The Quantitative Determination of Small Amounts[1] of Nitrile in Long Chain Fatty Amides

KATHERINE M. BUSWELL and W. E. LINK, Archer Daniels Midland Company, Minneapolis, Minnesota

Abstract

Small amounts of nitrile in long chain fatty amides can be determined by separating the nitrile from the amide on a silica gel column using chloroform as the eluting solvent. The amide stays on the column. After the chloroform is removed by evaporation, the nitrile can be dissolved in a suitable solvent and determined quantitatively either by IR spectrophotometry or by TLC. Determination by IR is much faster than by TLC, but when the nitrile is present at the level of 0.1% or lower, TLC gives more satisfactory results.

Introduction

TWO OF THE MOST FEASIBLE TOOLS for determining the presence of fatty nitriles in fatty amides are IR spectrophotometry and TLC each of which can be applied quantitatively. Chemical methods for nitrile determinations have been described (9,10), but none of them is applicable to nitriles in the presence of amides. Polarographic methods also are available (1,2, 6), but do not appear to have any advantage over IR or TLC techniques.

Nitriles exhibit an adsorption band at 4.42 μ which can be measured quantitatively (3,5). Since this band is relatively weak, it is difficult to measure in samples containing small concn of nitrile. When the nitriles are present in high purity long chain saturated amides, the difficulty is increased by the fact that these amides are only slightly soluble in most organic solvents (8). It is therefore necessary to concentrate the nitrile by separating it from the amide before subjecting it to IR analysis.

Mangold and Kammereck showed that nitriles could be separated from amides by TLC using Silica Gel G as the support and an ammonical benzene solution as the developing solvent (4). Purdy and Truter (7) described a method for quantitative thin layer analysis by measuring the size of the spots, in which the log of the weight of sample in a spot is a linear function of the square root of the area. This method was applied to the TLC analysis of nitriles and found to give results comparable in accuracy to that of the IR, with the advantage that trace amounts of nitrile can be measured by TLC which are not detected by the IR, used in the conventional manner.

With the TLC as with the IR method, it is necessary to conc the nitrile by removing the amide. This is accomplished by chromatographing a sample on a column packed with Silica Gel using chloroform as the eluent. The choice of chloroform was made after a study of other solvent systems, using TLC to follow the separations. Nitriles were eluted easily, amides were retained and the chloroform could be removed readily before subjecting the nitrile residue to analysis.

[1] Technical Paper, No. 270, ADM Co.

Experimental

Separation of the Nitrile From the Amide. Pack a chromatographic column, 28 cm in length and 2 cm in diam with Silica Gel (60–200 mesh) (Davison Chemical, Grade 950) to a depth of 20 cm. Weigh 1.0 g of amide into a 30-ml beaker and dissolve it in 20 ml of chloroform, warming gently if necessary. Quantitatively introduce the sample onto the dry column using a heat lamp directed at the top of the column to keep the amide in solution. The heat lamp is necessary only for saturated samples; unsaturated amides will stay in solution without heat. Using small amt of chloroform rinse down the sides of the column and let the chloroform soak in two or three times before adding more. Do not allow the solvent level to drop below the top surface of the Silica Gel. Elute with chloroform until 300 ml have been collected in a 400-ml beaker. Evaporate the sample to dryness over a steam bath with a stream of air. Quantitatively transfer the sample to a 30-ml beaker and evaporate to dryness again.

Infrared Method. A Perkin Elmer 221 IR spectrophotometer can be used with the following program:

Slit	38 μ
Attenuator speed	11
Gain	4.5
Speed	1 μ per min
Suppression	0
Scanning range	4.0 μ to 4.95 μ

Disolve the sample in exactly one ml of tetrachloroethylene in the 30-ml beaker and quickly transfer it to a 1.0-mm cell. Scan it on the IR spectrophotometer from 4.0–4.95 μ, using tetrachloroethylene in the blank. By the same method scan a known solution of nitrile standard. Measure the absorption (A) by the base line method and calculate the moles/kg of nitrile as follows:

Calculation of cell constant (K):

$$K = \frac{A}{\text{moles/liter of standard}}$$

$$\text{moles/kg of nitrile} = \frac{A}{K \times \text{sample wt in 1.0 ml}}$$

Thin Layer Chromatographic Method. Make a slurry of adsorbent by thoroughly mixing in a motar 30 g of Silica Gel G with 70 ml of distilled water. This makes a thin slurry which can be spread more evenly than

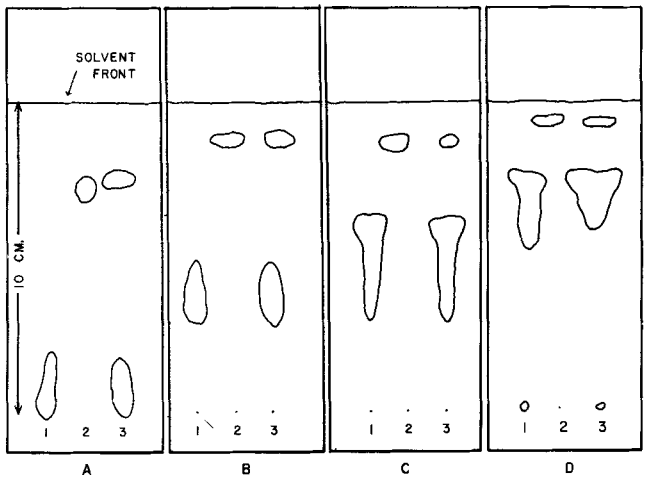

FIG. 1. Separation of lauronitrile from lauramide on Silica Gel G using A-chloroform, B-chloroform-methanol (95:5). C-acetone, D-acetone-conc ammonium hydroxide (98:2). 1-amide, 2-nitrile, 3-mixture.

one of thicker consistency. Pour the slurry over the glass plates (20 x 20 cm) which have been placed on a plastic board 22 x 113 cm with retaining ledges 1.8 cm wide along a short and long side. Using a Plexiglass applicator (Applied Science Laboratories) spread the slurry over the plates as evenly as possible. The Plexi-glass applicator was found to give more uniform layers than the conventional type.

Air-dry the chromatoplates for 10–20 min to allow the binder to set and place them in an oven at 110–120C for an hour. When cooled they are ready for use. The plates must be coated as evently as possible for quantitative use.

Using a standard nitrile of the same chain length as the sample, make solutions containing 0.1,0.2,0.4 and 0.8% nitrile in benzene. Separate the nitrile in the sample from the amide using a chromatographic column as described for the IR method. Use benzene as a solvent for the residue instead of tetrachloroethylene. Determine the number of ml used to dilute the nitrile from the number of mg of residue in the 30-ml beaker or from the percentage of nitrile expected;

% Nitrile expected	Nitrile residue wt in mg	Ml benzene used for dilution
.1– .2	1– 2	.5
.3– .5	3– 5	1
.6– .8	6– 8	2
.9–1.2	9–12	3
1.3–1.6	13–16	4
1.7–2.0	17–20	5

dilute larger amt accordingly. Add the benzene directly to the beaker immediately before the sample is to be spotted on the plate. All the spots, both standard and sample, must be the same volume. Use a 50-μl syringe such as Hamilton Model No. 705. Holding the syringe as close as possible to the plate, express 5 μl of the solution slowly from the syringe, in a single operation. Do not spot the sample a drop at a time for this leads to poor reproducibility of the size of the spots. The spots should be placed 1.5 cm apart. Make three spots of the sample and two spots of each of the nitrile standard solutions in the same manner. Place the chromatoplate in a tank containing a 1-cm layer of benzene-aqueous ammonia solvent, which is prepared by equilibrating 100 volumes of benzene at 20C with 10 volumes of 1 *N* aqueous ammonia, discarding the

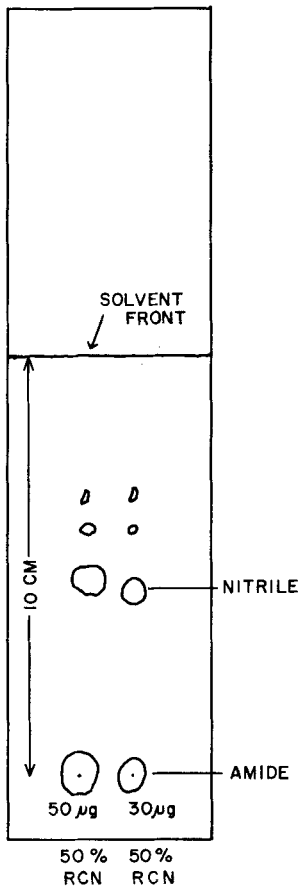

FIG. 2. Separation of caprinitrile from capramide on Silica Gel G, showing two impurities with R_f values > that of nitrile.

aqueous layer and drying the benzene for a few minutes with an excess of anhydrous sodium sulfate. Line the tank before the development process with filter paper which will act as a wick and saturate the jar with solvent vapor. Allow the solvent front to travel 100 mm.

To develop the spots, spray the plate with .05% Rhodamine B in 95% ethanol. After this has dried, place the plate in a tank containing iodine vapors for one or two min. Remove it and let it stand in the hood for five min to allow the excess iodine vapors to escape. Using an UV lamp to reveal the spots, carefully outline each spot exactly with a sharp pencil. The plate is now ready to be copied by the best means available. For this work a Xerox 914 Copier was used. If this is not available the spots can be covered with Scotch Tape and then traced as accurately as possible onto thin tracing paper.

Three methods for measuring the size of the spots were tried: measuring the area of the spots with a planimeter, tracing the spots on a thin graph paper with $\frac{1}{16}$-in. squares and counting the number of squares, and cutting each spot from the paper with a fine pair of scissors and weighing it on an analytical balance, the weight of the paper in mg representing the area of the spot. The best precision was obtained with the last method.

Calculations. Using semi-logarithmic graph paper plot the square root of 10 times the area ($\sqrt{A \times 10}$) against the weight (W) of the standards in micrograms (μg). This will give a straight line. Average the square roots of the areas of the three sample spots and read the wt of nitrile in the sample from the curve.

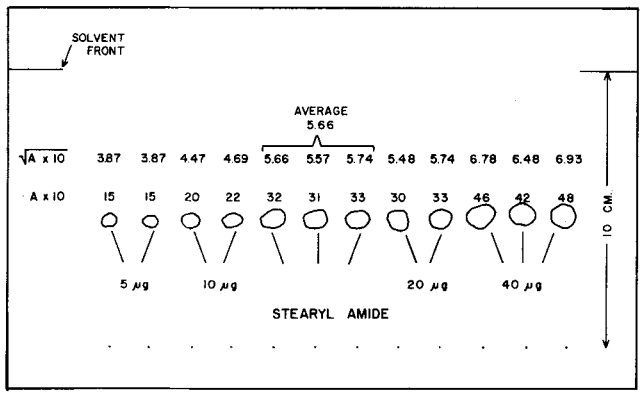

FIG. 3. Thin-layer plate of the nitrile portion from commercial stearamide and stearonitrile standards in sample sizes of 5,10,20 and 40 μg. Sample diluted with 2 ml benzene, 5 μl spots of standards and samples applied.

$$\% \text{ Nitrile} = \frac{\text{micrograms of nitrile in 5 } \mu\text{l}}{\text{micrograms of sample in 5 } \mu\text{l}} \times 100$$

When:

$$\text{micrograms of sample in 5 } \mu\text{l} = \frac{\text{(wt of sample introduced onto the column in micrograms)} \times \text{(no. of } \mu\text{l in spot)}}{\text{no. of } \mu\text{l used to dilute nitrile residue}}$$

Results and Discussion

The separation of a mixture of a fatty amide and nitrile may be accomplished by TLC with a variety of solvents, as shown in Figure 1. From the separation with chloroform we concluded that this solvent would be suitable in column chromatography to remove the interfering amide.

The R_f value of the nitrile in a solvent consisting of benzene equilibrated with aqueous ammonia was less than that in chloroform, permitting a separation of the nitrile from other impurities which might otherwise interfere with the measurement of the spot area. In Figure 2, two spots are seen above the nitrile, one of which, the upper, has been identified as a hydrocarbon. These compounds would not interfere if the final measurement of nitrile were made by the IR technique but would cause high results to be obtained for nitrile in the TLC technique if not separated from the nitrile. The nitrile can easily be identified by comparison with the standard nitriles on the same thin layer plate.

The R_f values in TLC are not exactly reproducible. With a mixture of a nonpolar solvent, such as benzene and ammonia, even less control over the distance traveled by the components is possible. Since it is desirable, when measuring areas, that the spots have R_f values of ca. 0.5, a means of controlling the polarity of the solvent is necessary. This was done by drying the benzene aqueous ammonia solution with sodium sulfate.

TABLE I

Recovery Data of Nitrile Determinations

% Nitrile added	% Nitrile determined by IR	% Nitrile determined by TLC
0.1	.08	.10
	.08	.14
	None detected	.11
	None detected	.09
0.5	.69	.49
	.42	.53
	.39	.40
	.39	.64
1.0	.86	.79
	.96	1.17
	.72	1.00
	1.09	.96

TABLE II

Results of Nitrile Determinations

	% Nitrile determined by IR	% Nitrile determined by TLC
Coco amide	.08	.14
	.11	.21
	.11	.21
	.24	.27
Oleamide	1.92	1.86
	1.70	1.82
	1.68	1.70
	1.83	1.69
Stearamide	1.64	1.40
	1.34	1.42
	1.32	1.59
	1.40	1.51

Samples containing known amt of nitrile were obtained by adding pure stearonitrile to pure stearamide. These were analyzed by the IR method and then by the thin layer method to determine the percentages of nitrile recovered. The thin layer separation is displayed in Figure 3, in which a sample was applied to a plate along with nitrile standards with greater and lesser amt than the sample. The area values of the spots are expressed in mg of paper cut from the facsimile of the chromatogram. The averages of the square roots of the areas plotted against the log of the wt of applied nitrile standards in μg give a straight line from which the wt of nitrile in the sample can be read.

The results of a recovery study are shown in Table I. From these data and others we believe that the TLC method is more reliable when the nitrile level is 0.1% or below and that either method is applicable at a higher level. Below the level of 0.1%, IR measurements can be made with the aid of scale expansion, but this accessory is not always available to the analyst. A statistical analysis of these data showed that none of the averages of the percentages of nitrile as determined by either method is significantly different from the percentages of nitrile added, when the level added is above 0.1%.

Commercial samples of coconut derived amide, oleamide and stearamide were also analyzed by both methods and the results are shown in Table II.

These methods are not restricted to amides, for they can be applied also to amines and amine acetates containing nitrile impurities. Cationic materials are easily removed with ion-exchange resins and the nonionic portion studied by TLC. With proper selection of solvent systems and supports, the determination of small amt of impurities other than nitriles in the presence of large amt of other fatty derivatives is possible. It is likely that this application of thin layer and column chromatography will be useful in problems where information regarding the type and amt of minor components is necessary.

REFERENCES

1. Bobrova, M. J., and A. N. Matveeva-Kudasheva, J. Gen. Chem. USSR *27*, 1219–24 (1957), CA *53*, 4022f.
2. Bobrova, M. J., and A. N. Matveeva-Kudasheva, Zhur. Obshchei Kim. *28*, 2929–32 (1958), CA *53*, 5976e.
3. Kitson, R. E., and N. E. Griffith, Anal. Chem. *24*, 334–7 (1952).
4. Mangold, H. K., and Rudolf Kammereck, JAOCS *39*, 201–206 (1962).
5. Nelson, J. P., L. E. Peterson and A. J. Milun, Anal. Chem. *33*, 1882–84 (1961).
6. Patsevich, I. V., A. V. Topchiev and V. Ya. Shtern, Zhur. Anal. Khim. *13*, 608–12 (1958), CA *53*, 5976f.
7. Purdy, S. Jean, and E. A. Truter, Analyst *87*, 802–809 (1962).
8. Ralston, A. W., C. W. Hoerr and W. O. Pool, J. Org. Chem. *8*, 473–88 (1943).
9. Whitehurst, Dorothy H., and J. B. Johnson, Anal. Chem. *30*, 1332–3 (1958).
10. Zaugg, H. E., and B. W. Harrom, *Ibid. 20*, 1026–29 (1948).

[Received April 27, 1964—Accepted August 5, 1964]