capable of being converted to HMF produced a positive TBA reaction for spray-processed and conventionally processed lactose. This is evident by the total HMF content of up to 16.39 mg./Kg. lactose for conventionally prepared and up to 41.52 mg./Kg. of lactose for spray-processed lactose. A definitive pattern of increase in total HMF with storage time for spray-processed lactose appears to exist, but no such relationship exists for conventionally processed lactose.

The data in these tables attempt to relate total HMF concentration to color development measured by reflectance readings for both conventionally and spray-processed lactose stored at ambient conditions. It is evident from these data that total HMF does not appear to be the criterion for color development since samples of conventionally processed lactose have considerably higher total HMF values than spray-processed lactose without affecting the color of the lactose. It would appear from these results that it is the concentration of free HMF in the samples that is related to color change, since none of the samples of conventionally processed lactose contained any detectable free HMF, while the spray-processed lactose samples contained varying amounts of free HMF.

It appears from these data that the presence of free HMF is an indication that the complex Maillard-type reaction has begun, and browning will take place once the concentration of HMF reaches a critical value. The data obtained by other investigators appear to substantiate this reasoning. Keeney and Bassette (14) made the general observation that once HMF in dry milk increased to a certain level, flavors associated with color change develop. Craig (12) reported that a quantitative relationship existed between storage stability and initial HMF concentration for vacuum foam-dried whole milk. Newth (16) also reported such a relationship for glucose solutions.

To evaluate the effect of the presence of HMF precursors in conventionally and spray-dried processed lactose on color development, samples of both sugars were heated at 80° for 24 hours. At the end of 24 hours, the total HMF present in conventionally processed lactose was approximately equal to that determined initially. However, in spray-dried lactose an increase in free and total HMF took place as illustrated in Table III. In addition, these samples exhibited a significant degree of darkening, evidenced by the decrease in reflectance values. The data presented in this table illustrate that, as HMF is formed, a concurrent production of coloring matter results.

In an attempt to determine whether the process of spray drying influences the HMF content of lactose, a sample of conventionally processed lactose, containing no free HMF and 13.42 mg./Kg. of total HMF, was spray dried. Testing of this spray-dried material for 1 year at ambient conditions gave no darkening. Spray drying a slurry containing 50% lactose, 5% dextrose U.S.P., and 5% galactose also showed no increase in total HMF after drying and no darkening after storage for 1 year.

These preliminary experiments indicate that the physical operations of spray drying and the addition of the common hydrolysis products of lactose give no additional quantities of HMF. It would appear that the simple hydrolysis products of lactose are not the major precursors of HMF in spray-processed lactose.

SUMMARY

The results obtained in this investigation implicate the presence of free HMF in lactose with resultant browning. Samples of conventionally processed lactose containing no free HMF and heated for 24 hours at 80° and stored at ambient conditions for 36 months showed no darkening. On the other hand, samples of spray-processed lactose, treated similarly, containing free HMF, darkened significantly.

The TBA reaction provides a suitable quantitative method for determining free HMF and should serve as a suitable technique for the quality control of lactose relative to browning possibilities.

REFERENCES

(1) Hodge, J. E., *Agr. Food Chem.*, 1, 928(1953).
(2) Patton, S., *ibid.*, 6, 132(1958).
(3) Ellis, G. P., *Advan. Carbohydrate Chem.*, 14, 63(1959).
(4) Dutra, R. C., Tarassuk, N. P., and Kleiber, M., *J. Dairy Sci.*, 41 1017(1958).
(5) Patton, S., *ibid.*, 38, 457(1955).
(6) Coulter, S. T., Jenness, R., and Geddes, W. F., *Advan. Food Res.*, 3, 45(1951).
(7) Danehy, J. P., and Pigman, W. W., *ibid.*, 3, 241 (1951).
(8) Aurand, L. W., Woods, A. E., and Roberts, W. M., *J. Dairy Sci.*, 42, 961(1959).
(9) Richards, E. L., and Chandrasekhara, M. R., *J. Dairy Res.*, 27, 59(1960).
(10) Aceto, N. C., Sinnamon, H. I., Schoppet, E. F., and Eskew, R. K., *J. Dairy Sci.*, 45, 501(1962).
(11) Adachi, S., and Patton, S., *ibid.*, 44, 1375(1961).
(12) Craig, J. C., Aceto, N. C., and Della Monica, E. S., *ibid.*, 44, 1827(1961).
(13) Keeney, M., and Bassette, R., *Science*, 126, 511(1957).
(14) Keeney, M., and Bassette, R., *J. Dairy Sci.*, 42, 945(1959).
(15) Keeney, M., *Proc. Flavor Chem. Symp.*, 1961, 183.
(16) Newth, F. H., *Advan. Carbohydrate Chem.*, 6, 83(1951)

Centrifugally Accelerated Thin-Layer Chromatography

By B. P. KORZUN and S. BRODY

Centrifugal force has been used to accelerate the development of thin-layer chromatograms and the results compared with data on the standard ascending development technique.

CENTRIFUGAL force has been employed to accelerate paper chromatographic separation of compounds in mixtures (1–4). This method was applied to thin-layer chromatography on circular glass plates or aluminum plates. Comparisons were made between standard thin-layer and accelerated thin-layer chromatography. The accelerated method was completed in about 10 minutes, compared to the 30 to 40 minutes required for the standard ascending method.

Circular glass or aluminum plates 26 cm. in diam-

Received July 17, 1963, from the Ciba Pharmaceutical Co., Summit, N. J.
Accepted for publication August 7, 1963.

TABLE I.—R_f VALUES AND TIME OBTAINED FOR SEPARATION OF DYES USING THE STANDARD AND ACCELERATED METHODS OF THIN-LAYER CHROMATOGRAPHY

Dyes	R_f Value—Accelerated	R_f Value—Standard
Light green SF yellowish	0.00	0.00
Sudan Black B	0.10	0.15
Indophenol blue	0.25	0.35
Sudan R	0.40	0.45
Oil Red O	0.55	0.65
Dimethylaminoazo benzene	0.65	0.70
Adsorbent—aluminum oxide G		
Solvent benzene-hexane (9:1)		
Development time accelerated—10 min.		
Development time standard—35 min.		

eter, 2.5 mm. thick, and with a 1.2-cm. centered hole to fit the Precision hi-speed chromatography[1] apparatus were used. The plates were coated with aluminum oxide G or silica gel G (with binder) by the spray technique of Bekersky (5) or the technique of Davidek and Prochazka (6). The plates were activated in the usual manner.

[1] Precision Scientific Co., Chicago, Ill.

The samples are spotted at a point 2.5 cm. from the center hole. Ten spots may be applied per plate. The inside of the apparatus was lined with heavy filter paper saturated with the solvent used for developing. The plate is secured to the spindle of the apparatus, and the rotation of the plate set at 500–700 r.p.m. The solvent flow was adjusted to permit a constant flow without overloading and possible washing away of the adsorbent.

Table I shows the R_f values and time obtained for separation of dyes using the standard and accelerated methods of thin-layer chromatography.

Accelerated thin-layer chromatography provides a method of decreasing the time necessary to achieve separation.

REFERENCES

(1) McDonald, H. J., Bermes, E. W., and Shepherd, H. B., *Chromatog. Methods*, 2(No. 1) (1957).
(2) McDonald, H. J., McKendell, L. V., and Bermes, E. W., *J. Chromatog.*, **1**, 259(1958).
(3) Herndon, J. F., Appert, H. E., Touchstone, J. C., and Davis, C. N., *Anal. Chem.*, **34**, 1061(1962).
(4) Miroslar Pavliček, Jan Rasmus, and Zdeněk Deyl, *J. Chromatog.*, **8**, 537(1962).
(5) Bekersky, I., *Anal. Chem.*, **35**, 261(1963).
(6) Davidek, J., and Prochazka, Ž., *Coll. Czech. Chem. Commun.*, **26**, 2947(1961).

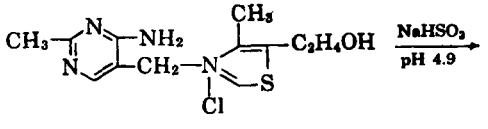
Estimation of Thiamine by Inverse Isotope Dilution

By ANTOINE A. NOUJAIM†, WAYNE V. KESSLER, JOHN E. CHRISTIAN, and ADELBERT M. KNEVEL

Thiamine hydrochloride was determined quantitatively by inverse isotope dilution in the presence of ascorbic acid, riboflavin, and nicotinic acid. The results obtained, using 100-mg. quantities, indicated an average error of 0.1 per cent and a reproducibility of 0.27 per cent.

THE PURPOSE OF THIS investigation was to study a thiamine assay by inverse isotope dilution analysis. Several advantages are inherent in this technique, including speed and simplicity. However, the most outstanding advantage is that quantitative isolation is not required at any point in the analysis. Thus, thiamine may be determined in the presence of other substances without quantitative extraction.

It was necessary to choose a suitable labeling reagent to react with thiamine to produce a chemically stable derivative. Williams, *et al.* (1), proved the structure of thiamine by a quantitative sulfite cleavage into two moieties. This reaction is

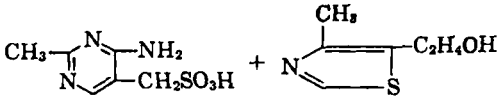


This reaction was used to perform an inverse isotope dilution analysis. Sodium sulfite-S-35 was used as the labeling reagent to form S-35 labeled 2-methyl-6-aminopyrimidyl-5-methanesulfonic acid as the derivative.

The specific activity of the derivative was determined indirectly by carrying out inverse isotope dilution analysis with a known amount of thiamine. The labeled derivative was then mixed with a known amount of carrier. The specific activity of the carrier-diluted derivative was determined by measuring the weight and radioactivity of a portion of the purified material. The weight of the derivative before adding the carrier was calculated by employing the standard inverse isotope dilution formula. The weight of thiamine analyzed was calculated from the weight of the derivative by a gravimetric factor.

EXPERIMENTAL

Optimum Conditions for Cleavage Reaction.—



Thiamine hydrochloride was cleaved under different conditions of temperature, pH, and reaction time. Cleavage at room temperature for 15 hours at a pH of 4.9 was chosen. These cleavage conditions are confirmed by the literature (1).

Physical Properties of Derivative.—The derivative crystallized as fine needle-like crystals insoluble

Received July 1, 1963, from the Bionucleonics Department, Purdue University, Lafayette, Ind.
Accepted for publication July 20, 1963.
The authors thank Hoffmann-LaRoche, Inc., Nutley, N. J., for supplying the thiamine hydrochloride used in this investigation.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.
† Fellow of the National Research Center, Dokki, Cairo, United Arab Republic.