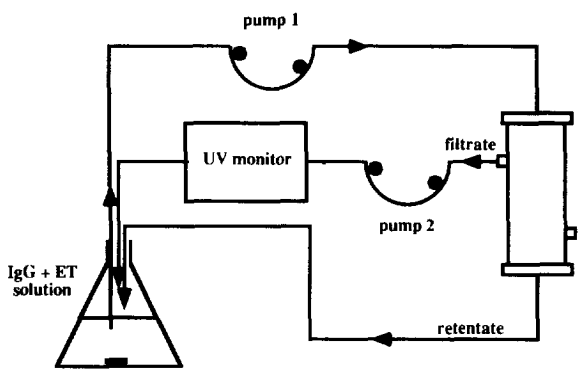

Fig. 1. Schematic of the experimental setup for affinity filtration experiments.

For the adsorption experiments, IgG and/or ET concentrations were monitored in the tank. The elution procedure was then run after a complete rinsing step [13]. Elution was achieved using increasing concentrations of NaCl buffer (0.1 *M*, 0.2 *M*, 2 *M*) using backflushing conditions (the filtrate compartment was filled with the elution buffer and fractions were collected on the retentate line after crossing the fibers).

2.5. IgG Concentration measurements

As the tested solutions contained only pure IgG, the absorbance at 280 nm was used to determine the IgG concentration, with a molar absorptivity of 14 for a 1% solution of IgG [14]. This measure was used as it has been standardized in some of our previous works. Good correlation was found between the pure IgG concentration determined by immunological methods and the absorption coefficient used here.

2.6. Endotoxins assay

ET concentration was measured either by the LAL COATEST® Endotoxin, which is a quantitative chromogenic limulus amebocyte lysate (LAL), choosing the end point method, or by the gelification method. ET from *E. coli* O111:B4 were used as standards.

3. Results and discussion

3.1. Preliminary results on IgG and ET adsorption on His-PEVA membrane

Matrix-linked histidine is well known to bind ET independently [7] and some subgroups of IgG (from human serum or monoclonal antibodies) [8–10], depending on the operating conditions. Nevertheless

data on depyrogenation of ET contaminated IgG solutions are not available (in contrast to other protein solutions such as albumin, TNF [11] and factor VIII [8]). In addition, all the previous studies were done only on soft gel matrices. Our results in this work show the affinity of the His-PEVA membrane towards both different IgGs and ET. We have thus explored various strategies for simultaneous depyrogenation of defined IgG solutions, based on the differences of adsorption and elution conditions for both the molecules.

Preliminary experiments on ET adsorption confirmed that His-PEVA membrane was able to bind pyrogens (63 EU/cm^2) best in the presence of 25 m*M* acetate buffer (pH 5) in agreement with the results from Matsumae et al. [11] and Minobe et al. [15] obtained with histidine linked to Sepharose beads (His-Sepharose 4B). Desorption conditions described in the literature indicated that a harsh treatment was necessary to remove ET from the support [15].

Table 1 lists the results on the conditions of adsorption and desorption of different types of IgG to membrane immobilized histidine. The given adsorbed amounts were obtained under less than saturation loading of the His-PEVA membrane, using operating conditions described elsewhere [12,13]. Human IgG was well adsorbed to the His-PEVA membrane, in 25 m*M* MOPS buffer (pH 6.5). The adsorption of prepurified human IgG was above 50% while that from total serum was about 34%, thereby indicating masking effects by other molecules in accordance with our previous data [16]. This type of interaction was medium affinity, with a K_D in dynamic mode of about 3.7×10^{-6} *M* for purified human IgG, as determined elsewhere using Langmuir description for adsorption isotherm [13]. Consequently the desorption conditions were very mild, since 0.1 *M* NaCl was sufficient to remove IgG from the membrane [6].

On the other hand, some monoclonal antibodies produced by hybridoma cell culture or by ascite fluid showed poor binding to the His-PEVA membrane. Among the studied examples, one mouse IgG_2 as well as MabCD4 were not adsorbed at all under the conditions employed here.

Competition between IgG and ET was anticipated with regard to their binding to immobilized histidine in some cases, while in others, only ET were supposed to adsorb.

Three different strategies for the depyrogenation of IgG were attempted: (1) binding of ET when IgG being depyrogenated does not bind to His-PEVA membrane (applicable to certain types of monoclonal IgG); (2) exploiting the different strengths of binding of ET and IgG and use of differential elution of IgG; (3) developing conditions for selective adsorption of ET in the cases where IgG (such as human IgG) also has affinity for His-PEVA.

3.2. Depyrogenation of a non adsorbing monoclonal IgG_1: MabCD4

Murine IgG_1 subclass monoconal antibody (MabCD4) is known for its lack of binding to histidine coupled matrices [17]. Hence, an ET contaminated preparation of MabCD4 was used in this study with His-PEVA membrane. It was found that

Table 1
Behaviour of His-PEVA hollow fiber membrane towards IgG adsorption

Species	Solution	Application conditions		Adsorption characteristics		Desorption conditions
		Buffer	pH	Injected mass (mg/cm^2)	Adsorbed mass (mg/cm^2)	
Whole IgG	Human serum	25 m*M* MOPS	6.5	0.085	0.029	100 m*M* NaCl
Whole IgG	Prepurified from human plasma	25 m*M* MOPS	6.5	0.08	0.045	100 m*M* NaCl
Mouse IgG_2	Ascite fluid	25 m*M* Tris	7.4	0.057	No adsorption	
Mouse IgG_3	Ascite fluid	25 m*M* Tris	7.4	0.075	$1.9\ 10^{-4}$	100 m*M* NaCl
MabCD4	Cell culture	25 m*M* Tris	7.4	0.24	No adsorption	

MabCD4 did not bind to His-PEVA in the presence of several different buffers (MOPS pH 6.5, MES pH 5.5, Tris–HCl pH 7.4 and 8.5, 25 m*M* acetate pH 5). These buffers were chosen for their decreasing ability to enhance human IgG adsorption to immobilized histidine [16].

Then the influence of different types of buffer (25 m*M* Tris pH 7.4, 25 m*M* phosphate pH 6.0, 10 m*M* MES pH 5.5, 25 m*M* acetate pH 5.0) on the ET adsorption was investigated. As immobilized histidine is known to bind ET at low ionic strength, chosen buffer concentrations were not higher than 25 m*M*. The results are summarized in Table 2. Different initial ET concentrations were used due to the difficulty of adjusting spiking solution concentrations. All experiments were run in the presence of MabCD4 (1 mg/ml). Acetate buffer (pH 5.0) seemed the most suitable for ET adsorption, followed by Tris–HCl, pH 7.5. In all cases no MabCD4 adsorption occurred. It may be mentioned that phosphate buffer was suggested by Matsumae et al. [11] for ET binding to His-Sepharose 4B, but was not found to be suitable with our system. This might be due to the higher ionic strength employed during binding.

The binding capacity for ET of the His-PEVA membrane may be compared to that found by Matsumae et al. with phosphate buffer using a chromatographic gel instead [10]. His-Sepharose 4B exhibited a maximum equilibrium capacity in pure water of 3.1×10^6 EU/g of wet adsorbent while the dynamic capacity decreased in the presence of TNF (pI near that of MabCD4) to 40.5 EU/g of dry gel (injection of 45 EU/g of gel). In our study, with acetate buffer, the dynamic capacity on the functionalized membrane was 63 EU/cm^2 (=33 000 EU/g) of membrane.

With initial concentration of ET as 228 EU/ml, high clearance rate of up to 81% was observed with reduction of endotoxins to the level of 44 EU/ml. However, with a lower initial ET concentration (25 EU/ml), complete depyrogenation of MabCD4 solution was achieved since the final content was below the LAL test detectable limit (Table 2). Thus, this method constitutes an excellent approach for the polishing step in depyrogenation of such monoclonal antibodies solutions.

The large scale module ($A=1$ m^2) was also used for scaling-up of the method. The respective concentration of MabCD4 and ET in the loading volume (2300 ml) were respectively 0.53 mg/ml and 27.2 EU/ml. Due to experimental and sterility constraints, it was not possible to take samples directly from the tank. The concentrations were measured both in filtrate and retentate lines. The results were similar to those obtained with the minimodule, with comparable initial ET concentrations. In addition, they provided more details about the ET removal process. The filtrate was totally devoid of endotoxins, while some of them remained in the retentate side: almost all the ET that crossed the membrane were adsorbed, while those whose mass was larger than 600 000 (nominal cutoff of the EVAL 4A membrane), were retained by the membrane. So, in the future, it is proposed to operate the filtration under dead-end conditions. In that case, ET that cannot cross the

Table 2
Buffer influence on the endotoxin adsorption on His-PEVA hollow fiber membrane minimodule ($A=41$ cm^2) in IgG solution (1 mg/ml)

Buffer	Molarity (m*M*)	pH	C_i (EU/ml)	Quantity adsorbed (EU/cm^2)	Endotoxins remaining in solution		IgG removal (%)
					C_{eq} (EU/ml)	% of C_i	
Tris	25	7.4	255	47.5	115	45	11
Phosphate	25	6.0	39	7	17	43	3
MES	10	5.5	75.6	11.2	42.7	56	4.5
Acetate	25	5.0	228	63	44	19	1.5
Acetate	25	5.0	25	8.5	0[a]	0	1

C_i: Initial concentration in the tank.
C_{eq}: tank concentration at the end of the experiment (after 1 h).
[a] Below the detectable limit.

membrane will be retained in the retentate compartment. Dead-end filtration could apparently be achieved without important membrane plugging, since with quasi dead-end filtration $Q_f/Q_i=0.85$), neither decrease of IgG sieving coefficient nor increase of transmembrane pressure were observed (unpublished data).

3.3. Simultaneous depyrogenation and adsorption of human IgG by differential elution

The treatment appeared to be more complicated in the case of human IgG when both IgG and ET were adsorbed on the membrane. In the case of human IgG, the strategy would be to obtain IgG without eluting ET in the first elution step, based on stronger adsorption of ET. The adsorption and elution of ET spiked human IgG was studied in the presence of either MOPS or Tris–HCl buffer (25 m*M*). MOPS was chosen because of its known ability to promote adequate human IgG adsorption, and Tris because it improved selectivity of linked-histidine towards IgG_1 and IgG_3 subclasses [12]. The results are summarized in Table 3.

The adsorbed amount of human IgG in this case was slightly lower than that obtained in the absence of ET. In the presence of ET it dropped to 119 $\mu g/cm^2$ with MOPS and 25 $\mu g/cm^2$ with Tris as compared to 144 $\mu g/cm^2$ and 96 $\mu g/cm^2$ for maximum dynamic capacity obtained in the absence of ET, respectively [12].

With regard to ET, no ET adsorption occurred with MOPS buffer and adsorption was poor (about 5 EU/ml) with Tris/HCl buffer in the presence of human IgG. In both cases, it seemed that the adsorption of IgG to the His-PEVA membrane prevented that of ET. This could be explained by the molar ratio between IgG and ET present in solution. If 1 000 000 was taken as the molecular mass for ET, an initial concentration of 50 EU/ml of ET and of 1 mg/ml of IgG corresponded to molar concentrations of 5×10^{-12} and 6.7×10^{-6} mmol/ml, respectively. Thus, on a molar basis, IgG were 10^6 times more concentrated than ET in solution, which was of course highly desirable in the preparation of pharmaceutical products. Hence it could be postulated that IgG adsorbed preferentially on immobilized histidine under these buffer conditions and that all adsorption sites were saturated by IgG, instead of ET, or that IgG adsorption displaced that of ET.

The amounts obtained by elution of this adsorbed IgG are given in Table 3. As expected, the complete elution of the adsorbed IgG (corresponding to the mass that disappeared from the tank) appeared with 100 m*M* NaCl. The eluted amount corresponded to the total recovery of the bound material. The results for ET differed from what was expected. First of all, the elution seemed to occur simultaneously, despite the fact that the ET were known to be very difficult to remove from a support. Secondly, the quantity of ET found in the elution peak was higher (>400 EU with MOPS and 800 EU with Tris) than that

Table 3
Influence of buffer type on adsorption and desorption of IgG/elidotoxin mixture on His-PEVA hollow fiber membrane (A=41 cm^2)

Buffer type		MOPS 25 m*M* pH 6.5	Tris–HCl 25 m*M* pH 7.5
Initial volume (ml)		10	10
Tank initial concentration	IgG (mg/ml)	1.0	1.0
	Endotoxin (EU/ml)	39.0	63.6
Tank final concentration	IgG (mg/ml)	0.5	0.75
	Endotoxin (EU/ml)	36.3	43.1
Elution NaCl 100 m*M*	IgG (mg)% of M_i	5 (50%)	1 (10%)
	Endotoxin (EU)% of EU_i	>400 (>100%)	800 (>100%)
	Volume (ml)	3.8	4.0

M_i: tank initial mass.
EU_i: tank initial ET content.

removed from the tank (23.4 EU and 203.5 EU respectively). These results obtained with the Coatest® method were also confirmed by the gelification test. It is possible that adsorption on the membrane has destroyed, at least partially, the ET oligomeric structure and hence released smaller units. As the activity of ET is located in the lipid A, it could be envisaged that more lipid A was accessible for the LAL reaction after the treatment.

In any case, it is obvious that this approach of differential elution for IgG and ET, though attractive in theory, cannot be efficiently employed in practice.

3.4. Selective adsorption of ET under non binding conditions for human IgG

In view of the above, the strategy was changed to exploring conditions under which only ET bound to His-PEVA. Therefore the influence of different buffers on human IgG adsorption was studied. The results are given in Fig. 2. The chosen buffers covered a pH range from 5.0 to 8.5. Some of these buffers were zwitterionic and are known to influence human IgG adsorption to His-PEVA membrane to a varying degree [16]. Phosphate buffer (pH 6.0–7.0) was the least favorable to IgG adsorption, followed by acetate buffer (pH 5.0–6.0) and Tris buffer (pH 7.5–8.5). Tris buffer had already shown a poor affinity for ET in human IgG solution. In the case of phosphate buffer, little ET adsorption was achieved, even in the presence of MabCD4 which did not adsorb (see Table 2). In contrast, acetate buffer combined the properties of enhancing ET adsorption (see Table 2) and limiting IgG binding to the membrane.

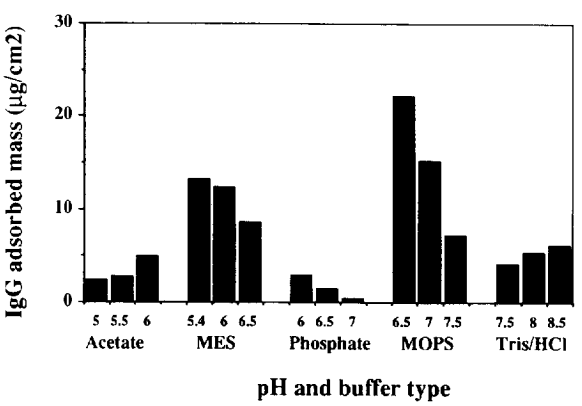

Fig. 2. Influence of buffer type and pH on human IgG adsorption onto a His-PEVA membrane.

Hence the depyrogenation step was conducted with acetate buffer pH 5.0. These experiments aimed at determining the capacity of the His-PEVA membrane for ET. Two different ET concentrations in 1 mg/ml IgG solutions were injected, always in a closed loop circuit as described in Section 2. The results are summarized in Table 4. The ET clearance rate increased as initial concentration decreased. However the quantity adsorbed with initial concentration of 856 EU/ml indicated a large capacity of the His-PEVA membrane minimodule (more than 9000 EU). Hence theoretically with an initial concentration of 228 EU/ml the totality of ET was expected to be adsorbed by the membrane module, since the ET total loading amount was less than half of the previously adsorbed one. But it was not the case. It may be suggested that a partition effect existed, perhaps indicating more than one equilibrium of binding. Nevertheless, these data and those obtained with ET spiked MabCD4 draw the estimation that 1 m^2 filtration area hollow fiber module should able to depyrogenate 3.5 l of IgG solution containing initially about 80 EU/ml of ET. This is a significant result as it again shows the success of this approach in the last step of depyrogenation (with low ET concentrations) which is the most difficult to achieve.

Now that depyrogenation was successful, we wanted to check the IgG recovery from the depyrogenated solution. Hence a second step of adsorption of human IgG unadsorbed in the ET adsorption experiment was performed on His-PEVA membrane in the presence of MOPS buffer (pH 6.5), which ensured the best human IgG adsorption [16]. After buffer exchange, 1 ml of depyrogenated IgG (0.4 mg/ml), the non-retained fraction of the previous step, was injected into a chromatographic column containing finely cut His-PEVA membrane. These IgG were quantitatively adsorbed on this column, exhibiting that IgG was not altered by the treatment. In addition, this also indicates that if one starts with crude human IgG solutions, this second step could serve as a purification step as well. Thus, this approach can combine depyrogenation and puri-

Table 4
Effect of initial concentration on human IgG and endotoxin adsorption onto His-PEVA hollow fiber membrane in the presence of acetate buffer, pH 5

Experiment	1	2
Initial volume V_i (ml)	15	14
IgG initial concentration (mg/ml)	1.03	0.79
IgG final concentration (mg/ml)	0.78	0.65
Initial endotoxin concentration (EU/ml)	856	228.3
Endotoxin concentration after 30 min (EU/ml)	730.8	47.3
Endotoxin concentration after 60 min (EU/ml)	238.6	44.0
Clearance rate	72.1%	80.7%
Quantity adsorbed (EU/cm^2)	226	63
Initial protein/endotoxin (mg/EU)	0.12%	0.35%
Final protein/endotoxin (mg/EU)	0.33%	1.48%
Enhancement factor	×2.75	×4.20

fication on the same module in two steps, in the case of human IgG.

4. Conclusion

The depyrogenation of parenteral and pharmaceutical products is a challenging job. In this study, we report two different strategies for ET removal from immunoglobulin G solutions based on histidine-linked hollow fibre membrane.

In the first and simpler case, a monoclonal antibody (MabCD4) was not adsorbed by the His-PEVA membrane. An appropriate buffer (acetate, pH 5.0) was chosen because it enhanced ET binding to immobilized histidine. The ET adsorption did not accompany any adsorption of this IgG.

The alternative approach for human IgG was to choose proper operating conditions which enhanced ET adsorption and did not allow IgG binding to the His-PEVA membrane, i.e., equilibration buffer acetate pH 5.0. If necessary, after the depyrogenation step, IgG could be adsorbed on His-PEVA membrane, in the presence of MOPS buffer pH 6.5, as already described, for purification purposes.

For the first step, as well as for MabCD4 depyrogenation, it was calculated that a module ($A=1$ m^2) would be able to reduce the ET level below the threshold limits of at least 3 l of IgG (1 mg/ml) with an initial ET concentration of 80 EU/ml.

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References

[1] S.K. Sharma, Biotech. Appl. Biochem., 8 (1986) 5.
[2] K.C. Hou and R. Zaniewski, Biotech. Appl. Biochem., 12 (1990) 315.
[3] E.A. Neidhardt, M.A. Lutherand and M.A. Recny, J. Chromatogr., 590 (1992) 255.
[4] K. Hanasawa, T. Tani, T. Oka, T. Yoshioka and H. Aoki, in Y. Nosé, P.S. Malchesky and J.W. Smith (Editors), Therapeutic Apheresis: a Critical Look, ISAO Press, Cleveland, OH, 1984, p. 167.

[5] S. Mitzner, J. Schneidewind, D. Falkenhagen, F. Loth and H. Klinkmann, Artif. Organs, 17 (1993) 775.
[6] S.M.A. Bueno, K. Haupt and M.A. Vijayalakshmi, J. Chromatogr. B, 667 (1995) 57.
[7] S. Minobe, T. Watanabe, T. Sato, T. Tosa and I. Chibata, J. Chromatogr., 248 (1982) 401.
[8] M. Ezzedine, F. Lawny, M.A. Vijayalakshmi, in C. Rivat and J.F. Stolz (Editors), Biotechnology of Blood Proteins, Colloque Inserm, Vol. 227, John Libbey Eurotext, Montrouge, 1993, p. 115.
[9] A. El-Kak and M.A. Vijayalakshmi, Bioseparation, 3 (1992) 47.
[10] S. Mandjiny and M.A. Vijayalakshmi, in C. Rivat and J.F. Stolz (Editors), Biotechnology of Blood Proteins, Colloque INSERM, Vol. 227, John Libbey Eurotext, Montrouge, 1993, p. 189.
[11] H. Matsumae, S. Minobe, K. Kindan, T. Watanabe, T. Sato and T. Tosa, Biotech. Appl. Biochem., 12 (1990) 129.
[12] S.M.A. Bueno, K. Haupt and M.A. Vijayalakshmi, Int. J. Artif. Organs, 18 (1995) 392.
[13] S.M.A. Bueno, C. Legallais, K. Haupt and M.A. Vijayalakshmi, J. Membrane Sci., 117(1–2) (1996) 45.
[14] A. ElKak and M.A. Vijayalakshmi, J. Chromatogr., 570 (1991) 29.
[15] S. Minobe, T. Watanabe, T. Sato and T. Tosa, Biotech. Appl. Biochem., 10 (1988) 143.
[16] K. Haupt, S.M.A. Bueno and M.A. Vijayalakshmi, J. Chromatogr. B, 674 (1995) 13.
[17] F.B. Anspach and D. Petsch, Bioseparation, 6 (1996) 165–184.