

NOTES

ION PAIR EXTRACTION OF
STREPTOMYCINH. HEDING, J. HARTVIG JØRGENSEN
and E. STEEN ANDERSENNovo Terapeutisk Laboratorium A/S,
Copenhagen, Denmark

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Several methods of isolation of streptomycin from fermentation broths have been described. Adsorption of the antibiotic on active carbon at neutral pH and subsequent desorption with dilute mineral acid are described by SCHATZ, BUGIE and WAKSMAN¹. Since carbon is a non-specific absorbent many other substances from the broth, some of which are toxic, are concentrated in the eluate.

Streptomycin is readily adsorbed on sulfonic acid ion exchange resins but the elution is difficult and causes contamination of the product with degradation products. Carboxylic acid ion exchange resins, for example Amberlite IRC-50, also readily adsorb streptomycin. The adsorption capacity of these resins is high and as they are weak acids the specificity is good and the streptomycin is easily desorbed giving a product of high purity².

Table 1. Distribution of monoesters at different pH in a system consisting of 10 ml isoamylalcohol+10 ml water.

500 mg ester was dissolved in the system.

	Per cent ester found in the aqueous phase					
	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
MBUP	8	32	74	85	84	84
MIAP	7	25	58	67	67	64
MIOP	2	2	15	28	31	32
MNDP	5	8	9	40	43	42
MLP	2	5	4	45	48	49
MCP	5	4	4	50	49	49
MCHP	10	26	64	76	77	77

MBUP=mono-butyl phthalate, MIAP=mono-isoamyl phthalate, MIOP=mono-2-ethyl-hexyl phthalate, MNDP=mono-*n*-decyl phthalate, MLP=mono-lauryl phthalate, MCP=mono-cetyl phthalate, MCHP=mono-cyclohexyl phthalate.

Ion pair extraction of streptomycin has also been described in the literature but has not found general application^{3,4}. We have found that mono-esters of dibasic carboxylic acids form ion pairs with streptomycin, some of which are readily extracted from an aqueous solution with common organic solvents. The specificity of the carriers was in all cases found to be comparable to the carboxylic acid exchange resins. The esters investigated were mono-butyl, mono-isoamyl, mono-2-ethyl-hexyl, mono-*n*-decyl, mono-lauryl, mono-cetyl and mono-cyclohexyl phthalate, succinate, maleate and glutarate.

Results and Discussion

The distribution of a number of mono-esters of phthalic acid in a binary system consisting of isoamylalcohol-water was investigated. 500 mg of ester was dissolved in 10 ml of amylalcohol and after the addition of 10 ml of water the pH was adjusted with 4N sodium hydroxide. The results are shown in Table 1.

The data shown in Table 1 indicate that mono-2-ethyl-hexyl and mono-*n*-decyl esters would be preferable as carriers in a large scale extraction procedure. The loss of carrier with the aqueous phase in the desired

Table 2. Distribution of monoesters of phthalic, succinic and maleic acid in three solvent systems

500 mg ester was dissolved in each of the systems and the pH adjusted to 8.5 with 4N sodium hydroxide.

	Per cent ester (as sodium salt) recovered in the aqueous phases		
	(1) 10 ml chloro- form+10 ml water	(2) 10 ml isoamyl- alcohol+10 ml water	(3) 10 ml ethylhe- xanol+10 ml water
MIOP	78	33	54
MNDP	64	40	52
MIOS	91	70	82
MNDS	73	80	80
MIOM	67	49	65
MNDM	91	70	+

MIOP=mono-2-ethyl-hexyl phthalate, MNDP=mono-*n*-decyl phthalate, MIOS=mono-2-ethyl-hexyl succinate, MNDS=mono-*n*-decyl succinate, MIOM=mono-2-ethyl-hexyl maleate, MNDM=mono-*n*-decyl maleate.

pH range is far less for these two esters than for the other compounds listed.

Table 2 shows the distribution of monoesters of phthalic, succinic and maleic acid with 2-ethyl-hexyl and *n*-decylalcohol in three solvent systems. Table 2 shows that the distribution of sodium mono-2-ethyl-hexyl phthalate into water is lower than any of the other compounds investigated when the binary system consists of equal volumes of isoamylalcohol and water.

Streptomycin contains two guanidino groups (pKa 11.5) and one secondary amino group (pKa 7.7). Mono-2-ethyl-hexyl phthalate is a weak acid (pKa 7.5). In agreement with these data we have found the distribution of streptomycin in a water-isoamylalcohol system containing mono-2-ethyl-hexyl phthalate to be very pH-sensitive. The most favorable extraction is obtained at pH 8. Analysis of a 5% solution of mono-2-ethyl-hexyl phthalate in isoamylalcohol saturated with streptomycin showed a molar proportion between streptomycin and mono-2-ethyl-hexyl phthalate of one to three.

The effectiveness of the extraction procedure is illustrated by the following example:

Twenty-four litres of filtered broth containing 68.5 g of streptomycin was subjected to a three step countercurrent extraction using 6 liters of a 10% solution of mono-2-ethyl-hexyl phthalate in amylalcohol. The pH was adjusted to 8.3 during the extraction. After the extraction the spent broth contained 0.6 g streptomycin (0.9% of the initial activity). The alcohol extract was reextracted with water at pH 3.0 in one step. The aqueous phase contained 66.4 g streptomycin (total yield, 97%).

Experimental

The monoesters investigated were prepared by reacting equivalent amounts of the alco-

hols with the corresponding anhydrides of the acids. The mixtures were kept overnight at a temperature of 110~115°C while stirring. The resulting reaction mixtures were used in our experiments without further purification. The yields of monoester were in all cases above 88%.

The preliminary extraction experiments were performed by mixing either a sample of a broth or an aqueous solution of pure streptomycin sulfate with a solution of ester in an organic solvent. The two phases were stirred vigorously while adjusting the pH to the desired value with sodium hydroxide. After stirring for a few minutes the mixture was centrifuged and the phases were separated.

Recovery of the extracted material was obtained by reextraction with dilute mineral acid at a pH between 1.0 and 2.0. Streptomycin in the neutralized reextract was assayed according to BOXER *et al.*⁵⁾

Literature

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