

Table III—Assay of Finished Capsules

Content Wt., mg.	Excipient	Acronine, mg./ Capsule	N	Precision (RSD), %
170	Microcrystalline cellulose	24.9	5	±1.63
290	Silica gel	23.0	5	±1.17
300	Mg. stearate and starch	23.7	4	±2.77
300	Stearic acid and starch	24.8	5	±1.16
300	Starch	25.3	5	±1.74
380	Talc	25.5	5	±1.59

dard solution. The peak height ratios were calculated and used to determine the precision of the method. These calculations gave a relative standard deviation of ±0.99%.

Accurately weighed amounts of acronine and talc were mixed in four different proportions and the simulated products were assayed for acronine by the described GLC method. The results of these assays are presented in Table I. The applicability of the method was further tested by assaying admixtures of acronine and seven different potential excipients. All samples were hand-mixed in a 1:1 ratio, and the results of these assays are shown in Table II, along with a comparative lot of acronine raw material. The recovery of acronine from all excipients except dipotassium phosphate was excellent. The use of dipotassium phosphate as an excipient resulted in consistently low assay results, indicating an apparent binding effect with the acronine.

The final evaluation of the method was made on six lots of acronine capsules prepared in our pharmaceutical pilot plant. Each lot of capsules contained the same amount of acronine (25 mg.) with varying amounts of different excipients. The results are reported in Table III and are based upon composite assays using 10 capsules.

Acronine is an alkaloidal compound with newly discovered antitumor activity. A simple, rapid, accurate, and precise method of determining acronine has been described. The method is suitable for the determination of acronine either as the raw material or in admixtures of pharmaceutical excipients. In general, extractions are clean and simple, and the assay can be performed with a minimum of familiarization with the method. It is believed that this method will find use in the assay of final pharmaceutical formulations and in further studies involving acronine.

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Studies with Ion-Exchange Resins on Cinchona Alkaloids III: Exchange Rates

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Abstract □ The exchange rates of four cinchona alkaloid sulfates with styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins of different degree of crosslinking and particle size have been studied and the results are discussed.

Keyphrases □ Cinchona alkaloids, exchange rates—sulfonic acid cation exchange resins □ Sulfonic acid cation exchange resins—degree of crosslinking and particle size.

Earlier (1) the effect of the ionic form of the sulfonic acid cation-exchange resins of different degree of crosslinking and of the added sulfuric acid on the equilibrium exchange of four cinchona alkaloid (quinine, quinidine, cinchonine, and cinchonidine) sulfates as well as the equilibrium uptake of the four cinchona alkaloid bases by the hydrogen form of the same resins from six aliphatic alcohols had been studied. This paper includes the study of the exchange rates of the four cinchona

alkaloid sulfates with sulfonic acid cation-exchange resins of different degree of crosslinking and particle size at 35 and 45°.

EXPERIMENTAL

Resins¹ (1–3)—Styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins of degree of crosslinking (percent nominal divinylbenzene content), $X = 1, 2,$ and 4 are referred to as Resins $X_1, X_2,$ and X_4 . Each resin was washed, conditioned, regenerated into hydrogen form, air dried, sieved, and stored. A large number (≈ 50 to 60) of air-dry particles of each resin was measured for the particle diameter of each fraction of the air-dry resin using a microscope with a mechanical stage at room temperature ($\approx 30^\circ$) and the values for the average particle diameter, a , thus obtained were; $X_1, 0.215$ mm.; $X_2, 0.215$ mm.; $X_4, 0.66$ mm., 0.43 mm., and 0.215 mm. The moisture content and the capacity of the air-dry resins were estimated (1, 4). The percentage moisture and the

¹ Dowex 50W, Dow Chemical Co., Midland, Mich.

capacity in milliequivalents per gram of the air-dry resins were: X1, 20.3, 4.18; X2, 20.4, 4.14; X4, 24.0, 3.89 (for each fraction).

Styrene divinylbenzene copolymer based sulfonic acid cation-exchange resin of relative degree of crosslinking about 20,² with an expanded structure was used. The resin was washed, conditioned, and regenerated into hydrogen form, air dried, sieved, and stored. The values for the average particle diameter, a , determined as given above, were 0.37 mm., 0.58 mm., and 0.84 mm. The percentage moisture and capacity per gram of the air dried resin were estimated. The percentage moisture and the capacity in milliequivalents per gram of the air dried resin were 28.1 and 3.44 for each fraction, respectively.

Chemicals and solutions (5)—Quinine sulfate (Government Quinine Factory, Madras, India), quinidine sulfate (P. B. Howard, London, England), cinchonine sulfate (B. D. H., London, England), and cinchonidine sulfate (Fluka, A. G., Switzerland) were crystallized several times from hot water.

The alkaloid sulfate (as $Q_2H_2SO_4 \cdot nH_2O$, where Q is the alkaloid base and therefore half of the molecular weight was taken as equivalent weight of the alkaloid sulfate) solutions were prepared by dissolving weighed amounts of the respective alkaloid sulfates in distilled water in volumetric flasks. The concentrations of the solutions were rechecked gravimetrically by sulfate estimation as barium sulfate and by UV absorption (5).

Procedure—A procedure, involving application of the second order law, similar to that used by Nachod and Wood (6) was used to obtain the relative values of the rate constants at 35 and 45°.

Earlier (2) the values of the equilibrium exchange for the hydrogen form of the resins used here with the four cinchona alkaloid sulfates were given and the percentage of the resin capacity exchanged at equilibrium was denoted by P_R and average value of P_R is denoted by P_{RA} . If C denotes the capacity of the resin in milliequivalents per gram of air dried resin, $C \cdot P_{RA}/100$ is termed as the effective exchange capacity of the resin and is denoted by C_{ef} . If b denotes the concentration of the alkaloid sulfate in milliequivalents per liter, and W , the weight in grams of the air-dry resin used per liter of the solution in a rate experiment, then W was always kept equal to b/C_{ef} . This means that the concentration of the alkaloid sulfate in aqueous solution in milliequivalents per liter was always kept equal to the concentration of the resin in milliequivalents per liter of solution, the effective capacity being used instead of the actual air-dry capacity of the resin in the calculation of the concentration of the resin in milliequivalents per liter. This condition was observed in all the rate experiments.

Under these conditions the rate constant, k is given by

$$k = \frac{10^3}{b \cdot t} \cdot \frac{x}{b - x}$$

where $(b - x)$ is the concentration in milliequivalents per liter at time $t = t$ and b is the concentration in milliequivalents per liter when $t = 0$. Hence the plot of $x/(b - x)$ against t should be linear, pass through the origin and the slope, S of the plot is given by $b \cdot k/10^3$. Therefore, S multiplied by $10^3/b$ gives the value of k . If the time is measured in minutes, the units of k are min.^{-1} (g. eq.)⁻¹ liter.

The experimental procedure for an exchange rate run was carried out in the following manner. A known amount of air dried resin (concentration of the resin in milliequivalents per liter, on the basis of the effective capacity equal to the concentration of the aqueous alkaloid sulfate solution in milliequivalents per liter) was kept in a clean, dry, three-neck (ground glass) round-bottom 500-ml. Pyrex flask, clamped in a constant-temperature bath ($\pm 0.02^\circ$). The central opening of the flask was fitted with a quickfit metallic piece with a Pyrex glass stirrer and the regulator of the stirrer controlled the speed. The other two openings of the flask were closed with ground glass stoppers. An aqueous alkaloid sulfate solution (450 ml. previously brought to the temperature of the bath) of required concentration, was poured into the flask with the help of a clean, shortstem glass funnel, through the side opening and the stopper replaced. The stopwatch was started when about half of the solution had been added. The stirrer was switched on as soon as the addition of the solution was complete (complete addition required about 20 sec.). After a definite time, the stirrer was switched off and 10 to 15 sec. (depending on the particle size of the resin) was

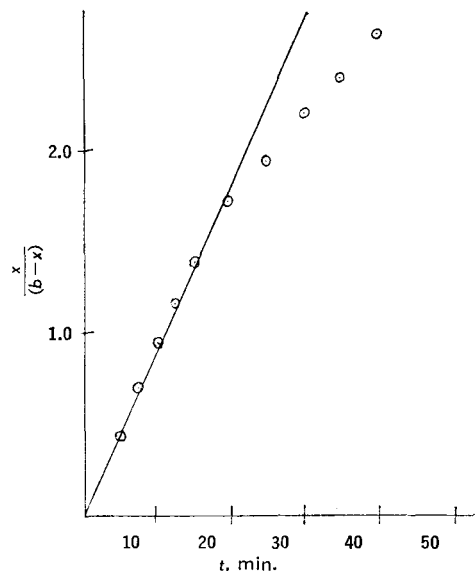


Figure 1—Illustration of the plot of $x/(b-x)$ against time, t (in minutes), for exchange of quinine sulfate with sulfonic acid cation-exchange resin X4 (average particle diameter, a , equal to 0.215 mm.) in aqueous solution at 35°; $b = 1.136 \text{ meq./l.}$

permitted to elapse in order to allow the resin to settle down. A suitable volume was quickly pipeted out (the pipet end just dipped in the solution to avoid the picking up of the resin particles) into well stoppered, clean, and dry test tubes, the stopper was replaced in the side opening of the reaction flask, and the stirrer restarted. Further samples were taken out similarly. In one run, not more than six samples of 5 ml. each or three samples of 10 ml. each (depending on the value of b) were withdrawn. To obtain more points, the run was repeated as many times as required. The contents of the test tubes were suitably diluted with distilled water and the absorbance, D_t , was observed at the invariant wavelength (296.5 μ m for quinine sulfate and quinidine sulfate and 294.5 μ m for cinchonine sulfate and cinchonidine sulfate) with a spectrophotometer (Beckman model DU) using 10-mm. matched quartz cells. A sample of the alkaloid sulfate solution from which some was used in the run was also diluted to the same extent and the absorbance, D_0 , was also measured at the same wavelength. Then $(D_0 - D_t)/D_t$ gives the value of $x/(b - x)$.

Preliminary work was done with different stirring speeds (400 to 1000 r.p.m.) and it was observed that if the rate of stirring was above 700 r.p.m. the rate of the reaction was practically independent of stirring rate. Hence, throughout the work, the rate of the stirring was kept about 800–900 r.p.m. The runs were always checked for reproducibility.

Figure 1 gives an illustration of the type of results obtained when $x/(b - x)$ was plotted against time, from values obtained by required repetitions of the run. It was, in general, observed that the initial plot was linear, after which there was a gradual deviation

Table I—Values of k^a for the Exchange Rate of Quinine, Quinidine, Cinchonine, and Cinchonidine Sulfates with Sulfonic Acid Cation-exchange Resins

Resin	a , mm.	k	
		35°	45°
X1	0.215	188	220
X2	0.215	135	158
X4	0.215	81	95
X4	0.215	81	95
X4	0.43	40	47
X4	0.66	26	31
IR-200	0.37	72	94
IR-200	0.58	47	61
IR-200	0.84	32	42

^a In liters equivalents⁻¹ minutes⁻¹.

² Amberlite 200, IR-200, Rohm & Haas Co., Philadelphia, Pa.

from linearity, which may be attributed to the gradual decrease in the diffusion during the later part of the exchange reaction. From the initial slope of the plot, the value of k was calculated.

RESULTS AND DISCUSSION

The values of the rate constant, k , were calculated using the procedure given above over a range of values of b from about 1.1 to 0.12 meq./l. It was observed that the value of k obtained was practically independent of the value of b . The value of k was also within about $\pm 5\%$ for the four cinchona alkaloid sulfates studied for each resin used. The values of k thus obtained are given for resins X1, X2, and the three particle sizes of X4 in Table I at 35 and 45°. The value of the apparent energy of activation for each of these resins was evaluated according to the Arrhenius equation (7) and is almost the same, 3.06 kcal.

Similarly, runs were carried out at 35 and 45° for the three particle sizes of the resin (Amberlite-200) with four cinchona alkaloid sulfates and the same conclusions were obtained. The values of k are given in Table I. The value of apparent energy of activation is higher, and is 5.19 kcal. The value of k is inversely proportional to the average particle diameter, a , as indicated by the values of k and a .

The observation that the value of k is almost the same for the four cinchona alkaloid sulfates indicates that for the expanded structure resin, the rate of exchange is practically the same although

the value of P_R (which gives the value of equilibrium exchange) (2) is higher for cinchonine sulfate and cinchonidine sulfate than that for quinine sulfate and quinidine sulfate.

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Polyamide-Kieselguhr Thin-Layer Chromatography of Antioxidants

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Abstract □ Eight fat antioxidants are identified on the polyamide-kieselguhr G (2:1) mixed layer, which is firmly bonded and easy to handle. The sharp separation is achieved by use of both aqueous and nonaqueous solvent systems.

Keyphrases □ Antioxidants—identification □ TLC—separation, identification □ UV light—TLC spot visualization □ Iodine vapor—TLC spot visualization

Thin-layer chromatography of fat antioxidants has been the subject of numerous investigations. The separation by TLC on alumina (1), silica gel (2, 3), silica gel-kieselguhr mix layer (4), acetylcellulose (5), polyamide (6), and polyamide-starch mix layer (7), has been reported, but the application of a mixed polyamide-kieselguhr layer in the chromatographic separation has not. In this note, the separation of eight fat antioxidants by this mixed layer procedure is described.

EXPERIMENTAL

Materials—The solvents and chemicals are reagent grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Thin-Layer Plates—Twenty-two grams of polyamide¹ chip was dissolved in a mixture (80:20) of 90% formic acid and distilled water.

¹ Nylon 6, type 1022B of UBE Industrial Ltd., Osaka, Japan. U. S. supplier: American Enka Corp.

Table I—Chromatographic Data

Substance	Group				
	N		A		
	Solvent ^a				
I	II	III	IV	V	
Time, hr. ^b					
	9	2	2.5	2.5	3
<i>n</i> -Propyl gallate	0.26	0.22	0.27	0.63	0.63
Isoamyl gallate	0.36	0.31	0.33	0.61	0.52
Lauryl gallate	0.56	0.54	0.48	0.44	0.45
Cetyl gallate	0.64	0.65	0.53	0.21	0.19
Stearyl gallate	0.72	0.67	0.56	0.08	0.08
Butylated Hydroxyanisole	0.69	0.87	0.74	0.70	0.54
Butylated Hydroxytoluene	0.80	0.95	0.63	0.10	0.14
Ethyl protocatechuate	0.42	0.38	0.37	0.55	0.64

^a I, isoamyl alcohol; II, isoamyl acetate-acetone (5:1); III, isoamyl acetate-xylene-ethanol (20:1:1); IV, acetone-water (5:3); V, dioxane-water-ethanol (10:7:5). ^b Required to ascend 10 cm. from origin.

After obtaining a homogeneous solution with gentle warming and stirring, 10 g. of kieselguhr G (E. Merck) was added. Two hundred milliliters of the above solution was added to a dish (14.5 × 19.5 × 2.5 cm.) and a glass plate (12 × 16 × 0.1 cm.) dipped into it. Both sides of the glass were covered evenly. The glass was placed over the dish for 2 min. to let the excess solution drain. It was then air-dried for 3 hr. and heated at 100° for 30 min.

Chromatographic Procedure—A 0.5% alcoholic solution of antioxidants was applied to the start line 1.5 cm. from the bottom of the layer and developed by ascending techniques. The chamber was equilibrated with the respective solvent for 30 min. before use.

Visualization—The layers were sprayed with 0.07% rhodamine B alcoholic solution and deep violet spots were observed under the