Surface active agents in paper chromatography

A recent note by ORME-JOHNSON AND SKINNER[1] on the separation of amino acids with butanol containing the liquid ion exchanger Amberlite LA-2 prompts us to describe work carried out along similar lines[2]. It was thought interesting to study the possible effects that surface active agents can have in various paper chromatographic problems, with a view to utilising paper chromatography as a simple orientating method in the study of foam adsorption and similar problems.

(1) *The effect of additions of surface active agents to the solvent in paper chromatography*

Paper chromatography was carried out using the solvent butanol–water–acetic acid (10:8:2) to which various amounts of cetyltrimethylammonium bromide, sodium lauryl sulphonate or "Neutronix 675" a neutral polyethyleneglycol surface active agent were added. The substances chromatographed were some inorganic anions and cations and a series of dyestuffs (those permitted in Italy as food colours).

The results are shown in Tables I, II and III. An increase in the R_F values is obtained in those cases where the "exchanger" (or surface active agent) has the charge opposite to that of the ion chromatographed and the effect is proportional to the amount of surface active agent added.

It is thus possible to remove an anion from a mixture of cations and anions or vice-versa by suitable additions of charged surface active agents to partition solvents.

TABLE I

R_F VALUES OF SOME DYESTUFFS AND INORGANIC IONS IN BUTANOL–ACETIC ACID–WATER WITH ADDITIONS OF CETYLTRIMETHYLAMMONIUM BROMIDE

Paper: Whatman No. 1. Method: ascending development. Solvent: butanol–acetic acid–water (100:20:80), equilibrated, and the amounts of cetyltrimethylammonium bromide indicated in the table added to the upper layer

Substance	*Grams of cetyltrimethylammonium bromide added to the solvent*				
	1.0	*0.5*	*0.1*	*0.05*	*0*
Iodide	0.75	0.59	0.34	0.31	0.31
Thiocyanate	0.77	0.53	0.47	0.46	0.41
Azorubin	0.92	0.82	0.52	0.37	0.31
Echtrot E	0.86	0.72	0.40	0.32	0.32
Amaranth	0.35	0.13	0.08	0.04	0.03
Ponceau 4R	0.79	0.53	0.13	0.10	0.08
Ponceau 6R	0.52	0.06	0	0	0
Scharlach GN	0.70	0.59	0.29	0.26	0.26
Erythrosine	0.90	0.90	0.90	0.89	0.86
Sunset Yellow	0.51	0.17	0.14	0.12	0.14
Tartrazine	0.09	0.02	0.04	0.06	0.05
C.I. Acid Yellow	0.06	0.02	0.02	0.02	0.02
	0.20	0.16	0.16	0.08	0.09
Indigo Carmine	0.08	0.06	0.06	0.04	0.05
C.I. Acid Blue 3	0.70	0.62	0.52	0.42	0.45
C.I. Food Black 1	0.03	0	0	0	0

TABLE II

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS IN BUTANOL–ACETIC ACID–WATER WITH ADDITIONS OF SODIUM LAURYLSULPHONATE

Paper: Whatman No. 1. Method: ascending development. Solvent: butanol–acetic acid–water (100:20:80), equilibrated, and the amounts of sodium laurylsulphonate indicated in the table added to the upper layer

Substance	*Grams of sodium laurylsulphonate added to the solvent*				
	1.0	*0.5*	*0.1*	*0.05*	*0*
Cu (II)	0.58	0.44	0.28	0.30	0.23
Ni (II)	0.65	0.45	0.19	0.17	0.14
Co (II)	0.65	0.44	0.21	0.17	0.14
Scharlach GN	0.15	0.16	0.20	0.24	0.26
Erythrosine	0.89	0.89	0.92	0.91	0.86
Sunset Yellow	0.09	0.12	0.16	0.16	0.14
Tartrazine	0	0.01	0.09	0.06	0.05
C.I. Acid Yellow 3	0.15	0.12	0.11	0.16	0.09
	0.03	0.01	0.02	0.02	0.02
Indigo Carmine	0.01	0.02	0.02	0.08	0.05
C.I. Acid Blue 3	0.42	0.40	0.43	0.39	0.45
C.I. Food Black 1	0	0	0	0	0

TABLE III

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS IN BUTANOL–ACETIC ACID–WATER WITH ADDITIONS OF NEUTRONIX 675 (A NEUTRAL SURFACE ACTIVE AGENT)

Paper: Whatman No. 1. Method: ascending development. Solvent: butanol–acetic acid–water (100:20:80), equilibrated, and the amounts of Neutronix 675 indicated in the table added to the upper layer

Substance	*Grams of Neutronix 675 added to the solvent*				
	1.0	*0.5*	*0.1*	*0.05*	*0*
Iodide	0.25	0.31	0.32	0.27	0.31
Thiocyanate	0.45	0.46	0.43	0.43	0.42
Cu (II)	0.25	0.21	0.25	0.23	0.23
Ni (II)	0.13	0.17	0.17	0.15	0.14
Co (II)	0.13	0.17	0.20	0.13	0.14
Azorubin	0.27	0.34	0.26	0.27	0.30
Echtrot E	0.25	0.28	0.26	0.23	0.31
Amaranth	0.02	0.02	0.01	0	0.04
Ponceau 4R	0.08	0.06	0.04	0.04	0.07
Ponceau 6R	0	0	0	0	0
Scharlach GN	0.24	0.25	0.25	0.20	0.27
Erythrosine	0.90	0.90	0.89	0.90	0.88
Sunset Yellow	0.11	0.08	0.09	0.08	0.17
Tartrazine	0.02	0	0	0.02	0.02
C.I. Acid Yellow 3	0.12	0.10	0.04	0.04	0.08
	0.02	0.02	0	0.02	0.02
Indigo Carmine	0.04	0.03	0.04	0.05	0.05
C.I. Acid Blue 3	0.46	0.39	0.35	0.36	0.44
C.I. Food Black 1	0	0	0	0	0

It should also be possible to gain information about the charge of an unknown substance by this method.

(2) The effect of surface active agents on the R_F value of substances when the surface active agent is present in the sample to be analysed

Artificial mixtures of solutions of surface active agents and dyestuffs or inorganic ions were prepared, placed on paper and developed with butanol–water–acetic acid (10:8:2).

As shown in Tables IV, V and VI as well as Fig. 1, two kinds of results may be obtained. The presence of the surface active agent of the opposite charge to that of the substance may produce either two distinct spots with the same substance or an elongated trail. Neutral or equally charged surface active agents have no effect.

The double spot phenomenon may be explained if we consider that the bulk of the surface active agent moves probably in a micellar form and that these micelles are capable of adsorbing some of the oppositely charged substance or alternatively that very strong ion association complexes are formed between the surface active agent and the ion.

The effect is of importance when substances *e.g.* dyestuffs are to be identified by their R_F values. This is clearly impossible where surface active agents are present in a sample.

(3) The effect of surface active agents in adsorption chromatograms on cellulose paper

Preliminary work with solvents containing surface active agentsindicated that these were adsorbed on the cellulose surface as are also the more insoluble "liquid ion exchangers" (see CERRAI AND TESTA[3]). In order to avoid a gradient effect due to a

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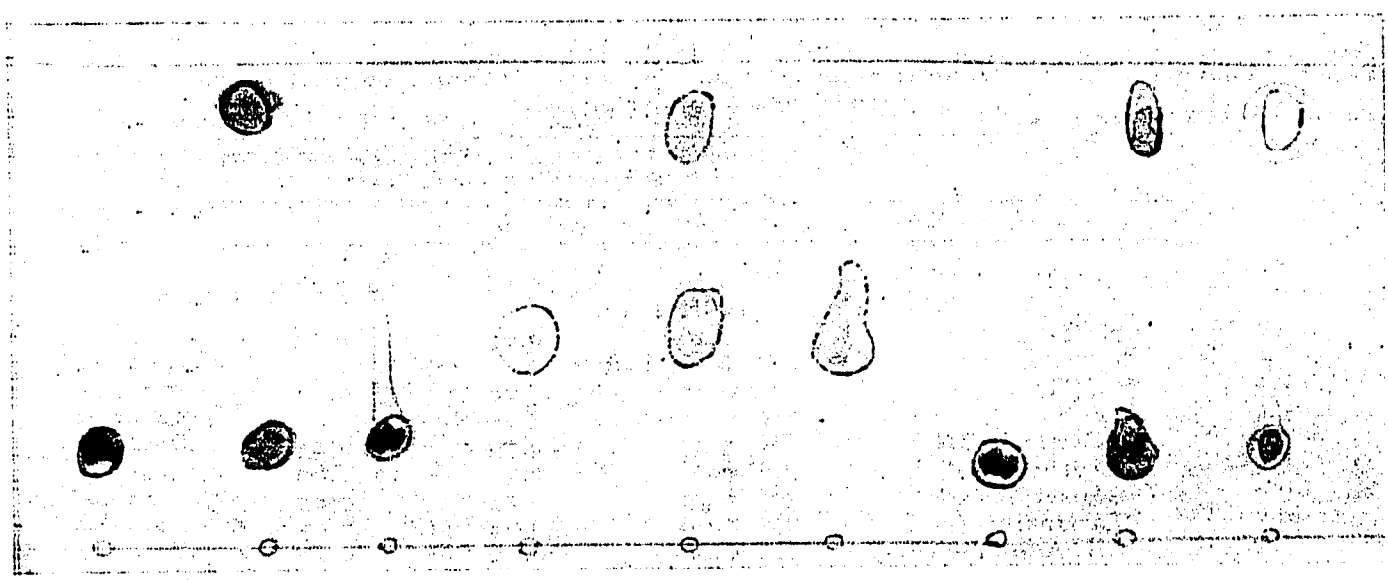

Fig. 1. Chromatogram of some dyestuffs mixed with cetyltrimethylammonium bromide. Solvent: butanol–acetic acid–water (100:20:80). From left to right: Scharlach GN; Scharlach GN mixed with a 10% solution of cetyltrimethylammonium bromide; Scharlach GN mixed with a 1% solution of cetyltrimethylammonium bromide; C.I. Acid Blue 3 alone and mixed with 10% and 1% cetyltrimethylammonium bromide solutions; Echtrot E alone and mixed with 10% and 1% cetyltrimethylammonium bromide. Bottom line: line of application of spots. Top line: liquid front.

TABLE IV

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS WHEN A MIXTURE OF THE SUBSTANCE WITH 10% OR 1% SOLUTIONS OF CETYLTRIMETHYLAMMONIUM BROMIDE ARE CHROMATOGRAPHED IN BUTANOL–ACETIC ACID–WATER

Paper: Whatman No. 1. Method: ascending development. Solvent: butanol–acetic acid–water (100:20:80) (upper phase).

Substance	*Concentration of cetyltrimethylammonium bromide mixed with the substance to be chromatographed*		
	10%	*1%*	*0*
Iodide	0.92, 0.33	0.46	0.30
Thiocyanate	0.90, 0.44	0.44	0.40
Azorubin	0.88, 0.30	0.88, 0.38	0.30
Echtrot E	0.85, 0.19	0.89, 0.29	0.31
Amaranth	0.85, 0.15	0.04, Tail	0.04
Ponceau 4R	0.80, 0.10	0.06, Tail	0.07
Ponceau 6R	0.86	0	0
Scharlach GN	0.86, 0.30	0.90, 0.39	0.27
Erythrosine	0.88	0.88	0.88
Sunset Yellow	0.83, 0.17	0.17	0.17
Tartrazine	0.24	0.07	0.02
C.I. Acid Yellow 3	0.79, 0.20	0.19	0.08, 0.02
Indigo Carmine	0.11	0.02	0.02
C.I. Acid Blue 3	0.83, 0.46	0.46	0.44
C.I. Food Black 1	0 Tail	0	0

TABLE V

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS WHEN A MIXTURE OF THE SUBSTANCE WITH 10% OR 1% SOLUTIONS OF SODIUM LAURYLSULPHONATE ARE CHROMATOGRAPHED IN BUTANOL–ACETIC ACID–WATER

Paper: Whatman No. 1. Method: ascending development. Solvent: butanol acetic–acid–water (100:20:80) (upper phase).

Substance	*Concentration of sodium laurylsulphonate mixed with the substance to be chromatographed*		
	10%	*1%*	*0*
Cu (II)	0.22 (with trail)	0.16	0.20
Ni (II)	0.26 (with trail)	0.07	0.13
Co (II)	0.22 (with trail)	0.08	0.14
Scharlach GN	0.25	0.30	0.25
Erythrosine	0.89	0.90	0.89
Sunset Yellow	0.15	0.15	0.15
Tartrazine	0.06	0.04	0.03
C.I. Acid Yellow 3	0.08	0.09	0.10
	0.05	0.06	0.02
Indigo Carmine	0.06	0.07	0.05
C.I. Acid Blue 3	0.43	0.44	0.45
C.I. Food Black 1	0.09	0.09	0

TABLE VI

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS WHEN A MIXTURE OF THE SUBSTANCE WITH 10% SOLUTIONS OF NEUTRONIX 675 (A NEUTRAL SURFACE ACTIVE AGENT) IS CHROMATOGRAPHED IN BUTANOL–ACETIC ACID–WATER

Paper: Whatman No. 1. Method: ascending development. Solvent: butanol–acetic acid–water (100:20:80).

Substance	*Concentration of Neutronix 675 mixed with the substance to be chromatographed*		
	10%	*1%*	*0*
Iodide	0.27	0.27	0.31
Thiocyanate	0.36	0.37	0.40
Cu(II)	0.25	0.24	0.23
Ni(II)	0.16	0.15	0.14
Co(II)	0.15	0.15	0.15
Azorubin	0.21	0.22	0.30
Echtrot E	0.22	0.23	0.31
Amaranth	0.01	0	0.03
Ponceau 4R	0.03	0.05	0.06
Ponceau 6R	0	0	0
Scharlach GN	0.20	0.21	0.23
Erythrosine	0.87	0.90	0.90
Sunset Yellow	0.14	0.14	0.15
Tartrazine	0.02	0.03	0.02
C.I. Acid Yellow 3	0.05	0.03	0.09
	0.02	0.03	0.02
Indigo Carmine	0.02	0.04	0.05
C.I. Acid Blue 3	0.44	0.44	0.44
C.I. Food Black 1	0	0	0

TABLE VII

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS ON PAPER IMPREGNATED WITH VARIOUS AMOUNTS OF CETYLTRIMETHYLAMMONIUM BROMIDE

Paper: Whatman No. 1 dipped in aqueous solutions of cetyltrimethylammonium bromide (concentrations indicated below) and air dried. Method: ascending development. Solvent: water–acetic acid (90:10).

Substance	*Concentration of cetyltrimethylammonium bromide used for impregnating the paper*					
	1%	*0.5%*	*0.1%*	*0.05%*	*0.01%*	*0*
Iodide	0.54	0.62	0.71	0.70	0.76	0.81
Thiocyanate	0.49	0.62	0.67	0.68	0.76	0.80
Azorubin	1.0,0	1.0,0	1.0,0	1.0,0	0.16	0.20
Echtrot E	1.0,0	1.0,0	1.0,0	1.0,0	0.25	0.29
Amaranth	1.0,0	1.0,0	1.0,0	0.34	0.27	0.36
Ponceau 4R	1.0,0	1.0,0	1.0,0	1.0,0	0.36	0.74
Ponceau 6R	1.0,0	1.0,0	1.0,0	1.0,0	0.48	0.90
Scharlach GN	1.0,0	1.0,0	1.0,0	0.18	0.39	0.60
Erythrosine	1.0,0	1.0,0	1.0,0	0	0	0
Sunset Yellow	1.0,0	1.0,0	1.0,0.17	0.25	0.25	0.62
Tartrazine	1.0,0	1.0,0	1.0,0.17	0.15	0.25	0.63
C.I. Acid Yellow 3	1.0,0	1.0,0	1.0,0	0.18	0.21	0.38
Indigo Carmine	1.0,0	1.0,0	1.0,0	0.15	0.25	0.38
C.I. Acid Blue 3	1.0	1.0	1.0,0.53	0.64	0.65	0.85
C.I. Food Black 1	1.0,0	1.0,0	1.0,0.04	0.03	0.04	0.12

TABLE VIII

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS ON PAPER IMPREGNATED WITH VARIOUS AMOUNTS OF SODIUM LAURYLSULPHONATE

Paper: Whatman No. 1 dipped in aqueous solutions of sodium laurylsulphonate (concentrations indicated below) and air dried. Method: ascending development. Solvent: water–acetic acid (90:10)

Substance	*Concentration of sodium laurylsulphonate used for impregnating the paper*				
	1%	*0.5%*	*0.1%*	*0.05%*	*0*
Cu (II)	1.0	1.0	1.0	1.0	1.0
Ni (II)	1.0	1.0	1.0	1.0	0.86
Co(II)	1.0	1.0	1.0	1.0	0.91
Scharlach GN	0.62	0.58	0.60	0.60	0.61
Erythrosine	0.90	0.90	0	0	0.90
	0	0			
Sunset Yellow	0.54	0.50	0.52	0.62	0.59
Tartrazine	0.61	0.59	0.60	0.63	0.63
C.I. Acid Yellow 3	0.55	0.55	0.54	0.38	0.38
	0.35	0.35	0.33		
Indigo Carmine	0.33	0.28	0.30	0.36	0.38
C.I. Acid Blue 3	1.0	1.0	0.89	0.85	0.85
C.I. Food Black 1	0.12	0.13	0.12	0.12	0.12

TABLE IX

R_F VALUES OF SOME INORGANIC IONS AND DYESTUFFS ON PAPER IMPREGNATED WITH VARIOUS AMOUNTS OF NEUTRONIX 675 (A NEUTRAL SURFACE ACTIVE AGENT)

Paper: Whatman No. 1 dipped into an aqueous solution of Neutronix 675 (concentrations indicated below) and air dried. Method: ascending development. Solvent: water–acetic acid (90:10).

Substance	*Concentration of Neutronix 675 used for impregnating the paper*			
	1%	*0.5%*	*0.1%*	*0*
Iodide	1.0	0.86	0.89	0.81
Thiocyanate	1.0	0.85	0.86	0.82
Cu (II)	1.0	1.0	1.0	1.0
Ni (II)	1.0	1.0	1.0	0.88
Co (II)	1.0	1.0	1.0	0.91
Azorubin	0.19	0.20	0.24	0.18
Echtrot E	0.26	0.25	0.27	0.29
Amaranth	0.33	0.32	0.34	0.36
Ponceau 4R	0.96	0.95	0.80	0.75
Ponceau 6R	0.99	0.96	0.90	0.90
Scharlach GN	0.59	0.59	0.56	0.60
Erythrosine	0.91	0.90	0.88	0.86
Sunset Yellow	0.37	0.40	0.49	0.62
Tartrazine	0.70	0.62	0.65	0.63
C.I. Acid Yellow 3	0.39	0.39	0.38	0.39
Indigo Carmine	0.40	0.38	0.36	0.38
C.I. Acid Blue 3	0.87	0.85	0.90	0.85
C.I. Food Black 1	0.13	0.10	0.11	0.12

liquid zone moving over paper poor in surface active agent (which could occur if the surface active agent were present in the solvent), we impregnated the papers by dipping them into aqueous solutions of the surface active agent and drying in air. The papers were then developed with aqueous solvents *e.g.* water–acetic acid (9:1).

Results with inorganic ions and dyestuffs are shown in Tables VII, VIII and IX. In general, above a certain concentration of the surface active agent, the dyestuff separates into two spots, one strongly adsorbed on the point of origin and another on the liquid front. Below that concentration the R_F value of the dyestuff is lower than the value obtained when no surface active agent is present. This effect is only observed when the surface active agent has a charge opposite to that of the substance chromatographed.

The phenomenon may be explained by considering that a certain amount of surface active agent can adsorb on the paper thus forming a solid ion exchanger with strong adsorptive power. The excess of surface active agent moves with the liquid front, probably in a micellar state and adsorbs some of the dyestuff into the micelles. There is a fair correlation between the concentrations at which the two spots form and the maxima of the surface tension–concentration curves for the surface active agent. Below the maximum surface tension in water, two spots do not form on the chromatogram.

Inorganic ions *e.g.* iodide and thiocyanate do not form double spots, the adsorption on the micelles perhaps not being strong enough. Their R_F values change, however, with the concentration of the (cationic) surface active agent, since adsorption is greater with increased concentration.

In such adsorption chromatograms, the charge on the surface active agent seems of great importance although one would expect the surface of the paper to be altered considerably even when covered by a neutral surface active agent.

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