MELTING POINTS WITH DECOMPOSITION AND THE HEAT STABILITY OF ATROPINE SULPHATE

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When an organic substance commences to decompose before it melts, the melting point is lowered by the products of decomposition, and depends on the time the sample has been subjected to the decomposition temperature. When the m.pt. forms part of an official specification, the results of different analysts may be widely divergent, and may lead to disputes regarding quality. Some alkaloid salts, and particularly sulphates, are sensitive to small variations in m.pt. procedure, and a study has been made of these factors as they affect atropine sulphate. The heat stability, or time taken for the substance to melt at a constant temperature well below its normal m.pt., is a useful indication of purity.

Introduction

A decomposition m.pt. will be higher the faster the substance is taken through the critical range of temperature. Thus, Kempf[1] observed that tyrosine melted at 280° C. when heated slowly, and at 314 to 318° C. when heating was rapid. Decomposition varies in nature and degree, until it reaches the point of explosion, as noted by Willgerodt[2] for iodoxybenzene. The apparatus of Dennis and Shelton[3] overcomes, to some extent, the difficulties inherent in the capillary tube method. The sample is laid in a thin trail along a copper bar heated at one end to establish a temperature gradient, and the temperature at the point at which the substance melts is measured by a thermocouple formed by touching the bar with a constantan wire. This apparatus was improved by Kofler[4], who called it a "heat bank," and among the m.pts. given by Kofler and Sitte[5] are acetylsalicylic acid, 143° C. (135° to 138° C.), arginine, 260° C. (238° C.) and morphine, 260° C. (254° C.), the figures in brackets being measured by the conventional method in capillary tubes. Since the latter is official in the various Pharmacopœias, it has been used exclusively in this investigation.

Alkaloidal Salts and Derivatives

All types of decomposition occur in the alkaloid field, from the darkening of aurichlorides and the frothing of amino-oxides to the explosion of certain picrates. Acetylatropine methylnitrate, immersed at 140° C. and heated at 3° C. per minute, melted sharply at 148° C. Acetylatropine methylbromide, with the same rate of heating, was strikingly anomalous. Immersed at 215° to 220° C. it melted at 223° C., lost acetic acid, resolidified, and melted again as apoatropine methylbromide at 265° C.

When immersed at 210° C., loss of acetic acid occurred before melting and the sample-remained solid to 265° C.

At elevated temperatures, atropine sulphate loses water, forming apoatropine sulphate. It is stable indefinitely at 50° to 60° C., but the B.P. test shows apoatropine after some weeks at 100° to 110° C., and after 2 hours at 150° C., and it is probable that a more profound decomposition also takes place. Homatropine sulphate is even more heat sensitive than the atropine salt, although it contains mandelic acid in place of tropic acid, and a similar dehydration cannot occur, whilst atropine hydrochloride, with the sulphate radical absent, is considerably more stable.

Official Standards and Methods

The methods and definitions of the B.P. 1953 regarding m.pts., differ from those of the U.S.P. XIV, and so do the standards for atropine sulphate. The sulphuric acid baths are similar, and the capillary tubes of the same dimensions, but the former specifies immersion 10° C. below the expected m.pt., and a heating rate of 3° C. per minute, whilst the latter requires immersion 30° C. below, and a heating rate of 3° C. per minute reduced to 1° C. per minute over the final 3 degrees. Apart from the manipulative difficulty of effecting this change of rate with precision, the U.S.P. sample is heated for a total of 12 minutes, against $3\frac{1}{3}$ minutes in the B.P. test. This is reflected in the official m.pt. standards, the lower limits being 188° C. and 191° C. respectively.

Variability of Results

The difficulty in obtaining agreement on the m.pt. of atropine sulphate is illustrated by a series of tests made on one sample in 3 different laboratories, the means of several determinations in each case being 188° C., 190° C. and 191·5° C., the B.P. method being adopted for all tests. This led to a statistical investigation, in which 6 separate measurements were made by each of 8 experienced analysts on a B.P. sample of zero rotation. Using the B.P. method, the mean of the 48 tests was 191·4° C., with a range of 2·5° C. and a standard deviation 0·5° C. A similar series under U.S.P. conditions gave a mean of 188·9° C., range 4·2° C. and standard deviation 0·75° C. Simplication of the U.S.P. method by continuing the 3° C. per minute rate up to the time of melting raised the m.pt. 1° C., and reduced the range. A check series of 48 determinations on hyoscine hydrobromide, which does not decompose, was made by the same team, giving a range of only 1° C., and standard deviation 0·3° C. Analysis of the results for atropine sulphate shows that, if the chance of a sample being wrongly approved is not to exceed 0·001, the least acceptable mean of two measurements must exceed the B.P. lower limit by 1·4° C., and the U.S.P. by 2·2° C.

Effect of Variable Factors

Preliminary series, each of 10 tests, showed that the m.pt. was not appreciably affected by the use of soda-glass or Pyrex capillaries, by variations in their diameter from 0·5 to 1·5 mm., by method of packing,

or by the rate of stirring the bath. The two factors of importance were the method of drying, and, to a predominant degree, the rate (and consequently, the time) of heating.

(*a*) *Method of Drying.* Atropine sulphate crystallises with one molecule of water and in the B.P. melting point test the sample is dried for 15 minutes at 135° C. The U.S.P. requires 4 hours at 105° C. Comparative test series gave the same mean m.pt. after 15 minutes at 135° C. and 2 hours at 105° C., but a fall of 0·8° C. when the time at 105° C. was extended to 4 hours.

(*b*) *Rate of Heating.*

TABLE I

M.PT. OF ATROPINE SULPHATE IN B.P. APPARATUS WITH INCREASING RATE OF HEATING

Heating rate ° C./minute	Immersed at ° C.	Time heated Minutes	M.pt. ° C.
1	180	8	188
2	180	5	190
3	180	3·8	191·4
4	180	3	192
5	180	2·7	193·4
10	175	2·0	195·3
20	170	1·35	197

When the time of heating falls below 1·5 minutes melting is delayed, since heat transfer through the capillary takes an appreciable time.

Atropine Sulphate: Stability Tests

When the bath is held at a constant temperature somewhat below the normal m.pt. the sample melts after a definite time which depends upon its purity. Each of the times in Table II is the mean of 6 measurements, which did not vary more than 7 per cent. on either side of the mean. The atropine sulphate was the B.P. sample of m.pt. 191·4° C. The figures for this sample in Table II may be compared with the results for one which failed to comply with the B.P. standard, and melted at 190° C. This melted in 17 minutes at 175° C., 6·3 minutes at 182° C., and 3·9 minutes at 184° C.

TABLE II

TIME TAKEN FROM IMMERSION TO MELTING AT VARIOUS TEMPERATURES

Temperature ° C.	Mean time to melting Minutes
175	34·5
180	16·0
182	10·0
184	8·3
186	7·3
188	5·4
190	4·2
192	2·4
193	2·1
194	1·8
195	1·4
196 and over	1·0

From this, and from Table I, the true m.pt of this sample, if decomposition products do not interfere, is apparently close to 195° C.

Other Alkaloidal Salts

The hydrobromides and hydrochlorides of hyoscine, hyoscyamine and homatropine have m.pts. which are not greatly affected by small variations

in time and rate of heating. In Table III the stability of atropine sulphate is shown to be much less than that of the hydrochloride. Homatropine sulphate, in which dehydration of the mandelic acid cannot occur, is even less stable than atropine sulphate. It is concluded that the sulphate radical has a profound destructive effect on the ester-alkaloids, and that the instability of atropine sulphate is not explained simply by the formation of apoatropine.

TABLE III

HEAT STABILITY OF ALKALOIDAL SALTS COMPARED.
TEMPERATURE OF BATH CONSTANT

Number of ° C. between immersion temperature and m.pt.	Time in minutes to melting for		
	Atropine sulphate	Atropine hydrochloride	Homatropine sulphate
9	10·0	—	12·1
7	8·3	27·0	7·0
5	7·3	21·0	4·7
3	3·2	14·0	1·5
Actual m.pt.	191·4°	171·5°	220·0°

SUMMARY

1. Decomposition melting points are affected mainly by rate and time of heating, and this has been illustrated for atropine sulphate.

2. In two series of tests on atropine sulphate, m.pts. following the B.P. technique were more concordant than those carried out according to the U.S.P.

3. The results were less divergent with experienced analysts, but, even with the B.P. technique, considerable replication is desirable to ensure a reliable figure.

4. The sulphates of these ester-alkaloids appear to be more heat-sensitive than the hydrobromides and hydrochlorides.

5. The "heat stability" of decomposable substances may give a useful indication of purity.

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