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Amberlite XAD-4 is a convenient tool for removing Triton X-100 and Sarkosyl from protein solutions

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ABSTRACT

Amberlite has been shown to be an appropriate material for the adsorption of organic contaminants from aqueous solutions. In addition, Amberlite XAD-2 has been successfully used, as an alternative to Bio-Beads, to remove Triton X-100 from protein solutions, such as from samples of solubilized membrane proteins. However, Amberlite has not been tested as an adsorbent when a mixture of detergents is necessary to solubilize and refold a target protein. Here the authors show that Amberlite XAD-4 can be appropriately used to aid the purification process of proteins solubilized from inclusion bodies with the ternary detergent system consisting of Sarkosyl, Triton X-100 and CHAPS.

TWEETABLE ABSTRACT

The authors devised a time-saving and cost-effective method for the removal of detergents from proteins refolded after their solubilization from inclusion bodies.

METHOD SUMMARY

Amberlite XAD-4 can be successfully used to remove detergents that are used to solubilize recombinant proteins overexpressed in *Escherichia coli* as inclusion bodies. In particular, the authors show that the detergents Sarkosyl, Triton X-100 and CHAPS can be adsorbed by Amberlite XAD-4 when present as a ternary mixture in protein solutions. Remarkably, the adsorption of proteins to Amberlite XAD-4 was found to be negligible.

KEYWORDS:

Amberlite XAD-2 • Amberlite XAD-4 • CHAPS • *Escherichia coli* • inclusion bodies • N-lauroylsarcosine • Triton X-100

Detergents are essential for the solubilization, purification and crystallization of membrane proteins. Usually, nonionic detergents are considered to be the most suitable agents for the isolation of membrane proteins under conditions preserving their native properties.

Triton X-100 is a neutral surfactant commonly used for the solubilization of membrane proteins [1,2]. However, the low critical micellar concentration of Triton X-100 (0.22–0.24 mM [3,4]) translates into its difficult removal from protein solutions by dialysis or size exclusion chromatography. Therefore, a number of alternative procedures have been defined to remove this detergent from solubilized proteins. Among these, the efficient adsorption of Triton X-100 by Bio-Beads or Amberlite XAD-2 via on-column or in-batch methods has been reported [5–7].

The overexpression in *Escherichia coli* of proteins whose natural copy number is low represents an invaluable technology to obtain and purify these proteins. Nevertheless, the overexpression in *E. coli* of a target protein can lead to its aggregation *in vivo* in the form of inclusion bodies [8,9], therefore demanding the solubilization of the protein of interest. Quite recently, a mild method to recover overexpressed proteins from inclusion bodies was devised [10]. According to this procedure, the anionic detergent N-lauroylsarcosine (also denoted as Sarkosyl) is used as the solubilizing agent, at concentrations ranging from 1% to 10% (w/v) [10]. Remarkably, it was reported that proteins solubilized with Sarkosyl can maintain their secondary structure [11], with the subsequent removal of Sarkosyl representing the only further step necessary to obtain their refolding. To this aim, Triton X-100 and the zwitterionic detergent CHAPS (Figure 1) are added to the solution containing proteins and Sarkosyl [10]. By this means, the presence of Triton X-100 and CHAPS favors the exchange of Sarkosyl from the solubilized proteins to mixed micelles [10]. It should indeed be noted that both Sarkosyl and CHAPS feature a relatively high critical micelle concentration (CMC) at 25°C (13–14 mM [11] and 6.5 mM [12], respectively).

Nevertheless, removal of the Sarkosyl–Triton–CHAPS (STC) detergent triad from target proteins is a difficult task, the accomplishment of which has not been inspected. Conversely, a simplified procedure for the recovery of aggregated proteins from inclusion bodies has been investigated [13]. In particular, overexpressed subunits of *E. coli* RNA polymerase were recovered from inclusion bodies using Sarkosyl, which was subsequently removed by dialysis [13]. To devise a simple and inexpensive procedure for the removal of the STC detergent triad or Sarkosyl only from protein solutions, the authors of the present study thought it of interest to investigate the use of Amberlite XAD-4. Indeed, both Amberlite XAD-2 and Amberlite XAD-4 are polystyrene-divinylbenzene copolymers, but the latter features

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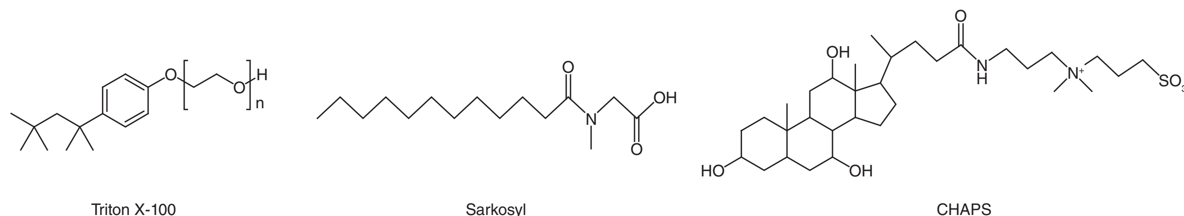

Figure 1. Structures of the surfactants considered in the present work.

a much higher surface area (about 300 vs ≥750 m^2/g, respectively). Accordingly, the authors report here on the competence of Amberlite XAD-4 to adsorb Triton X-100, Sarkosyl and CHAPS, when individually present in solution or as components of a ternary mixture.

To detect the adsorption by Amberlite XAD-4 of Triton X-100, Sarkosyl and CHAPS (Figure 1), the authors selected a spectroscopic analytical method. Accordingly, they first determined the UV absorption spectra of these detergents over the 200–400 nm wavelength interval (Figure 2A). They then tested the adsorption by Amberlite XAD-4 of Triton X-100 at 20°C and observed that the resin did rapidly adsorb the detergent at 0.1% concentration (w/v, 1.6 mM) in 50 mM Tris-HCl, 50 mM NaCl, pH 7.5 (Figure 2B). In addition, a relatively fast and almost complete adsorption of Sarkosyl from a 1 % (w/v, 34.1 mM) solution was also observed under the same conditions (Figure 2C), whereas the performance of Amberlite in removing CHAPS (10 mM starting concentration) was limited to approximately 40% (Figure 2D). Quite a number of years ago, it was shown that Amberlite XAD-2 is very efficient in removing Triton X-100 from aqueous solutions [6]. Therefore, the authors of the present study decided to compare the competence of Amberlite XAD-4 in adsorbing Triton X-100, Sarkosyl and CHAPS with the corresponding performance of Amberlite XAD-2. As expected, the removal of 1.6 mM Triton X-100 by 1.9 g of Amberlite XAD-2 was observed to occur within 30 min after addition of the resin to the sample (Figure 2B). This implies that Amberlite XAD-2 is as efficient as Amberlite XAD-4 in the adsorption of Triton X-100. Surprisingly, the authors detected poor adsorption of Sarkosyl by Amberlite XAD-2, with Amberlite XAD-4 performing much better under the same conditions (Figure 2C). In the case of CHAPS, Amberlite XAD-2 removed only 10% of this detergent (Figure 2D), whereas Amberlite XAD-4 did adsorb ∼40% of the zwitterionic surfactant (Figure 2D). According to the observations obtained with Triton X-100, Sarkosyl and CHAPS, Amberlite XAD-4 appears to feature a wider competence in adsorbing detergents when compared with Amberlite XAD-2, the action of which seems quite specific only for Triton X-100.

When the procedure proposed by Park *et al.* [14] is used to solubilize overexpressed proteins from *E. coli* inclusion bodies, the target protein/enzyme is finally recovered into a solution containing 0.8% (w/v, 27.3 mM) Sarkosyl, 0.1% (w/v, 1.6 mM) Triton X-100 and 0.06% (w/v, 1 mM) CHAPS (08-01-006 STC triad) [14]. Therefore, the authors of the present study evaluated the effectiveness of Amberlite XAD-4 in adsorbing detergents when 50 ml of the 08-01-006 STC triad in 50 mM Tris-HCl and, 50 mM NaCl (pH 7.5) was incubated with 1.9 g of the resin. Moreover, to determine if proteins were adsorbed by Amberlite XAD-4 when the resin was used to remove the detergents of the STC triad from an aqueous solution, the authors supplemented the sample with bovine serum albumin (BSA) at 1 mg/ml, obtaining the STC–BSA mixture. Under these conditions, and similarly to what was previously observed (Figure 2B), they detected an efficient displacement of Triton X-100 from the protein-containing sample (Figure 3A). Notably, they did not detect a significant adsorption of BSA, the concentration of which in solution appeared to be unaffected by the presence of Amberlite XAD-4, as revealed by SDS-PAGE and absorption spectrum analyses (Figure 3B & C). Remarkably, the spectroscopic analysis did also show that Triton X-100 was completely adsorbed by Amberlite XAD-4: over the 250–350 nm wavelength interval, the absorption spectrum of the sample exposed to the resin for 240 min did indeed reveal the presence of BSA only (Figure 3C). It is also interesting to note that the inability of Amberlite XAD-4 to adsorb BSA is in contrast with observations obtained with Amberlite XAD-8, which was shown to efficiently adsorb BSA from aqueous solutions [15].

To estimate the residual concentration of Sarkosyl in the STC–BSA mixture exposed for 240 min to Amberlite XAD-4, the authors performed multicomponent analyses using previously reported deconvolution procedures (Supplementary Figure 1). Taking into account that Amberlite XAD-4 did not adsorb BSA, the authors first subtracted the absorption spectrum of the protein from that of the STC–BSA mixture (Supplementary Figure 2A). Finally, using the appropriate derivative spectrum of the sample (Supplementary Figure 2B) and the calibration curve for Sarkosyl (Supplementary Figure 1E), they calculated the residual concentration of this detergent to be equal to 5.1 ± 0.4 mM, with this observation indicating that the presence of BSA did not affect the efficiency of Sarkosyl removal by Amberlite XAD-4 (*cf.* Figure 2C). Notably, when they assayed the removal by Amberlite XAD-4 of the STC triad from a solution also containing recombinant CRM197 (solubilized from *E. coli* inclusion bodies), they observed a behavior similar to that detected with the STC–BSA mixture (Figure 4). It is also interesting to note that Amberlite XAD-2 performed poorly on the STC–BSA mixture (Supplementary Figure 3 & *cf.* Supplementary Figure 2A).

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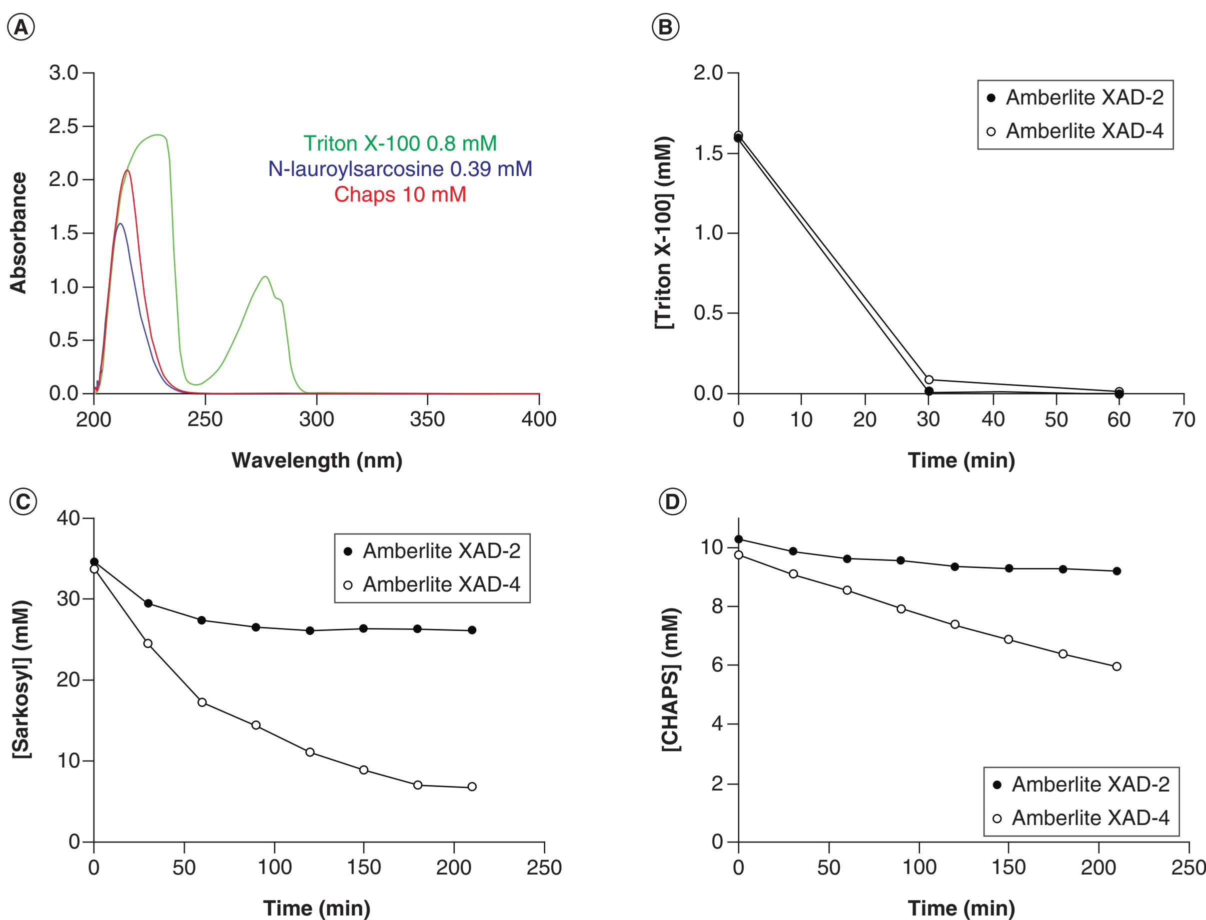

Figure 2. Absorption spectra of Triton X-100, Sarkosyl and CHAPS and their removal by Amberlite XAD-4 or Amberlite XAD-2 from aqueous solutions. **(A)** Absorption spectra of Triton X-100, Sarkosyl and CHAPS in distilled water at the indicated concentrations. **(B)** Removal of Triton X-100 (starting concentration: 1.6 mM) by 1.9 g Amberlite XAD-4 (empty circles) or by the same amount of Amberlite XAD-2 (filled circles) from an aqueous solution (final volume: 50 ml; temperature: 20°C) containing 50 mM Tris-HCl and 50 mM NaCl, pH 7.5. **(C)** Removal of Sarkosyl (starting concentration: 34.1 mM) by 1.9 g Amberlite XAD-4 (empty circles) or by the same amount of Amberlite XAD-2 (filled circles) under the same conditions used for the assay shown in Figure 2B. **(D)** Removal of CHAPS (starting concentration: 10 mM) by 1.9 g Amberlite XAD-4 (empty circles) or by the same amount of Amberlite XAD-2 (filled circles) under the same conditions used for the assay shown in Figure 2B.

According to the observations reported here, Amberlite XAD-4 appears to be an efficient tool to get protein solutions free from Triton X-100. Moreover, it is important to note that when exposing the STC–BSA mixture to Amberlite XAD-4,the concentration of Sarkosyl decreases to a level (about 5 mM) two- to three-times lower than the CMC of this surfactant. Further, it should be considered that the concentration of CHAPS (1 mM) used to solubilize proteins from inclusion bodies according to the procedure proposed by Park *et al.* [14] is much lower than the CMC of this detergent. Therefore, the authors of the present study suggest the use of Amberlite XAD-4 in protein purification operations requiring the use of detergents to remove Triton X-100 when overexpressed proteins are solubilized from inclusion bodies and then refolded according to the strategy introduced by Tao *et al.* [10] and to lower the concentration of Sarkosyl below its CMC when this surfactant is used alone or in detergent mixtures to solubilize overexpressed proteins from inclusion bodies.

Finally, it should be noted that Amberlite resins were applied to remove organic [16] and trace metal pollutants [17,18] from aqueous solutions. Moreover, Amberlite XAD-4 has been recognized as a safe material and has been successfully used for the hemoperfusion of plasma affected by acute drug intoxication [19,20]. Accordingly, and considering the observations reported here, the use of Amberlite XAD-4 to remove detergents from proteins of pharmaceutical relevance represents a convenient and inexpensive aid for industrial processes.

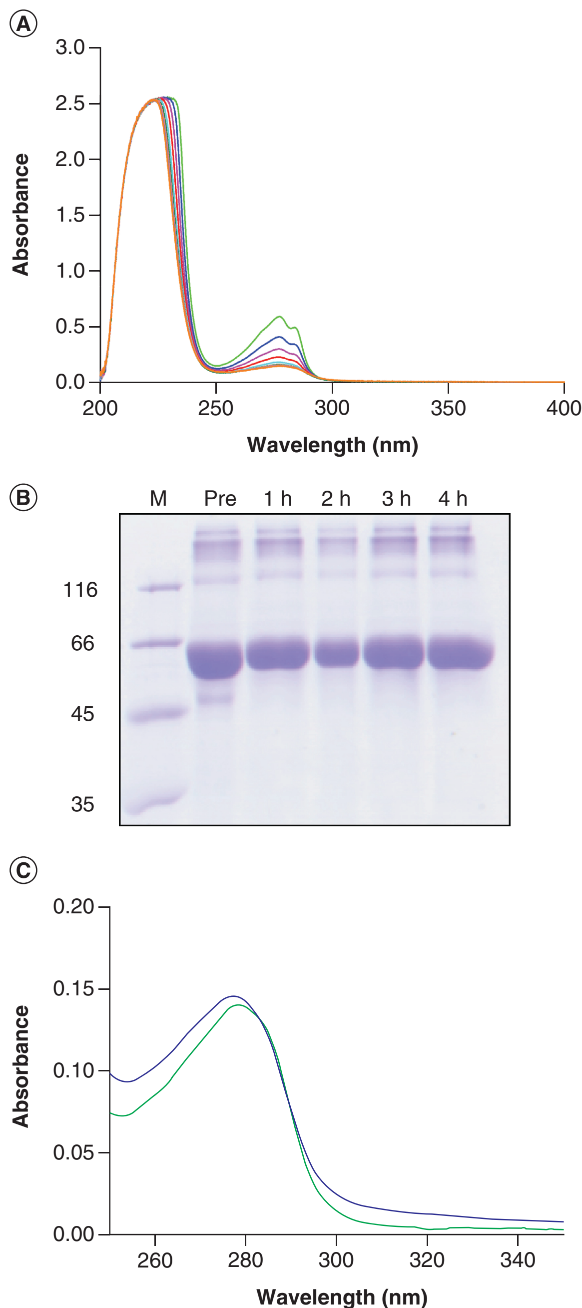

Figure 3. Action of Amberlite XAD-4 in removing detergents from an aqueous solution containing Triton X-100, Sarkosyl, CHAPS and 1 mg/ml bovine serum albumin (BSA). (A) The green line represents the absorption spectrum of a 1:5 dilution of a detergent mixture containing 0.8% (w/v, 27.3 mM) Sarkosyl, 0.1% (w/v, 1.6 mM) Triton X-100, 0.06% (w/v, 1 mM) CHAPS and 1 mg/ml BSA in 50 mM Tris-HCl and 50 mM NaCl, pH 7.5 (final volume: 50 ml; temperature: 20°C). The absorption spectra shown with blue, magenta, red, cyan, dark green, pink, light blue and orange lines were recorded 30, 60, 90, 120, 150, 180, 210 and 240 min after the addition of 1.9 g Amberlite XAD-4, respectively. **(B)** SDS-PAGE analysis of BSA exposed to Amberlite XAD-4 under the conditions of the assay shown in Figure 3A. **(C)** Absorption spectra of 0.2 mg/ml BSA (green line) and of the quaternary mixture (1:5 dilution) containing 1.6 mM Triton X-100, 27.3 mM Sarkosyl, 1 mM CHAPS and 1 mg/ml BSA after 240 min of incubation with 1.9 g Amberlite XAD-4 (blue line). M: Molecular mass markers (mass in kDa reported at the left); Pre: Sample before the addition of Amberlite; 1h, 2h, 3h, 4h: Samples obtained 1, 2, 3 and 4 h after the addition of Amberlite to the aqueous solution.

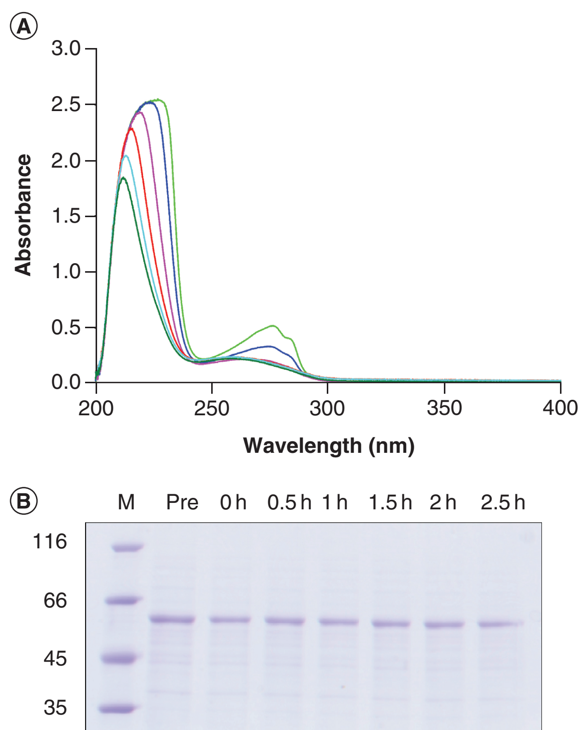

Figure 4. Action of Amberlite XAD-4 in removing detergents from an aqueous solution containing Triton X-100, Sarkosyl, CHAPS and 0.25 mg/ml recombinant CRM197. (A) The green line represents the absorption spectrum of a 1:5 dilution of a detergent mixture containing 0.8% (w/v, 27.3 mM) Sarkosyl, 0.1% (w/v, 1.6 mM) Triton X-100, 0.06% (w/v, 1 mM) CHAPS and 0.25 mg/ml recombinant CRM197 in 50 mM Tris-HCl and 50 mM NaCl, pH 7.5 (final volume: 50 ml; temperature: 20°C). The absorption spectra shown with blue, magenta, red, cyan and dark green lines were recorded 30, 60, 90, 120 and 150 min after the addition of 1.9 g Amberlite XAD-4, respectively. **(B)** SDS-PAGE analysis of recombinant CRM197 exposed to Amberlite XAD-4 under the conditions of the assay shown in Figure 4A.
M: Molecular mass markers (mass in kDa reported at the left); Pre: Aliquot of the recombinant CRM197 purification batch used in the assay; 0, 0.5, 1, 1.5, 2 and 2.5 h: Samples obtained immediately before or 30, 60, 90, 120 and 150 min after the addition of Amberlite to the aqueous solution.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2022-0098

Author contributions

L Scutteri and G Maltoni: investigation; A Hochkoeppler: supervision, writing – reviewing and editing.

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Data sharing statement

The data reported in the present study are available from the corresponding author upon request.

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