Efficient extraction of bile acid conjugates with tetraheptylammonium chloride, a liquid ion exchanger

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SUMMARY Ethyl acetate or chloroform solutions of tetraheptylammonium chloride, an oil-soluble quaternary amine, quantitatively extract polar, anionic lipids such as steroid or bile salt conjugates from aqueous solution by a process of anion exchange.

KEY WORDS extraction · bile acid conjugates · tetraheptylammonium chloride · liquid ion exchanger · alkaloid glucosiduronates · alkyl sulfates

FOR THE LIQUID–LIQUID EXTRACTION from water of polar, acidic lipids such as steroid sulfates or glucosiduronates, alkyl sulfonates or sulfates, or glycine or taurine conjugates of bile acids, few solvents offer satisfactory partition coefficients. This note presents experiments which demonstrate that the addition of tetraheptylammonium chloride (THAC) to chloroform or ethyl acetate permits the quantitative extraction of such polar anionic lipids, and further, that the favorable quenching characteristics of THAC suggest its use in liquid scintillation spectrometry.

Theory. When a solution of THAC is equilibrated with an aqueous solution of a salt, Na^+R^-, ion exchange occurs as follows (1, 2):

$$\frac{THA^+\,Cl^-\ \text{nonaqueous}}{Na^+\,R^-\ \text{aqueous}} \longrightarrow \frac{THA^+\,R^-\ \text{nonaqueous}}{Na^+\,Cl^-\ \text{aqueous}}$$

where THA^+ is the tetraheptylammonium ion and R^- is a lipid anion.

Materials. THAC (compound 9505, Distillation Products Industries, Rochester, N. Y.) is a yellow viscous oil of negligible water solubility; it was used as purchased. A colorless preparation of THAC was obtained from tetraheptylammonium iodide (compound 7630, Distillation Products Industries) by crystallization and ion exchange. 25 g of tetraheptylammonium iodide was dissolved in 200 ml of hot ethyl acetate; on cooling, a quantitative yield of colorless crystals was obtained. A solution in ethanol–water 7:3 was passed over an anion exchange column (Biorad Ag 21-K, see below)

Abbreviation: THAC, tetraheptylammonium chloride.

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that was in the chloride form. Concentration of the effluent solution yielded a syrup of colorless THAC in which no iodide ion could be detected. An equimolar mixture of sodium taurocholate, taurodeoxycholate, and taurochenodeoxycholate was synthesized from an equimolar mixture of the free bile acid sand taurine-1-^{14}C (New England Nuclear Corp., Boston, Mass.) by the mixed carboxylic–carbonic anhydride method of Norman (3), and purified by solvent extraction (4) to thin-layer chromatographic purity (5). A similar equimolar mixture of sodium glycocholate, glycodeoxycholate, and glycochenodeoxycholate was prepared with glycine-1-^{14}C (New England Nuclear Corp.). Ion exchange resins were purchased from California Corp., for Biochemical Research, Los Angeles, Calif.: (anionic) Biorad AG 21-K, 16–20 mesh; and (cationic) AG-1, 16–20 mesh.

Affinity of THAC for Anionic Lipids. In initial experiments designed to compare the affinity of THAC for anionic lipids with that for the chloride ion, a volume of mixed labeled taurine-conjugated bile salts or mixed labeled glycine-conjugated bile salts (10 mM total) was extracted with an equal volume of THAC in chloroform (10 mM). Over 99% of the radioactivity entered the chloroform phase in two extractions, which indicates the selective nature of the binding of THAC to anionic lipids. Chloroform without added THAC extracted less than 3% of the radioactivity.

Choice of Extraction Solvent. Extraction with THAC solutions could not be performed with certain solvents such as petroleum ether or benzene, since the resulting THA^+R^- salts (where R^- was a glycine or taurine conjugate of a bile acid) formed insoluble oils at the liquid–liquid interface. Ethyl acetate, chloroform, methylene chloride, and methyl isobutyl ketone showed good solvent properties; of these, ethyl acetate and chloroform were chosen for further study.

Efficiency of Extraction. The efficiency of extraction was determined by the following types of extraction experiments on radioactive model mixtures: (*a*) extraction of the labeled compounds from water, (*b*) recovery of radioactivity after the model mixtures were added to serum, and (*c*) extraction of radioactivity from the bile and serum of patients previously given radioactive bile acid parenterally. The aqueous phase was extracted with an equal volume of the extraction solvent (5 g of THAC per 100 ml). Each extract was blown to dryness with a stream of air and dissolved in a toluene-based scintillation solvent,[1] and its radioactivity was determined. After three extractions, an aliquot of the aqueous phase was counted in the dioxane-based scintillation solvent described by Bray (6). In every case, three extractions with chloroform or ethyl acetate solutions of THAC (5 g/100 ml) removed 99% or more of the label from the aqueous phase. Typical results are shown in Fig. 1. Similar results were obtained with morphine glucosiduronate, dodecyl sulfate, and dodecyl sulfonate.

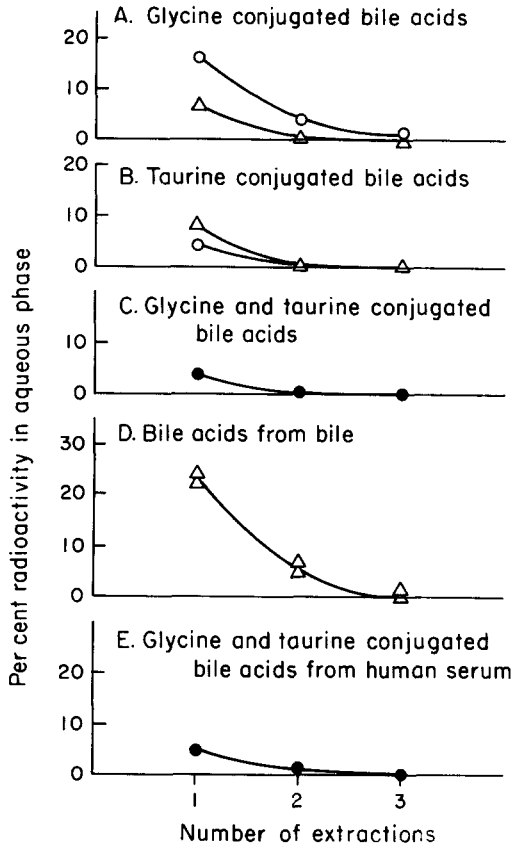

FIG. 1. Completeness of extraction versus number of extractions with THAC (5 g/100 ml) in △, chloroform; ○, ethyl acetate; or ●, the chloroform phase of the Folch procedure (7).

Quenching Characteristics of THAC. Aliquots of THAC were added to the toluene-based scintillation solvent or the dioxane-based scintillation solvent described by Bray (6). The results, shown in Fig. 2, indicate that 2 g of THAC, equivalent to 40 ml of extraction solvent, produces only 30% quenching of ^{14}C radioactivity in 18 ml of toluene-based scintillation solvent. Since the dioxane-based system manifested greater quenching, and since THAC salts have good solubility in toluene, it is recommended that toluene-based systems be used.

THAC, as purchased, is yellow. A colorless preparation of THAC made as described above quenched less (Fig. 2), but the improvement was small.

Comparison of THAC with Another Liquid Ion Exchanger. THAC was superior to a commercially available liquid ion exchanger of the secondary amine type, Amberlite XLA-3 (Rohm and Haas Co., Philadelphia, Pa.).

[1] Liquifluor, Pilot Chemicals, Inc., Watertown, Mass. Contains 2,5-diphenyloxazole (PPO), 4 g/liter, and *p*-bis[2-(5-phenyloxazolyl)]-benzene (POPOP), 0.05 g/liter.

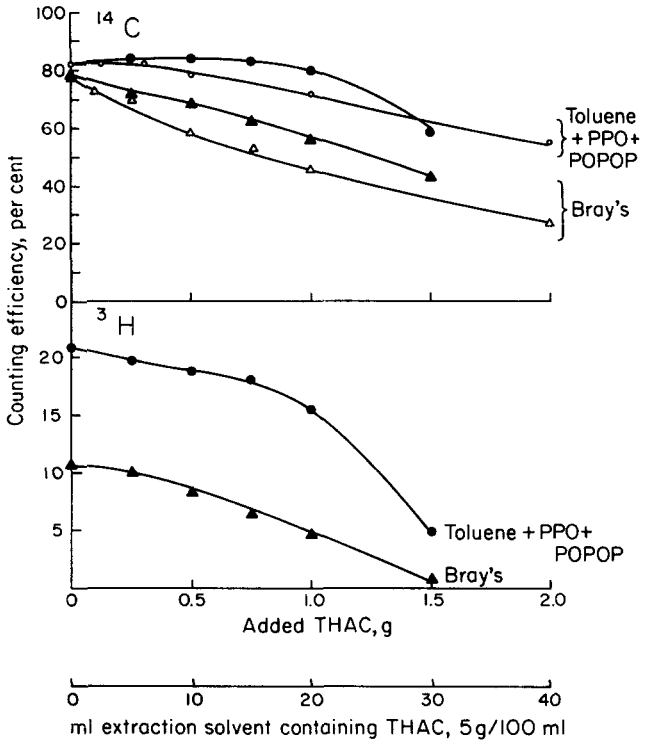

FIG. 2. Quenching effect of added THAC in grams per 18 ml of scintillation solvent for ^{14}C (above) and ^{3}H (below). Counting was performed in a toluene-based system (●, ○) or the dioxane solution described by Bray (6) (▲, △). In order to determine whether the yellow color of THAC caused quenching, commercially available THAC (○, △) was compared with colorless THAC prepared from the iodide salt (●, ▲).

Extraction of an aqueous solution containing 30 μmoles of mixed taurine-conjugated bile acids with an equal volume of a chloroform solution containing 5 g of XLA-3 per 100 ml removed 20% of the radioactivity in two extractions. Even after acidification of the aqueous phase to pH 1, two extractions removed only 80% of the radioactivity. Since THAC is a quaternary amine, it may be used for extraction of aqueous solutions of pH 2–11 without pH adjustment.

Procedure for Routine Use. The following extraction schemes have proved consistently satisfactory: (*a*) for dilute aqueous solutions, three extractions are performed with an equal volume of ethyl acetate or chloroform containing 5 g of THAC per 100 ml, and (*b*) serum or biological fluids containing proteins or micellar aggregates are first extracted with a suitable chloroform–methanol mixture (7–9), with the modification that the chloroform contains THAC, 5 g/100 ml, and then extracted again with "Folch lower phase" containing THAC, 5 g/100 ml.

The binding of anionic lipids to THAC is nonspecific; any anionic lipid is extracted to some extent by solvents containing THAC.

Removal of THAC from Extract. The extracted anionic lipid may be isolated from THAC by a second ion exchange procedure. The solvent is evaporated from the pooled extracts. The residue is dissolved in ethanol–water 1:1 and passed over a cation exchange column that is in the hydrogen form and that has been packed in the same solvent mixture. The THA^+ is quantitatively bound; the extracted anionic lipid passes through the column and may be quantitatively recovered from the eluent, which also contains hydrochloric acid because of the liberation of H^+ from the resin by the THA^+ cations. 1 ml (wet volume) of the cation exchange resin AG 21-K is stated by the manufacturer to bind 1.3 meq, or the THA^+ present in 580 mg of THAC. In these experiments a 6-fold excess of the resin was used, viz. 50 ml (65 meq) of AG 21-K for 5 g (11.2 meq) of THAC. The THA^+ may be recovered from the cation exchange resin by elution with 1 N HCl in ethanol–water 1:1.

Thin-Layer Chromatography of Extract. If the ratio of THAC to sample is not extremely high in the extract (<10,000:1, by weight), thin-layer chromatographic examination is possible. With a solvent system that gives good resolution for polar lipids (5), the THA^+ salts dissociate, and the THAC moves with an R_f of 0.9; many polar lipids with this solvent system have lower mobilities and can therefore be detected.

Applications. Many applications of this compound can be suggested. For example, it should be possible to separate free and conjugated steroids from urine by an initial extraction with ethyl acetate followed by an extraction with ethyl acetate containing THAC. Countercurrent distribution with solvents containing THAC could be applied to the separation of polar lipids such as alkaloid conjugates, bile pigments, or alkyl sulfates. Finally, THAC (especially as the hydroxide) supplements the use of Hyamine hydroxide [*p*-(diisobutylcresoxyethoxyethyl) dimethyl benzyl ammonium hydroxide] (10) in liquid scintillation counting for the preparation of toluene-soluble derivatives of water-soluble compounds.

The use of an oil-soluble amine, THAC, for the extraction of polar anionic lipids is similar, in some respects, to the use of a water-soluble amine salt, e.g. pyridinium sulfate, for the transfer of steroid sulfates into chloroform from water (11, 12). Further work to assess the relative advantages of these complementary methods is necessary.

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