

CHROMATOGRAPHIC BEHAVIOR OF SOME QUINONES AND RELATED COMPOUNDS

By

SELMA BANDEMER

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## CHROMATOGRAPHIC BEHAVIOR OF SOME QUINONES AND RELATED COMPOUNDS

Chromatographic separation, since it was first described by Tswett in 1906, has become one of the most important techniques for the resolution of mixtures, and, in many cases, the only one. The resolution of stereo-isometric mixtures into individual isomers, the isolation of new compounds from mixtures supposedly pure, the concentration of substances which occur only in trace amounts in materials, and the purification and check for purity in the standardization of commercial products are well known applications of chromatography.

The literature on chromatography has become very extensive in the past few years, but relatively few references are found regarding quinones and their related products. Isomeric nitrophenols have been separated on columns of alumina and calcium carbonate, where these compounds produced bands in the order of their adsorbability- para, meta, and ortho, with the first being the greatest (22). The separation and purification of Vitamin K<sub>1</sub>, (2-methyl-3-phytyl-1,4-naphthoquinone) (6,8) and the isolation of phthiocol (2-hydroxy-3-methyl-1,4-naphthoquinone) by Wagner-Jauregg (36), were accomplished chromatographically from the crude products. Both the synthetic and natural tocopherols, which are considered as derivatives of hydroquinone, have been purified and separated into the alpha, beta, and gamma isomers by chromatography. (13, 14, 15, 20, 21, 36). Boletol, isoboletol, and chrysophanol, all anthroquinones, were separated from their crude products by this procedure (50).

Most of the chromatography with the quinones and their related hydroxy compounds has been done with the addition of easily removable groups, generally chromatophoric in nature. W. Bielenburg and co-workers (2, 3, 4, 5) separated a great many hydroxybenzenes, such as phenol, catechol, resorcinol, phloroglucinol, cresols, para-xyleneol, carvacrol, thymol, guaiacol, and numerous others, by preparing the diazonium chloride of the para-nitroaniline of each one and then chromatographing on alumina, flolidin XXF, and tonsil A. C. The original hydroxybenzene was regenerated after separation was effected. Separation of resorcinol, catechol, and phloroglucinol has been accomplished by treating the aqueous solutions with ferric chloride and chromatographing the deeply colored solutions (50). Mono- and di-acetyl ethers of duroquinone were separated on an alumina column from a benzene solution, the mono-ether being adsorbed by the column, the di-ether passing into the percolate (19). Ruggli and Jensen found the sodium salt of 2-hydroxy-naphthol-4-sulfonic acid more strongly adsorbed than the corresponding salt of 1-hydroxy-naphthol-4-sulfonic acid, and the same order was true for the dye derivatives of alpha and beta naphthol (28,29).

The structure of the molecule is very important, since adsorption affinity appears to be dependent upon its shape and the presence or absence of certain groups in the compound. Such groups are called functional groups, and their position and number profoundly affect the adsorption of a compound. In order to systematize the methods of chromatographic analysis, more information relating the behavior of a compound to its chemical constitution is required. Therefore, the following

problem was undertaken with the hope of contributing additional information to this type of analysis.

#### Materials and Experimental Procedure

The solvents used for this project were hexane, absolute alcohol, and distilled water. Skelly-solve B, procured from the Skelly Oil Co., Bartlesville, Okla., was used as a source of hexane. It was purified by passage thru a large Tswett tube, 120 cms. long and 4 cms., wide, containing freshly ignited silica gel, and the percolate was collected in 2-300 ml. lots. Each lot was examined separately for transparency down to 230 millimicrons on a Beckman spectrophotometer and a transparency of 96 per cent was considered satisfactory. Approximately one liter of hexane could be obtained from one charge. The alcohol was obtained in five gallon lots from the Stores Department of Michigan State College, and redistilled before use. The resulting product had an acceptable transparency.

Various mixtures of these solvents, used for adsorption and elution are indicated in the tables and figures by the numbers in the brackets following the statement of the solvent mixture. Thus hexane-ethanol (98-2) means two per cent by volume in the hexane.

The adsorbents were activated alumina, florisil, magnesium silicate, and anhydrous sodium sulfate crystals. The alumina was the Alcoa brand minus 80 mesh, produced by the Aluminum Ore Co., East St. Louis, Ill. The activity was not determined, as the same lot was used for all the experiments. The florisil, 60/100 mesh was supplied by the Floridin Co.,

Warren, Pa. The magnesium silicate was furnished by the Philadelphia Quartz Co., Berkeley, Cal. The anhydrous sodium sulfate crystals were purchased from the J. T. Baker Co., Phillipsburg, Pa.

The adsorption apparatus originally used was the simple type Tswett tube. After a few preliminary experiments, this was abandoned in favor of a Hennessy tube, having a 25 ml. reservoir, and a six inch tube, with a capillary tip, obtained from Wilkens-Anderson Co., Chicago. The advantage of this tube over the older type was the very small quantities of adsorbent required to provide a column of adequate length. The tubes were filled in the following way: small glass wool plugs were tamped down, the adsorbent was added in small portions, the tube tapped on the table to pack the material after each addition, and a small glass wool plug placed on top. The reservoir was wiped clean of any adhering adsorbent. The amount of adsorbent used was not measured, but in as much as the tubes were filled to approximately the same level each time, the variation in the different tubes was slight. They required approximately one gram of florisil and 1.6 grams of alumina.

Because preliminary experiments showed that many of the compounds being chromatographed were affected by light, the procedures were carried out in low actinic glassware, preferably the red glass of the Corning Glass Co., where it was available. Holders for the Hennessy tubes were made by cutting the bottoms from large brown bottles with narrow necks, into which the tubes were set. Covers for the reservoirs extending above this neck, were made by cutting the tops from smaller brown bottles, and placing them over the tube and neck of the large container. Similarly,

large bottles from which the bottoms had been removed, were stoppered and served as bell-jars, under which light sensitive substances could be kept.

The liquid chromatogram was used, and the filtrate was collected under the brown jar in volumetric flasks into which the capillary tip of the Hennessy tube had been fitted. Either ten or twenty-five ml. glass-stoppered flasks were used. The adsorption spectrum was obtained for each fraction in order to determine the amount of elution of the adsorbate and to observe any changes in structure that might have occurred during adsorption. Suitable blanks were made in exactly the same way, using the same procedure with the solvents alone.

Absorption curves for all the compounds in the various solvents used were obtained by plotting the wave-length against the extinction values determined with the Beckman quartz spectrophotometer, for the range of 230-300 millimicrons, using quartz cells. Concentration curves were made at the wave-length showing maximum absorption by plotting the extinction values obtained for various concentrations against those concentrations. A straight line was obtained, from which concentrations of unknown solution strength could be read.

The compounds chromatographed were: menadione(2-methyl-1,4-naphthoquinone), phthiocol(2-hydroxy-3-methyl-1,4-naphthoquinone), both from Merck and Co., New York, and used without additional purification. The other compounds manufactured by the Eastman Kodak Co., Rochester, N. Y., were recrystallized from hexane: alpha-naphthol, beta-naphthol, ortho-, meta-, and para-dihydroxybenzene, 1,2- and 1,4-naphthoquinone, 1,3-naphthoresorcinol, and quinone.

## Results

1. alpha-naphthol. This product was completely adsorbed from a hexane solution by florisil and alumina, and was not eluted from either column by washing with this solvent. The addition of one per cent ethanol produced a very rapid elution from florisil and a slightly slower one from alumina (Table I). In both cases, practically complete recovery was obtained. No change was observed in the absorption curve of the naphthol, following adsorption and subsequent elution, when it was compared to that obtained with the same solvent (Fig. 1).

2. Beta-naphthol. The behavior of this compound was almost exactly the duplicate of the alpha-naphthol, except the elution occurred more slowly from both florisil and alumina (Table I). No change in the absorption curve of the eluted material compared to that of the naphthol in the same solvent was noted (Fig. 2).

3. Ortho-dihydroxybenzene or catechol. This compound was readily adsorbed from both activated alumina and florisil. It was very slowly eluted from florisil by hexane containing one per cent ethanol, but much more rapidly when two per cent was used. When the hexane contained five per cent ethanol, about 80 per cent of the adsorbed material was removed in the first volume (Table 2). The recovery was practically complete, and no change was found in the compound, as shown by the absorption curve (Fig. 3). Catechol was slowly eluted from alumina with hexane-ethanol mixtures, more rapidly with ethanol, but very rapidly if five per cent water was added. The compound was not changed by the alumina.



It was observed that there was a slight variation in the absorption curves of the freshly prepared solution of catechol in hexane, and the same solution after it stood for several weeks, (Fig. 4). The minimum at 280 and the slight maximum at 282 millimicrons of the freshly prepared solution disappeared and only a wide shoulder remained. The effect of light was excluded, since the solution was kept in red glassware.

4. Meta-dihydroxybenzene or resorcinol. Resorcinol is completely adsorbed from hexane and hexane containing one per cent ethanol by florisil and alumina. It was eluted from florisil with hexane containing two per cent ethanol, but more slowly than catechol; when the concentration of ethanol was increased to five per cent, the elution was much more rapid, but still less than that for catechol (Table 2). No elution was obtained from alumina with any ethanol-hexane mixture tried, but ethanol-water (five per cent) mixtures proved effective eluants. The compound was not changed by adsorption and subsequent elution as evidenced by the absorption curves, (Fig. 5).

5. Para-dihydroxybenzene or hydroquinone. Because of the slight solubility of hydroquinone in hexane alone, ethanol was added to make its concentration in hexane two per cent, thus insuring that an adequate amount of hydroquinone was in solution. If less than this amount was used, it was observed that long needle-like crystals extending out into the solution from the sides of the container, appeared on standing. Examination showed these to be pure hydroquinone, which did not darken on exposure to light as rapidly as did the original solution. This method was used to obtain pure hydroquinone for the experiments.

Hydroquinone that has been adsorbed on florisil, magnesium silicate, or sodium sulfate crystals, was readily eluted by washing the column with the solvent mixtures. The compound was unaltered as shown by the absorption curves (Fig. 6). The amount adsorbed from solution and the volume of solvent required for elution depended on the ethanol concentration. With florisil, practically all the hydroquinone was removed from a two per cent ethanol solution and it required 100 ml. of this solvent for elution. If the ethanol concentration was increased to five per cent, only 40 ml. were required (Table 3). With sodium sulfate crystals, magnesium silicate, and super-filtrol (Johns-Manville Co.), very little was retained from a solution containing five per cent or more ethanol, and this little was eluted with the first volume of eluant.

When activated alumina was used as the adsorbent, the results were entirely different. The hydroquinone was completely adsorbed from the two per cent ethanol solution, none appearing in the percolate. As the column was washed with the solvent mixture, however, the eluate was found to contain some substance with an absorption curve entirely different from that of the hydroquinone. With the seventh volume of the solvent mixture, very little of this material was extracted, and the total amount thus recovered represented about 18 per cent of the total amount adsorbed. The column was then eluted with ethanol. When this eluate was examined with the spectrophotometer, it was found to be hydroquinone. Similar results were obtained with an eluant containing five per cent ethanol, (Table 4). If, after the hydroquinone had been adsorbed on alumina, it was eluted with a solvent mixture containing twenty per cent or more

ethanol, then only hydroquinone was found in the eluate, according to spectrophotometric analysis, (Fig. 7).

6. Quinone. After a number of compounds had been examined, it was determined that this unidentified substance eluted from the alumina column on which the hydroquinone had been adsorbed, was quinone. In Fig. 7, it is seen that the substance eluted from the alumina has an identical absorption curve to that of quinone. A series of experiments was then carried out with quinone, similar to those with hydroquinone. It was determined that if the hexane contained two per cent or more ethanol, quinone was eluted as such readily from florisil and sodium sulfate crystals. If the eluant contained but one per cent ethanol, then only about 75 per cent of the quinone could be eluted from the florisil. In order to recover the remaining 25 per cent, ethanol was used as the eluant. When examined spectrophotometrically, this fraction was found to be hydroquinone, (Fig. 8).

Analogous results were obtained with alumina. Adsorbed quinone was eluted with mixtures containing 25, 50, 75 per cent ethanol in hexane, and ethanol. As the ethanol concentration increased, the absorption curve gradually changed its shape from that characteristic of quinone to that of hydroquinone, (Fig. 9). An alcoholic solution of quinone was percolated thru an alumina column; the percolate had a hybrid-shaped absorption curve, but that of the ethanol eluate was almost identical to that of hydroquinone, except the maximum was still slightly displaced towards the left (Fig. 10).

7. 1,2-naphthoquinone. This compound was completely adsorbed from hexane by florisil, sodium sulfate crystals, and alumina. As the

concentration of ethanol in the solution increased, the amount adsorbed decreased. The material eluted from florisil and sodium sulfate had the same absorption curve as the original solution, showing the material was unaffected by adsorption (Fig. 11). The eluate from alumina did show a slight difference, in that the maximum at 251 millimicrons was depressed below that at 246, and was rounded rather than sharp as in the original (Fig. 12).

8. 1,4-naphthoquinone. This compound behaved very much like the preceeding one. It was adsorbed from hexane by florisil, sodium sulfate crystals, and alumina, but not by the first two, if two per cent ethanol was present in the hexane. Naphthoquinone eluted from florisil with hexane containing two per cent ethanol was unchanged, except its adsorption curve was flattened at 241 millimicrons, (Fig. 13). This same flattening was also observed in this region when the compound was dissolved in hexane which contained four per cent ethanol (Fig. 14).

If this solution containing two per cent ethanol was chromatographed on alumina, 81 per cent was recovered in the percolate, and was unchanged by its contact with the alumina, as it passed thru the column. The portion adsorbed by the column was eluted by additional volumes of the solvent. The absorption curve of this eluate was considerably changed, indicating that this compound had undergone some changes in constitution, similar to that experienced by hydroquinone. When the elution was carried out with higher concentrations of ethanol, the absorption curve tended to be more like the original curve, (Fig. 15).

9. 1,3-naphthoresorcinol. Like the previous compounds, this one was also completely adsorbed from hexane by florisil, sodium sulfate crystals,

and alumina. It was eluted from florisil and sodium sulfate with hexane containing two per cent or more ethanol without change.

It was also completely adsorbed from a hexane solvent containing high concentrations of ethanol by alumina, and was not eluted by any of these mixed solvents, until the ethanol concentration was 90 per cent, and then the elution was slight. With ethanol, the elution increased somewhat, but if 10 per cent water was added, the elution was rapid and complete. The absorption curve for this eluate was very different from that of the original solution, but very similar to that of 1,3-naphthoresorcinol in 90 per cent ethanol, (Fig. 16). An aqueous solution of this compound was percolated thru an alumina column. Some of the material passed thru into the percolate, and had experienced some change on contact with the alumina, (Fig. 17). The adsorbed material eluted by additional volumes of water, showed even greater changes and closely resembled the eluate obtained from alumina with ethanol.

10. 2-methyl-1,4-naphthoquinone or menadione. When alumina was the adsorbent, this compound was removed from hexane, or hexane containing one per cent ethanol. If more than one per cent ethanol was present in the solution, the material passed thru the column and appeared in the percolate. This same concentration of ethanol was sufficient to elute any of the naphthoquinone that had been adsorbed on the alumina. The absorption curve of the adsorbed and eluted compound was identical with that of the original solution. (Fig. 18). The same result was obtained with florisil, sodium sulfate, and magnesium silicate.

11. 2-hydroxy-3-methyl-1,4-naphthoquinone or phthiocol. This compound was completely adsorbed by florisil, sodium sulfate, magnesium silicate, and alumina, from hexane and all hexane-ethanol mixtures tried up to ethanol alone. It was completely eluted from florisil and sodium sulfate by ethanol, and the absorption curve of the eluate was the same as that of the original material, showing it had not been changed by adsorption and elution. It was eluted very slowly from alumina with ethanol, but rapidly if 10 per cent water was added, or if a one per cent ammonium hydroxide solution was used as an eluant. The absorption curve of the aqueous eluate was entirely different from that of the original hexane solution, but was identical with the ammoniacal solution of the compound, (Fig. 12). Magnesium silicate behaved the same way.

The spectrophotometric determination of this eluate can be made in two ways. Either the extinction can be determined directly on the alkaline eluate and compared to a curve obtained on a solution made by shaking out the original hexane solution with a definite volume of one per cent ammonium hydroxide; or the alkaline eluate can be slightly acidified, extracted with hexane, which recovers the naphthoquinone quantitatively and unchanged, and the extinction determined on this solution.

It has generally been recommended that the column be wetted with the solvent used for the solution that is to be chromatographed. Some experiments were conducted to determine the effect of this prewetting. Definite volumes of a solution such that the solute was completely adsorbed, were percolated thru a prewetted and dry column of the adsorbent. The adsorbed material was then eluted with a suitable solvent and collected in volumetric flasks (10 ml.). The concentration of the eluted material in each

fraction was determined spectrophotometrically. There was no difference in the total amount eluted, but the distribution in the different fractions varied greatly (Table 5).

### Discussion

The liquid chromatogram was used to determine the behavior of eleven compounds on several different adsorbents, and the course of adsorption and elution was followed with the Beckman quartz spectrophotometer. The compounds studied were: alpha- and beta- naphthols, ortho-, meta-, and para-dihydroxybenzene, quinone, 1,2- and 1,4-naphthoquinone, 1,5-naphthoresorcinol, 2-methyl-1,4-naphthoquinone, and 2-hydroxy-3-methyl-1,4-naphthoquinone. The adsorbents were florisil, sodium sulfate crystals, magnesium silicate and activated alumina.

When alpha and beta naphthols were adsorbed from hexane on florisil, they were completely eluted by hexane containing one per cent or more ethanol, without any change. The same was true for these compounds adsorbed on alumina, but the elution was somewhat slower (Fig. 1, 2). The beta form was adsorbed more strongly than the alpha, and thus was eluted more slowly; in other words, more volumes of eluant were required to elute it completely (Table I). This greater adsorbability is due to the substitution of the hydrogen in the beta position. It has been shown by previous workers (28, 29), that if a hydroxyl or amino group occupy the beta position, salts of the naphtho-4-sulfonic acid are more strongly adsorbed than if such groups are in the alpha position. This same relationship is carried over into the dye derivatives of these alpha and

beta compounds. Such a difference could be made the basis of a chromatographic separation of these compounds and their derivatives.

The three dihydroxybenzenes were completely adsorbed by florisil and alumina from hexane and hexane containing one per cent ethanol. The ortho and meta forms were eluted from florisil by two per cent ethanol in hexane, the ortho compound readily, the meta considerably less so, (Table 2). Neither compound was changed by adsorption and elution (Fig. 3, 5). The para compound, on the other hand was completely adsorbed from the two per cent ethanol solution, but was completely eluted, if a sufficient volume of the solvent was used. This adsorbability is in direct relationship to the di-pole moments of the three compounds, as suggested by Arnold (1), with the ortho form having the smallest, and the para, the greatest (23). Under such conditions, separation of the three isomers could be obtained.

When alumina was used as the adsorbent, this relationship no longer held. Catechol (ortho) and resorcinol (meta) were eluted as such (Fig. 3, 5), from alumina with the proper eluant (ethanol for the former, 95 per cent ethanol for the latter). Hydroquinone (para) experienced some change on adsorption and elution. As is seen from the absorption curves (Fig. 7), when hydroquinone was eluted from alumina with a non-polar solvent, containing just sufficient ethanol to produce elution, it was recovered as quinone. As the concentration of ethanol was increased, the absorption curve changed in character, until, when ethanol alone was used as the eluant, the curves showed that hydroquinone had been eluted (Fig. 7). If, however, the hydroquinone was percolated thru an alumina column



containing just sufficient ethanol to prevent adsorption, then the percolate contained hydroquinone as such. This indicates that contact with alumina alone is not sufficient to produce this shift from hydroquinone to quinone; there must be adsorption and subsequent elution, with a non-polar, or at least, a very slightly polar solvent.

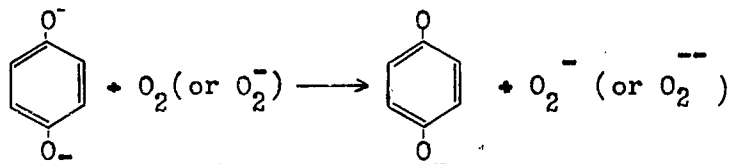
Quinone likewise experienced similar changes. When it was adsorbed on florisil or alumina, and eluted with solvents containing low concentrations of ethanol, it was recovered as quinone (Fig. 8). When the eluant contained increasing amounts of ethanol, the absorption curves of the eluates more and more approached that of hydroquinone, until with ethanol alone, the curve was almost identical with it, (Fig. 9). When an alcoholic solution of quinone was percolated thru an alumina column, the percolate, which had had only slight contact with the alumina, shows a hybrid-type curve, which was neither that of quinone nor hydroquinone. The ethanol eluate on the other hand had almost the true hydroquinone curve (Fig. 10). Florisil reacted similarly with quinone (Fig. 8).

It is not known at present whether these hybrid curves resulted from mixtures of quinone and hydroquinone, or were the absorption curves of some entirely different substances. However, quinhydrone which is composed of one molecule each of quinone and hydroquinone, did not show this hybrid characteristic, but rather had the two distinct portions of the absorption curves of both (Fig. 20). On this basis, it is felt the curves represent separate compounds.

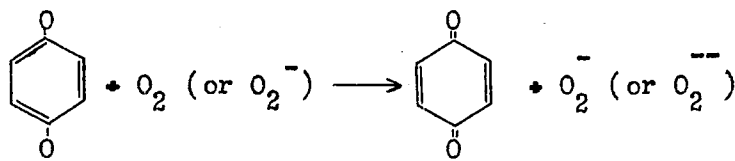
The mechanism whereby this change occurs in solution has been investigated by Weissberger and co-workers (17, 18, 27, 39), and is considered

to be as follows: hydroquinone  $\rightleftharpoons$  semiquinones  $\rightleftharpoons$  quinone. This autoxidation proceeds in two steps.

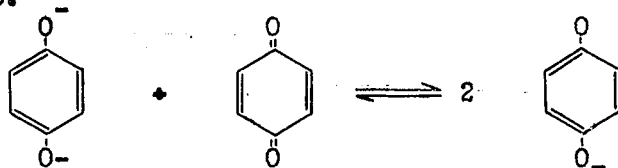
1. The divalent hydroquinone ion loses an electron and yields a semiquinone ion.



2. The semiquinone ion gives off an electron, forming the quinone.



3. The semiquinone ion is also formed by the reaction of hydroquinone ion with quinone.



This reaction occurs in an alkaline solution with molecular oxygen, which in this case the alumina could provide. It is known that alumina contains about one per cent sodium oxide, and it has been suggested that this is responsible for some of the reactions occurring on the alumina(7). Other workers (30, 31) have reported that that part of the alumina important for adsorption can not be leached out with water. Washing the column first with the solvent did not prevent this change, and it has been shown that the compound must be adsorbed by the alumina before the change can take place, (Fig. 7)

Only a certain amount of the hydroquinone was changed into the quinone, and was about 18 per cent, as shown in Table 4. The other

reaction product was either water or hydrogen peroxide, both of which are adsorbed by alumina. It had been shown that alumina did not decompose peroxides nor change them into other products, merely adsorbed them (11).

As Weissberger et al (17, 18, 27, 39) have shown, the rate of oxidation decreased as the concentration of ethanol increased. This then, would favor the formation of hydroquinone, as the concentration of ethanol was increased in the solution or eluate.

A second possible explanation for this phenomenon is offered by Weitz (40, 41). Many compounds serve as donors of unshared electrons to the atoms of the adsorbent possessing incomplete octets. The formation of color upon adsorption from a colorless solution has been explained on this basis. The molecule thus adsorbed undergoes extensive polarization and forms a strongly resonating structure, which could react with adsorbed oxygen. It should be emphasized that such changes are found only in the para forms, the ortho and meta forms not being affected in this way. The 1,4-naphthoquinone experienced a similar change, which did not appear in the ortho form nor in the 1,3-naphthoresorcinol. The change did not appear with the 2 methyl, and the 2-hydroxy-3-methyl-1,4-naphthoquinones, because of the substitution of the hydrogen on the nucleus. It has been reported that the autoxidation of hydroquinone is due to the activity of the hydrogen atoms attached to the nucleus, but disappears if other groups such as methyl are substituted (18).

Many interactions between adsorbant and adsorbate are known. To cite a few of the more recent ones, there is the conversion of 3-formoxy

steroids to 3-hydroxy steroids, which proceeds quantitatively (9, 19, 25, 26); the production of styrene in yields up to 86 per cent by the vapor-phase dehydration of methyl-phenyl-carbinol (38); both with alumina columns. The interconversion of the stereo-isometric stilbenes on neutral filtrol to the extent of 11 per cent (48, 49); the isomerization of olefins by silica gel (16); the almost complete isomerization of pinene to polytarpinene on Fuller's earth (12) show that this reaction is not found on alumina columns alone, other adsorbents also possess this quality. In general, the elimination of alcohol, water, or a hydrocarbon radical, the liberation of a base from a salt, the conversion of one salt into another, the splitting of addition compounds dimerization, and polymerization are some of the more common changes that occur on chromatographic columns (32, 33, 34, 35, 42, 43, 44, 45, 46, 47, 50).

When 1,2-naphthoquinone was chromatographed on both florisil and alumina with low concentrations of ethanol in solution, the percolate then contained most of the compound, and the rest was easily eluted, unchanged, with the first volume of solvent (Fig. 11). The only change observed in the absorption curve of this compound was a lowering and rounding off of the maximum at 250 millimicrons by passage thru the alumina column (Fig. 12).

The 1,4-naphthoquinone adsorbed from hexane by florisil, was eluted unchanged except for the flattening of the curve at 241 millimicrons (Fig. 13). A four per cent ethanol in hexane solution of this naphthoquinone showed the same flattening in this area, and after adsorption and elution, it had no further change (Fig. 14). When this solution was

passed thru an alumina column, the percolate also had this slight flattening; but the adsorbed material eluted from the column by hexane with low concentrations of ethanol was altered to the extent that the absorption curve was shifted towards the shorter wave-lengths (Fig. 15). As the concentration of ethanol in the hexane increased, the curve more nearly approached that of the material.

The 1,3-naphthoresorcinol was adsorbed from hexane solution by florisil and sodium sulfate crystals, and eluted therefrom by hexane containing two per cent ethanol without change. Its adsorbability on alumina was so great that it could not be eluted with any hexane-ethanol mixtures, and only slightly with ethanol. If the ethanol contained 10 per cent water, the elution was rapid and complete. The absorption curve for this eluate varied greatly from that of the original solution, but agreed very well with that of a 90 per cent ethanol solution, (Fig. 16).

Aqueous solutions percolated thru alumina experienced some change on contact, but the change was more pronounced after adsorption and elution (Fig. 17).

This compound experienced considerable change, when kept in solution for any length of time, altho not exposed to light. The differences in the absorption curves of the compound dissolved in hexane, are shown in Fig. 21. The curve of the aged solution approaches that of the dihydroxybenzenes, with the maximum in the region above 270 millimicrons, but the curve of the freshly prepared solution is very similar to that of quinone.

When ethanol was employed as the solvent, this difference in the absorption curves is still present. Whereas the freshly prepared solution

has one maximum at 236 millimicrons, the aged solution shows two - one at 231, the other at 269 millimicrons, (Fig. 22).

It is not known at present what causes this change, but it is very probably that it is similar to that occurring with hydroquinone in solution, with a shift from the quinone to the resorcinol form.

The 2-methyl-1,4-naphthoquinone was adsorbed from hexane by all the adsorbents tried, but if the solution contained five per cent or more ethanol, this compound appeared in the percolate unchanged (Fig. 18). The adsorbed material was easily eluted with solvents containing this amount of ethanol.

The 2-hydroxy-3-methyl-1,4-naphthoquinone was adsorbed by all the adsorbents tried even from solutions with high concentrations of ethanol. It could be eluted from alumina only with 90 per cent ethanol or dilute ammonium hydroxide (37). The eluted material had an entirely different adsorption curve from the original, but was the same as the curve for the dilute alkaline solution (Fig. 19). The material adsorbed by florisil was eluted unchanged with ethanol.

2-hydroxy-3-methyl-1,4-naphthoquinone is very stable as compared to the 2-methyl-1,4-naphthoquinone. It can be exposed to strong sunlight for long periods without showing any change in the absorption curve. On the contrary the 2-methyl compound is extremely sensitive to light, and marked changes occur in the absorption, the amount of change varying with the amount of exposure.

This increased adsorbability of the 2-hydroxy-3-methyl-1,4-naphthoquinone must be due to the substitution of the hydrogen in the 3-position.

As was mentioned above, 1,3-naphthoresorcinol was adsorbed more strongly than the 1,2- and 1,4-naphthoquinones, and the meta-dihydroxybenzene more than the ortho- and para- isomers. Vitamin K<sub>1</sub>, which is 2-methyl-3-phytyl-1,4-naphthoquinone could not be recovered from alumina (8), although the 2-methyl-1,4-naphthoquinone was not adsorbed at all, if small concentrations of ethanol were present in the solution. Thus it appeared that the substitution of the hydrogen in the 3-position in the ring is responsible for this increased adsorbability, and not the particular group that is substituted.

The pretreatment of the column was determined to be of little importance. For one adsorbent, however, the total amount of adsorbate eluted was the same, but the distribution in the different fraction of the eluate was very different, (Table 5).

All these experiments were conducted at room temperature, since it has been shown that the rate of movement in a chromatographic column is practically independent of the temperature at temperatures from 25°-35° C.(24).

It should also be mentioned that anhydrous powdered sodium sulfate used to dry non-aqueous solutions showed no adsorptive capacity for any of the compounds used.

#### Summary

The adsorption behavior of some quinones and their related compounds on several adsorbents using liquid chromatographic procedure has been investigated by spectrophotometric methods.

It has been shown that the behavior of these compounds is determined by their structure. Beta-naphthol has a greater adsorption than the alpha-naphthol; para-dihydroxybenzene has a greater adsorbability than the ortho and meta isomers; the substitution of the hydrogen in the 3-position in the ring by other groups, increased the adsorbability over the unsubstituted; and hydroquinone and benzoquinone, adsorbed on alumina, shift between these two forms when eluted, depending on the concentration of ethanol in the eluant.

In general, adsorbents such as florisil, magnesium silicate, and anhydrous sodium sulfate crystals did not react with any of the compounds used with one exception. This exception was the shift of quinone to hydroquinone when eluted from florisil with ethanol after adsorption.

The same amount of adsorbate was recovered from a given adsorbent regardless of whether it was prewetted with the solvent or used dry, but the distribution of the adsorbate in the eluate varied.

Possible methods of separations of isomers of the compounds used have been suggested.



TABLE I

Elution from florisil and alumina with 10 ml. portions of hexane-ethanol (99-1) of alpha-naphthol (0.015 g./liter) and beta-naphthol (0.02916 g./liter)

Solvent Fraction Number	Florisil % eluted		Alumina % eluted	
	alpha	beta	alpha	beta*
1	97.60	0.38	0.50	0.07
2	2.00	95.00	37.00	0.11
3	0.20	2.00	49.20	0.12
4	0.00	1.00	13.50	37.50
5	0.00	0.38	0.00	31.50
6			----	8.00
7				4.50

\* Not carried to completion.

TABLE 2

Dihydroxybenzenes eluted from florisil by one 10 ml. portion of hexane-ethanol mixtures.

Hexane-ethanol mixtures, % by volume	% eluted		
	catechol (0.0496 g./l) ortho	resorcinol (0.042 g./l) meta	hydroquinone (0.0255 g./l) para
100-0	0.0	0.0	0.0
99-1	0.6	0.0	0.0
98-2	32.4	3.2	0.6
95-5	78.5	19.6	68.5

TABLE 3

Elution from florisil with 10 ml. portions of hexane-ethanol mixtures of hydroquinone (0.0255 g./l).

Solvent Fraction Number	% Hydroquinone eluted hexane-ethanol mixtures	
	98-2	95-5
1	0.6	68.5
2	8.3	31.4
3	16.9	0.0
4	26.9	
5	26.9	
6	13.6	
7	3.6	
8	1.1	
9	0.7	
10	0.2	
Total	98.8	99.9

TABLE 4

Hydroquinone (0.0255 g./l) adsorbed on alumina. Eluted with hexane-ethanol mixtures in 10 ml. fractions. Calculated as per cent hydroquinone, altho compound is quinone.

Number of eluate fraction	% hydroquinone recovered		
	Hexane-ethanol mixtures in % by volume		
	98-2	98-2	95-5
1	3.44	4.99	5.04
2	5.04	5.55	5.58
3	4.60	4.27	4.01
4	4.30	2.00	2.32
5	0.76	0.90	1.09
6	0.16	0.43	0.90
7	0.10	0.20	0.46
Total	18.40	18.29	19.40

TABLE 5

Effect of prewetting the adsorbent in the column on the elution of the adsorbate. 10 ml. eluate fractions. And % of material eluted

Adsorbate	Concentration g./liter	Eluate Fraction	Adsorbent					
			Florisil		Alumina		Na <sub>2</sub> SO <sub>4</sub>	
			wet	dry	wet	dry	wet	dry
Hydroquinone	0.0255	1	0.19	0.22	76.40	93.80		
		2	0.75	0.88	22.60	5.80		
		3	93.25	94.19	---	---		
2-methyl-1,4-naphthoquinone	0.02048	1	1.95	0.0	77.80	96.40	1.95	80.80
		2	70.80	1.95	3.60	1.95	95.75	8.70
		3	26.50	97.70	----	----	1.95	2.35

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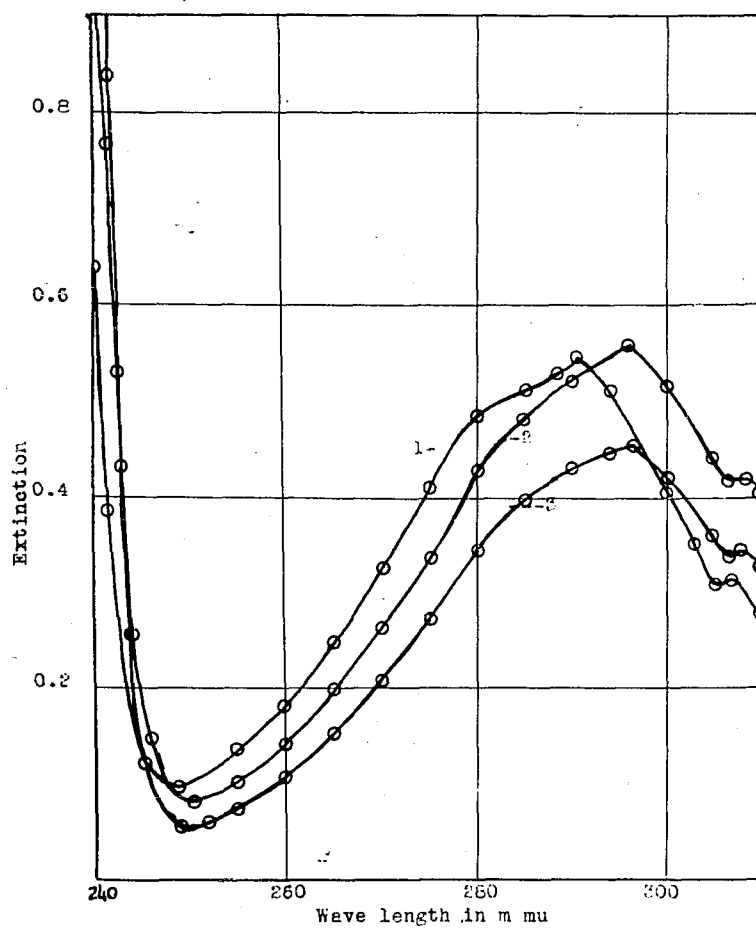


Fig. 1. Absorption curves for alpha-naphthol

1. Alpha-naphthol (0.01547 g./liter) in hexane
2. Alpha-naphthol (0.01547 g./liter) in hexane-ethanol (98-2)
3. Eluate of alpha-naphthol adsorbed on alumina obtained with hexane-ethanol (98-2)

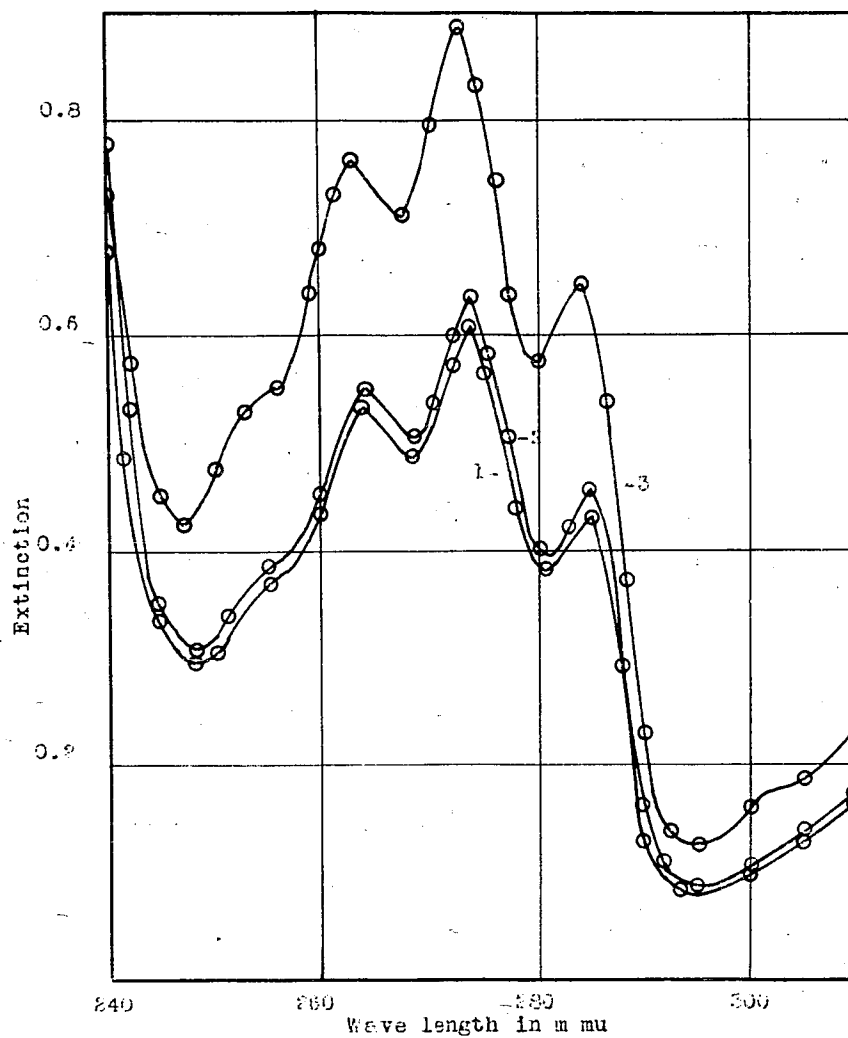


Fig. 2. Absorption curves for beta-naphthol

1. Beta-naphthol (0.02916 g./liter) in hexane
2. Beta-naphthol (0.02916 g./liter) in hexane-ethanol (98-2)
3. Eluate of beta-naphthol adsorbed on alumina obtained with hexane-ethanol (98-2)

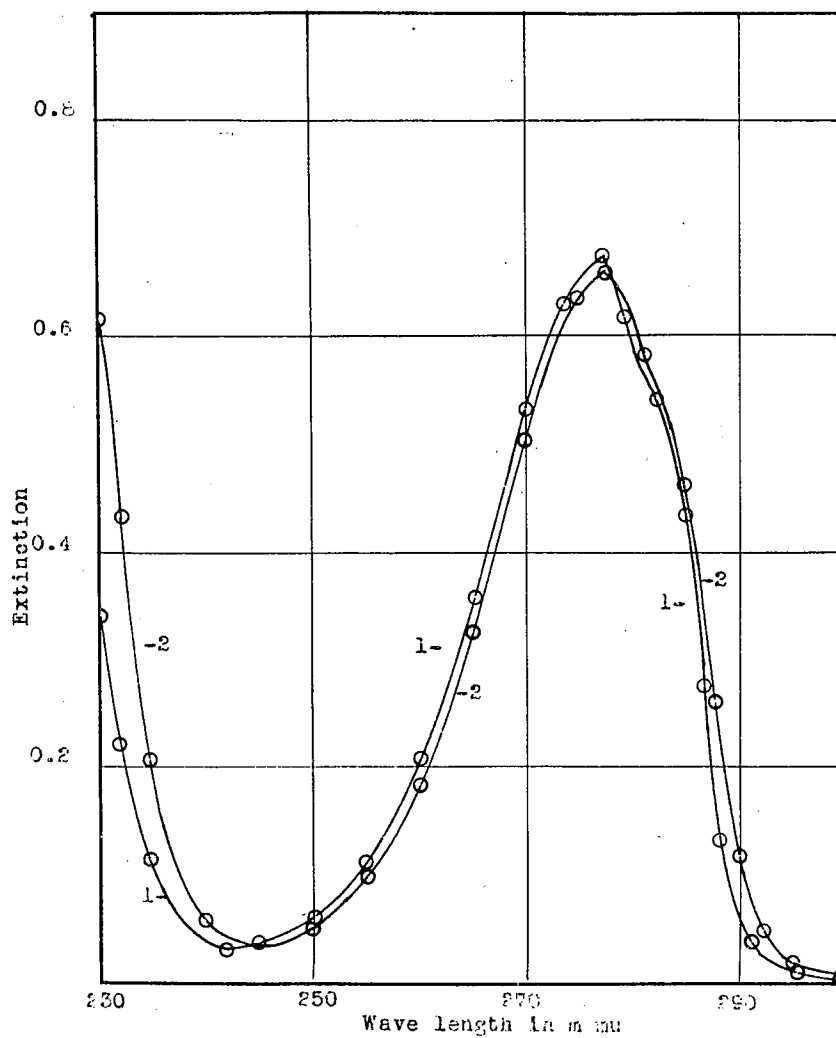


Fig. 3. Absorption curves for catechol

1. Catechol (0.0496 g./liter) in hexane-ethanol (95-5)
2. Eluate of catechol adsorbed on florisil, obtained with hexane-ethanol (95-5)

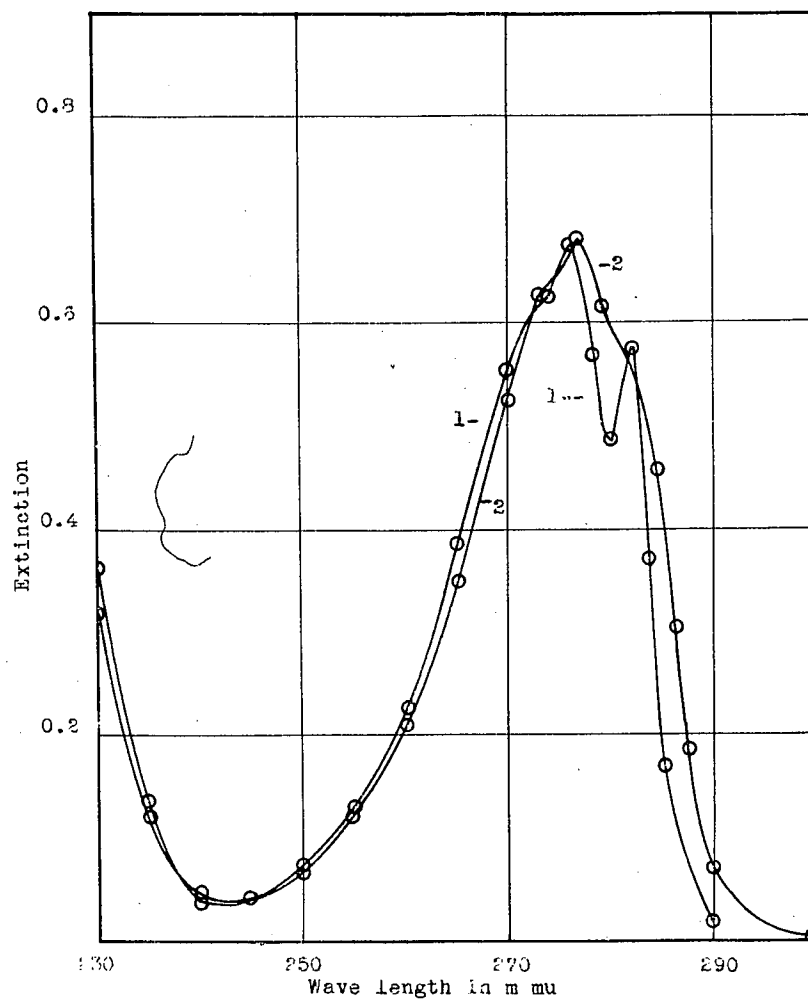


Fig. 4. Absorption curves for catechol

1. Catechol (0.0496 g./liter) in hexane, freshly prepared.
2. Same solution after several weeks storage

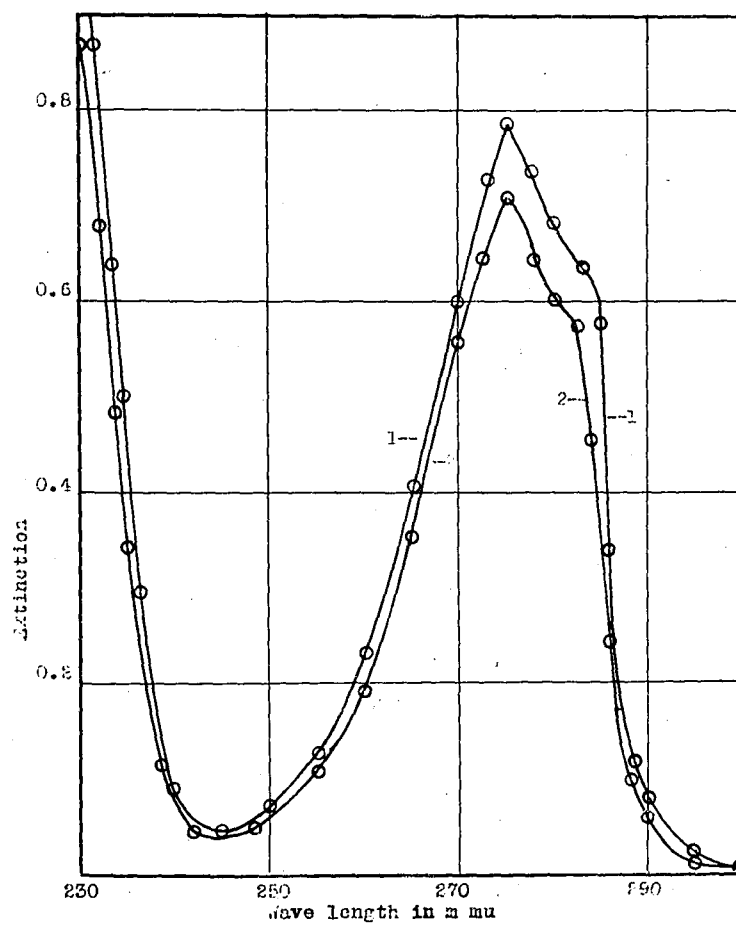


Fig. 5. Absorption curves for resorcinol

1. Resorcinol (0.042 g./liter) in hexane
2. Eluate of resorcinol adsorbed on florisil, obtained with hexane-ethanol (98-2)

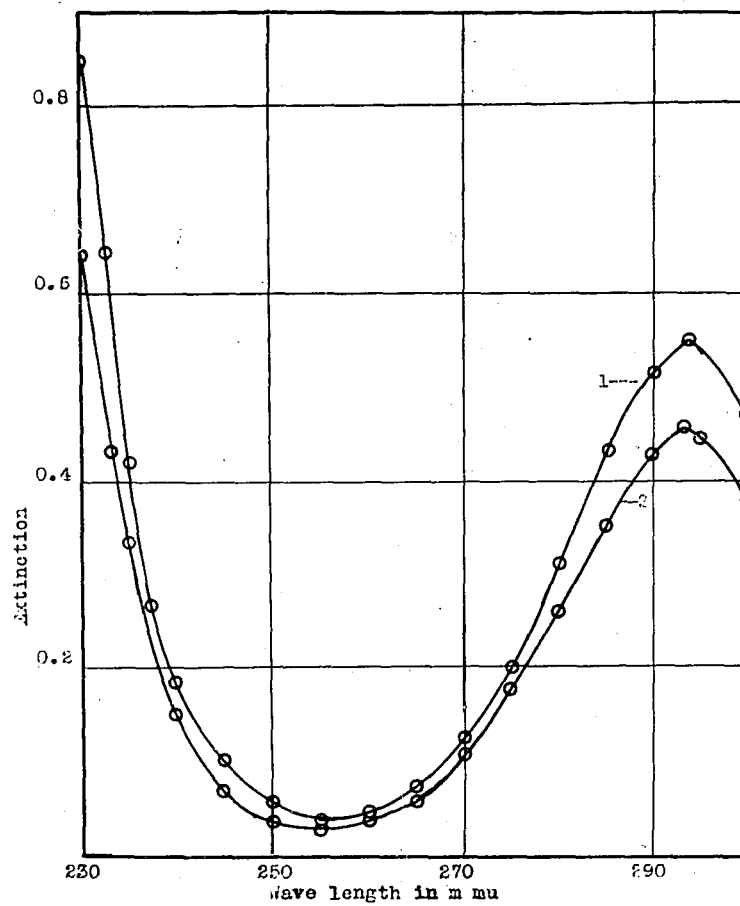


Fig. 6. Absorption curves for hydroquinone

1. Hydroquinone (0.0255 g./liter) in hexane-ethanol (98-2)
2. Eluate of hydroquinone adsorbed on florisil, obtained with hexane-ethanol (98-2)

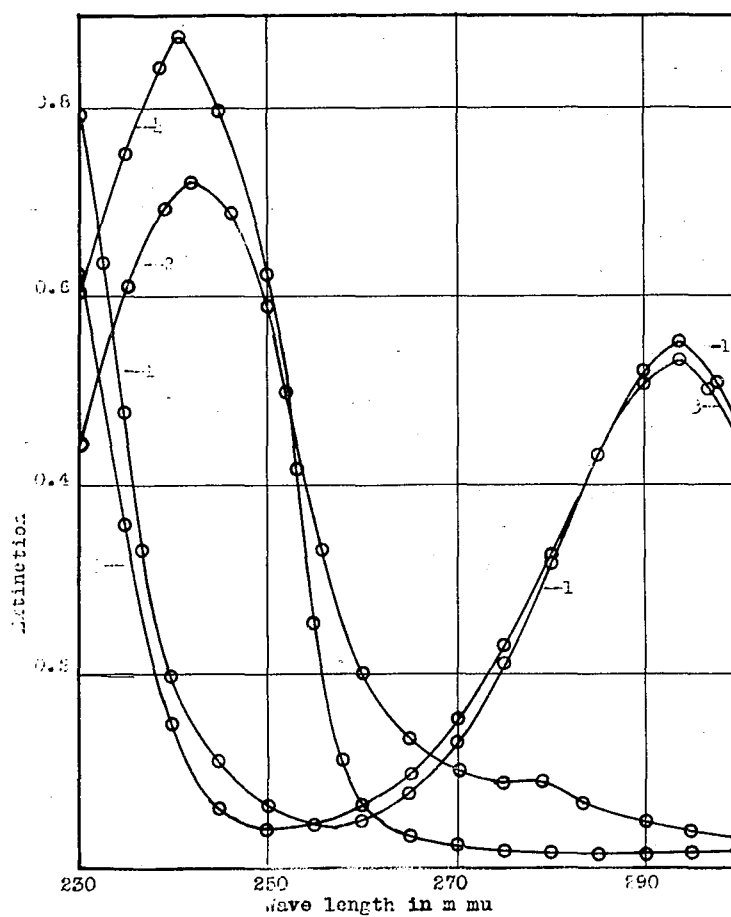


Fig. 7. Absorption curves for hydroquinone

1. Hydroquinone (0.0255 g./liter) in hexane-ethanol (98-2)
2. Eluate of hydroquinone adsorbed on alumina obtained with hexane-ethanol (98-2)
3. Eluate of hydroquinone adsorbed on alumina obtained with ethanol
4. Quinone (0.0049 g./liter) in hexane-ethanol (98-2)

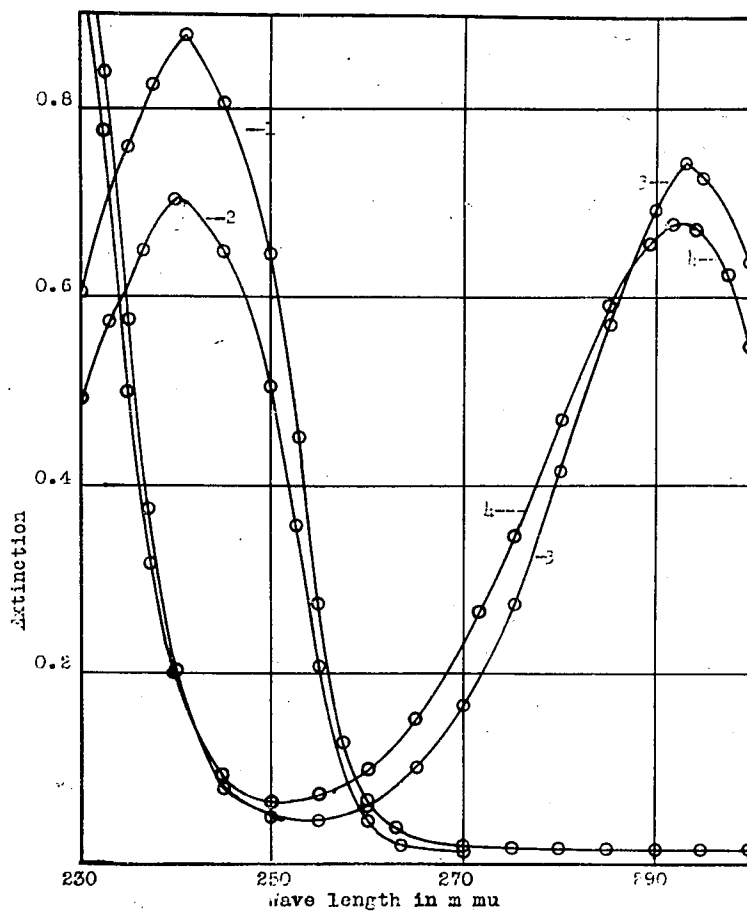


Fig. 8. Absorption curves for quinone

1. Quinone (0.0049 g./liter) in hexane-ethanol (99-1)
2. Percolate thru florisil of quinone in hexane-ethanol (99-1)
3. Eluate of quinone adsorbed on florisil, obtained with ethanol
4. Hydroquinone (0.0315 g./liter) in ethanol



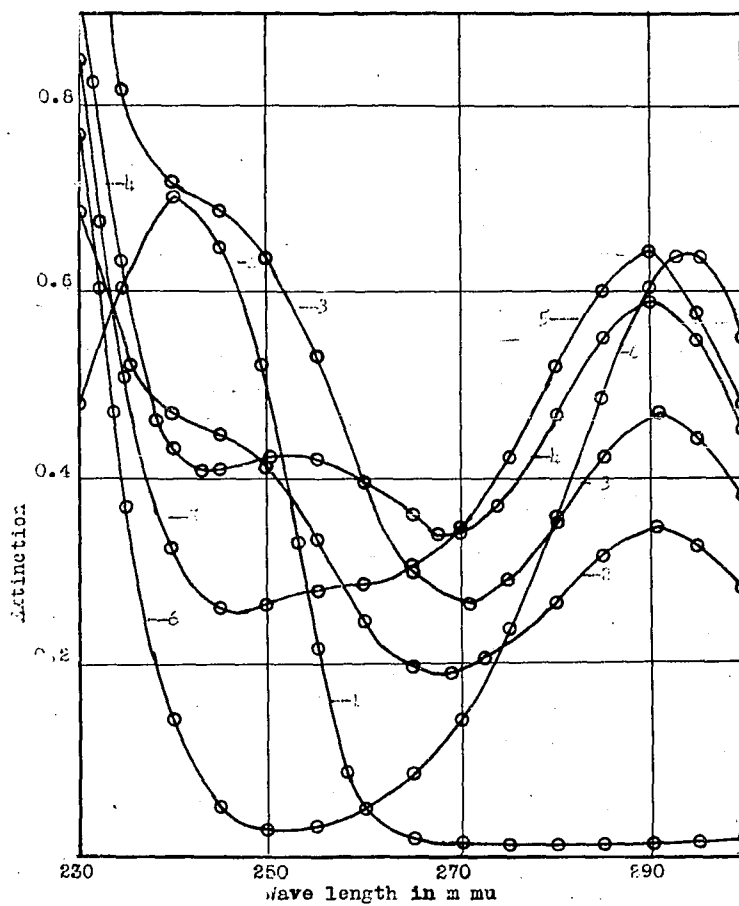


Fig. 9. Absorption curves for quinone adsorbed on alumina

1. Quinone (0.0049 g./liter) in hexane-ethanol (99-1) before adsorption
2. Eluate obtained with hexane-ethanol (75-25)
3. Eluate obtained with hexane-ethanol (50-50)
4. Eluate obtained with hexane-ethanol (25-75)
5. Eluate obtained with ethanol
6. Hydroquinone (0.0225 g./liter) in ethanol (for comparison)

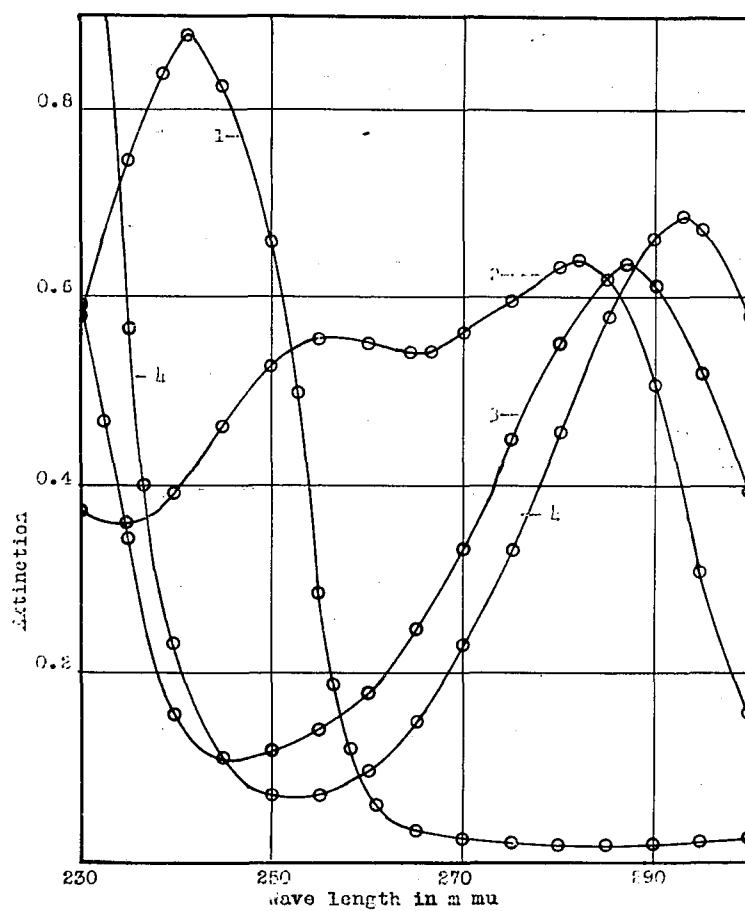


Fig. 10. Absorption curves for quinone

1. Quinone (0.005 g./liter) in ethanol
2. Percolate of quinone in ethanol thru florisil
3. Eluate of quinone adsorbed on florisil, obtained with ethanol
4. Hydroquinone (0.0255 g./liter) in ethanol

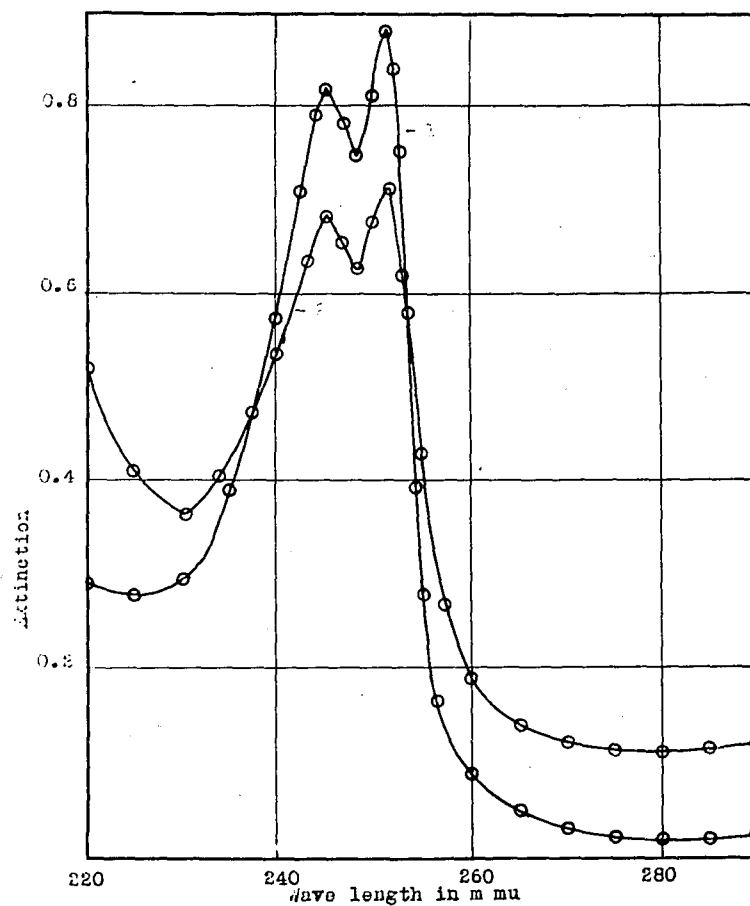


Fig. 11. Absorption curves for 1,2-naphthoquinone

1. 1,2-naphthoquinone (0.0063 g./liter) in hexane-ethanol (98-2)
2. Percolate of 1,2-naphthoquinone in hexane-ethanol (98-2) thru florisil

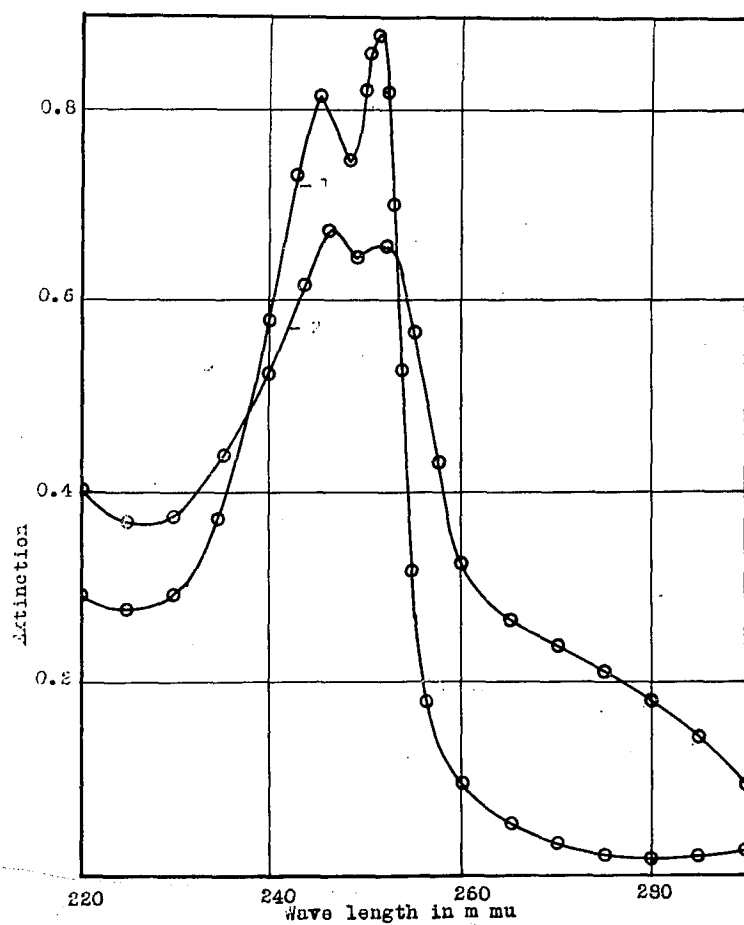


Fig. 12. Absorption curves for 1,2-naphthoquinone

1. 1,2-naphthoquinone (0.0053 g./liter) in hexane-ethanol (98-2)
2. Percolate of 1,2-naphthoquinone in hexane-ethanol (98-2) thru alumina

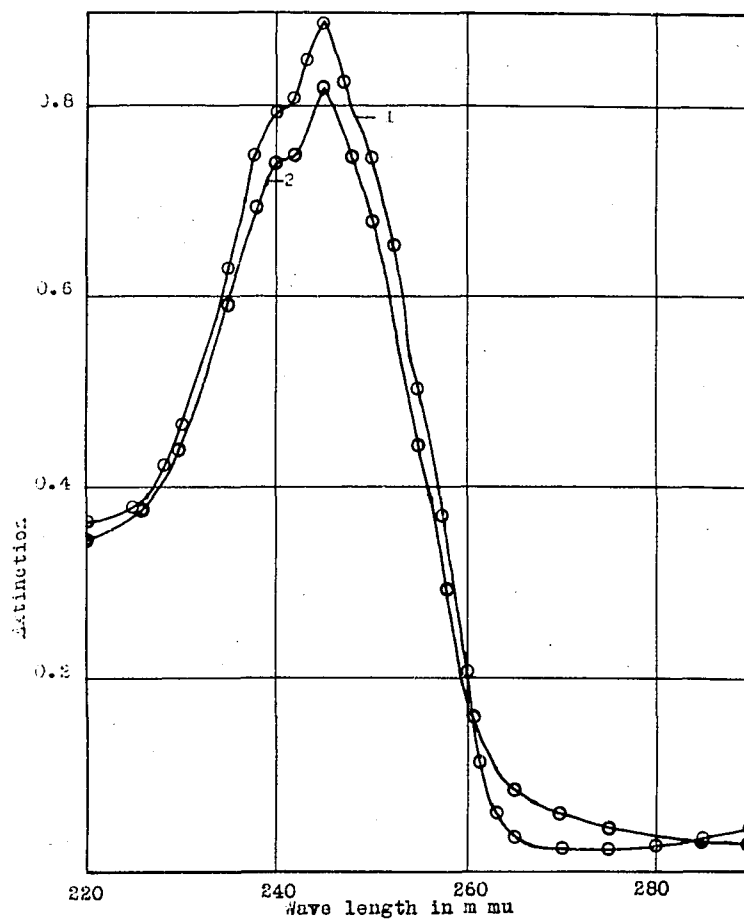


Fig. 13. Absorption curves for 1,4-naphthoquinone

1. 1,4-naphthoquinone (0.0065 g./liter) in hexane-ethanol (96-4)
2. Percolate of 1,4-naphthoquinone in hexane-ethanol (96-2) thru florisil

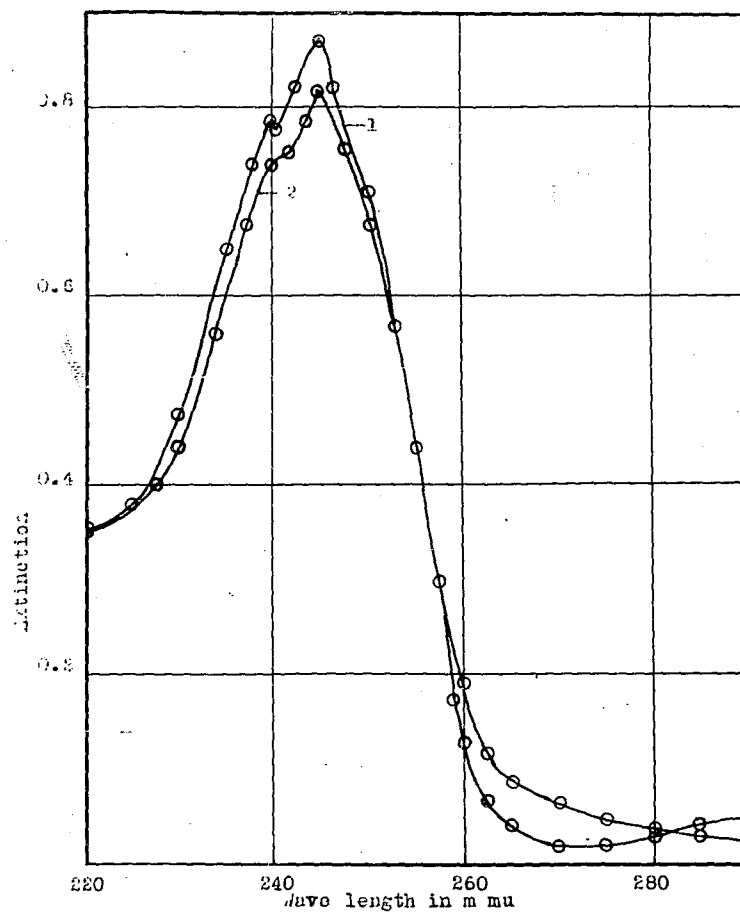


Fig. 14. Absorption curves for 1,4-naphthoquinone

1. 1,4-naphthoquinone (0.0055 g./liter) in hexane-ethanol (98-2)
2. 1,4-naphthoquinone (0.0055 g./liter) in hexane-ethanol (96-4)

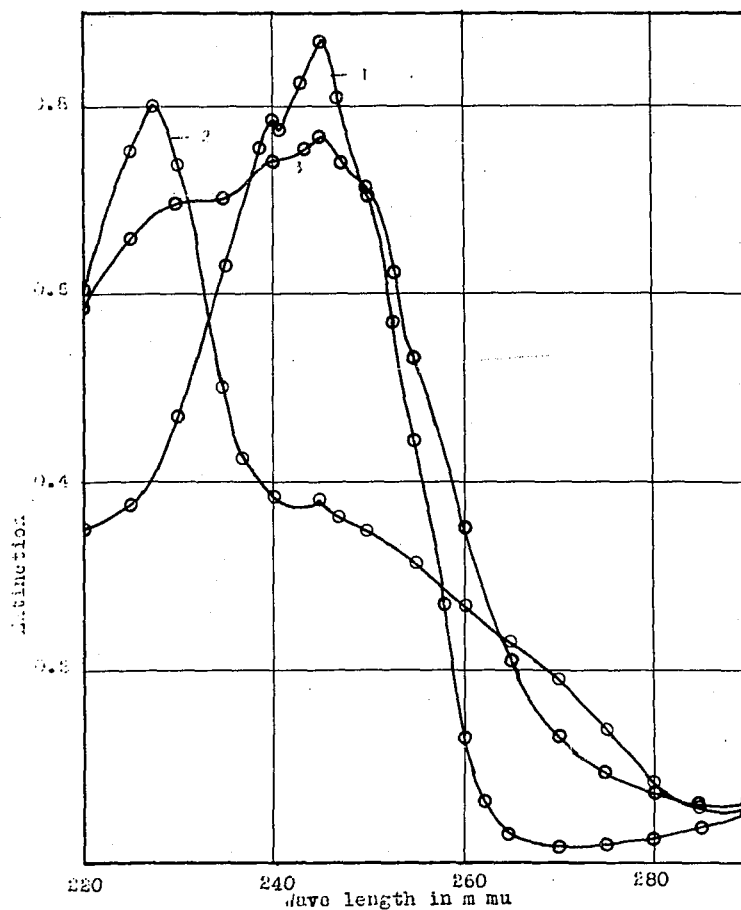


Fig. 15. Absorption curves for 1,4-naphthoquinone

1. 1,4-naphthoquinone (0.0065 g./liter) in hexane-ethanol (98-2)
2. Eluate of 1,4-naphthoquinone adsorbed on alumina obtained with hexane-ethanol (98-2)
3. Eluate of 1,4-naphthoquinone adsorbed on alumina obtained with hexane-ethanol (30-70)

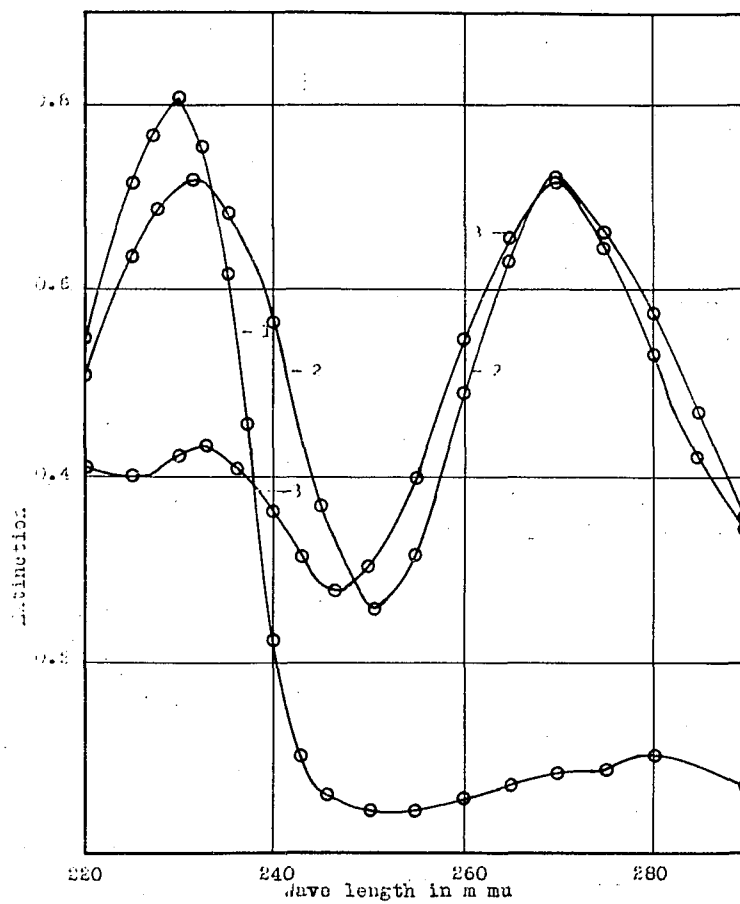


Fig. 16. Absorption curves for 1,3-naphthoresorcinol

1. 1,3-naphthoresorcinol (0.00395 g./liter) in hexane
2. 1,3-naphthoresorcinol (0.00395 g./liter) in ethanol
3. Eluate of 1,3-naphthoresorcinol adsorbed on alumina obtained with ethanol



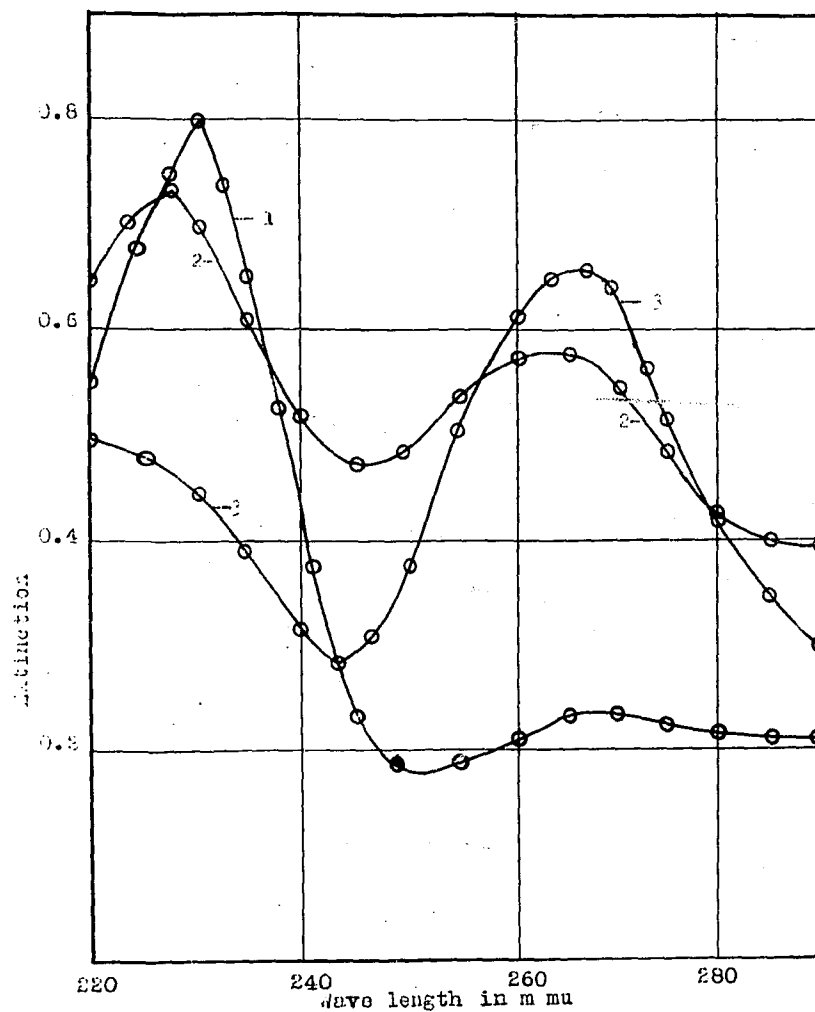


Fig. 17. Absorption curves for 1,3-naphthoresorcinol

1. 1,3-naphthoresorcinol (0.00395 g. /liter) in water
2. Percolate of 1,3-naphthoresorcinol in water thru alumina
3. Eluate of 1,3-naphthoresorcinol adsorbed on alumina obtained with water

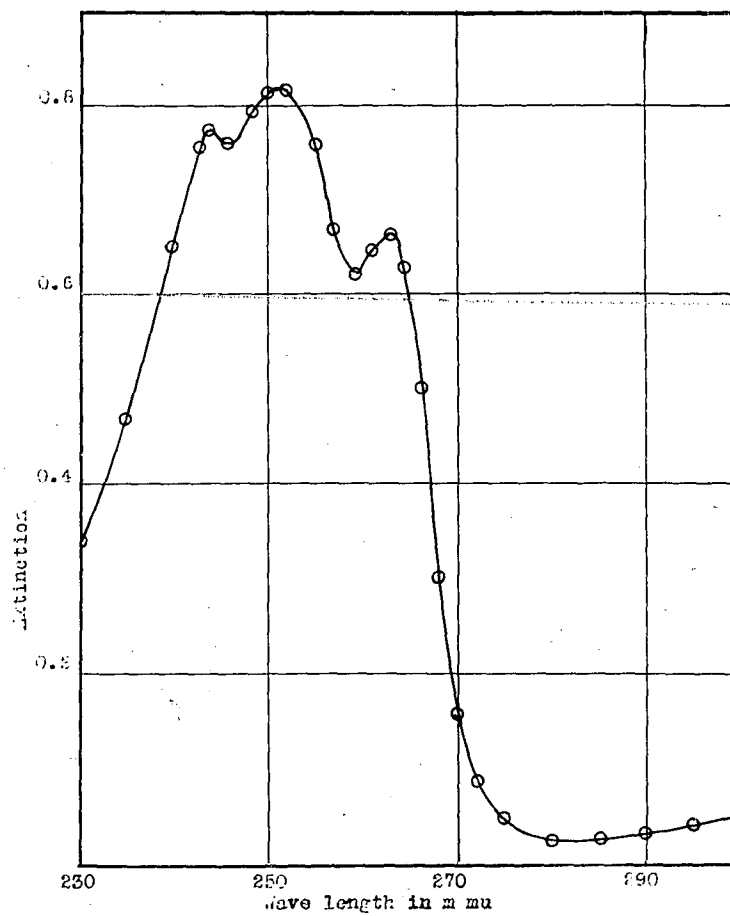


Fig. 18. Absorption curve for 2-methyl-1,4-naphthoquinone (0.0067 g./liter) in hexane

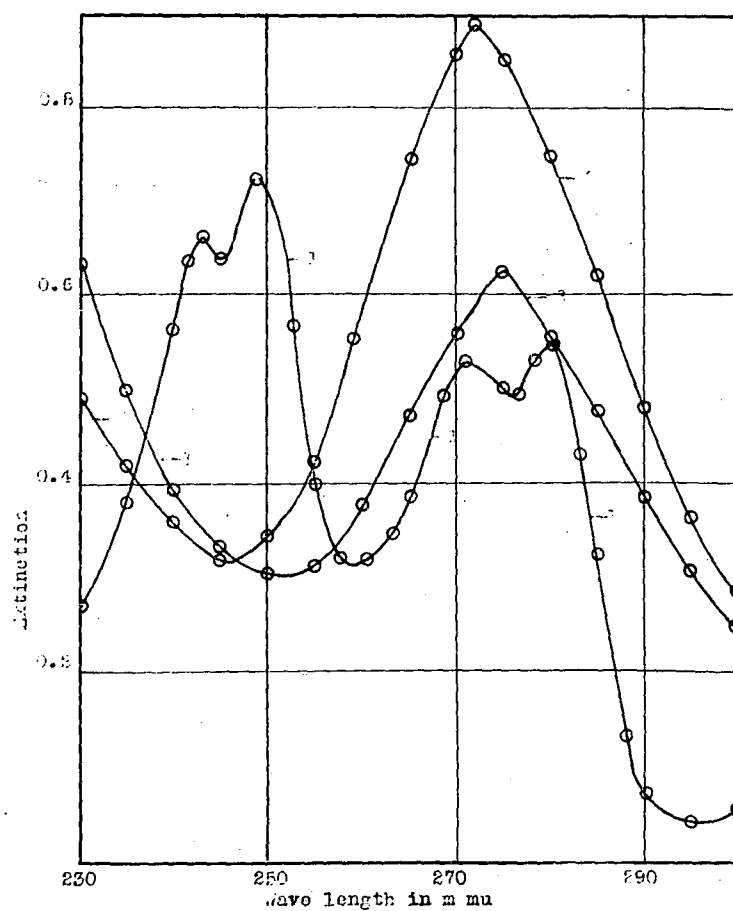


Fig. 19. Absorption curves for 2-hydroxy-3-methyl-1,4-naphthoquinone (Phthiocol)

1. Phthiocol (0.00686 g./liter) in hexane
2. Phthiocol (0.00686 g./liter) in 1% ammonium hydroxide
3. Eluate of phthiocol adsorbed on alumina obtained with 1% ammonium hydroxide

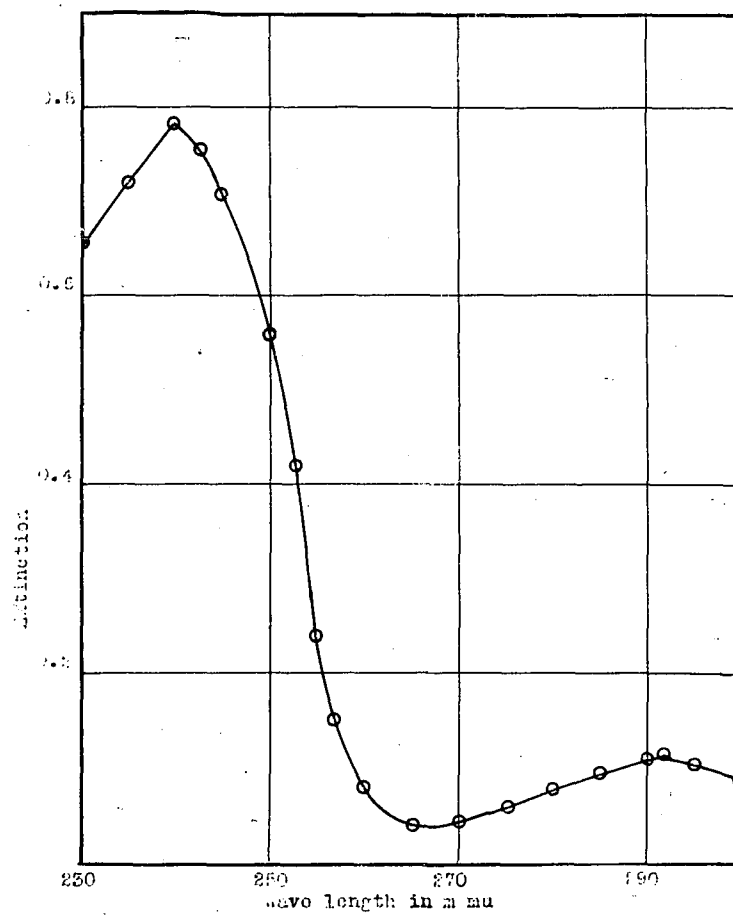


Fig. 20. Absorption curve for quinhydrone (0.009 g./liter) in hexane-ethanol (98-2)

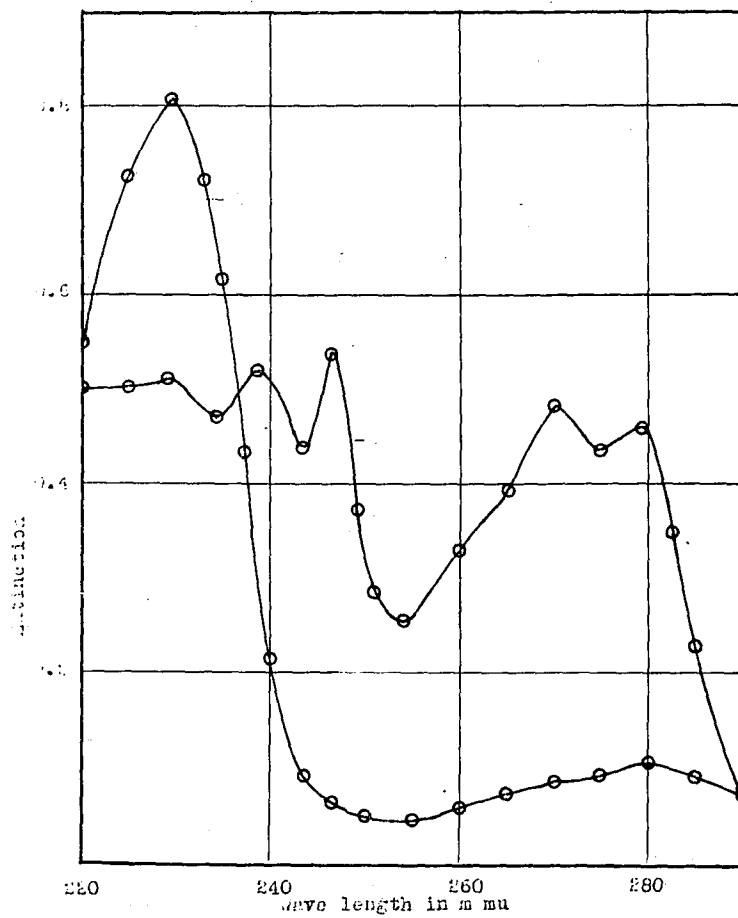


Fig. 21. Absorption curve for 1,3-naphthoresorcinol

1. 1,3-naphthoresorcinol (0.00395 g./liter) in hexane freshly prepared
2. Same solution after several months storage

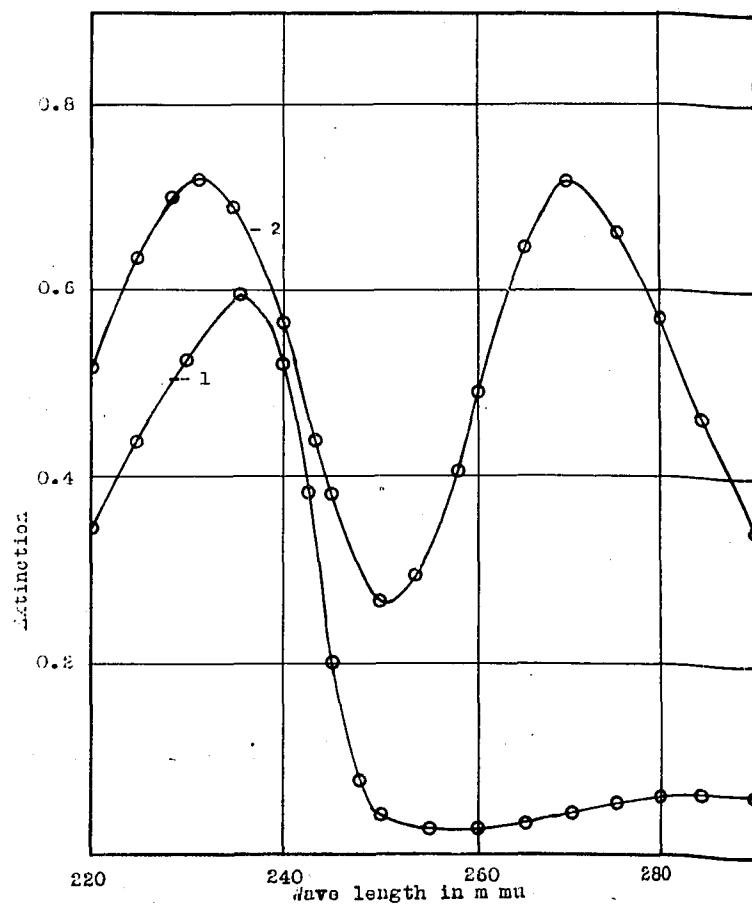


Fig. 22. Absorption curves for 1,3-naphthoresorcinol

1. 1,3-naphthoresorcinol (0.00288 g./liter) in ethanol, freshly prepared
2. 1,3-naphthoresorcinol (0.00395 g./liter) in ethanol, after several months storage