Enhancement of Ampicillin Partition Behavior

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Abstract □ Several ion-pair or adduct forming additives that enhanced ampicillin partition behavior were identified and evaluated. At pH 3, picric acid and trichloroacetic acid increased the ampicillin aqueous–octanol partition coefficient 250 and 30 times, respectively. At pH 7, quaternary compounds gave the most significant increases in the partition coefficient. Values for an aqueous pH 7 chloroform system increased from zero in the absence of additives to 2.28, 1.86, 1.82, and 1.70 for equimolar amounts of benzalkonium, tetraheptylammonium, benzethonium, and cetalkonium chlorides, respectively. Extraction of ampicillin from aqueous pH 7 solution was possible by adding a quaternary agent in an equimolar amount. However, extraction of ampicillin from plasma required large molar excesses. Tetraheptylammonium chloride was added at a molar concentration 10^3 times greater than that of the ampicillin. Plasma samples spiked at the 3-μg/ml level gave 93% recovery (*CV* 6.7%, $n = 16$) when extracted three times. The extracts were quantitated by TLC.

Keyphrases □ Ampicillin—aqueous–octanol partition coefficient, effect of various ion-pair or adduct forming additives □ Partition coefficients, aqueous–octanol—ampicillin, effect of various ion-pair or adduct forming additives □ Ion-pair forming agents—effect on aqueous–octanol partition coefficient of ampicillin □ Adduct forming agents—effect on aqueous–octanol partition coefficient of ampicillin □ Antibacterials—ampicillin, aqueous–octanol partition coefficient, effect of various ion-pair or adduct forming additives

The aqueous–octanol pH partition profile of ampicillin in the pH 3–8 range was determined previously (1). The apparent partition coefficient was <0.01 and independent of pH because of the ampholytic character of the drug.

Common anionic penicillins usually have been extracted as the acids at pH ~2 with solvents such as chloroform (2). In some cases, solutions at pH up to 5 have been extracted after the addition of ammonium sulfate for its salting-out effect (3). An improved extraction was obtained by adding ion-pair or adduct forming agents (4). However, there has been no apparent attempt to enhance the partition behavior of ampholytic penicillins, such as ampicillin, with ion-pair or adduct forming agents.

Several acids and quaternary ammonium compounds were identified and evaluated for their ability to enhance ampicillin partitioning. The results were used to develop a specific chemical determination of plasma ampicillin levels.

EXPERIMENTAL

Materials—Ampicillin trihydrate[1] and tetradecylamine[2], practical grade, were used as received. All other reagents and solvents were analytical reagent, reagent, or compendial grade and were used directly. Phosphate (0.1 *M*) buffer solutions were prepared at pH 7.0.

Additives to Enhance Partitioning—Equimolar amounts (5.7×10^{-3} *M*) of picric acid or trichloroacetic acid and ampicillin were combined in a buffer solution before extraction with octanol. After separation, the octanol phase was extracted three times with a buffer solution. The ampicillin content of the combined extracts was determined by a colorimetric assay (5).

Solutions of quaternary additives were prepared in chloroform or ethyl acetate and shaken with buffer solutions of ampicillin. Quaternary additives and ampicillin were used in equimolar quantities (5.7×10^{-3} *M*). This procedure gave higher distribution ratios than when the quaternary additive was dissolved in the buffer prior to extraction. After equilibration, an aliquot of the organic phase was evaporated to dryness and reconstituted with buffer. This extract and the aqueous phase were assayed by a colorimetric method (5).

Preparation of Tetraheptylammonium Acetate—Equimolar amounts of silver acetate and tetraheptylammonium iodide were added to water and shaken with a known volume of chloroform for 30 min. The mixture was centrifuged, and the chloroform phase was withdrawn.

Preparation of Tetraheptylammonium Hydroxide—A small, analytical, straight glass column was packed with anion-exchange resin[3] and washed extensively with 0.1 *N* NaOH until a portion of the eluate acidified with nitric acid showed no precipitate when silver nitrate solution was added. The column was then washed with 500 ml of water. An aqueous solution of tetraheptylammonium chloride was added to the column and eluted with more water. The acidified eluate yielded no precipitate with silver nitrate. The eluate was shaken with chloroform to transfer the tetraheptylammonium hydroxide into the chloroform layer.

Assay of Quaternary Cation Concentration—An aliquot of chloroform or the aqueous phase was transferred to a glass-stoppered erlenmeyer flask. Fifteen milliliters of 6 *N* H_2SO_4 was added, and the water and chloroform volumes were each adjusted to 30 ml. Finally, 1 ml of 0.01% methyl yellow in alcohol was added. The quaternary ammonium compound was titrated with sodium lauryl sulfate solution to a salmon-pink color in the chloroform layer.

The titrant was standardized in the same fashion with known amounts of the individual quaternary ammonium compounds. Calculations of concentrations in each case were based on this standardization.

Spiked Plasma Extraction Procedure—A 12-μl aliquot of a 1-mg/ml ampicillin aqueous solution was added to 4 ml of plasma in a small separator and mixed to give a concentration of 3 μg/ml of plasma. Four milliliters of a 6–10-mg/ml tetraheptylammonium chloride in chloroform solution was added, and the separators were stoppered and gently shaken by hand for 3 min. After the phases separated, the organic phase was drained into a 35-ml conical centrifuge tube. This procedure was repeated twice with 4-ml portions of the chloroform solution, and the pooled chloroform was evaporated to dryness with the aid of a nitrogen stream. The residue was transferred to a 1-ml volumetric flask and diluted to volume with chloroform.

TLC Assay—Any GLC, TLC, or high-pressure liquid chromatographic (HPLC) procedure with adequate specificity and sensitivity could be applied to the extraction residues. In this study, extracts were analyzed by a TLC method based on a previously developed procedure[4]. Ampicillin standard solution and reconstituted extracts were applied to separate labeled channels of a silica gel plate. The developing solvent was ethyl acetate–acetone–water–formic acid (65:15:13:9).

RESULTS AND DISCUSSION

Additives to Enhance Partitioning—At low pH, the ampicillin amine cation (pKa = 7.25) is present while at high pH the carboxylic anion (pKa = 2.66) is present. At pH 4.9, the compound exists almost completely as the zwitterion. It has been claimed that the anionic species is the most efficiently partitioned (6). Since ampicillin exists either in ionized or zwitterion forms at common pH values, the search for compounds to enhance its partition behavior centered on potential ion-pair formers.

Modin and Schroder-Nielsen (4) reported ion-pair partitioning for penicillin V, penicillin G, and other anionic penicillins. To determine if

[1] USP grade.

[2] Eastman.

[3] Dowex 1-X8, J. T. Baker Chemical Co.

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Table I—Aqueous Chloroform or Aqueous Ethyl Acetate Apparent Partition Coefficient Values for Ampicillin with the Addition of Several Quaternary Ammonium Chlorides at pH 7.0 ([Ampicillin]$_i$ and [Quaternary Ammonium Chloride]$_i$ = 5.7×10^{-3} *M*)

Quaternary Compound	Partition Coefficient, Chloroform	Partition Coefficient, Ethyl Acetate
Benzalkonium	2.28	—
Benzethonium	1.82	1.45
Tetraheptyl-ammonium	1.86[a]	0.67[a]
Cetalkonium	1.70	0.30[a]
Cetylpyridinium	0.62	—

[a] Two or more determinations.

Table II—Chloroform Apparent Partition Coefficient Values of Ampicillin at pH 7.0 with Tetraheptylammonium and Various Anionic Counterions ([Ampicillin]$_i$ and [Tetraheptylammonium]$_i$ = 5.7×10^{-3} *M*)

Anion	Partition Coefficient[a]
Acetate	4.78
Hydroxide	1.74
Chloride	1.80
Bromide	0.46
Iodide	0.016

[a] Average of two determinations except iodide.

ion-pair partitioning of ampicillin would be possible, many candidate cationic and anionic compounds were screened.

At pH 3, the anionic compounds trichloroacetic acid, a carboxylic acid (pKa = 0.70), and picric acid, a phenol (pKa = 0.38), increased the apparent partition coefficient in octanol about 30 and 250 times, respectively. Pamoic, 1,2-ethanedisulfonic, bromoacetic, 3-bromopropionic, chloroacetic, benzilic, and diphenylacetic acids offered no enhanced extraction of ampicillin into octanol at pH 3. Trichloroacetic and picric acids increased the aqueous–chloroform partition coefficient from 0 to 0.010 and 0.011, respectively, and the aqueous–ethyl acetate partition coefficient from 0 to 0.062 and 4.64, respectively.

Several long chain (>six carbons) alkylammonium cationic compounds were evaluated at pH 7. The apparent partition coefficient values determined upon quaternary compound addition to aqueous chloroform and aqueous ethyl acetate systems are shown in Table I. The apparent slight variation in chloroform partition coefficients compared to the fivefold difference in ethyl acetate partition coefficients between cetalkonium and benzethonium is not understood at this time. Chloroform, a good solvent for ion-pairs, may have had a leveling effect.

Aqueous chloroform partition coefficient values increased when the concentration of quaternary compound added to the organic phase was increased while the ampicillin concentration in the aqueous phase was kept constant. When the ratio of the initial tetraheptylammonium chloride concentration to the initial ampicillin concentration was increased from 1 to 2.08 to 3.30, the aqueous chloroform partition coefficient increased from 1.85 to 4.90 to 6.77. Similarly, when the ratio of tetraheptylammonium acetate to ampicillin was increased from 0.95 to 1.85 to 2.76, aqueous ethyl acetate partition coefficient values increased from 3.0 to 7.5 to 8.9.

No significant increase in the partition coefficient in octanol, chloroform, or ethyl acetate systems was found for urea; proline; guanosine; hexadecylamine; tetradecylamine; tetrabutylammonium hydroxide, chloride, bromide, and iodide; nonyltrimethylammonium bromide; and tridodecylammonium acetate. For example, with added nonyltrimethylammonium bromide, the aqueous–chloroform and the aqueous–ethyl acetate partition coefficient values were only 0.05 and 0.04, respectively. With added tridodecylammonium acetate, the aqueous–chloroform partition coefficient value was 0.07.

Effect of Quaternary Counterion—Tetraheptylammonium was purchased or prepared with various counterions. The water–chloroform partition coefficient values as a function of the associated anion are shown in Table II. Although the largest partition coefficient was observed with acetate, a two-order of magnitude increase in the partition coefficient occurred as the halogen molecular weight decreased.

A similar variation with the halogen species was previously reported (7) in the dye extraction of long chain amines. Anion interference was observed for the analysis of long chain amines by the dye extraction method because of competition between the anions and the dye for extraction into the organic phase. Acetate did not interfere. For the other anions studied, the degree of interference followed the order $I^- > Br^- > Cl^-$. This order correlated with that for the extraction of the anions into chloroform by ammonium ions (8). Since a similar interference occurs for ampicillin–tetraheptylammonium chloride extraction, the observed partition coefficient values in the presence of different anions are in the anticipated order.

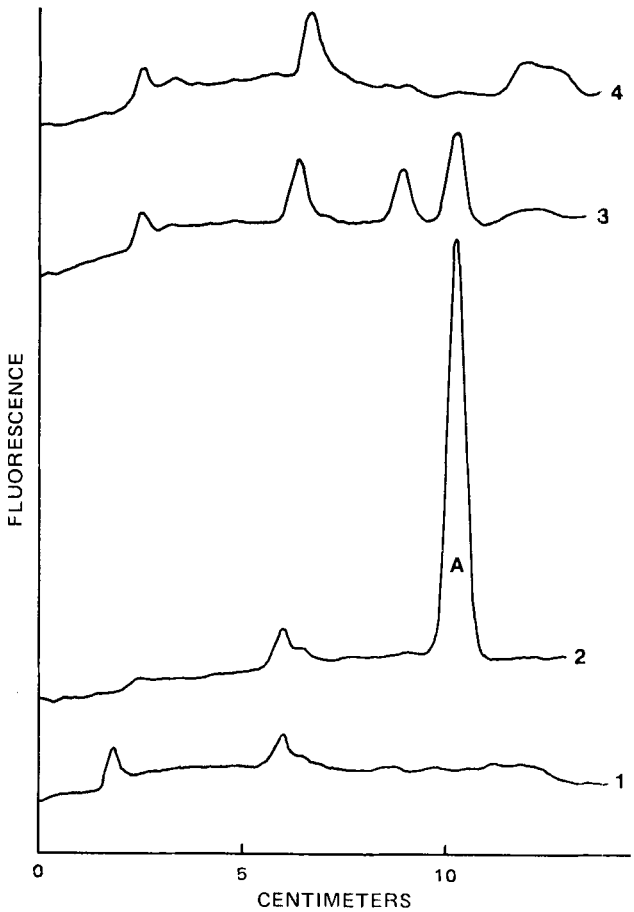

Figure 1—*Thin-layer chromatograms of ampicillin (A). Key: 1, extract of blank human plasma; 2, ampicillin standard spotted from water; 3, extract from plasma of ampicillin partially degraded in acid before spiking; and 4, extract from plasma of ampicillin totally degraded in base before spiking.*

Extraction of Ampicillin from Plasma—Human plasma and dog plasma and serum were spiked at the 3-μg/ml level. Tetraheptylammonium chloride at a molar ratio of about 2000:1 ampicillin was the optimum level for extractions from human plasma. No apparent advantage occurred when the tetraheptylammonium chloride concentration was raised above this level. However, below this level, recoveries of ampicillin fell off quickly. Neither cetalkonium nor benzalkonium at molar ratios up to 2000:1 aided plasma ampicillin extraction. Tetraheptylammonium acetate showed a partition coefficient 2.5 times greater than that of tetraheptylammonium chloride in aqueous systems. It could be considered for plasma extraction, but it is not commercially available.

The percent recovery from human plasma was 60% after a single extraction, 88% after two extractions, and 93% after three extractions. The coefficient of variation for three extractions (n = 16) was 6.7%. After three extractions, recovery was 92% from dog serum and 84% from dog plasma. Possibly more interfering substances are present in plasma than in serum. However, the dog serum and plasma data were from single determinations. With the outlined procedure, quantitation of a concentration of 0.5 μg of ampicillin/ml of plasma was possible. By suitable alterations of the procedure, this quantitation limit might be lowered by a factor of two or three.

Extractions of decomposed samples of ampicillin gave anticipated recoveries of the intact species. Some decomposition products were also extracted but not quantitatively (Fig. 1).

Analysis of Plasma Ampicillin Extracts—GLC, TLC, HPLC, and adsorption chromatographic procedures have been developed for ampicillin. However, these methods cannot be adapted for the detection and quantitation of ampicillin and its metabolites in plasma since sensitivities are not adequate. The best sensitivity attained was 10 μg/ml by HPLC (9). Fluorescence methods with a sensitivity at 0.1 μg/ml have been used (10–12). However, these methods at best are specific for intact ampicillin but do not measure decomposition products or metabolites. Enhanced extraction with tetraheptylammonium chloride followed by chromatography offers the possibility of quantitating plasma ampicillin levels in the 1-μg/ml range.

Metabolites could also be extracted, followed by identification and quantitation. The procedure could be modified for the separation of ampicillin from its prodrugs. For example, pivampicillin could be easily separated with a prior chloroform extraction. Ampicillin would then be quantitated using the described procedure.

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COMMUNICATIONS

Spectrophotometric Analysis of Binary Mixtures of Antazoline and Naphazoline

Keyphrases □ Antazoline hydrochloride—spectrophotometric analysis simultaneously with naphazoline nitrate in pharmaceutical formulations □ Naphazoline nitrate—spectrophotometric analysis simultaneously with antazoline hydrochloride in pharmaceutical formulations □ Spectrophotometry—analyses, antazoline hydrochloride and naphazoline nitrate, simultaneously in pharmaceutical formulations □ Antihistaminics—antazoline hydrochloride, spectrophotometric analysis simultaneously with naphazoline nitrate in pharmaceutical formulations □ Adrenergic agents—naphazoline nitrate, spectrophotometric analysis simultaneously with antazoline hydrochloride in pharmaceutical formulations

To the Editor:

Two-component spectrophotometric analysis (1) is based on solving a set of two linear equations. Glenn (2) formulated the solution of the two linear equations in terms of absorbance ratios that are independent of concentration. Pernarowski *et al.* (3) used absorbance ratios for the analysis of binary mixtures and derived an equation similar to Glenn's equation. However, these authors (3) assumed that an isoabsorptive point must be chosen to apply their equation. The equation published by Cho and Pernarowski (4) to obtain absolute concentration was derived under the impression that an isoabsorptive point must be present to apply the equation of Pernarowski *et al.* (3). The three published equations (2–4) are consistent with each other and can be applied using any suitably chosen pair of wavelengths. None of the selected wavelengths needs to be an isoabsorptive point.

According to Glenn's limitations (2), if the contribution of one component to the absorption curve of the total mixture is low, erroneous results are obtained whenever the two-wavelength method of analysis is applied. In this connection, we suggest the use of least squares (5) to minimize the instrumental errors during the analysis of the minor component in binary mixtures.

These methods were applied to the determination of antazoline hydrochloride (I) (0.5% w/v) and naphazoline nitrate (II) (0.025% w/v) in nasal drops (6–8) also containing chlorobutanol (0.5% w/v) and sodium chloride (0.6% w/v). Thus, by diluting 1 ml of the nasal drop solution to 50 ml with 0.1 *N* H_2SO_4, measuring the absorbances of 1-cm pathlengths at 281 and 295 nm[1], and applying the Glenn and Cho and Pernarowski equations, the mean percentage recoveries for I were 99.5 ± 1.05 and 99.7 ± 1.24, respectively, for 10 samples; for II, they were 107.1 ± 2.35 and 107.1 ± 2.64, respectively, for 10 samples. The recoveries obtained using the two equations were consistent. The relatively high percentage recoveries obtained for II were due to its low contribution to the absorption curve of the total mixture at the concentration used.

To improve the accuracy and precision for the determination of II, the method of least squares (5) was applied. Fifteen absorbances measured at the wavelengths of 267–295 nm at 2-nm intervals gave the best results (mean ± *SD* = 100.7 ± 1.10) for the determination of II in the same 10 samples. The final wavelengths used covered the peak characteristics of II (8). Neither chlorobutanol nor sodium chloride interfered with the determinations.

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