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Thin-layer Chromatography of Plant Extracts

I. A Solvent Series Based on Dielectric Constant

ESKIL HULTIN*

Department of Pharmacology, University of California Medical Center, Los Angeles, Calif. 90024, U.S.A.

Solvent mixtures previously used for developing thin-layer chromatograms of particular classes of chemical compounds have now been tested for other compounds and other plant material fractions than those they were originally intended for. Some were selected and arranged in order of increasing dielectric constant on a logarithmic scale for general use in the thin-layer chromatographic analysis of preparations containing substances of corresponding polarity without regard to chemical composition.

A new solvent mixture with dielectric constant about 20, differing substantially in acidity from previously used mixtures with dielectric constant above 15, has been developed. Also, a new series of solvent mixtures with dielectric constant below 2.7, containing *t*-amyl alcohol, has been studied.

The aim of this series of investigations is to explore thin-layer chromatographic separation of substances in plant extracts in connection with a program of screening plants pharmacologically for substances, of any chemical composition, which may prove to have strong specific effects upon living organisms.

A goal for the chemical analysis in such a general screening program is the isolation of new substances which should be further tested pharmacologically, whereas commonly used chemical methods for the analysis of plants are predominantly intended for the isolation of and the assay of particular chemical substances or different classes of chemical substances.[1]

Separation work on chromatographic columns and by thin-layer chromatography has so far usually been carried out in order to separate the components of such mixtures as are obtained by isolating some specific class of chemical compounds. A list of suitable adsorbents and solvent mixtures for the column chromatographic fractionation of substances in each of various groups of

* On leave of absence from University of Stockholm, Sweden.

chemical compounds was published in a review article by Cassidy [2] and in a recent handbook edited by Lederer.[3] Methods for thin-layer chromatographic analysis, including suggestions of adsorbents and solvent mixtures for the separation of similar substances, for each of various chemical classes of compounds, have been worked out and have been collected particularly by Stahl.[4]

Thin-layer chromatographic procedures give a relatively good resolution of multicomponent mixtures, but mixtures which include very lipophilic and very hydrophilic substances, as, *e.g.*, unfractionated alcoholic plant extracts usually do, cannot be resolved completely in one step with any solvent mixture used. Some components would remain at the starting point and some would, for most developing solvents, move with the solvent front. Therefore, if a wide range of substances from plants are to be surveyed by thin-layer chromatography, it is important first to divide the extract into a series of fractions with different polar properties.

For a thin-layer chromatographic analysis of such fractions of plant material a series of solvents selected to be used for specific classes of chemical substances would not be so generally useful as a series of solvent mixtures prepared to be used for extract fractions with different polar properties.

The polar properties of substances can hardly be defined strictly. Dipole moment, acidity, hydrogen bonding and dielectric constant have a great influence on the interaction of solvents with solutes. Hence, solvents do not come in exact the same order in Trappe's [5] eluotropic series and in Hecker's [6] mixotropic series. However, for practical purposes the dielectric constant is convenient for arranging solvents and solvent mixtures in a series as to their polar properties.

A large number of solvent mixtures [4,7-10] were tested for the development of thin-layer chromatograms of various fractionated plant extracts. Those solvents which proved worthy of consideration for routine use are listed in Table 1 in order of increasing dielectric constant on a logarithmic scale. The dielectric constants of the solvent mixtures were computed as the weighted mean values of the dielectric constants for their components.

EXPERIMENTAL

Material. Green parts of the following plant species, and the following substances of plant origin were used in this work: *Mortonia scabrella* A. Grey (Celastraceae) and *Feijoa sellowiana* Berg (Myrtaceæ); Senna leaves (*Cassia* sp. (Leguminosæ)), obtained from Horton & Converse, Los Angeles, Calif.; and commercial preparations of digitoxin, oleandrin, quercitrin, rhamnetin, and rutin. Silica Gel G was the only adsorbent included in this work on routine methods, as the use of this adsorbent is very predominant.

New solvent mixtures for developing thin-layer chromatograms. Three new solvent mixtures were developed during this work, and they are included in Table 1. The solvent mixture consisting of chloroform, tetrahydrofuran, tertiary butanol and water (10:6:50:14 v/v) is particularly worth mentioning, as this is a solvent mixture which in acidity differs substantially from other solvent mixtures with the dielectric constant above 15, used in thin-layer chromatography. A new possibility to develop solvent mixtures with very low dielectric constant for thin-layer chromatography of lipophilic substances was noticed during this work. Tertiary amyl alcohol, the dielectric constant of which is as small as 5.82, has proved useful for preparing solvent mixtures with a dielectric constant less than about 2.7 and still with a relatively high concentration of hydroxyl groups, without carboxylic acids added.

Table 1. A selection of solvents and solvent mixtures for thin-layer chromatography listed in order of increasing dielectric constant ε on a logarithmic scale. For mixtures, volumes of the components are given, and also page references to Stahl's monograph.[4] New solvent mixtures suggested here are designated by an asterisk *. Abbreviations: Acone acetone, Bzne benzene, BuOH butanol, Buone butanone, Chlf chloroform, cHxne cyclohexane, EtOAc ethyl acetate, EtEth ethyl ether, EtOFo ethyl formate, EtOH ethanol, HOAc acetic acid, HCOOH formic acid, iPrEth isopropyl ether, iPrOH isopropanol, MeOH methanol, Pene pentane, tPeOH tertiary amyl alcohol, tBuOH *t*-butanol, THF tetrahydrofuran, Tlne toluene, w water: S & S Stahl and Schorn.

	ε	ε	Solvent mixtures for thin-layer chromatography
Pene	1.8		
cHxne	2.0	2	95 Pene + 5 EtEth (154)
Bzne	2.3		90 Pene + 10 EtOAc *
Tlne	2.4		85 Pene + 15 tPeOH *
			70 Pene + 30 5EtEth + 2 HOAc (154)
		3	
			95 Bzne + 5 MeOH (201, 320)
iPrEth	3.9	4	75 Bzne + 24 EtOFo + 1 HCOOH (388) (S & S IV)
EtEth	4.2		
Chlf	4.7		90 Chlf + 10 HOAc (271, 280)
		5	
tPeOH	5.8		
EtOAc	6.0	6	10 Chlf + 90 EtOAc (283) (Görlich I)
HOAc	6.2		
EtOFo	7.2		
THF	7.6		
		8	75 iPrEth + 25 Acone (282) (Tschesche *et al.*[7])
		10	50 Tlne + 40 EtOFo + 10 HCOOH (388) (S & S III)
tBuOH	11.7		50 Chlf + 40 EtOAc + 10 HCOOH (388) (S & S II)
		15	
BuOH	17.1		
iPrOH	18.1		(Görlich II)
Buone	18.5	20	10 Tlne + 2 HOAc + 80 Buone + 5 MeOH + 6 w (283)
Acone	20.7		10 Chlf + 6 THF + 50 tBuOH + 14 w *
			50 EtOAc + 30 Buone + 10 HCOOH + 10 w (388) (S & S V)
EtOH	24.3		
			10 EtOAc + 2 HCOOH + 3 w (Hänsel & Hörhammer [8])
		30	15 HOAc + 60 BuOH + 25 w (Partridge & Westall [9]; Smith [10])
MeOH	32.6		70 iPrOH + 6 HCOOH + 24 w (400)
		40	
HCOOH	58.5	60	
w	78.5	80	

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