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Improvement in the polyethylene glycol–Cibacron Blue purification method

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Abstract

A new approach was developed for the purification of polyethylene glycol (PEG)–Cibacron synthesized from PEG 8000 and Cibacron 3G-A, using an NH_2-silica anion exchanger for separation of the unreacted PEG 8000 from the other compounds of the reaction mixture. The adsorption of PEG–Cibacron and Cibacron under acidic conditions (pH 4.7) was 22.4 mg (Cb and PEG–Cb) per gram of silica and 100% elution was achieved with 1% (v/v) ethanolamine solution at pH 9. The silica exchanger can be reused several times without loss of performance. An extraction step with chloroform was used to separate the PEG–dye from the non-bonded Cibacron, which completely partitioned into the aqueous phase. After drying, a dark-blue solid polymer with a yield of 7.6% in relation to the initial mass of PEG was obtained, which is an increase of 50% in relation to earlier work.

1. Introduction

Two-phase aqueous systems have been studied and used in separations of microbial cells and cellular debris [1], in the purification of proteins and enzymes [2] and in bioconversion systems [3]. Affinity partitioning with the objective of increasing the specific purification of biological products (*e.g.*, enzymes and proteins) has also been used, based on two-phase aqueous systems [4]. Synthetic dyes are common and advantageous affinity ligands, Cibacron Blue (Cb) bound to polyethylene glycol (PEG) being one of the most studied ligands [5] for extraction with aqueous two-phase systems.

Different reactions and mainly three purification methods [6–8] (Table 1) have been used to obtain pure PEG–Cb. The aim of this work was to contribute to the improvement of the purification of PEG–Cb by eliminating the first step (Table 1) and to replace DEAE-cellulose with another anion exchanger, namely a silica carrier modified by silanization [9] with a primary amine group.

2. Experimental

2.1. Materials

Cibacron Blue 3GA and polyethylene glycol (PEG) of average molecular mass 8000 were obtained form Sigma (St. Louis, MO, USA). Silica gel (XWP 500 MP) with a pore diameter of 50 nm and a particle size between 0.5 and 1.0 mm was purchased from Grace (Worms, Ger-

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Table 1
PEG–CB purification methods

Ref.	1st step	2nd step	3rd step	PEG 8000 yield (%)
Kopperschlager and Johansson [7]	Drying	Chloroform extraction	Methanol recrystallization	4.2
Johansson *et al.* [8]	Chloroform extraction	DEAE ion exchange	Chloroform extraction	5.0
Johansson and Joelsson [6]	Dilution or dialysis	DEAE ion exchange	Chloroform extraction	5.0

many). 3-(Triethoxysilyl)propylamine (99%) and ethanolamine (98%) were obtained from Merck (Hohenbrunn, Germany). Sephadex G-75 was supplied by Pharmacia (Uppsala, Sweden). Other reagents were of analytical-reagent grade.

2.2. Analytical methods

Cb, PEG–CB, ethanolamine and PEG determinations. The absorbance of the Cb and PEG–Cb complex was measured at 612 nm [6]. Ethanolamine was measured by spectrophotometry at 310 nm. The concentration of PEG was determined using the method of Skoog [10] by reading the absorbance at $\lambda_{max} = 546$ nm.

Amine groups in silica. Silica amine groups were determined by the 2,4,6-trinitrobenzenesulphonic acid (TNBS) method [11] after alkaline solubilization.

Preparation of PEG–Cb. The covalent binding of Cb to PEG was based on the method described by Johansson and Joelsson [6]. A solution of PEG and Cb was prepared by mixing 20 g of PEG, 0.66 g of Cb and 20 ml of water. The reaction was started with 16 ml of a solution of 14% (w/v) NaOH and 6% (w/v) $NaSO_4$. After 7 h of agitation at room temperature the reaction was stopped by lowering the pH with pure acetic acid (99.8%).

3. Results and discussion

3.1. Adsorption conditions

The acidic reaction mixture obtained from PEG–Cb preparation produced a PEG–salt-type aqueous two-phase system after settling. The purest top phase was used in further processing; however, this phase was diluted fivefold to decrease its viscosity.

Adsorption of top-phase Cb and PEG–Cb on a silica anion exchanger was tested at different pH values and amine levels. The adsorption capacity was highest at the lowest pH in the range 4–7 (Fig. 1) without adsorption of PEG.

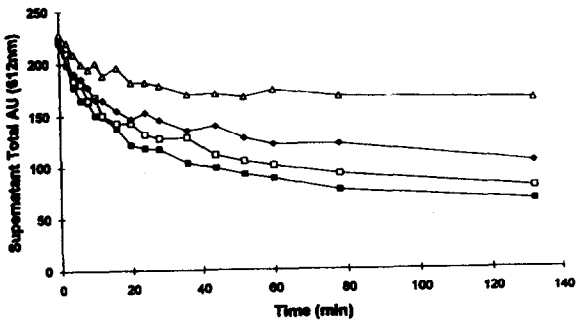

Fig. 1. Cibacron and PEG–Cb batch adsorption at 23°C on modified silica (1 g with 12.5 mg NH_2) at different pH values: (△) pH 7; (◆) pH 6; 9□) pH 5; (■) pH 4.

Adsorption was done at pH 4.7 owing to the buffer capacity of acetic acid at this pH [12].

The capacity of the modified silica was improved by increasing the amine group loading (Fig. 2). There was no adsorption on the unmodified silica and with the aqueous silanization method [9] the best binding was 22.4 mg (Cb and PEG–Cb) per gram of silica for an average amine level of 13.6 mg NH_2 per gram of silica.

3.2. Elution conditions

The method described in the literature [6,8] uses 2 *M* KCl to destabilize the ionic interaction between DEAE-cellulose and PEG–Cb. It could be verified that using silica modified with a primary amine group, which is a milder ion exchanger than the DEAE group, it was possible to elute Cb and PEG–Cb with an alkaline buffer (pH 9) (Fig. 3) by neutralizing the electric charge of amine groups on the silica surface. Ethanolamine solution (1%, v/v) was chosen as the eluent as it efficiently regenerates the modified silica and presents a buffer capacity at high pH.

3.3. Extraction

PEG–Cb was extracted from the silica eluate in a two-phase system (aqueous chloroform) with a volumetric phase ratio of 10:1. Ethanolamine

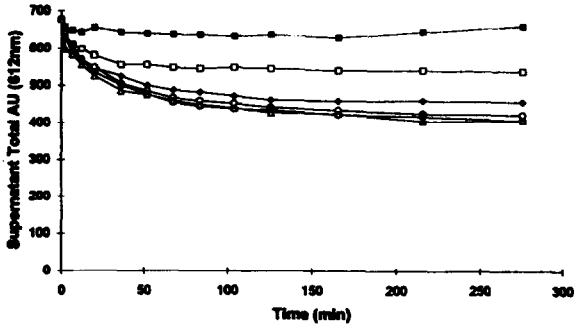

Fig. 2. Cibacron and PEG–Cb batch adsorption at 23°C on modified silica with different amine levels (1 g with different amounts of NH_2) at pH 4.7. NH_2: (■) 0.0; (□) 5.3; (◆) 11.3; (○) 12.5; (●) 13.0; (△) 14.2 mg.

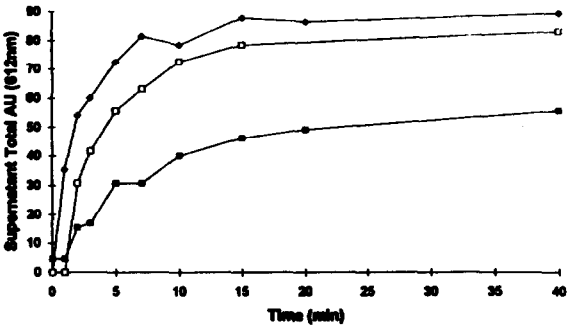

Fig. 3. Cibacron and PEG–Cb batch elution from modified silica (1 g with 5.3 mg NH_2) with 2.3% ethanolamine at different pH values. (■) pH 8; (□) pH 8.5; (◆) pH 9.

(partition coefficient 16.4) and Cibacron partition favourably to the aqueous phase, but total phase separation was only possible if 0.4 *M* KCl was present in the aqueous phase.

PEG–Cb was obtained as solid product after drying the chloroform phase with anydrous sodium sulphate and vacuum evaporation at 40°C.

3.4. PEG–Cb purity and yield

A purification strategy, shown in Fig. 4, was tested to obtain the PEG–Cb complex. Several cycles of column adsorption and elution were

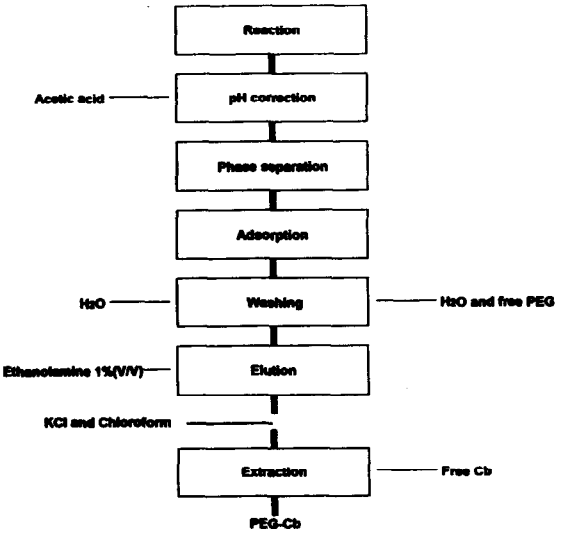

Fig. 4. PEG–Cb purification process.

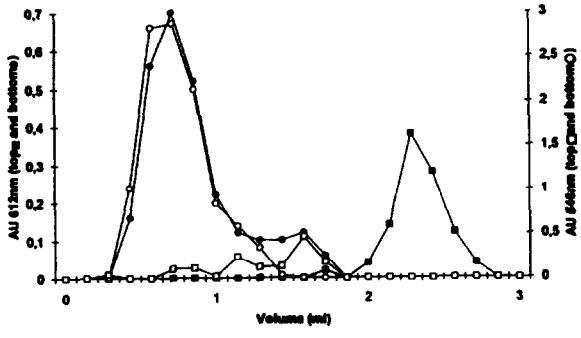

Fig. 5. Chloroform extraction of PEG–Cb demonstrated by gel filtration chromatography. PEG–Cb is present in the bottom phase (signal at 546 nm, ○; and 612 nm, ●), and in a parallel run Cb is only present in the top phase (signal at 612 nm, ■; and no signal at 546 nm, □).

used to purify a total reaction batch (20 g of PEG reacted with 0.66 g of Cibacron Blue 3G-A). The NH_2-silica exchanger could be totally recycled using 1% (v/v) ethanolamine solution at pH 9. These adsorption–elution cycles were extended to more than ten runs without any apparent loss of adsorption and elution capacity.

The efficiency of the chloroform extraction step in separating PEG–Cb and Cb was tested by gel filtration chromatography using a Sephadex G-75 column (8 cm × 0.4 cm I.D.). From fig. 5, it can be seen that PEG–Cb was collected in the first fractions and Cb in the last fractions. It can also be concluded that Cb is present only in the top phase and PEG–Cb in the bottom phase.

The final yield was 0.076 g of PEG–Cb per gram of initial PEG, based on dry mass measurements, whereas the value of monosubstituted PEG 8000 (0.117 mmol Cb per gram of polymer) found experimentally is similar to the theoretical value (0.125 mmol Cb per gram of polymer). These results compare favourably with those in the literature (Table 1).

4. Conclusions

A two-step procedure was achieved for PEG–Cb purification. The PEG–Cb was adsorbed on an NH_2-silica anion exchanger and was easily and completely eluted with 1% (v/v) ethanolamine solution, leading to a higher (50%) purification yield in comparison with previous procedures. The anion exchanger can be reused several times (>10) without a decrease in capacity.

5. References

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