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HEAT-TREATED MAGNESIA AS AN ADSORBENT FOR THE CLASS SEPARATION OF ALIPHATIC MONOCARBONYL COMPOUNDS

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SUMMARY

In order to control the activity of magnesia as an adsorbent, the effect of temperature was studied. The activity at various temperatures of treatment showed two regions. During the first, in the range 106–320°, the activity increased with increasing temperature, whereas at temperatures above 320° the activity decreased with increasing temperature.

The iodine number of heat-treated magnesia correlated well with its affinity for 2,4-dinitrophenylhydrazones of carbonyls.

By heating several batches of magnesia at graphically determined temperatures it was possible to obtain adsorbents with the same activity for class separation.

INTRODUCTION

The importance of carbonyl compounds as flavour constituents of many food products is well established. Of the various methods for the isolation and separation of these compounds, the most commonly used is their conversion into 2,4-dinitrophenylhydrazones, either before or after the extraction.

The separation of 2,4-dinitrophenylhydrazone derivatives into the individual homologues can be accomplished by liquid–liquid column chromatography[1,2], thin-layer chromatography[3], gas chromatography[4–6], direct insertion of the sample into the source of a mass spectrometer[7] or gas chromatography of the free carbonyls regenerated in an injector–reactor[8,9]. Nevertheless, the great variety of carbonyls that is found in a sample, particularly in dairy products, may prevent the use of these methods and there is therefore still a need for preliminary fractionation into classes. This separation has been performed by several types of thin-layer chromatography[3,10] and by the Schwartz *et al.*[11] column chromatographic method. Later workers described a method with Magnesia 2665 (activated magnesia) as an adsorbent but applications in food flavour research have often been carried out with the activated magnesia Sea Sorb 43. Unfortunately, the activity of this magnesia is very variable between batches and it is necessary to modify the strength of the eluting solvent according to the activity of the batch of adsorbent.

Although Sea Sorb 43 magnesia was in many instances used as received[12,13] it has also been used in a heat-treated form as an adsorbent in class separations. Several workers have referred to heat treatment at 400° for 24 h[14] or 48 h[15] and others to heat treatment at 150° for 36 h[16]. Although the activity of heat-treated magnesia was indicated in some publications, the actual effect of heat treatment on the activity of magnesia has never been considered.

This apparent diversity in heat treatments and the likelihood that the heating conditions might not be optimal in view of the variable activity of different batches led us to study the effect of temperature on the activity of magnesia in order to improve the reproducibility of the method by means of accurate control of the activity of the adsorbent.

MATERIALS AND METHODS

Heat treatment of magnesia

Unless otherwise specified, Sea Sorb 43 magnesia (Fisher Scientific, Pittsburgh, Pa., U.S.A.), was heated isothermally in a muffle furnace for 24 h. A regulation device consisting of proportional–integral circuits assured the control of temperature to within ± 0.5°. The temperature was checked by means of a mercury thermometer.

The loss of water during heat treatment was determined by weighing magnesia samples before and after heating.

Control of activity of magnesia

The activity of the adsorbent was measured according to two different methods. The first consisted in determining the activity by iodine adsorption of magnesia and the second in defining the activity as the equivalent retention volume for 2-propanone 2,4-dinitrophenylhydrazone.

Determination of iodine number. Iodine numbers were determined by the Fisher Scientific method. A 2-g sample of magnesia was transferred into a 250-ml erlenmeyer flask and 50 ml of 0.10 *N* iodine in carbon tetrachloride solution were added. After sealing, the mixture was agitated for 25 min and the resulting magnesia suspension was filtered through Whatman No. 1 filter-paper, discarding the first 5 ml. Ten millilitres of filtrate were pipetted into a glass-stoppered erlenmeyer flask and the residual iodine titrated to a colourless end-point with 0.10 *N* sodium thiosulphate solution. The sample was agitated between additions of the titrant.

The iodine number, reported as milliequivalents of iodine adsorbed by 100 g of magnesia, is given by the relationship

$$\text{Iodine number} = A \cdot 2500 - (B \cdot 250 \cdot V)$$

where A = normality of iodine solution, B = normality of sodium thiosulphate solution and V = millilitres of sodium thiosulphate solution.

Measurement of equivalent retention volumes. One gram of Celite 545 (dried in an oven for 24 h at 150°) and 1 g of magnesia were slurried together with 6 ml of chloroform. The slurry was immediately poured into a 12.7-mm diameter chromatographic tube. The column was first packed by a vibrating action and then under a 0.1 bar pressure of nitrogen. A 0.25-μM aliquot of 2-propanone 2,4-dinitrophenylhydrazone in chloroform solution was applied to the column and the development was accomplished with chloroform at a flow-rate of 20 ml/h. The chloroform used was saturated by shaking it in a separating funnel with an equal volume of distilled water, for the reason discussed below.

On measuring the retention volumes on magnesia, two main difficulties were encountered. Firstly, in the washing of magnesia of high activity with a large volume of solvent (the retention volume increases with increasing activity) and for the aromatic 2,4-dinitrophenylhydrazone derivatives irreversible sample adsorption as described by Snyder[17] may occur. In order to prevent this effect, the solvent was saturated with water. Further, the very different activities of the treated samples made it difficult to obtain both retention of the sample on low-activity magnesia and retention times that were not too long on high-activity magnesia with the same solvent. By using chloroform, this problem was overcome as retention was observed from the initial activity to the 500° activity level.

Class separation

Adsorption chromatography on Celite–magnesia adsorbent was used for the measurement of equivalent retention volumes. A 25.4-mm diameter chromatographic tube was packed with 7 g of Celite and 7 g of heat-treated magnesia. The dry adsorbent was slurried with 40 ml of the first solvent (15% chloroform in *n*-hexane), which was found to be the most suitable volume for producing correct packings. A too fluid slurry tended to separate the two constituents of the adsorbent; a too firm slurry tended to trap air bubbles. Class separation was then carried out according to the method of Schwartz *et al.*[11], the chromatograms being developed by combinations of chloroform in *n*-hexane at a flow-rate of 80 ml/h.

RESULTS AND DISCUSSION

Effect of heat treatment on activity of magnesia

Samples from three batches of magnesia (A, No. 702969; B, No. 735505; C, No. 740441) and from an aged batch H that had not been efficiently protected from moisture were treated at various temperatures.

According to the specifications of the manufacturer, the initial activity of each batch had been maintained at an iodine number above 80. The activity of the untreated magnesia was in fact found to be above 80, but it had significantly different values in the three batches: 119.4 (A), 84.2 (B) and 103.8 (C).

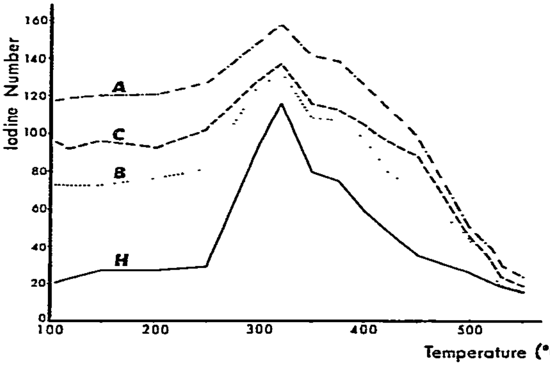

Fig. 1. Effect of a 24-h heat treatment on the activity of magnesia expressed as the iodine number.

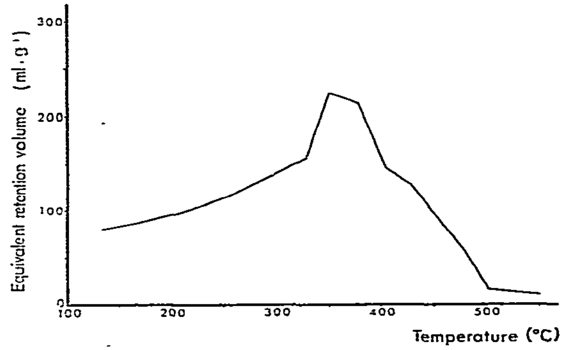

Fig. 2. Effect of a 24-h heat treatment on the activity of magnesia (batch A) expressed as the equivalent retention volume for the 2,4-dinitrophenylhydrazone derivative of 2-propanone.

In Fig. 1, the activity of magnesia (iodine number) is plotted against treatment temperature. In the range 106–200°, increasing temperature did not significantly affect the activity, which remained at the initial level. From 200 to 320° the activity increased with increasing temperature, slightly over the range 200–250° then markedly over the range 250–320°. Above 320° the activity decreased with increasing temperature. The relative activity levels of the different batches remained approximately constant over the whole temperature range.

Fig. 2 shows the activity of magnesia determined as the equivalent retention volume of 2-propanone 2,4-dinitrophenylhydrazone when the adsorbent was heated at various temperatures. Over the range 132–348° the activity increased with increasing temperature, with an acceleration between 255 and 348°. Above 348° the activity decreased with increasing temperature. These results confirm those obtained for the iodine number.

These results should be compared with those reported by Schwartz *et al.*[18], who obtained partial deactivation of magnesia by heat treatment at 525 ± 25°. These results may appear to contradict those of Snyder[17], who found a continuous decrease in activity with increasing temperature of treatment. Nevertheless, it should be noted that in the latter instance no measurements were made in the range 300–500°.

The procedure based on the determination of the equivalent retention volume

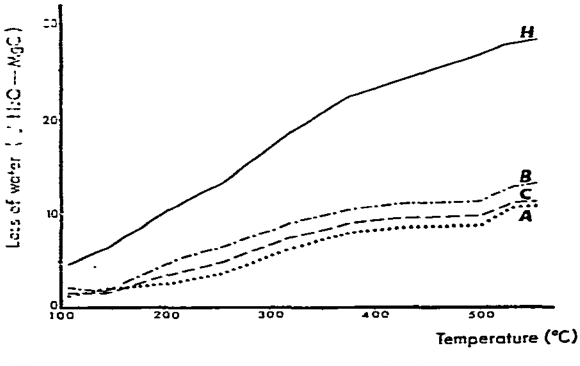

Fig. 3. Amounts of water (in g-% of the initial adsorbent) liberated by magnesia during heat treatments.

for a carbonyl 2,4-dinitrophenylhydrazone derivative must be considered as a reference test for measuring the activity of magnesia. A comparison between Figs. 1 and 2 shows that this test can be replaced by the iodine number test, which is easier to use.

Fig. 3 shows the loss of water observed during heat treatment of the four batches of magnesia. The amount of water liberated increased with increasing temperature but the rate of water loss (the amount liberated by an increase of 1°) decreased as the temperature increased. In considering the relationship between water content and activity of magnesia, it was found that the lower the initial activity, the greater was the water loss during heat treatment. In the first region of magnesia activity, the water loss is related to an increase in activity, whereas in the second region it is related to a decrease in activity.

Although the aged lot H was found to have a lower initial activity it regained its activity markedly in the maximum region.

Heat treatments and class separation

By appropriate adaptation of the heat treatment, it is possible to obtain an adsorbent with constant activity from several batches. First the activity is plotted against temperature over a range that includes the activity level chosen for class separation, then the temperature required to yield the expected activity is determined graphically for a particular batch.

For class separation, an adsorbent of iodine number 16–20, which can be obtained by heating above 500°, was chosen. Magnesia treated at such a temperature is reported to give an adsorbent with superior performance[17]. In addition, an adsorbent of the same activity level as Magnesia 2665 is produced, which makes it possible to perform the initial stepwise elution using *n*-hexane–chloroform combinations[11].

As illustrated in Fig. 4, the activity of magnesia decreased rapidly over the interval 15 min–6 h as the period of treatment was increased; thus, after a 6-h and a 16-h treatment, the decrease was found to represent 90.5% and 96.0%, respectively, of that resulting from a 24-h treatment. Hence, reducing the period of treatment to 16 h (overnight) or to 6 h will require only a slight increase in temperature to yield an adsorbent of similar activity.

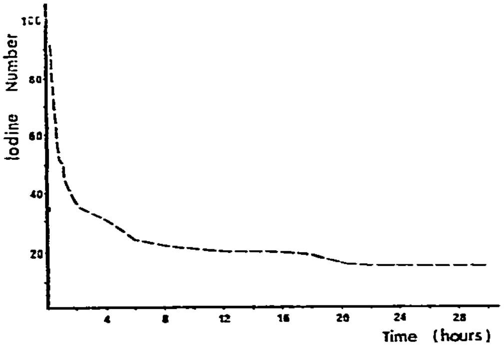

Fig. 4. Activity (expressed as the iodine number) of magnesia (batch D, No. 743579) treated at 569° for various periods.

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REFERENCES

1 E. A. Corbin, D. P. Schwartz and M. Keeney, *J. Chromatogr.*, 3 (1960) 322.
2 L. J. Papa and L. P. Turner, *J. Chromatogr. Sci.*, 10 (1972) 747.
3 G. Pradel and J. Adda, *Lait*, 55 (1975) 603.
4 R. J. Soukup, R. J. Scarpellino and E. Danielczik, *Anal. Chem.*, 36 (1964) 2255.
5 H. Kallio, R. R. Linko and J. Kaitaranta, *J. Chromatogr.*, 65 (1972) 355.
6 L. J. Papa and L. P. Turner, *J. Chromatogr. Sci.*, 10 (1972) 744.
7 J. B. Stanley, D. F. Brown, V. J. Senn and F. G. Dollear, *J. Food Sci.*, 40 (1975) 1134.
8 H. Halvarsen, *J. Chromatogr.*, 57 (1971) 406.
9 D. P. Schwartz and C. R. Brewington, *Microchem. J.*, 17 (1972) 63.
10 A. R. Keen, P. M. Boon and N. J. Walker, *N. Z. J. Dairy Sci. Technol.*, 11 (1976) 180.
11 D. P. Schwartz, O. W. Parks and M. Keeney, *Anal. Chem.*, 34 (1962) 669.
12 D. P. Schwartz, H. S. Haller and M. Keeney, *Anal. Chem.*, 35 (1963) 2191.
13 A. P. Hansen and P. G. Keeney, *J. Food Sci.*, 35 (1970) 37.
14 J. Schormüller, M. Walther and W. Wachs, *Z. Lebensm.-Unters.-Forsch.*, 139 (1969) 273.
15 D. F. Anderson and E. A. Day, *J. Agr. Food Chem.*, 14 (1966) 241.
16 C. K. Dartey and J. E. Kinsella, *J. Agr. Food Chem.*, 19 (1971) 771.
17 L. R. Snyder, *J. Chromatogr.*, 28 (1967) 300.
18 D. P. Schwartz, M. Keeney and O. W. Parks, *Microchem. J.*, 8 (1964) 176.