Separation of hydroxyanthraquinones by chromatography

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Chromatographic properties of hydroxyanthraquinones have been examined. Good separation was achieved using new solvent systems for paper and thin-layer chromatography on common and impregnated chromatographic support materials. Commercial reagents were analyzed by the newly-developed procedures.

Было изучено хроматографическое поведение гидроксиантрахинонов. Хорошее разделение было достигнуто при использовании предложенных новых хроматографических систем: бумажная хроматография смесью уксусной кислоты и воды на простой бумаге или бумаге импрегнированной оливковым маслом, тонкослойная хроматография на целлюлозе импрегнированной диметилформамидом и на силикагеле без или с импрегнацией щавелевой или борной кислотами.

Anthraquinones constitute an important class of organic substances. They are produced industrially as dyes [1] and occur also in natural products [2]. The fact that some hydroxyanthraquinones react with metal cations to give colour chelates has been utilized in analytical chemistry [3].

Anthraquinone and its derivatives can be determined spectrophotometrically [4—6] and by polarography [4]. The determination of anthraquinones is frequently preceded by a chromatographic separation the purpose of which is to prepare a chemically pure substance.

For chromatographic separation of anthraquinone derivatives common paper [7—9] and paper impregnated with dimethylformamide or 1-bromonaphthalene has been used [10, 11]. Dyes derived from anthraquinone have also been chromatographed on thin layers of cellulose containing 10% of acetylcellulose [12]. Thin-layer chromatography on silica gel has been applied in the separation of dihydroxyanthraquinones [13], di- and trihydroxycarboxylic acids of anthraquinones [14] and anthraquinones occurring in nature [15]. Some substances of this class have been separated by liquid chromatography [16, 17].

The purpose of this work was to improve the existing above-mentioned chromatographic separation methods which were not found quite satisfactory, and to find new separation systems suitable for the chromatographic analysis of analytical reagents of this class.

Experimental

Chemicals

2,3- and 1,4-Dihydroxyanthraquinones were prepared from the corresponding dihydroxybenzene and phthalic anhydride [18]. Other chemicals were commercial products (Lachema, Brno) of reagent grade purity and were used as supplied.

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Paper chromatography was carried out on Whatman No. 1 and 3 chromatographic filter paper. Thin-layer chromatography was carried out on microcrystalline cellulose LT, silica gel L 5/40 (Lachema, Brno) and Silufol® (15 × 15 cm) silica gel coated aluminium foils (Kavalier, Votice).

Instruments and accessories

A commercial Desaga spreader and standard developing tanks were used. Ultraviolet detection was carried out using a Desaga UVIS lamp. Spectrophotometers Cary 118-C (Varian) and VSU 2-P (Zeiss, Jena) were used.

Chromatography

The samples were applied in a benzene—ethanol (1 1) mixture (5 mg/ml) using a 5 µl pipette. The atmosphere of the paper and thin-layer developers was saturated with vapours of the system at 21 ± 1°C for 5 and 1 hrs for paper chromatography and thin-layer chromatography, respectively. Impregnation of Whatman No. 1 paper was carried out by a subsequent immersion in a 5 and 10% (v/v) olive oil solution in toluene with a 10 sec drying following each impregnation. The process was repeated (reversed order of concentrations) and the papers were dried at room temperature for 24 hrs. The separation results using acetic acid—water (3 1) as the mobile phase are given in Table 2. Prior to qualitative analysis Whatman No. 3 paper was rid of metal impurities by elution with acetic acid (24 hrs, descending technique).

The self-made thin layers were prepared by the application of a 0.2 mm thick film of silica gel or cellulose. For this purpose microcrystalline cellulose (15 g) was shaken with water (60 ml) for 1 min and spread onto five glass plates (20 × 20 cm). The cellulose thin layers were impregnated with dimethylformamide in acetone (5 and 10%, v/v). For this purpose dimethylformamide was purified by passing through a column of activated alumina [19]. The systems and the respective results are given in Table 3. The silica gel layers were made in a similar manner from a slurry of silica gel (27.5 g), starch (2.5 g), and water (90 ml) which was shaken for 1 min, heated to 80—90°C for 1 min and poured onto five glass plates (20 × 20 cm). When acid-impregnated silica gel layers were prepared, 0.2 N oxalic or 0.1 N boric acid (60 ml) was used in place of water. Silufol® pre-coated foils were impregnated by their development in the solution of the acid or sodium tetraborate. The mobile phases and the results are summarized in Tables 4 and 5. The detection was achieved by illumination with ultraviolet light ($\lambda_{max} = 254$ nm) and by spraying with 8% potassium hydroxide in methanol [5]. The produced spot colours are given in Table 1.

Results and discussion

Paper chromatography

The separation of anthraquinone and its derivatives on a common chromatographic paper using petroleum ether saturated with methanol [7] or cyclohexane—pyridine (25 1) mixture [20] is not satisfactory when the active substances are to be determined, subsequent to the elution of the substance, by spectrophotometry. Under the conditions described elsewhere [20] 6 µg of a mixture of alizarin and purpurin is the approximate maximum amount which can be applied as a single spot onto Whatman No. 3 paper. Since the compounds of the class under investigation migrate when acetic acid is used as the main component of the mobile phase in the reversed-phase chromatography on nonimpregnated paper [10] (Table 2), systems based on acetic acid were tried.

An important advantage of acetic acid as the mobile phase is the separation capacity. When acetic acid—water (3 1) and Whatman No. 3 paper was used, the spot corresponding to 35 μg of alizarin was still round and the purity of the commercial product could be checked on Whatman No. 3 paper in a similar manner. The identity of the components was confirmed spectrophotometrically [21].

The results of the reversed-phase chromatography using dilute acetic acid as the mobile phase are given in Table 2.

The order of mobility of the substances under investigation on the olive oil-impregnated paper is reversed, when compared to the mobility on nonimpregnated paper. The nonpolar substances (anthracene, phenanthrene) show the smallest R_F values. 1,4-Dihydroxyanthraquinone behaves similarly, which can be explained by the formation of stronger hydrogen bonds compared to those present in 1,2-dihydroxy substances [22].

For practical analyses of anthraquinone and its hydroxy derivatives the use of Whatman No. 1 paper impregnated with 5% olive oil solution in toluene as the stationary and acetic acid—water (3 1) as the mobile phase can be recommended.

Table 1

Colour-forming detection of anthraquinone derivatives

Compound	Ultraviolet detection 254 nm	Detection with 8% KOH
Anthracene	Blue	—
Anthraquinone	Green	—
1,2-Dihydroxyanthraquinone	Brown-red	Blue
2,3-Dihydroxyanthraquinone	Pink	Purple
1,4-Dihydroxyanthraquinone	Pink	Blue
1,2,4-Trihydroxyanthraquinone	Pink	Purple
1,2,5,8-Tetrahydroxyanthraquinone	Brown-red	Blue
1,2,4,5,8-Pentahydroxyanthraquinone	Pink	Pink

Table 2

Separation of anthraquinone derivatives by paper chromatography

Compound	Whatman No. 1	
	nonimpregnated	impregnated with 5% of olive oil
Anthracene	76	—
Phenanthrene	—	—
Anthraquinone	80	—
1,2-Dihydroxyanthraquinone	69	67
2,3-Dihydroxyanthraquinone	60	60
1,4-Dihydroxyanthraquinone	74	53
1,2,4-Trihydroxyanthraquinone	61	60
1,2,5,8-Tetrahydroxyanthraquinone	65	60
1,2,4,5,8-Pentahydroxyanthraquinone	48	51

Table 3

Separation of anthraquinone derivatives by thin-layer chromatography on impregnated cellulose

Compound	hR_F		
	S_1	S_2	S_3
Anthracene	95	79	83
Anthraquinone	88	76	70
1,2-Dihydroxyanthraquinone	5	4	8
2,3-Dihydroxyanthraquinone	0	1	7
1,4-Dihydroxyanthraquinone	88	71	76
1,2,4-Trihydroxyanthraquinone	5	5	7
1,2,5,8-Tetrahydroxyanthraquinone	0	2	12
1,2,4,5,8-Pentahydroxyanthraquinone	—	—	7

S_1 — cellulose (5% dimethylformamide) cyclohexane.
S_2 — cellulose (10% dimethylformamide) cyclohexane.
S_3 — cellulose (10% dimethylformamide) cyclohexane—carbon tetrachloride (9 1).

Table 4

Separation of anthraquinone derivatives by thin-layer chromatography on silica gel

Compound	hR_F					
	S_1		S_2		S_3	S_4
	I	*II*	*I*	*II*	—	—
Anthracene	72	69	74	70	68	78
Anthraquinone	19	20	19	20	0	0
1,2-Dihydroxyanthraquinone	34	32	35	33	43	48
2,3-Dihydroxyanthraquinone	29	27	27	28	43	47
1,4-Dihydroxyanthraquinone	70	66	68	65	67	68
1,2,4-Trihydroxyanthraquinone	30	28	30	30	43	48
1,2,5,8-Tetrahydroxyanthraquinone	34	23	29	21	46	53
1,2,4,5,8-Pentahydroxyanthraquinone	13	10	—	8	31	32
1,2,4,5,6,8-Hexahydroxyanthraquinone	—	—	—	—	26	25

S_1 — benzene—acetone—acetic acid (50 : 5 : 0.8).
S_2 — benzene—acetone—acetic acid (50 : 5 : 0.4).
I — Silufol ®; *II* self-made layers.
S_3 — silica gel (0.2 N oxalic acid) benzene—carbon tetrachloride—acetone (50 : 60 15).
S_4 — silica gel (0.2 N oxalic acid) benzene—acetone (9 1).

Thin-layer chromatography on cellulose

Sharp separation of components have been achieved by thin-layer chromatography of anthraquinone derivatives on microcrystalline cellulose with dimethylformamide and non-polar solvents as the stationary and mobile phase, respectively. A similar system has been used by *Gasparič* [10]. At the applied impregnating concentration (Table 3) distortion of the migration, observed during chromatography on a paper containing higher concentration of dimethylformamide [23], did not occur.

The formation of intramolecular hydrogen bonds between the hydroxyl group and the carbonyl group in the *peri*-position of 1,4-dihydroxyanthraquinone manifested itself, due to the less pronounced interaction of the substance with the stationary phase, by larger R_F values compared to *o*-dihydroxy- and polyhydroxyanthraquinones. The latter substances move at a lower rate and show close R_F values.

Thin-layer chromatography on silica gel

The interaction of the substances with the additives present in a common support material interferes with the chromatography of dihydroxyanthraquinones on silica gel. Starch was therefore used exclusively as the binder. The unwanted effects, resulting in the formation of chelates and bad separation, which occur due to the dissociation of weakly acidic substances,

Table 5

Separation of anthraquinone derivatives by thin-layer chromatography on Silufol® and silica gel impregnated with 0.1 N boric acid

System	Layer	1,2-Dihydroxy-anthraquinone		1,4-Dihydroxy-anthraquinone		1,2,4-Trihydroxy-anthraquinone	
		I	*II*	*I*	*II*	*I*	*II*
n-Butanol with	1	21	11	78	76	23	10
4% ammonia	2	23	6	78	78	27	0
n-Butanol with	1	34	15	81	76	35	13
6% ammonia	2	24	11	82	77	24	9
n-Butanol with	1	28	21	72	65	28	22
10% ammonia	2	20	17	69	64	23	17

I — Silufol®; *II* — self-made layer.
1. Nonimpregnated layer; 2. impregnated layer.

Table 6

The content of alizarin as found in a commercial sample (Lachema, Brno) according to different methods

Support material	Content of alizarin %	s_r %
Whatman No. 3	93	3.4
Whatman No. 3 pre-washed	99	1.7
Silufol®	77	0.88

s_r — relative standard deviation for $n = 3$.

can be largely overcome by an addition of stronger acids either to the mobile or stationary phase (impregnation). Table 4 shows the results obtained during such experiments.

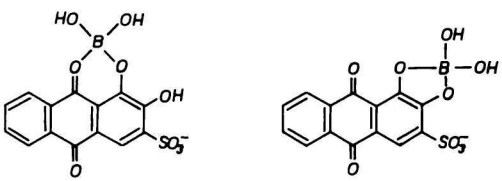
Acetic acid has been most frequently used as an acidic component of mobile phases. Reexamination of the work by *Voyatzákis et al.* [13] showed that the addition of an acid to the neutral organic solvent is necessary; the class of the substances under investigation can, however, be equally well chromatographed using benzene—acetone—acetic acid (50 : 5 : 0.4—0.8), *i.e.* in a system containing ~ 10 times less of the acid than originally suggested [13]. Virtually the same results, as to the relative mobility and resolution, were achieved using self-made and commercial Silufol® thin layers.

Compared to the paper chromatography alizarin produced an additional pink (ultraviolet detection) spot. It can be assumed, in view of the molecular weight of the compound corresponding to this spot determined by gel chromatography, that the substance is 1-hydroxyanthraquinone — an impurity commonly present in alizarin [3]. The isomeric 2-hydroxyanthraquinone having the same molecular weight would show a lower R_F value as it would easier form hydrogen bonds with the silanol hydroxyl groups.

The considerable difference between the R_F of 1,4-dihydroxyanthraquinone and the other polyhydroxyanthraquinones is apparent at the first sight. The mobility of 1,4-dihydroxyanthraquinone is governed by the strong intramolecular hydrogen bond. Anthraquinone itself, bearing free carbonyl groups, is, in the same solvent system, strongly adsorbed by silica gel.

It was suggested by *Stahl* [24] that for the chromatographic separation of acidic substances silica gel impregnated with oxalic acid should be used. The compounds under investigation produce in this way (Table 5) nicely shaped spots while the complex-formation is minimized due to the presence of metalic impurities in the support material.

Identification of polyhydroxy substances having an *o*-dihydroxy arrangement has been carried out by paper chromatography [26] and thin-layer chromatography on silica gel [27] with boric acid as a stationary phase. To verify the possibility of using similar systems for the identification of variously substituted title substances three model compounds bearing different hydroxyl group arrangement, namely alizarin (1,2-), quinazarin (1,4-), and purpurin (1,2,4-) were chromatographed on Silufol® and on self-made silica gel thin layers impregnated with boric acid. The results obtained with the solvent systems based on *n*-butanol containing variable amount of ammonia, found satisfactory in paper chromatography [26], are summarized in Table 5. From a comparison of the R_F values on plain silica gel with those found on silica gel impregnated with boric acid it follows that the addition of the acid results in a considerable and selective retention of the *o*-hydroxy derivatives. The phenomenon can be attributed to the complex formation of the substances with the impregnating agent, since it is known that boric acid forms chelates with polyalcohols and polyphenols. These have been studied by *Havelková* and *Bartušek* [25] who concluded that Alizarin S and boric acid can give two forms of chelates (Scheme 1): In the absence of water



I *II*

Scheme 1

chelate *I* is formed whereas the form *II* is produced in dilute water solutions. The procedure is, however, less suitable for quantitative work as the investigated substances easily undergo oxidation under alkaline conditions.

There is a little difference between the basic mechanism of the separation on impregnated and nonimpregnated silica gel thin layers, as can be seen from a comparison of the R_F values. The polyhydroxyanthraquinones containing the *o*-dihydroxy arrangement show in both instances lower R_F values than does 1,4-dihydroxyanthraquinone, as a result of strong intramolecular hydrogen bonds between the carbonyl and hydroxyl group in the *peri*-position of the latter compound which, consequently, does not form chelates with boric or silicic acid.

Chromatography of alizarin and purpurin combined with the spectrophotometric evaluation after elution

Chromatographically pure alizarin was obtained by several recrystallizations from glacial acetic acid. Molar absorption coefficients found for its ethanol solutions (10—60 μg/ml) agreed well with the literature data [21].

Samples of commercial alizarin (5 μl of a ∼0.4% benzene—ethanol (1 1) solution) were chromatographed on Whatman No. 3 paper with acetic acid—water (3 1) and on Silufol® with benzene—acetone—acetic acid (50 : 5 : 0.8). The zones corresponding to alizarin were cut out, eluted with ethanol and the amount of the substance was determined spectrophotometrically in the usual manner.

It follows from the results in Table 6 that the modification of the paper is an important factor in the quantitative analysis. The lower values observed on Silufol® or unpurified paper can be explained by a nonquantitative migration and/or by elution of alizarin which reacts on the base line as well as during migration with the metal impurities present in the support material. The best results were obtained on the paper pre-washed with acetic acid, which operation removes most of the interfering impurities.

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