die Peaks schon zu eng zusammenrücken. Die Trennungen an dem gelartigen Lewatit® S 1080 verlaufen zwar ähnlich, aber mit geringerer Auflösung und grösseren Elutionsvolumina.

Bei den basischen Ionenaustauschern machten sich in den chromatographischen Trenneigenschaften die Unterschiede zwischen makroporösen und gelartigen Ionenaustauschern noch deutlicher bemerkbar. Nach Fig. 2 werden *p*-Aminobenzoesäure, Salicylsäure und Gallussäure an dem makroporösen Lewatit® MP 5080 bei 38° am besten und bei den anderen Temperaturen hinreichend getrennt. An dem gelartigen Lewatit® M 5080 trennen sich *p*-Aminobenzoesäure und Salicylsäure nur bei 50° andeutungsweise auf. Gallussäure lässt sich bei allen Temperaturen abtrennen, wobei sich allerdings der Peak mit abnehmender Temperatur stark verbreitert. Allgemein verringern sich auch bei diesem Trennbeispiel die Elutionsvolumina mit steigender Temperatur.

Die Ursache für die besseren Trennungen an makroporösen Ionenaustauschern kann nur in der grösseren Porenweite und dem starreren Aufbau der Matrix liegen, wodurch bei den Austauschvorgängen die Gel-Kinetik zugunsten der Film-Kinetik zurückgedrängt wird. Weiterhin ist anzunehmen, dass bedingt durch die grosse Oberfläche an makroporösen Ionenaustauschern zusätzliche Verteilungsvorgänge eher auftreten als an gelartigen Ionenaustauschern.

Grössere Trennschärfe hat man bisher durch Verringerung der Korngrösse von gelartigen Ionenaustauschern bis herunter auf *ca.* 1 μ erreicht, wobei man einen grösseren Druckabfall in der Säule und damit Chromatographie unter Druckanwendung in Kauf nehmen musste. Makroporöse Ionenaustauscher eröffnen nach den hier vorliegenden Ergebnissen die Möglichkeit, dass gute Trennungen unter einfachen apparativen Bedingungen schon an Harzen mit Durchmessern von 100–300 μ zu erreichen sind.

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Detection and estimation of cyclamate by thin-layer chromatography

With the rapid growth in the consumption of low-calorie and dietetic foods, a great deal of attention is being focussed on non-nutrition sweeteners. While a number of artificial sweeteners have been identified and developed, cyclamates and saccharin are the main sweeteners of commercial importance.

Chemically cyclamate is the sodium or potassium salt of cyclohexyl sulfamic acid which is susceptible to bromination due to its unsaturation[1-3]. KORBELAK[4] has separated and identified saccharin, cyclamates, dulcin and P-4000 on silica gel by thin-layer chromatography (TLC). KOJIMA[5] employed a $AgNO_3$–hydroquinone reagent for the identification of the salts of saccharin and cyclamate and used many solvent systems for their separation. SALO[6] used a mixture of acetylated cellulose and polyamide as the adsorbent for the separation of sweeteners and identified them under UV light. WOIDICH *et al.*[7] have separated saccharin and cyclamate on acetylated cellulose–polyamide adsorbent and employed dichlorofluorescein as the spray reagent before visualisation under UV light.

In our method, Silica Gel G is used as the adsorbent and fluorescein solution as the spray reagent after bromination. The thin-layer plates coated with Silica Gel G and containing cyclamate are subjected to bromine vapour when bromination takes place. The plate is then sprayed with 0.05% fluorescein solution when eosin is formed; unchanged fluorescein only remains on those parts of the layer where there are other compounds which can be brominated. In visible light, pink spots are seen on a yellow background. Detection by UV light is even more sensitive, the background fluorescence is quenched, and only the zones where there are cyclamates glow yellow green. Instead of the above procedure the cyclamate spots can also be developed on 0.04% sodium fluorescein impregnated Silica Gel G layers and then subsequently detected by bromination. The sensitivity is a little bit higher in this case.

The pink spots can be extracted with ethanol and the fluorescence measured quantitatively with a spectrophotometer or fluorimeter.

Experimental

Extraction of cyclamate from the sample. 50 ml of sample is acidified with 10 ml HCl and extracted with two 25 ml portions of ether. The combined ether extract is washed three times with 5 ml water. The ether solution is concentrated and made up to a volume of 10 ml.

If the sample is solid or semisolid 10 g of the sample is blended with 50 ml water, 10 ml HCl is added and the above procedure is then followed.

Preparation of the thin-layer plates. 10 × 20 cm glass plates are coated with a slurry of Silica Gel G in water (1:2) to a thickness of 250 nm and dried at room temperature for ½ h. The plates are activated at 110° for 1 h in an air oven and then in a desiccator.

Solvent system. Ethyl acetate–isopropyl alcohol–acetone–methanol–water (50:15:15:4:16).

Spray reagent. 0.05 g sodium fluorescein is dissolved in 10 ml absolute alcohol.

Preparation of standard cyclamate solution. 0.1 g of sodium cyclamate is dissolved in 100 ml of distilled water. 10 μl of this solution is equivalent to 10 μg of cyclamate.

Spotting and development. The Silica Gel G plates are spotted with 20 μl of the ethereal extract of the cyclamate along with standard cyclamate solutions containing 10, 20, 30, 40 and 50 μg of cyclamate leaving 1 cm from the base line. The plates are developed in the ascending manner with the mobile solvent mentioned above. When the solvent front reaches 10 cm from the starting point, the plate is removed from the solvent and dried in air for ½ h.

Detection of spots. Bromine vapour is applied to the plate by allowing bromine vapour from a litre flask containing one or two drops of bromine to pour over the surface; excess bromine should be avoided. The plate is kept at room temperature for 5 min to remove excess bromine and then sprayed with the 0.05% alcoholic fluorescein solution when pink spots on yellow background become visible (R_F = 0.56).

Quantitative analysis. The portions of the silica gel containing the pink spots are carefully scraped off with the sharp end of a knife and placed in test tubes. 10 ml of ethyl alcohol is added to each test tube and filtered. The filtrate is collected in 25 ml volumetric flasks. The residue is washed with ethanol and made up to volume with ethanol. The fluorescence is measured in a spectrophotometer (Zeiss type) at a wavelength of 540 mμ (Table I).

TABLE I

SPECTROPHOTOMETER READINGS AT 540 mμ

The unknown is calculated from the equation $y = -0.6141 + 1236.86x$.

Concentration of cyclamate (y) (μg)	*Optical density mean (x)*	*Standard deviation*	*C. V. (%)*
10	0.010	0.0014	14.0
20	0.016	0.0022	13.8
30	0.023	0.0038	16.5
40	0.034	0.0068	20.0
50	0.041	0.0094	22.0

The plot of concentration of cyclamate (in μg) is plotted against optical density and the unknown concentration of cyclamate is read by intrapolation from the standard line.

Conclusions

With the widespread use of cyclamate as an artificial sweetener in dietetic candies, jellies, canned fruits and particularly in carbonated beverages, a rapid and sensitive method is needed for its detection and quantitative estimation. The solvent system used here has the advantage that it separates cyclamate from other sweetening agents *viz.* sucrose (R_F = 0.25) and saccharin (R_F = 0.19).

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