Table III. Killing action of group A bacteriocin No. 5 against *S. marcescens* indicator isolate No. 1 in the presence or absence of rifampin

Time (min) following exposure to bacteriocin	Bacteriocin No. 5 added to cells of *S. marcescens* indicator (i) isolate No. 1			
	1 iS[a]	1 iS + 100 µg/ml rifampin	1 iR[a] + 100 µg/ml rifampin	1 iS (control)
0	1.3×10^4 [b]	1.3×10^4	1.4×10^4	1.3×10^4
10	2.0×10^1	5.0×10^1	1.0×10^1	—
30	1.5×10^1	1.5×10^1	0	1.3×10^4

[a] S denotes sensitive to rifampin, R indicates resistant to rifampin. [b] Number of colony-forming units/ml (survivors).

finding that high-titered bacteriocin preparations could overcome the immunity of bacteriocinogenic cells to their own bacteriocins confirms previous observations[11–13]. The failure of rifampin to abolish the immunity of exposed bacteriocinogenic cells to their own group A bacteriocins was probably due to the fact, that these cells had synthesized adequate amounts of immunity susbtance[14] before their exposure to rifampin. Conceivably the catabolic turnover of this immunity substance is rather slow, so that sufficient amounts of this inhibitor were stlil present after exposure of the bacteriocinogenic cells to rifampin.

Zusammenfassung. Rifampin blockierte die Induktion von Gruppe A Bakteriozinen in bakteriozinogenen *Serratia marcescens* Zellen; demnach scheint die Induktion dieser Bakteriozine auf funktionstüchtiger, DNS-abhängiger RNS-Polymerase der Wirtszellen zu beruhen. Rifampin hatte keinen Einfluss auf die Empfindlichkeit von Indikatorzellen auf Gruppe A Bakteriozine; demzufolge scheint die bakterizide Wirkung dieser Bakteriozine unabhängig von Proteinsynthese durch Indikatorzellen zu sein.

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PRO EXPERIMENTIS

Thin Layer Chromatography of Metal Ions on a New Synthetic Inorganic Ion-Exchanger

Ion-exchange thin layer chromatography of inorganic species has been reviewed by LEDERER[1]. However, very few attempts have been made with the use of synthetic inorganic ion-exchangers in thin layer chromatography[2–4]. QURESHI et al.[5–11] have recently synthesized a few new inorganic ion-exchangers and papers impregnated with some of these ion-exchangers have been used successfully in chromatographic separation of metal ions[12–15]. As far as we are aware, stannic arsenate a new synthetic inorganic ion-exchanger[11] has not been used for thin layer chromatography of metal ions. The present work was therefore undertaken to study the chromatographic behaviour of 38 metal ions on combined thin layers of stannic arsenate gel and silica gel using 15 buffer solutions. As a result, a number of useful and interesting separations have been developed, and some important separations are summarized in this paper.

Experimental. Apparatus: A 'Quickfit' thin layer apparatus was used. Development was performed in 22 × 24 cm circular glass tanks using the ascending method.

Reagents: Chemicals and solvents were either E. Merck or Riedel. Silica gel GF (E. Merck) was used.

Preparation of ion-exchanger plates: 0.05 M solutions of stannic chloride and sodium arsenate were mixed together in volume ratio (3:2). The mixture was continuously stirred during which a white gel was formed, the pH of the gel was adjusted to 1 using 6 M nitric acid. This gel was left overnight. The gel was washed 3 or 4 times with distilled water. The supernated liquid was removed completely. Now 50 cm^3 of this wet gel was mixed with 14 g of Silica gel GF. This slurry was used to coat 5 clean glass plates (20 × 20 cm) using the Quickfit apparatus preset to give an applied layer 0.25 mm thick. These plates were dried in a hot oven at 105 °C–110 °C for 1 h and then were stored in an oven at room temperature, after that they were used as such.

Cation solutions: 0.2 M solution of chlorides, nitrates or sulphates of most of the cations were prepared in 0.2 M solution of the corresponding acids. Antimony(III) and Bismuth(III) chlorides were prepared (0.1 M) in 30% (v/v) HCl solution. Selenium dioxide was dissolved in water and made alkaline with 1 M KOH solution. Ceric sulphate was prepared in 3 N H_2SO_4. Mercuric nitrate solution was prepared in 0.5 N HNO_3, As_2O_3 and $Be(NO_3)_2$ were dissolved in 1% HNO_3, and 1% solution of the Gold(III) chloride was prepared in 4 M hydrochloric acid.

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Table I. Separation of 1 cation from numerous metal ions on ion-exachange plates as predicted by Rf values

Metal ion separation (R_T-R_L)	Solvent system	Ions that interfere	Time (min)
V^{+5} (0.55–0.86) from 28 cations	1 *M* Ammonium nitrate + 0.5 *M* nitric acid (1:1)	Fe^{+2}, Fe^{+3}, Cu^{+2}, Ce^{+3}, Mo^{+3}, Ce^{+4}, UO^{2+2}, As^{+3}, Cd^{+2}, Hg_2^{+2}.	10
Ge^{+4} (0.18–0.41) from 30 cations	0.25 *M* Ammonium oxalate + 0.1 *M* oxalic acid (1:1)	Bi^{+3}, Sb^{+3}, Fe^{+3}, Fe^{+2}, Zr^{+4}; Se^{+4}, Ce^{+4}.	8
Ba^{+2} (0.00–0.00) from 33 cations	2 *M* HBr + 2 *M* Ammonium bromide (1:1)	Ge^{+4}, Fe^{+3}, Fe^{+2}, Sb^{+3}, Ag^{+}, W^{+6}.	13
W^{+6} (0.00–0.09) from 33 cations	2 *M* HBr + 2 *M* NH_4Br (1:1)	Ba^{+2}, Ge^{+4}, Fe^{+3}, Fe^{+2}, Sb^{+3}, Ag^{+}.	13

Table II. Separations practically achieved on ion-exchanger Plates

Solvent system	Separations achieved (R_T-R_L)	Time (min)
1 *M* Ammonium acetate + 1 *M* acetic acid (1:2)	Pb^{+2} (0.00–0.23) Cd^{+2} (0.91–1.00)	15
	Cr^{+3} (0.00–0.18) Mn^{+2} (0.86–1.00)	15
	Ag^{+} (0.00–0.00) Ce^{+3} (0.80–0.86) Hg^{+2} (0.95–1.00)	15
1 *M* Citric acid + phenol (600 cm^3 + 0.5 g) + NH_3 solution till a pH of 4.5	Se^{+4} (0.00–0.45) Hg^{+2} (0.91–1.00)	17
	Pb^{+2} (0.14–0.64) Hg^{+2} (0.91–1.00)	17
	Ag^{+} (0.00–0.00) Bi^{+3} (0.36–0.95)	17
	As^{+3} (0.64–0.77) Mo^{+6} (0.91–1.00)	17
1 *M* Ammonium nitrate + 0.5 *M* HNO_3 (1:1)	Sb^{+3} (0.00–0.04) As^{+3} (0.64–0.73) Cd^{+2} (0.82–0.95)	10
	Ag^{+} (0.00–0.00) Pb^{+2} (0.04–0.27) Hg^{+2} (0.91–1.00)	10
	Pb^{+2} (0.06–0.27) Mn^{+2} (0.91–1.00)	10
1 *M* NH_4Cl + 0.1 *M* HCl (1:1)	Ag^{+} (0.00–0.00) Se^{+4} (0.04–0.36) Pd^{+2} (0.82–1.00)	12
	Pb^{+2} (0.00–0.36) Pd^{+2} (0.82–1.00)	12
3 *M* Citric acid + 1 *M* ammonium citrate (1:2)	Ag^{+} (0.00–0.00) Pb^{+2} (0.04–0.50) Cu^{+2} (0.82–0.95)	44
	Se^{+4} (0.00–0.32) Mo^{+6} or Au^{+3} (0.87–0.91)	44
0.1 *M* Ammonium molybdate + 0.2 *M* nitric acid (1:1)	Cd^{+2} (0.77–0.85) Zn^{+2} (0.88–0.95)	11
	Pb^{+2} (0.00–0.09) Zn^{+2} (0.86–0.95)	11
	Pb^{+2} (0.00–0.09) As^{+3} (0.73–0.76) Hg^{+2} (0.86–0.95)	11

Detectors: 0.1% alcoholic solution of Alizarine red S was used to detect Ce^{+3}, Ce^{+4}, Zr^{+4} and Ca^{+2}. 5% aqueous solution of sodium sulphide was used to detect Ag^{+}, Pb^{+2}, Hg_2^{+2}, Hg^{+2}, Bi^{+3}, Tl^{+}, Cd^{+2}, As^{+3}, Sb^{+3}, Pd^{+2} and Cu^{+2}. Stannous chloride in concentrated HCl was used to detect Au^{+3}, Pt^{+4}, Mo^{+6}, W^{+6} and Se^{+4}. Al^{+3} and Be^{+2} were detected by 1% alcoholic solution of aluminon. Diphenylcarbazide was used to detect Mn^{+2}, Cr^{+3}, Ir^{+4} and Zn^{+2}. Fe^{+3}, V^{+5}, UO_2^{+2} were detected with aqueous $K_4Fe(CN)_6$ solution. Mg^{+2} was detected with quinalizarine. A fresh 5% aqueous solution of sodium rhodizonate was used to detect Ba^{+2} and Sr^{+2}. Ge^{+4} was detected by phenylfluorone solution.

Procedure: The test solutions were applied on the plate using thin glass capillaries. The solvent was allowed to ascend 11 cm from the starting line on the plate in all cases. Time of the development has been specified with each solvent. After the development was over, the plates were dried in the air oven and the cations were detected with respective reagents.

Results. The ion-exchanger stannic arsenate is so selective that in many cases one metal ion can be separated from numerous metal ions. Few of them are recorded in Table I. A large number of binary and ternary important separations were found possible and were practically achieved. Some of them are reported in Table II. To give a clear picture of the spot, instead of simply giving the Rf value of the spot, the Rf of the front limit (R_L) and Rf of the rear limit (R_T) are given.

Discussion. It is clear from the results that thin layers of the stannic arsenate combined with silica gel are exceedingly useful for metal ion separations. Attempts to prepare the thin layers with stannic arsenate gel alone were not successful. Tungsten can be separated in 13 min from 33 metal ions including Mo^{+6}, Cr^{+3} etc. which interfere in its determination. The separation of Germanium from numerous metal ions reported here is an improvement over the available paper chromatographic method[15] and is probably the fastest separation of Ge^{+4} yet reported. Important binary and ternary separations have also been achieved with great ease, to mention a few Sb^{+3}-As^{+3}-Cd^{+2}, V^{+5}-Mn^{+2}, Cd^{+2}-Zn^{+2} etc. Thin layer chromatography is known for its speedy separations. However, when a synthetic inorganic ion-exchanger is employed fast, selective and clear separations are obtained. The separation potential of this ion-exchanger in mixed organic solvent systems, offers further study and the work is in progress[16].

Zusammenfassung. Neues, für die Trennung anorganischer Ionen nützliches Trägermaterial für Dünnschichtchromatographie.

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