CHROM. 3728

Thin-layer chromatography of anthocyanins on mixed layers of polyvinylpyrrolidone and cellulose

Thin-layer chromatography (TLC) on various substrates is increasingly being used to separate anthocyanins. SOMERS[1] used cellulose TLC as a supplement to paper chromatography in separating the anthocyanins of *Vitis vinifera*, variety Shiraz. DEIBNER[2] preferred cellulose TLC to paper chromatography in separating diglucosides of malvidin and peonidin; both systems resolved the two pigments but TLC resulted in a more "distinct" separation. ASEN[3] separated anthocyanins on a preparative scale using plates layered with a mixture of silica gel and cellulose. CONRADIE AND NEETHLING[4] used silica gel TLC for separating anthocyanins of *Vitis vinifera* as did MORTON[5] for blackcurrant anthocyanins. QUARMBY[6] chromatographed anthocyanidins and other flavonoids and phenolics on polyvinylpyrrolidone (PVP) thin-layer plates. BIRKOFER *et al.*[7,8] have separated anthocyanins on polyacrylonitrile–polyamide and on alumina.

In this communication, TLC of anthocyanins on layers of PVP–cellulose mixtures is described. Incorporation of PVP in the cellulose layer resulted in more compact spots without markedly affecting the R_F value.

Experimental

Preparation of plates

Cellulose. Cellulose plates of 0.25 mm thickness were prepared by spreading a homogenate of 20 g of cellulose powder (MN-300, without binder) and 110 ml of distilled water on five 20 × 20 cm plates. The coated plates after setting at room temperature for 1–4 h were dried at 100° for 30 min and stored in a desiccator.

Cellulose–PVP. Plates of mixtures of PVP and cellulose were prepared in the same manner using the following proportions of PVP to cellulose: 1:19 (5%), 1:9 (10%), and 1:3 (25%). Insoluble PVP (polyclar AT) obtained from General Aniline and Film Corp., New York, was purified as described by LOOMIS AND BATTAILE[9] to remove trace amounts of hydrogen peroxide. Particle size of 100–150 mesh was used.

PVP. 100% PVP plates were prepared as recommended by QUARMBY[6] except that soluble PVP (Plasdone K29–32, General Aniline and Film Corp.) rather than polyvinyl alcohol was used as a binding agent.

Silica gel. Plates were prepared as described by MORTON[5].

Polyamide. The procedure of DAVÍDEK AND DAVÍDKOVÁ[10] was followed in preparing plates.

Sources of anthocyanins

Purified anthocyanin extracts of rhubarb (cyanidin-3-rutinoside, cyanidin-3-glucoside—WROLSTAD AND HEATHERBELL[11]), Bing cherries (cyanidin-3-rutinoside, cyanidin-3-glucoside—LYNN AND LUH[12]), red raspberries (cyanidin glycosides—DARAVINGAS AND CAIN[13]), strawberries (pelargonidin-3-glucoside and cyanidin-3-glucoside—ROBINSON AND ROBINSON[14] and LUKTON, CHICHESTER AND MACKINNEY[15]) and strawberry anthocyanidins (pelargonidin chloride and cyanidin chloride) were chromatographed. Anthocyanins were dissolved in 0.01% methanolic HCl and the aglycones were isoamyl alcohol solutions. Sample size was 1 μl.

Chromatography

Plates were developed in AWHCl, glacial acetic acid–water–conc. hydrochloric acid (15:82:3), and BAW 415, less-dense phase of *n*-butanol–glacial acetic acid–water (4:1:5). Silica gel plates were also developed in BEBF, *n*-butanol–ethyl acetate–benzene–formic acid (1:1:1:1, saturated with solid paraformaldehyde), polyamide plates in 80 % methanol and 100 % PVP plates in Forestal, glacial acetic acid–water–conc. hydrochloric acid (30:10:3). Development was carried out at 21 ± 1° in the dark.

Results and discussion

In our laboratory cellulose has been used in preference to other substrates for TLC of anthocyanins. Cellulose TLC plates are not as fragile as silica gel or polyamide. Also, satisfactory separations on cellulose TLC can generally be achieved using the same solvent systems one would use for paper chromatography; this is a considerable advantage when one considers the wealth of data published on paper chromatography of anthocyanins. Paper chromatography is preferred by many for preparative work, and, since it is possible to use the same solvent systems, is very convenient.

The R_F value of some anthocyanin pigments separated on cellulose and PVP–cellulose plates with BAW 415 and AWHCl are shown in Table I. PVP affected the R_F values to a small degree in the polar solvent system while in BAW 415 the effect was more profound. Incorporation of PVP into the cellulose plate resulted in a reduction of spot size. After development in AWHCl the area of the pelargonidin-3-glucoside spot measured approximately 50 mm² on cellulose and 5 % PVP–cellulose plates and 30 mm² on 10 and 25 % PVP–cellulose plates. The smaller spot size is

TABLE I

R_F VALUES OF ANTHOCYANIN PIGMENTS SEPARATED BY CHROMATOGRAPHY ON CELLULOSE AND CELLULOSE–POLYVINYLPYRROLIDONE THIN LAYERS

Pigment	*Plate composition*	*R_F values*	
		AWHCl	*BAW 415*
Cyanidin-3-glucoside	Cellulose	0.30	0.34
	5 % PVP–cellulose	0.31	0.22
	10 % PVP–cellulose	0.29	0.20
	25 % PVP–cellulose	0.28	0.21
Cyanidin-3-rutinoside	Cellulose	0.50	0.26
	5 % PVP–cellulose	0.50	0.09
	10 % PVP–cellulose	0.47	0.11
	25 % PVP–cellulose	0.44	0.11
Pelargonidin-3-glucoside	Cellulose	0.43	0.52
	5 % PVP–cellulose	0.44	0.36
	10 % PVP–cellulose	0.42	0.28
	25 % PVP–cellulose	0.37	0.20
Pelargonidin	Cellulose	0.18	
	5 % PVP–cellulose	0.17	
	10 % PVP–cellulose	0.11	
	25 % PVP–cellulose	0.08	

particularly advantageous for two-dimensional chromatography and for densitometry work. To achieve compact spot size without markedly affecting the R_F values the 10% PVP–cellulose mixture is recommended.

The polyamide, PVP, and silica gel TLC plates were unsatisfactory with the BAW 415 and AWHCl solvent. The anthocyanins streaked severely on the polyamide plates; the 80% methanol system was more satisfactory, however, resolution was not as good as with cellulose and PVP–cellulose systems. In the silica gel–AWHCl system the pigments moved with the solvent front and in BAW 415 they were not separated. The BEBF system while not completely resolving the pigments gave a more reasonable chromatogram.

We were unsuccessful with 100% PVP plates using all three systems. The solvent front was irregular; the pigments were not separated and spread in an irregular manner during development.

Acknowledgements

The suggestions of Mrs. TERYL PUTNAM and the technical assistance of Miss KATHY JOHNSTON are appreciated.

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1 T. C. SOMERS, *J. Sci. Food Agr.*, 17 (1966) 215.
2 L. DEIBNER, *J. Chromatog.*, 34 (1968) 425.
3 S. ASEN, *J. Chromatog.*, 18 (1965) 602.
4 J. D. CONRADIE AND L. P. NEETHLING, *J. Chromatog.*, 34 (1968) 419.
5 A. D. MORTON, *J. Chromatog.*, 28 (1967) 480.
6 C. QUARMBY, *J. Chromatog.*, 34 (1968) 52.
7 L. BIRKOFER, C. KAISER, H. A. MEYER-STOLL AND F. SUPPAN, *Z. Naturforsch.*, 176 (1962) 352; *C.A.*, 57 (1962) 52856.
8 L. BIRKOFER, C. KAISER AND M. DONIKE, *J. Chromatog.*, 22 (1966) 303.
9 W. D. LOOMIS AND J. BATTAILE, *Phytochem.*, 5 (1966) 423.
10 J. DAVÍDEK AND E. DAVÍDKOVÁ, J. *Chromatog.*, 26 (1967) 529.
11 R. E. WROLSTAD AND D. A. HEATHERBELL, *J. Food Sci.*, in press.
12 D. R. C. LYNN AND B. S. LUH, *J. Food Sci.*, 29 (1964) 735.
13 G. DARAVINGAS AND R. F. CAIN, *J. Food Sci.*, 30 (1965) 400.
14 G. M. ROBINSON AND R. ROBINSON, *Biochem. J.*, 26 (1932) 1650.
15 A. LUKTON, C. O. CHICHESTER AND G. MACKINNEY, *Nature*, 176 (1955) 790.

Received August 5th, 1968