

Changes occurring in active alumina on storage

We have examined the chromatographic behaviour of rotenone on neutral alumina in ether-petroleum ether solvent mixture. Under these conditions part of the rotenone is irreversibly adsorbed by the alumina and a study of this phenomenon forms the basis of this communication.

Experimental

Materials. Active alumina was prepared from commercial material (Peter Spence, Type H, γ - Al_2O_3), by acidification (pH 2) of an aqueous suspension followed by washing until the aqueous supernatant was neutral. The alumina was then filtered off and dried on plates in an oven at 100° for 3 days. The "dry" active alumina contained approximately 5% water and prior to use water (7% v/w) was added and the sample of deactivated alumina rolled in a closed container for 2 h. The solvents used were dried by distillation, except the solvent ether which was distilled from sodium metal. The rotenone used had m.p. 161.5° , λ_{max} 237 m μ , ϵ_{max} 14,580.

Chromatographic method. A column (15 cm \times 1.5 cm) was packed with alumina (50 g Grade III), using solvent ether-petroleum ether (b.p. 40 - 60°) (1:1) mixture (100 ml) in the usual manner. A solution of rotenone (60 mg) in the eluent mixture (25 ml) was then introduced onto the column followed by elution with the same eluent mixture. Fractions (10 ml) of the eluate were collected and an aliquot (0.05-0.5 ml) of each fraction was evaporated with a stream of nitrogen and then dissolved in spectroscopic ethanol (95%, 10 ml) by warming in a water bath at 40° . The optical density at 237 m μ was determined for each diluted fraction. The amount of rotenone recovered from the column was calculated from the expression:

$$\text{rotenone (mg)} = \Sigma[(x - y) \times \text{dilution factor}] \times \frac{3.94 \times 1000}{14,580}$$

where x = optical density of diluted aliquots,

y = optical density of diluted aliquots of eluent mixture.

In later experiments, it was found more convenient to collect a bulk fraction (250 ml) after 100 ml of eluate had left the column. This fraction contained all the rotenone.

Storage of alumina. The dried active alumina was removed from the oven (time = zero), and stored in a well-filled closed glass container. At intervals, 50 g was removed, deactivated with water and used for the chromatography of rotenone. Fig. 1 shows the variation in the rotenone recovery obtained using deactivated alumina prepared from two batches of "dry" alumina stored for various time intervals. The general shape of the curves obtained for the two batches was similar although the positions of the maxima and minima differed considerably.

Discussion

We have found that when rotenone is chromatographed on alumina in dry ether-petroleum ether solvent mixture (1:1), part of the rotenone is irreversibly adsorbed (decomposed). The decomposition product remained on the column since the ultra-violet absorption characteristics of the eluate were those of rotenone, and further-

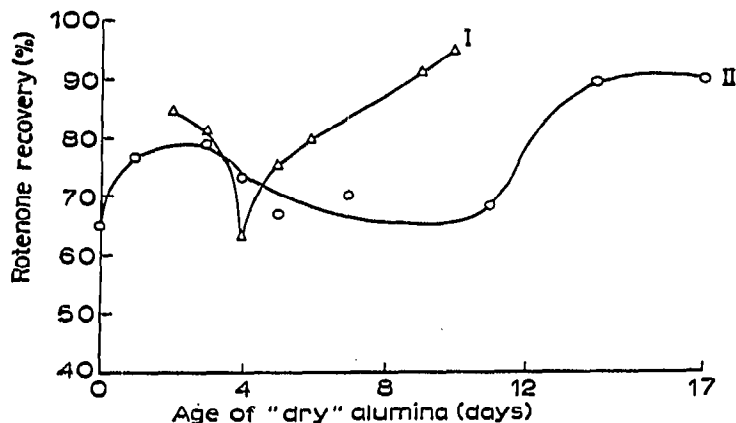


Fig. 1. Rotenone recoveries from deactivated stored "dry" alumina.

more the elution curve was symmetrical and did not show tailing (see Fig. 2). The recoveries of rotenone from the column were quantitative when solvent ether previously saturated with water was used in the solvent mixture.

The activity of alumina is directly related to its water content¹ since the addition of water to dry active alumina decreases the activity of the alumina by reducing the number of available adsorption sites². The solvent used in our work has a high affinity for water and presumably some of the physically adsorbed water molecules on the alumina surface are removed, exposing an active surface on which part of the rotenone is irreversibly adsorbed. This view receives support from the work of SNYDER who has shown that non-chemically bound water in samples of alumina containing more than 4-5 % water may be partially removed by a weaker hydrophilic eluent such as benzene².

It has been further noted that the extent to which the rotenone is irreversibly adsorbed by the alumina is dependent upon the age of the "dry" alumina from the

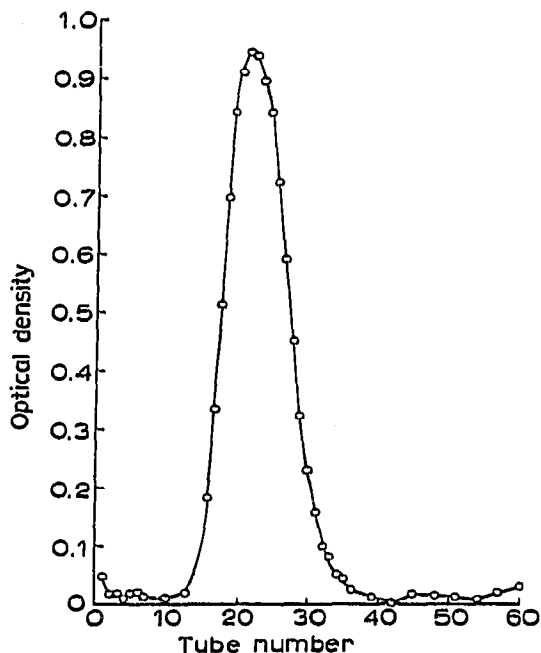


Fig. 2. Elution curve for rotenone on deactivated alumina.

time of its preparation (see Fig. 1). KIPLING AND PEAKALL have shown that active alumina (γ - Al_2O_3) in contact with water vapour irreversibly adsorbs water molecules to give a gibbsite-like surface ($\text{Al}_2\text{O}_3 \cdot 3\text{H}_2\text{O}$)³. We suggest that the "dry" alumina which contains a small proportion of water (5%) undergoes similar changes at the surface on storage, and that as hydration proceeds the dipolar character of the ($-\text{Al}-\text{O}-$) bonds at the surface are altered, and the dipolar attractive forces between the surface of "dry" alumina and the added water molecules are modified accordingly. Elution of the water molecules at the surface by hydrophilic solvent mixture will become easier as these attractive forces decrease, leading to increased rotenone decomposition and *vice versa*. However, this explanation does not account for the complex nature of the curves in terms of the species γ - Al_2O_3 , $\text{AlOH}(:\text{O})$ and $\text{Al}_2\text{O}_3 \cdot 3\text{H}_2\text{O}$, and the phenomenon is not clearly understood.

Now this interpretation of our results requires further comment since from an examination of free moisture in stored deactivated alumina WOHLLEBEN⁴ has concluded that the chromatographic activity of alumina is not influenced by storage. We have reconciled these two views by showing that although the irreversible adsorption of rotenone is very sensitive to the changes occurring at the alumina surface on storage, the activity of the alumina to a reversible adsorption process is unchanged by storage.

Stored "dry" alumina was deactivated with water in the usual manner and then washed with the same volume of dry ether-petroleum ether (1:1) mixture (100 ml) as was used to prepare the column in the experiments with rotenone. The alumina was then air-dried and the activity determined by measuring the distance travelled by the front of the Sudan-Yellow dye band in the BROCKMANN method of grading⁵. A control was conducted when the activity of the deactivated alumina was directly determined by the BROCKMANN method (see Table I). The preliminary washing of the

TABLE I
BROCKMANN GRADING OF STORED "DRY" ALUMINA AFTER DEACTIVATION

Age of "dry" alumina (days)	1	1 ¹ / ₂	3	5	7	10
Distance travelled by dye-front (cm)						
Unwashed	3.5	3.2	3.3	3.9	3.6	3.3
Washed	3.2	3.4	3.0	3.3	3.8	3.3

alumina with the ether-petroleum ether solvent did not appreciably increase its activity towards reversible adsorption of Sudan-Yellow, and furthermore the activity was independent of the age of the "dry" alumina.

The rotenone was readily eluted from the column and not subjected to the more fully exposed alumina surface to be expected after the passage of all the solvent used in the experiment (500 ml). It seemed of interest to amplify the previous experiment to see how a more fully exposed alumina surface affected the reversible and irreversible adsorption process being studied.

Rotenone was passed down a freshly-used column from which a 89% recovery of rotenone had been noted in the usual manner. All of the rotenone was irreversibly adsorbed on the column. Control experiments without the rotenone showed that the

BROCKMANN grading of the alumina could have differed only slightly before and after the first batch of rotenone was passed down the column since the distances travelled by the Sudan-Yellow dye were 3.7 and 3.3 cm respectively.

The amount of alumina surface exposed by the solvent in the first run was sufficient to considerably affect the irreversible adsorption of rotenone but insufficient to appreciably affect the average activity of the alumina as determined by the BROCKMANN method.

It can be concluded from this work that whereas reversible adsorption processes are unaffected by the changes occurring in "dry" alumina on storage, the chromatographic recoveries of small quantities of materials which are labile to alumina may be profoundly affected when dry strongly hydrophilic solvents are being used.

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A simple elution device for the automatic stepwise chromatography of lipids*

Several reproducible column chromatographic techniques are available which employ discrete changes in mobile phase composition for the analysis of lipid extracts. However, the frequent attention of the operator is required for the addition of each discrete mobile phase. Also, inconsistency in the addition of the phase solvents may decrease the reproducibility of these methods.

A simple reservoir apparatus was designed for use in conjunction with an automatic fraction collector to facilitate the use of these methods. This device is best employed with a chromatographic system requiring five or less mobile phase changes.

The different solvent phases are placed into each numbered reservoir, as illustrated in Fig. 1. The reservoirs are filled in reverse order, starting with number 5. If all five reservoirs are not needed for the system utilized, the remaining unused upper reservoirs can be left empty without affecting the efficiency of the device. After the required reservoirs are filled, the center capillary tube is cleared of mixed solvents by opening the stopcock carefully to allow flushing of the tube with solvent from the uppermost reservoir. The device is then connected to the column so that the standard taper outlet enters onto the column. When the stopcock is opened, the mobile phase

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