that those solvents had been unable to separate glucose from its formate esters was excluded by two-dimensional paper chromatography. A solvent suspected of causing hydrolysis was used for the first dimension. For the second dimension, any solvent from Table I was chosen, a solvent definitely capable of separating glucose from its formate esters. Under these conditions with aniline hydrogen phthalate only one spot was detected, whose R_F values matched those of glucose. The presence of formic acid, acetic acid, or pyridine in the chromatography solvent did not cause any hydrolysis.

Lactic acid, acetic acid, and chloroacetic acid were tried as ester-forming reagents with glucose but these failed to produce any compounds different from glucose, as monitored by paper chromatography. In addition, these three acids were rather poor solvents for glucose as compared to formic acid. D-Mannose and L-rhamnose were also studied and found to react with formic acid in like manner. Mannose in 80 % formic acid yielded three spots while rhamnose, lacking a hydroxyl group on position number six, produced only two spots. Whatman No. 1 paper with ascending flow was used for all the chromatograms.

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The separation of Alizarin Complexan from impurities by paper chromatography

Alizarin Complexan (3-[di-(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone) is the most important reagent for the spectrophotometric determination of fluoride[1–3]. The reagent is synthesized by Mannich condensation[4] from alizarin, iminodiacetic acid and formaldehyde in strong alkaline media[5]. The yield of the synthesis is very satisfactory, but the reagent may be contaminated by small amounts of the starting products. The different solubilities found for various samples of Alizarin Complexan confirms this opinion. Furthermore, other reagents that are synthesized

in a similar manner, *e.g.* Xylenol Orange, are also contaminated by the starting products[6].

In the present study, alizarin and iminodiacetate were separated from Alizarin Complexan; volatile formaldehyde is not expected in the final product. Paper chromatography was employed, because absorbents used for thin-layer separations may cause decomposition of products prepared by Mannich condensation[6].

Experimental

Paper. Chromatographic paper Whatman No. 1 was used for the separations.

Solvents. The following solvents were used: (S1) *n*-butanol–conc. HCl–water (8:1:1); (S2) *n*-propanol–conc. HCl–water (6:1:1).

Detection. Larger spots of alizarin are visible; smaller ones were detected by ammonia vapours, which give an intense violet colour. Iminodiacetate was detected by spraying the chromatograms with a solution containing 175 mg of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ and 10 g of KSCN in 100 ml. White spots on a red background, which turn to orange during 24 h, appear on the chromatograms.

Spots of Alizarin Complexan are readily visible. They may be detected by ammonia vapours, which give a violet colour, or by spraying with lanthanum nitrate, acetate buffer and sodium fluoride solutions according to a photometric procedure for the determination of fluoride[7].

Chromatographic procedure. About 50 mg of Alizarin Complexan are suspended in 5 ml of water and dissolved by the addition of 0.05 ml of concentrated ammonia. 0.05 ml of glacial acetic acid is then added and the solution diluted to 10 ml with water. This solution is applied on the longer side of the chromatogram (24 × 15 cm) in about 10 μl portions. The paper is rolled up to a cylinder, fastened by clips[8], and the chromatograms are developed by the ascending technique until the front has travelled 10 cm (about 2 h). Alizarin and iminodiacetate are chromatographed in the same manner. The chromatograms are dried under an infrared lamp and treated with the detection reagents.

Results and discussion

Solvent S1 (*n*-butanol–conc. HCl–water (8:1:1) proved to be the best of the various solvents examined. When the content of *n*-butanol was changed significantly, very diffuse and elongated spots were obtained. Changes in the content of hydrochloric acid had no influence on the separation. Substitution of HCl for acetic acid, which may lead to better separation of organic reagents from starting substances[6], resulted in double spots of iminodiacetate, which interfered with the spots of Alizarin Complexan. On examining other organic solvents, an excellent mixture for the separation of alizarin from Alizarin Complexan was found, *viz.* solvent S2 (*n*-propanol–conc. HCl–water, 6:1:1). Very sharp spots of alizarin at the front of the mobile phase are obtained. The R_F values for both solvents mentioned are given in Table I.

Solvent S1 may be recommended for the preparation of highly pure Alizarin Complexan, *e.g.* by column cellulose chromatography. This solvent may also be used for the determination of iminodiacetate in samples of Alizarin Complexan. For the determination of alizarin in this reagent, solvent S2 may be employed. We have determined alizarin and iminodiacetate in two samples of Alizarin Complexan. The determination was carried out by comparing spots of standards with spots of both

TABLE I

R_F VALUES OF ALIZARIN COMPLEXAN AND ITS IMPURITIES

No. of the spot	*R_F value*		*Substance*
	S1	*S2*	
1	0.18	0.37	iminodiacetate
2	0.39	0.52	Alizarin Complexan
3	0.98	1.00	alizarin

TABLE II

THE DETERMINATION OF ALIZARIN AND IMINODIACETATE IN ALIZARIN COMPLEXAN

Samples: (I) Hopkin & Williams, Ltd., Great Britain; (II) Siegfried S.A., Zofingue, Switzerland.

Substance determined	*Detection limit*	*Content in the sample (%)*	
	(μg)	*I*	*II*
Alizarin	0.1	1.6	4.2
Iminodiacetate	0.4[a]	3.8	0

[a] For an orange colour on a red background.

substances obtained in the separation of samples. The results are given in Table II, which also includes the detection limits for both substances. The results indicate a new way of washing Alizarin Complexan after it has been synthesized; the sample with a higher content of alizarin contains less iminodiacetate and *vice versa*. The determinations of alizarin and iminodiacetate in freshly prepared and 4-month-old solutions of Alizarin Complexan showed that the content of alizarin increased by 45 %, while that of iminodiacetate only increased by 20 % during this period. This indicates that solutions of Alizarin Complexan may be stored much longer than was previously[2] supposed. The recently observed high stability of lanthanum–Alizarin Complexan reagent in acetone–water media[7] may be due to the stability of the solutions of Alizarin Complexan.

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