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Synergistic complexation effects in the thin-layer chromatography of certain triglycerides on silica impregnated with silver nitrate

The co-ordination of olefins with certain metal ions and with silver ions in particular has long been recognised and studied. Using a partition method WINSTEIN AND LUCAS[1] showed that the complexation of silver with olefins and acetylenes was both reversible and rapidly attained. The nature and physical chemistry, including the correlation with bond order, steric hindrance and electron releasing effects have been studied by FUENO et al[2], TRAYNHAM AND SCHNERT[3] and by GARDNER et al.[4]. NICHOLS[5] first proposed that the distribution between a hydrocarbon solvent and a methanolic silver nitrate solution might be used to separate cis–trans isomers. This was later confirmed experimentally for fatty acid esters by SCHOFIELD et al.[6]. The order of stability of silver complexes according to KIRCHER[7] is cyclopropenes > cis-olefins > alkynes > trans-olefins > allenes. This variation in stability constants forms the basis of chromatographic separation of unsaturated lipid materials. The chromatographic separation of lipids using argentation has been reviewed in detail by MORRIS[8] and only the main points will be mentioned herein.

MORRIS[9] and DE VRIES[10] reported simultaneously that adsorbents impregnated with silver nitrate separate lipids according to the degree and geometry of their unsaturation, using thin-layer chromatography and column chromatography. At the same time BARRETT et al.[11] reported the separations of glycerides on thin layers impregnated with silver nitrate. Further work has been described by DE VRIES AND JURRIENS[12], GUNSTONE AND PADLEY[13] and by DEN BOER[14]. The order of separation of triglycerides in benzene–ether mixtures was reported by GUNSTONE AND PADLEY[13] as 000, 001, 011 and 002, 111, 012, 112, 022, 003, 122, 013, 222, 113, 023, 123, 223, 033, 133, 233 and 333 where the numbers refer to the numbers of cis double bonds in the fatty acid chain. The separations are complicated where there are isomers present, R_F values being a function of the number of double bonds as well as the stereochemistry. DE VRIES[15] has shown that trans double bonds give rise to significantly higher R_F values than cis in triglycerides, MORRIS[8] has reported on the effect of the position of the double bond in the fatty acid chain and RENKONEN AND RIKKINEN[16] on the effects of the position of the unsaturated fatty acid in the triglyceride. In addition to the molecular solute variables indicated above there are variables due to the chromatographic conditions. DALLAS[17] has discussed the general factors which affect R_F values in adsorption TLC and STEIN AND SLAWSON[18] silver nitrate impregnated chromatography in particular.

JONES[19] has described how certain olefin hydrocarbon such as cyclooctene complex very strongly with silver ions. The present communication considers the synergistic complexation of silver with benzene and cyclooctene and cyclohexene and subsequent reduction of retention of certain triglycerides on silver nitrate impregnated silica thin-layer plates using benzene–cyclic olefin eluants. The effects are related to those described by FEATHERSTONE AND SORRIE[20] on the solubility of straight chain olefins–silver fluoroborate solution in benzene. The effects of increase in chain length in the linear and cyclic olefins are similar.

Experimental

Triglycerides: tristearin, trielaidin, triolein, trilinolein and trilinolenin all being of 95–100% purity. These were made up as 0.5% w/v solutions in chloroform with 0.005% BHT as antioxidant and stored at 4°.

Solvents: cyclooctene (cis[21]), cyclohexene and benzene, all of Analytical Reagent grade.

TLC materials: Silica Gel G (Merck); silver nitrate A. R.

Spray reagent: dichlorofluorescein, 0.01% in methanol.

Plates: 10 × 2.5 cm.

Procedure

The thin layers of impregnated Silica Gel G were prepared following the technique of BARRETT et al.[11] slurrying the silica gel (30 g) with 60 ml of a 12.5% w/v solution of silver nitrate, giving a layer 300 μ thick with 20% w/w $AgNO_3$. The resulting plates were activated by heating at 110° for 45 min and then stored over silica gel, in the dark, until required. The triglyceride solutions were applied in 1 μl aliquots, which was found to be the optimum loading and the chromatogram developed at 20° in the appropriate solvent mixture. The walls of the tank were lined with filter paper to provide an atmosphere saturated with solvent, to improve reproducibility. The chromatograms were dried at 60°, in a current of air, taking care to remove all the solvent; cyclooctene being particularly difficult to dry, taking about 10 min for complete removal of the solvent. The chromatograms were visualised by spraying with dichlorofluorescein reagent and examination under 365 nm UV light.

The results obtained are reproducible if determinations are carried out using the same batch of plates, there are some batch variations which give rise to differences in R_F values.

Results

All the results given in the tables, if expressed graphically, produce smooth curves.

Discussion

The results reported in Tables I and II show that little or no movement of certain triglycerides takes place in the pure cyclic olefins or in pure benzene, whilst higher R_F values are obtained in mixtures of the two. This indicates that synergistic

TABLE I

R_F VALUES USING THE CYCLOOCTENE–BENZENE SOLVENT SYSTEM AT 20°
Values are the mean of 3 determinations.

Compound	% benzene												
	0	2	5	10	15	40	50	60	70	80	90	95	100
Tristearin	0	0.80	0.82	0.87	0.87	0.86	0.90	0.88	0.90	0.79	0.75	0.71	0.64
Trielaidin	0	0.78	0.84	0.94	0.93	0.92	0.87	0.91	0.92	0.75	0.64	0.59	0.31
Triolein	0	0.82	0.85	0.93	0.92	0.90	0.92	0.90	0.91	0.74	0.64	0.57	0.11
Trilinolein	0	0.85	0.81	0.80	0.29	0.04	0.04	0.05	0.05	0.04	0.05	0.07	0
Trilinolenin	0	0.86	0.84	0.81	0.64	0	0	0	0	0	0	0	0

TABLE II

R_F VALUES USING THE CYCLOHEXENE–BENZENE SOLVENT SYSTEM AT 20°
Values are the mean of 3 determinations.

Compound	% benzene												
	0	2	5	10	15	40	50	60	70	80	90	95	100
Tristearin	0.12	0.16	0.40	0.59	0.71	0.98	0.98	0.84	0.82	0.79	0.71	0.70	0.64
Trielaidin	0.18	0.29	0.20	0.28	0.44	0.76	0.74	0.64	0.60	0.54	0.50	0.40	0.31
Triolein	0.06	0.23	0.20	0.24	0.32	0.69	0.69	0.64	0.56	0.50	0.36	0.30	0.11
Trilinolein	0.05	0.15	0.21	0.31	0.30	0.33	0.35	0.29	0.09	0.04	0.06	0.06	0
Trilinolenin	0.06	0.12	0.16	0.16	0.18	0.10	0.09	0.07	0.07	0.04	0.05	0.05	0

TABLE III

EFFECT OF THE NUMBER OF DOUBLE BONDS UPON THE R_F VALUES
5 % benzene in cyclohexene, 20°; results obtained on a single plate.

Compound	Number of double bonds	R_F value
Tristearin	0	0.350
Trielaidin	3 (trans)	0.215
Triolein	3 (cis)	0.200
Trilinolein	6	0.185
Trilinolenin	9	0.140

TABLE IV

TIME (min) FOR THE SOLVENT MIXTURE TO RUN 8 cm

Mixture	% benzene											
	0	2	5	10	15	40	50	60	70	80	90	100
Cyclooctene	35	34.5	35	34.5	31	21.5	20	16.5	16.0	15	12	10.5
Cyclohexene	19	15	10.5	11	10.5	10.5	10.5	10.5	10.5	11.0	10.0	10.5

complexation of the silver in the thin-layer plates with benzene and cyclic olefin is taking place, thus releasing the triglycerides into the mobile phase. Similar synergistic effects have been reported earlier in a study of the solubility of straight chain olefins in aqueous silver fluoroborate in the presence of aromatic hydrocarbons by FEATHERSTONE AND SORRIE[20]. The synergistic effect was found to be larger the longer the chain length of the olefin. In the present work a similar effect is found with cyclic olefins as R_F values are considerably higher in cyclooctene–benzene mixtures than in cyclohexene–benzene mixtures. A linear relationship was observed between the number of double bonds present in the triglycerides studied and R_F values as illustrated by the results recorded in Table III.

At the loading used very little tailing was observed and satisfactory separations of the triglycerides were obtained using the appropriate mixture of solvents, from the

tables, for the compounds under examination. Whilst the systems employed do not give a separation of the triglycerides as great as that obtained by BARRETT et al.[11] using chloroform (99.5) and acetic acid (0.5) it does provide some information on the nature and number of double bonds present in triglycerides.

The effect of temperature between 4 and 20° upon the R_F values was examined and found to be slight, the major effect being an increase of running from 20 to 45 min at the lower temperature. The running time was also dependent on the solvent composition (Table IV); this effect is under further investigation.

The control of R_F values using the synergistic complexation effect would appear to allow the development of novel separations of lipids by argentation chromatography.

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