A STUDY OF NEW SOLVENTS IN ALKALOIDAL ASSAYING

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Introduction

During the past few years a considerable number of new solvents have been introduced by the chemical industry, many of which are commercially available in such quantities as to make them economically useful for many purposes. Many of these modern solvents have been used in certain pharmaceutical operations, such as, the extraction of oils, fats, waxes, resins, balsams, and other plant and animal principles.

Several excellent articles[1,2,3] have appeared in recent years on the general subject of new solvents and their uses. However, no study of the value of any of these solvents in extracting alkaloids quantitatively from vegetable drugs has been reported. It is the purpose of this investigation, therefore, to determine the value of certain of these solvents in the quantitative determination of the alkaloidal content of certain drugs.

For several reasons isopropyl ether and methylene chloride have been selected for this study. In the first place, these solvents are sufficiently insoluble in water to be classified under the general heading of "immiscible solvents". Also, the isopropyl ether possesses pro-

perties somewhat similar to ethyl ether, which is now used in many of the assay processes, with certain additional advantages such as, lower vapor pressure, higher boiling point, higher flash point, less solubility in water, and in many cases somewhat higher solvent power. Methylene chloride has a lower boiling point than chloroform, and a specific gravity of about 1.33 as compared to 1.48 for chloroform. It is also practically insoluble in water. Thus, either one of these solvents may be used alone, or combined with the other in any proportion, as immiscible substances to extract alkaloids from aqueous solution.

Historical Introduction

I. Alkaloidal Assaying By the Immiscible Solvent Method.

Many papers on the quantitative estimation of alkaloids by means of immiscible solvents and on the difficulties encountered in such estimations have been published, mostly within the past fifty years. C. Kippenberger[4] published a paper in 1897 in which he discussed the possibilities of errors in alkaloidal assays by hydrolysis of the salt with the liberation of free alkaloid. In discussing the solvents most suited to alkaloidal assays he suggested chloroform, or chloroform containing alcohol. In 1900 this same author published a paper[5] in which he dealt with the subject of alkaloidal analysis more scientifically. He studied ether and chloroform as immiscible solvents to be used in this connection and extended the work to include

several alkaloids. In certain instances salt was added to the acidified alkaloidal solution and its effect studied.

The procedure used by Kippenberger was as follows: The alkaloid was dissolved in 70 cc. of water by the use of an excess of acid, and treated with 50 cc. of the immiscible solvent. The mixture was shaken in a separatory funnel for a period of about three minutes, allowed to separate, and the immiscible solvent layer drawn off. The solvent was next evaporated on a water bath and the residue dried in a desiccator over sulphuric acid. The amounts of alkaloid and alkaloidal salt were then determined by dissolving the residue in an excess of fiftieth normal acid and titrating the excess acid with fiftieth normal alkali. In this way the amount of free alkaloid in the residue could be calculated, and the amount of hydrolysis determined.

The alkaloidal solution was then made alkaline with NaOH solution and again extracted with the immiscible solvent. To determine the amount of alkaloid present as a salt, the immiscible solvent was evaporated, the residue dissolved in standard acid and the excess acid titrated as before. The amount of free alkaloid was then subtracted from the amount of total alkaloid to obtain the amount present as a salt. As a result of this work Kippenberger concluded that during an alkaloidal determination some of the alkaloidal salt is hydrolyzed, thus leading to error in the results.

In 1901, Proeless[6] published a paper on the behavior of alkaloidal solutions toward different solvents. This work was carried out to determine the best solvent for alkaloids in general, and also to determine the best solvent for certain individual alkaloids. The solvents studied were ether, chloroform, benzene, and mixtures of these with alcohol added in some cases. He concluded that any of the solvents studied were satisfactory for extracting atropine from aqueous solution; that ether is best for brucine in an aqueous solution made alkaline with sodium carbonate-ammonium hydroxide mixture; that benzene, chloroform or chloroform plus alcohol are best for codeine; that any solvent studied was satisfactory for colchicine; that alcohol plus chloroform from potassium carbonate mixture was best for morphine; that ether was best for picrotoxin from sodium carbonate-ammonium hydroxide solution; that chloroform, alcohol plus chloroform, or benzene are best for strychnine; and that chloroform, ether plus chloroform, or benzene, are satisfactory for removing veratrine from aqueous solution made alkaline with ammonia water.

Simmer[7] published a paper in 1906 on the behavior of the salts of the common alkaloids toward extraction by chloroform and other solvents. In this paper the author also reported on the question of alkaloidal decomposition when treated with chloroform, and on the reducing action of certain alkaloids. The method used was to prepare an aqueous solution of the alkaloidal salts containing 0.2

gram of the free alkaloid in 50 cc. of solution, representing 0.4 per cent of the free alkaloid. The alkaloids were then converted into the salts by treating with different strengths of acid, after which they were extracted with chloroform for one hour. After this treatment the layers were separated and the chloroform evaporated. The amount of free alkaloids and alkaloidal salts were then determined. Simmer carried out similar experiments using benzene as the immiscible solvent. The results obtained indicate that certain neutral alkaloidal salts are extracted from their aqueous solutions by chloroform and benzene. In some cases more salt is extracted from a strongly acidified solution than from the corresponding neutral solution. Tables are given in the article to substantiate these conclusions.

Experiments were also carried out by Simmer to determine if alkaloids decompose chloroform when the latter is used as a solvent and the alkaloidal solution evaporated to dryness. The procedure used was to extract a mixture of 50 Gm. of water and 2 Gm. of the powdered alkaloid with 50 Gm. of chloroform for several hours. At the end of the time period the water layer was tested and always showed a cloudiness with silver nitrate solution and in most cases gave no test for alkaloids. The chloroform layer was then evaporated to dryness, and the residue taken up in water acidulated with sulphuric acid. Silver nitrate was then added, and when a precipitate was obtained, it was puri-

fied, collected in a crucible and its weight taken.

The conclusions drawn from these experiments were that the amount of decomposition is exceedingly small in most cases and none at all in a few cases. Thus, it is evident that the decomposition of chloroform by alkaloids is not to be considered as a serious objection to the use of this solvent as has been claimed by some.

Marden and Elliott[8] published a paper in 1914 in which they dealt with the methods of extraction by use of immiscible solvents from the standpoint of distribution ratios of certain alkaloids between water and the immiscible solvents, ether and chloroform. They used ammonium hydroxide to make the solutions alkaline. These investigators pointed out that by use of the distribution coefficient and an algebraic formula, the number of extractions necessary to extract practically all of the alkaloid from aqueous solution may be calculated. Distribution ratios for several alkaloids between the systems water and ether and water and chloroform are listed. The authors state in conclusion that the value of the research in alkaloidal assaying by the immiscible solvent method is apparent when one considers that in practice the question of the number of extractions with the immiscible solvent has not heretofore been predictable. In other words, when the distribution ratio is known, the number of extractions necessary to extract practically all of the alkaloid can be calculated. Such data should make it possible to com-

plete an assay without testing for complete extraction by some such method as the use of Mayer's reagent, and thus eliminate this source of error and at the same time effect a saving of time and of solvent. The above article by Marden and Elliott will be reviewed in greater detail later in this paper.

Beal and Lewis[9] published a paper in 1916, in which they gave the partition of several alkaloids between the acid layer and the immiscible solvent. These investigators worked with different acids, and under different conditions of concentration, with the idea of determining which salt was most soluble in chloroform or ether, and what concentration of acid most suitable to use. They determined which acid and in what concentration, removes the alkaloid most completely from its solution in chloroform or ether. In general, they found that (a) neutral sulphates and tartrates in aqueous solution are hydrolyzed to a certain extent with the subsequent formation of free alkaloid and acid (b) that with an increase in the acidity of the solution, the hydrolytic action becomes less and the amount of alkaloid taken up in the free state decreases with the increase in acidity (c) that many of the acid sulphates and tartrates are removed as salts to a slight degree by chloroform and ether, and (d) that the alkaloidal hydrochlorides tend to be quite insoluble in chloroform, and in such cases the solubility increases with the acidity of the solution in all cases studied.

Several tables appear in the article which show the experimental data. In conclusion they state that the most practical method for the determination of alkaloids involves the extraction of the alkaloids from an aqueous solution by means of an immiscible solvent, such as chloroform or ether; that it involves the purification of the alkaloidal solution by removal of gums, coloring matter, etc., by similar methods, and finally, that unless conditions are carefully guarded loss of alkaloid as salt or in the free state will occur during the extraction.

In 1920, Beal and Hamilton[10] published their findings on the effect of clarification and "salting out" in the estimation of alkaloids in drugs and their preparations. They pointed out that when a drug, or preparation of a drug, is extracted of its alkaloidal content, it will be accompanied by other extractives, such as, proteins, fats, volatile oils, acids, gums, resins, colors and carbohydrates, and that some of these namely, fats, volatile oils, organic acids, and acid resins may be removed by shaking an acid aqueous solution of the extract with the immiscible solvent which will extract these non basic compounds, but which will not theoretically remove the alkaloidal salt. It is pointed out further that, unless these substances are removed, they will form emulsions with the immiscible solvents which are very troublesome and may pass to the final alkaloidal residue and contaminate it.

Experiments were carried out on caffeine, quinine, strychnine and morphine. The method of procedure in the case of the first three was to dissolve the alkaloid in four per cent acetic acid and add ten per cent lead acetate solution. The lead was removed with hydrogen sulphide, the solution made alkaline with ammonia water and extracted with five 10 cc. portions of chloroform, or until the alkaloid was completely extracted. In some cases a half-saturated sodium chloride solution was used and its effects studied. The conclusions drawn as a result of the experiments were: (a) lead acetate when used as a clarifier for alkaloidal extracts has no harmful effect upon the extraction of the alkaloid by immiscible solvents, (b) the addition of sodium chloride to such extracts after clarification increases the quantity of alkaloid removed at a single extraction, and (c) the assay of powdered nux vomica is greatly facilitated by the use of lead acetate as a clarifying agent upon a dilute acid extract of the drug, which was then immediately made alkaline and shaken out with the solvent. These authors also determined the conditions for obtaining a residue of anhydrous morphine. The procedure followed was to extract the drug with six portions of hot amyl alcohol, (30, 20, 10, 10, 5 and 5 cc.), the first portion being added just before making the solution very slightly alkaline with ammonia water. When the solvent was evaporated in air about 7.5 per cent resinous matter was formed, however, when

evaporated at 100°C. in a current of CO_2 practically no resin was formed. Evaporation in air at 40°C. left about 3 per cent resin. Lead acetate as a clarifier and sodium chloride for "salting out" introduced no error in the determination of morphine.

Palkin and Watkins[11] published a paper in 1924 on alkaloidal assaying. Working specifically with nux vomica, they pointed out the difficulties encountered in the assay of preparations of this drug, and called attention to the isolation of an alkaloid in nux vomica, strychnicine, by Boorsma which possesses different properties from strychnine and brucine. The official method of assay was condemned on the basis that too many extractions are required in the different steps of the assay, thus making it time consuming, and also because of the formation of emulsions in many cases. Experiments were carried out, and recorded in tabular form, to show the number of extractions necessary to completely extract strychnine, brucine and a mixture of the two with chloroform. It is pointed out that even though alkaloids are somewhat soluble in the presence of an excess of ammonia, complete removal is possible in aqueous solution by chloroform.

Experiments were carried out to determine the effect that varying the amount of ammonia would have on extraction with chloroform. Tables given in the article show that the optimum alkalinity for exhaustion of the alkaloids is approximately that point where an amount of

ammonia equivalent to the total alkaloid present has been added beyond the point neutral to methyl red.

These authors also found that even though strychnine and brucine are more soluble in chloroform than in alcohol, addition of alcohol to the chloroform, extracts these alkaloids as well as chloroform alone, if not better. It was pointed out further that the two additional steps in the U. S. P. assay to the original exhaustion of the alkaloids from the nux vomica preparations, necessary to render the alkaloids clean enough for determination by titration are time and labor consuming, and it was suggested that a shorter method would be desirable. In the light of these facts a short method suitable for certain nux vomica preparations was perfected by Palkin and Watkins. The method used is as follows:

Fluid Extract:

> Pipette 25 cc. of sample into a 50 cc. volumetric flask. Add about 3 cc. normal sulphuric acid and evaporate on a steam bath (using air blast to hasten evaporation) to a volume of about 10 cc. To the residue add about 30 cc. of water while rotating the flask, cool to room temperature and dilute to volume. Allow to stand five minutes and filter through dry filter paper. The major portion of contaminating extractive matter will have precipitated in a flocculent form. Pipette 20 cc. (equivalent to 10 cc. original sample) into a separatory funnel (this is done in duplicate), add 1 cc. of ammonium hydroxide and extract the alkaloids with equal volumes of chloroform until extraction is complete, testing the final extracted residue with Mayer's reagent."

The chloroform is evaporated, the residue taken up in 10 cc. of neutral alcohol, and the amount of alkaloids determined volumetrically in the regular way.

Tables are given in which the results obtained by the above method are compared to those obtained by the U. S. P. method. These tables show that the results in case of the extract, fluid extract, or tincture of nux vomica obtained by the modified assay method compare very favorably with those obtained by the official assay method. And it is a conclusion that the modified method reduces the tendency to emulsify.

Dean and Edmonton[12] working with extracts and fluid extracts of nux vomica made the initial extractions with benzene instead of chloroform and the final extraction with chloroform. They obtained better results in this way and attributed it to loss by emulsification in the other method. They found no difference in the results when sodium carbonate and sodium hydroxide were used to liberate the alkaloids.

Watkins and Palkin[13] have made a study of automatic devices for extracting alkaloidal solutions as applied to nux vomica and belladonna alkaloids. Liquid preparations of these drugs were used and it was shown that under conditions of dealcoholization and subsequent prolonged hot extraction no ammonium sulphate is carried over to the alkaloidal concentrate; that almost all of the alkaloid is extracted in the first 30 minutes; that with variations in the conditions of the experiment, such as variation in ammonia concentration, does not affect the quantity of titratable alkaloids extracted. In some cases it was

shown that a greater quantity of alkaloid can be extracted by means of the automatic devices than by means of a separatory funnel.

Watkins, Murray and Palkin[14] working with improved types of automatic extraction apparatus obtained some very encouraging results on preparations of certain alkaloidal containing drugs. They described two types of extractors (a) for solvents lighter and (b) for solvents heavier than water. In a later article[15] Palkin and Watkins described another type of automatic extraction apparatus designed for powdered materials, and used the method to extract several powdered drugs of their alkaloidal content. In working with fluid extracts and tinctures, the sample was first de-alcoholized and partially purified as follows: 25 cc. fluid extract (for tinctures 100 cc. samples were used) and 3 cc. of 1 N. sulphuric acid were evaporated on a steam bath to about 10 cc. and the resulting concentrate diluted to 50 cc. and filtered. After this preliminary purification the preparation was extracted in the automatic extractor and at the same time another sample of the preparation was determined according to the U. S. Pharmacopoeia Assay. Such preparations as fluid extracts, tinctures, and solutions of tablets when determined with the automatic extrator for aqueous liquids gave very satisfactory results and in some cases, notably fluid extract of ipecac, much higher results were obtained than by the official method. For powdered drugs, such as hyos-

cyamus, ipecac, belladonna leaves, stramonium, and nux vomica, the modified automatic extractor gave results that compared favorably with the official methods, and in some cases, notably hyoscyamus, the results were over twice as high. Solvents used in this work were chloroform, ether and benzene.

Rasmussen and Christensen[16] recommended the use of 0.05 N sodium borate instead of sodium hydroxide to be used in the back titration of acid in alkaloidal assays. They list experiments to show that it is equally satisfactory, and point out the advantage that borax is not so sensitive to carbon dioxide as sodium hydroxide.

Enz and Jordan[17] reported their findings on the extent of emulsification of alkaloid-containing preparations with immiscible solvents at different degrees of pH. Five official alkaloidal-containing preparations were treated respectively with varying amounts of N KOH and N HCL. These portions were then shaken with a definite volume of water and an immiscible solvent, and the time that emulsification persisted observed in each case. The preparations studied were Fluidextract of Belladonna Leaves, Fluidextract of Cinchona, Fluidextract of Hydrastis, Tincture of Stramonium, and Tincture of Nux Vomica. The immiscible solvents used were chloroform, ether, amyl alcohol, benzene, and petroleum benzin.

The procedure used by Enz and Jordan was to titrate 250 cc. of the alkaloid-containing preparation

at a pH of exactly 7.0 using the quinhydrone electrode. The total volume was then divided into five portions of 50 cc. each and varying amounts of normal acid or alkali added to four of the portions. Each of the alkaloidal-containing preparations was shaken with the five immiscible solvents under each of the five pH degrees and the time that emulsification persisted in minutes recorded. They concluded that there is no general uniformity in the time that emulsification persists. Fluidextract of Belladonna showed in general least emulsification at the neutral point. Tincture of Stramonium showed least emulsification in acid solution and the remaining three drugs studied showed no uniformity. Petroleum benzin showed the least amount of emulsification in acid solution except in the case with Fluidextract of Cinchona.

Caines and Evers[18] found that a mixture of four volumes of ether and one volume of chloroform gave much less troublesome emulsions than a mixture of three volumes of ether and one volume of chloroform, as suggested in the U.S.P. X for the assay of belladonna leaves.

Thus, an examination of the literature reveals the fact that a great deal of work has been done on the quantitative estimation of alkaloids by the "immiscible solvent" method, and many helpful suggestions have been offered. Most of the sources of error have been dealt with and suggestions offered, whereby some of these could be overcome. The idea of securing better solvents for al-

kaloidal assay procedures has been mentioned from time to time, but not a great deal of work has been done with this as the primary purpose of the investigation. As was pointed out earlier in this review benzene has received some attention as a solvent to replace ether and chloroform, and different proportions of these latter two solvents have been suggested in certain assays. Also, alcohol as a solvent in alkaloidal assays has been investigated somewhat, as has been pointed out.

II

THE DEVELOPMENT OF ALKALOIDAL ASSAY METHODS

Gravimetric Procedures

The determination of alkaloids in drugs gravimetrically is a rather old practice. The method used, in short, is to extract the alkaloid or alkaloids from the drug by means of a solvent, the most common of which is ether, chloroform, or a mixture of these; subject the extracted alkaloids to a process of purification by shaking out with dilute acid, in order to remove the alkaloids as salts from the organic solvent, leaving much of the dissolved impurities behind; decomposing the alkaloidal salt with alkali (usually ammonia water), and reextracting with an organic solvent. Finally, the organic solvent is evaporated, the alkaloidal residue dried to constant weight, and the amount of alkaloids determined by direct weighing.

As pointed out by Herzig,[19] there are several advantages in the gravimetric method of analysis by direct weighing. There is no reaction to be considered; the alkalinity of solvents does not interfere; inaccuracies due to poor indicators are avoided; volatile bases are lost during evaporation of the solvent, and finally a volumetric factor to be used in the calculations is eliminated.

In some cases where the alkaloid is a weak base, volumetric analysis cannot be used, thus making it essential that a gravimetric procedure be followed.

Various methods have been proposed whereby, the alkaloids are precipitated as salts with certain reagents, the salts weighed and by use of a factor the amount of alkaloid caluclated.

Silicotungstic acid has been used to determine alkaloids quantitatively. Various formulas have been ascribed to this substance, however, they all have the same content of $Si\ O_2 . 12\ WO_3$, differing only in the content of water. The contact of water should not make any difference in the results if the compounds formed with the alkaloids are ignited to constant weight. However, Jensen[20] states that the silicotungstic acid used for quantitative alkaloidal determinations has to be prepared so that it will be definite in composition.

The principle upon which this method is based is the formation of a precipitate when the alkaloidal solution is treated with the silicotungstic acid. The precipitate is practically insoluble in water and dilute acids, and after standing, in some cases for several hours, is collected on a filter, washed, dried, and weighed. In some methods the precipitate is weighed after drying, and in other cases it is ignited before weighing. In case the precipitate is ignited before weighing no difficulty is encountered in securing uniform precipitates; however,

if the precipitate is dried at 120°C the reagent must contain a definite amount of water, in order that the precipitate obtained will also contain a definite amount of water. Chapin[21] determined nicotine quantitatively by precipitating with an excess of 12 per cent silico-tungstic acid solution in a solution rendered acid with hydrochloric acid, washing the precipitate obtained and drying at 120°C to constant weight. He gives as the formula for such a precipitate ($2\ C_{10}\ H_{14}\ N_2 .\ 2H_2O .\ Si\ O_2 .\ 12\ WO_3$).

Bertrand[22] used a 5 per cent solution of silico-tungstic acid as the precipitating agent with the formula ($12\ WO_3 .\ SiO_2 .\ 2H_2O$). He obtained flocculent, white or yellowish white precipitates. These precipitates were difficultly soluble and contained after drying at 30°C varying amounts of water of crystallization, all of which was not given off upon drying at 125°C. This author also reported that caffeine and theobromine are precipitated completely, only from a weak acid solution, as acid salts.

Ecalle[23] used Bertrands method for the determination of aconitine, but could not obtain satisfactory results. Later Bertrand and Javillier used it for the determination of nicotine; however, they decomposed the nicotine silicotungstate ($12\ WO_3 .\ SiO_2 .\ 2H_2O .\ 2C_{10}H_{14}N_2$) with an alkali or magnesium oxide, distilled the nicotine and determined it volumetrically. Javillier also analyzed conicine, sparteine, and atropine and used as the precipitating agent a 10 per cent solution of silico-tungstic

acid or its salt in neutral or alkaline solution; however, he did not report good results in most of these cases.

As late as 1924 Beal and North[24] investigated the use of silicatungstic acid as a volumetric reagent for the determination of certain alkaloids. They reported that the method gives results which compare favorably with those obtained by the gravimetric method, and by the volumetric method when 0.1 N sulphuric acid and 0.1 N sodium hydroxide is used. The same procedure was used to extract the alkaloids from the drug and in the case of Cinchona, for example, the sulphuric acid titration showed an alkaloidal content of 5.84 per cent, whereas, the silicotungstic acid determinations averaged 5.82 per cent. The same sample contained 6.00 per cent of alkaloids when determined gravimetrically. These authors concluded, therefore, that standard solutions of silcotungstic acid in aqueous solution may be used to titrate alkaloidal salts in the presence of free hydrochloric or sulphuric acid, using malachite green as an outside indicator.

Several other papers have appeared in the literature dealing with the estimation of alkaloids by precipitation with silico-tungstic acid. Generally speaking, good results have not always been obtained. The conditions necessary for concordant results are such that the method has never been considered as of much value except in certain isolated cases, and consequently has been largely discarded.

Kemp published a paper[25] in which he discussed the behavior of organic bases with picric acid. Later, Hager[26] investigated picric acid as a precipitating agent for determining alkaloids quantitatively. He pointed out that this acid could be used to precipitate alkaloids, and further, that the alkaloidal picrates could be used for the separation of individual alkaloids. Some alkaloids, for example, atropine and caffeine, could not be precipitated with this reagent, while on the other hand, it was pointed out that some drugs when extracted and the alkaloids precipitated with this reagent, give too high results on account of other materials being precipitated also. Thus, Hager analyzed cinchona bark for total alkaloids, but found the results to be too high when samples of known alkaloidal content were used. It was pointed out also, by Van der Burg[27] that other substances are precipitated along with the alkaloids, thereby causing the results to run high.

Because of the difficulties encountered when picric acid is used to precipitate alkaloids quantitatively, it has not come into general use for this purpose. However, other substances, having properties similar to picric acid have been proposed for the purpose of determining the amount of alkaloids in plants. Chief among these is dinitrophenyl-methylpyrazolon or picrolonic acid.

Matthes and Rammstedt[28] first used picrolonic acid for determining alkaloids quantitatively. The alka-

loidal picrolonates prepared by these investigators were of a definite melting, or decomposition point. They experimented with nux vomica and its galenical preparations, and stated that strychnine and brucine are precipitated quantitatively from ether-chloroform solution by this acid.

The reagent is formed by the action of nitric acid upon phenyl-methyl-pyrazolone. Alkaloids experimented with by these investigators, other than strychnine and brucine, are morphine, codeine, hydrastine, and pilocarpine. The method used was to treat the alkaloidal solution with picrolonic acid, collect the precipitated alkaloidal picrolonate on a weighed Gooch crucible, wash, dry and weigh. Since the alkaloidal picrolonates are definite in chemical composition, they serve as compounds from which to estimate the alkaloids quantitatively.

Several other papers appeared from time to time indicating the value of picrolonic acid as an alkaloidal precipitant. Warren and Weiss[29] prepared the picrolonates of several alkaloids, determined their melting and decomposition points and described their crystalline forms. They also prepared pure alkaloids through the decomposition of the alkaloidal picrolonates.

Richter[30] determined berberine quantitatively by the picrolonate method. The method he used was to extract the alkaloid from the drug with alcohol, distill off the alcohol, take up the residue in a little water and add sodium hydroxide and ether. After treating the

mixture with tragacanth and shaking, a part of the ether was drawn off and the alkaloid precipitated with picrolonic acid solution. The alkaloidal picrolonate was collected on a filter, washed with alcohol and ether, dried and weighed. Good results were reported.

It is evident upon comparison of data reported in the literature that concordant results are not always obtained by the picrolonic acid method, and also, that the alkaloids cannot be recovered quantitatively when known samples are used, therefore, it becomes clear why this method has not met with greater success.

Jonescu and Thoms[31] published a paper on the precipitation and quantitative estimation of alkaloids by potassium bismuthous iodide. These authors found that various alkaloids can be estimated by use of this reagent and recommended it especially for quinine alone, or in mixtures. It was pointed out that the reagent yields with quinine a yellowish-red precipitate, and that this precipitate may be decomposed with sodium hydroxide solution to liberate free quinine. Puckner[32] points out that the alkaloidal bismuth iodides are of variable composition, and states therefore, that the reagent is only of value in separating alkaloids from other bodies and not as a means of directly estimating them.

Volumetric Procedures

Perhaps the first volumetric procedure used in

determining alkaloids quantitatively was to titrate them directly with standard acid. One of the first things discovered about alkaloids was that they form salts with acids, thus, it was natural that this property should have been early utilized in their estimation. In the volumetric procedure, as in the gravimetric, the principle of extracting the alkaloids from the drug is the same. It has to be extracted by means of a suitable solvent, and carried through a process of purification before it can be determined by any method. After purification, the alkaloids may be determined by direct titration with standard acid, using a suitable indicator. However, there are reasons why this method is not actually used and these will be pointed out later.

Schlössing[33] in 1847 extracted nicotine from tobacco, and titrated it with 0.01 N sulphuric acid, using litmus paper as the indicator. Glenard and Guilliermond[34] introduced a different procedure in 1860 when they dissolved cinchona alkaloids in a measured excess of sulphuric acid, and determined the excess acid with standard ammonia, using brazil wood as the indicator.

This method has undergone a number of refinements and has been greatly improved since the researches of Schlössing mentioned above; however, it has remained essentially the same. The choice of indicator in alkaloidal analysis has received much attention from time to time. Wales[35] published a paper in 1926 dealing with this

phase of the problem, which seems to be the outstanding work along this line to date.

The solvents used to extract alkaloids from plants have also received a great deal of attention. There seems to be no ideal solvent for the purpose. Ether will not dissolve all alkaloids sufficiently. Chloroform has been shown to react with some alkaloids with the liberation of chloride ion, etc. Schmidt[36] pointed out in 1899, that when alkaloids are extracted from drugs with chloroform and the chloroformic solution evaporated the residue contains some chloride ions and even chloroform in certain cases. Also, the tendency of chloroform to form emulsions during the extraction and purification process causes trouble in many cases, especially if the drug contains fat. Beckurts[37] and later Schweissinger and Sarnow[38] recommended ammonia for the liberation of the alkaloids before extracting with chloroform in order to lessen this tendency.

The volumetric procedure where the alkaloids are caused to combine directly with standard acid and the excess acid titrated with standard base has much to recommend it. It is simple and relatively fast, and furnishes the true alkaloidal content of many drugs.

In 1861, R. Wagner first introduced a method based on the fact that alkaloids form insoluble compounds with iodine in acid solution. A 0.1 N. potassium diiodide was used as the precipitating agent and was added in excess

to the alkaloidal solution in order to titrate the excess iodine with 0.1 N sodium thiosulphate. The reaction as explained by Wagner was as follows:

Alkaloid + HCl + KI + I_2 = Alkaloid. Hi. I_2 + KCl; however, it was soon found by other workers, that the general formula would not hold for all alkaloids. In fact, different conditions gave rise to different results, and too, other basic substances often present as impurities reacted with the reagent causing errors. For these and other reasons, the periodides formed with Wanger's reagent and alkaloids did not furnish a method of great value for the determination of the latter.

Another method which received a great deal of attention in the early development of alkaloidal analysis is that where Mayer's reagent is used to titrate the alkaloids. Mayer's reagent is a solution of 13.456 Gm. mercuric chloride and 49.8 Gm. of K I in one liter of water. This reagent reacts with alkaloids to give precipitates similar to those formed with Wagner's reagent. i. e.,

Alkaloid + HCl = Alkaloidal. HCl

Alkaloid. HCl + KI = Alkaloidal. HI + KCl

Alkaloid $(HI)_m$ + $(HgI_2)_n$ = Alkaloid $(HI)_m \cdot (HgI_2)_n$

The precipitates produced between alkaloids and Mayer's reagent will form in neutral or alkaline solution, however, ammonia and acetic acid cannot be present since they tend to dissolve the precipitate. Alcohol and glycerine also influence the precipitation.

Titration with Mayer's reagent is carried out by adding the solution from a burette to an alkaline solution of known concentration until a drop of the mixture, after the precipitate has settled, will not show any cloudiness when viewed on a watch glass over a black background. Kippenberger[40] modified the method somewhat by completing the titration with ammonium sulphide, and by allowing a few drops of the solution filter from one piece of filter paper to another. In case the second strip gave a black color with Mayer's reagent, it indicated incomplete reaction between the alkaloid and reagent. Results obtained with Mayer's reagent indicate that the method has never been highly satisfactory.

Gordin[39] modified the Mayer's procedure. He assumed that the alkaloidal precipitate always contains a constant quantity of HI and that only the content of HgI_2 is a variable. When the isolated alkaloid is dissolved in an excess of normal acid, precipitated with Mayer's reagent and filtered, the filtrate will contain the excess acid not used for neutralization. This acid in the absence of the alkaloid can be titrated with standard base, phenolphthalein as the indicator. The modification was found not to be satisfactory.

Heikel[42] worked out a method for the estimation of alkaloids by means of potassium-mercuric iodide which was a modification of Mayer's method. He used a 0.05 N solution containing 6.775 Gm. of $HgCl_2$ and 25 Gm. of KI

per liter, and recommended this reagent for rapid and fairly accurate determinations of alkaloids. Since the amount of reagent required to precipitate a definite amount of alkaloid depends upon the concentration of the reacting substances, it was proposed to use a method of residual titration. The alkaloid was first precipitated in acid on neutral solution by an excess of Mayer's reagent, the excess of mercury estimated by adding a known amount of 0.05 N KCN solution to form undissociated $Hg\ CN_2$, and the excess of KCN titrated with 0.05 N solution of $Ag\ NO_3$. Several alkaloids were determined by this method, among them being quinine, atropine, strychnine, brucine, cocaine, veratrine, etc. with results that indicate the method is reasonably accurate. However, several critics of the method have stated that the original method of Mayer is to be preferred over Heikel's modification.

Phospho-molybdic acid (Sonnenschein's reagent) and silico-tungstic acid have been used to determine alkaloids quantitatively in a volumetric procedure. Sonnenschein's reagent was introduced in 1857[43] and was prepared by precipitating ammonium molybdate with sodium acid phosphate. The yellow precipitate obtained is washed, suspended, and dissolved in water by use of sodium bicarbonate. The solution is then evaporated, and the residue ignited in order to get rid of the ammonia. The precipitate is then treated with nitric acid and again ignited, after which it is suspended in water, acidified with nitric acid

and diluted to a definite volume. The resulting reagent is a clear yellow solution. This reagent was used in much the same way as Mayer's reagent for the volumetric determination of alkaloids, but with little success because of the inconsistency of the alkaloidal precipitates. Sonnenschein first, and Dragendorff in his book on plant analysis pointed out the fact that other plant principles will also react with the reagent and therefore cause errors in the analysis. Snow[44] was unable to obtain good results with Sonnenschein's reagent, and so were several other investigators who were interested in the problem before 1900.

Silicotungstic acid has also been used for the quantitative determination of alkaloids. Heiduschka and Wolf[45] found that the reagent would give good results under certain conditions; however, the disadvantages connected with the method outweigh any advantages.

Potassium ferrocyanide as a reagent in volumetric alkaloidal analysis was proposed by Dunstan and Short.[46] These authors used this reagent as a means of separating strychnine and brucine in sulphuric acid solution. Strychnine is completely precipitated with potassium ferrocyanide, while brucine remains undissolved. They dissolved not more than 0.2 Gm. of the two alkaloids in 10 cc. of 5 per cent sulphuric acid, diluted the solution to 175 cc. and finally added 25 cc. of 5 per cent potassium ferrocyanide solution, making a total volume of 200 cc. After shaking and allowing to stand from three to six hours, the precipitate was

filtered and washed with one-fourth per cent sulphuric acid until the filtrate ceased to be bitter. The ferrocyanide-strychnine thus obtained was found to decompose under the influence of light and air into strychnine ferricyanide, free strychnine and water, therefore it was immediately decomposed with strong ammonia and the strychnine extracted from the ammoniacal solution with chloroform. After evaporation of the chloroform the anhydrous strychnine was weighed as such. The original acid solution of the potassium ferrocyanide was next supersaturated with ammonia, and the brucine also extracted with chloroform and weighed after evaporation of the solvent. Thus, it is seen that these authors worked out a method for the determination of strychnine and brucine in mixtures.

The above method was found by other investigators to give too high percentages of strychnine and too low results for brucine, the reason being that varying amounts of brucine-ferrocyanide is formed, depending on concentration, etc.

Beckurt and Holst[47] modified the Dunstan-Short method somewhat by changing the concentration of the alkaloidal solution and also, by not adding an excess of potassium ferrocyanide, thus preventing the precipitation of any brucine-ferrocyanide. It was pointed out that the precipitation of the strychnine-ferrocyanide is rapid and quantitative, and that as a result no brucine-ferrocyanide is precipitated. These authors determined the ratio of

strychnine and brucine in various preparations of nux vomica with good results. However, Kremel[48] could not obtain results that checked with those of Buckert and Holst, the reason offered being insufficient purification of the alkaloids.

Gordin and Prescott[49] precipitated the nux vomica alkaloids by shaking with potassium ferrocyanide, decomposing the mixture of strychnine and brucine ferrocyanides with zinc sulphate, in which case only the strychnine ferrocyanide is changed to the sulphate. The strychnine sulphate was then titrated with Wagner's reagent and the amount present calculated. The method has not gained favor and apparently is of not much value.

Picric acid has been used in the volumetric determination of alkaloids. Kleinstuck[50] first applied a volumetric procedure to this reagent. Without reviewing in detail the work that has been done along this line, it is sufficient to say that picric acid is not a suitable volumetric reagent for most alkaloids, and its use as such has not been recognized.

Ionescu and Spirescu[51] worked out a method for the determination of alkaloids which is based on the titration of the mercuric ion with chlorine ion. They precipitated the alkaloidal material with an acid solution of mercuric potassium iodide, then dissolved the precipitate in a mixture of nitric and sulphuric acids, destroyed the excess of nitric acid with potassium permanganate, and deter-

mined the mercury with standardized sodium chloride, using sodium nitroprusside as indicator.

Colorimetric Procedures

Color reactions may be used in quantitative analysis if the intensity of color is such that a conclusion may be reached as to the quantity of substance present. In alkaloidal analysis it has been used to some extent, however, impurities are usually present in alkaloids extracted from their sources, which more or less interfere with the determination of the alkaloids colorimetrically. To purify such an alkaloidal extract would involve time to such an extent that the method would cease to be practical. In some cases, however, where the amount of alkaloid present in a drug is so small, the colorimetric method may be used to advantage, disregarding the expense of special apparatus needed for the determination.

One of the earliest colorimetric methods is that reported by Stein[52] in 1869. The work was based on the principle that morphine reduces hydriodic acid to iodine and produces a yellow color, intensified by the addition of ammonia. The opium extract was diluted until the color of the iodine in the chloroform, used for shaking out, could just be detected. The sensitivity limit has to be determined first and is used for comparison, instead of a standard prepared from morphine and iodine in chloroform. The method served only to estimate the minimum quantity of morphine in opium.

Mylius[53] extended the method of Stein by determining how much alkaloid is present. He added to both the extract and a comparative test solution iodic acid and a few drops of sulphuric acid, also 5 cc. of carbon disulphide and shook the mixtures vigorously. The carbon disulphide with the greater intensity of color was diluted with more carbon disulphide until equal in color intensity with the other. From the quantities of carbon disulphide added and the concentration of the standard, the morphine content could be calculated.

Various other investigators have modified and extended Stein's method in the determination of morphine. While the method seemed to give satisfactory results in the hands of those who developed it, it has never become of much practical importance, and it is perhaps safe to say never will, because of the advantages offered by other procedures.

Another alkaloid that received some attention, because it reacts with certain reagents to form more or less definite colors, is brucine. Douzard[54] worked out a colorimetric procedure based on the red color produced when brucine is treated with nitric acid. He used as standard a solution containing 0.16 Gm. strychnine and 0.16 Gm. brucine in 100 cc. of approximately 2 per cent sulphuric acid. He then dissolved 0.1 Gm. of an isolated pure alkaloid mixture of strychnine and brucine in 50 cc. of 2 per cent sulphuric acid, and to this and the compara-

tive solution he added 5 cc. of concentrated nitric acid, and after standing for five minutes, compared the red color of the two solutions in a colorimeter, taking the mean of six readings for the calculation. Other investigators have worked along the same line as Douzard in an attempt to determine quantitatively brucine in the presence of strychnine, but the method has too many limitations to be of much value. Likewise, attempts have been made to determine other alkaloids in mixtures colorimetrically, but with not much success.

Refractometric Method

It has been found that refractometric methods for determination of alkaloids are complicated and may be applied only within certain limits. As is the case with some of the other methods proposed, the alkaloid has to be entirely pure and this is always hard to accomplish, however, if it is accomplished, other methods, such as direct weighing, would be more expedient.

The general procedure used in most refractometric methods for alkaloids is as follows: The pure alkaloid is dissolved in water or some other solvent, the refractive index of which is known before hand, and the refractive index of the alkaloidal solution determined by means of a refractometer. Then, with the refractive index of the solvent and that of the alkaloidal solution at hand, the percentage of alkaloid in the solution can be calculated.

Utz[55] did considerable work along the line of the refractometric method of determining alkaloids. He experimented with caffeine, morphine, and brucine, and found, as had previously been reported by other investigators, that the refractive indices of the alkaloidal solutions were proportional to the amount of alkaloid in solution. In some cases, where the alkaloid was not sufficiently soluble in water, he used some other liquid for dissolving the alkaloid. In such cases, of course, the refractive index of the liquid had to be known before calculations were made.

Polarimetric Method of Analysis

A considerable number of alkaloids are optically active. Therefore, some attention has been directed to the quantitative determination of certain alkaloids by means of the polariscope. Oudemanns[56], Hesse[57] and Lenz[58] did some early work along this line by determining the optical rotations of the most common alkaloids. They observed that the optical rotation depends largely upon the type of solvent, the concentration of the solution, and the temperature. Solvents used in the work were water, alcohol, dilute acids and chloroform-alcohol mixtures. The conclusions reached were that in the case of cinchona alkaloids the specific rotation could not be used for their determination, because in the first place there is a mixture of alkaloids and unless the composition is known in advance no results can be calculated, and in the second place,

there is a natural coloring principle in the extract which interferes with observations in the polariscope.

It was also pointed out that a mixture of two alkaloids can be determined, provided the polarization constants of the two are known. Such a procedure might be applied to any two optically active alkaloids when in combination and in a chemically pure condition, however, it is so difficult to obtain absolute purity of alkaloidal extracts that the polariscopic method will never serve as a practical means of determining alkaloids in crude drugs or their galenical preparations.

III

A HISTORICAL REVIEW OF THE USE OF SOLVENTS IN THE UNITED STATES PHARMACOPOEIA

Belladonna

The eighth revision of the Pharmacopoeia was the first to carry an assay for Belladonna Leaves and Roots. It required that not less than 0.35 per cent of mydriatic alkaloids be present in the leaves and not less than 0.5 per cent of the same alkaloids in the roots. The assay procedure was the same for the leaves and roots. The solvent used to extract the alkaloids from the drug was a mixture of one part of chloroform and four parts of ether by volume, and chloroform was the final immiscible solvent used to extract the alkaloids from the alkaline aqueous solution.

The method consisted of exhausting the drug of its alkaloidal content with the mixed solvent by percolation, removing the alkaloids from the solvent with weak sulphuric acid and finally extracting the alkaloids from the aqueous solution with chloroform.

The weakness of this assay seems to have been the lack of a method to test for the complete extraction of the alkaloids from the original solvent with dilute acid, and also, from the acid aqueous solution. This weakness is noted in many of the earlier assay processes,

making it difficult to understand how concordant results could have been obtained, especially by different workers using somewhat different techniques.

The ninth revision required that Belladonna Leaves yield not less than 0.3 per cent and that the Root yield not less than 0.45 per cent of the total alkaloids. The initial solvent in this assay was a mixture of two volumes of ether and one volume of chloroform, and the final immiscible solvent chloroform. This is an aliquot part method, whereas, we will see that in the next revision (U.S.P.X), belladonna leaves and roots are assayed by Type Process B, or the total extraction method.

The U.S.P. X directs that the solvent used to exhaust the drug consist of a 3:1 mixture of ether-chloroform and that the alkaloids be extracted from the aqueous solution with chloroform.

The U.S.P. XI assay for Belladonna permits a continuous extraction method for the first time. A 2:1 ether-alcohol mixture is used to macerate the drug and ether is used to extract the alkaloids. Chloroform is used as the immiscible solvent to extract the alkaloids from the aqueous solution. It is also observed that an alternative process is included for the assay of this drug which is essentially the total extraction method of the previous revision. The initial solvent used to macerate the drug in the alternative method is a 2:1 mixture of ether-chloroform, while a 3:1 ether-chloroform mixture

is used to exhaust the drug of alkaloids. The final organic solvent is chloroform.

Cinchona

The first assay method to appear in the U.S.P. for Cinchona was in the sixth decennial revision (1880), however, the fourth and fifth revisions required that this drug contain a minimum per cent of alkaloids which yielded crystalline salts.

The sixth revision permitted the bark of any species of cinchona (Nat. Ord., Rubaceae) containing at least 3 per cent of its peculiar alkaloids. The bark was assayed for total alkaloids and also for quinine. The assay for total alkaloids consisted of treating the cinchona with lime in water suspension, drying the mixture and extracting with alcohol.. The alcoholic extract was evaporated to expell the alcohol, the residue collected on a small filter, washed with dilute sulphuric acid, and soda solution added to render it strongly alkaline. The precipitated alkaloids were then collected, washed, dried and weighed.

The above assay while long and tedious served to evaluate the alkaloidal content of the drug at the time, however, further research revealed the fact that lime acted to reduce the yield of alkaloids. Masse made a study of the effects of lime on the yield of alkaloids and recommended the use of ammonia in its place. Other invest-

igators reported similar results, and in the seventh revision lime was replaced by ammonia.

Also, in the seventh revision the solvent was changed to one consisting of a mixture of 19 volumes of alcohol and 5 volumes of chloroform, whereas, the solvent used in the previous revision was alcohol. Ether was used to determine the amount of quinine in the total alkaloids, and the method appears to have been an improvement over the older precipitation method. The assay in the seventh revision was changed in regard to the alkaloidal requirements. It required that cinchona yield not less than 5 per cent of total alkaloids, and at least 2.5 per cent of quinine. The revision carried an assay for total alkaloids and one for quinine.

In the eighth revision (1900) is found the statement that Cinchona should yield not less than 5 per cent of total anhydrous cinchona alkaloids, and at least 4 per cent of anhydrous ether-soluble alkaloids when assayed by the official process. Thus, the assay in this revision was designed to determine the amount of anhydrous cinchona alkaloids and also the ether-soluble alkaloids. The assay underwent other changes between 1890 and 1900. The determination of total alkaloids in the seventh revision was changed to anhydrous cinchona alkaloids in the eighth revision and the assay for quinine was changed to ether-soluble alkaloids, which included quinine, quinidine, and cinchonidine. The initial solvent used in the seventh re-

vision assay consisted of a mixture of alcohol and chloroform, and the final immiscible solvent was chloroform. However, in the eighth revision, the first solvent was changed to one consisting of five parts of ether and one part of chloroform and the alkali used to liberate the alkaloids from the acid aqueous solution was ammonia water instead of sodium hydroxide solution. The final solvent used to extract the alkaloids from acid aqueous solution in the determination for anhydrous cinchona alkaloids was a mixture of one volume of ether and three volumes of chloroform, and finally chloroform alone.

The U.S.P. IX required that Cinchona yield not less than 5 per cent of alkaloids. In this revision, for the first time, general directions for alkaloidal assays were given under Proximate Assays. However, the assay was still given in detail under the drug. In this revision, also, the drug directed to be used was in a number 40 powder. The initial solvent was a mixture of one volume of chloroform and two volumes of ether, while the final solvent was chloroform.

The assay requirement of Cinchona in the U.S.P. X did not differ from that in the previous revision, however, there were certain changes made in the assay. The fineness of the drug was changed from a No. 40 powder to a No. 60 powder. The drug was heated with dilute hydrochloric acid for a period of time in order to convert all of the alkaloids into the hydrochlorides and thus render them water

soluble. The initial solvent was a 3:1 ether-chloroform mixture, and the final immiscible solvent chloroform. The U.S.P. XI assay for Cinchona is essentially the same as in the previous revision.

Hydrastis

The first assay requirement for hydrastis was introduced in the U.S.P. VIII. The requirement was not less than 2.5 per cent of hydrastine when assayed by the official process. The process consisted of extracting the alkaloids from the drug with ether, shaking out the ethereal solution with dilute sulphuric acid to purify, and finally extracting the alkaloids from the acid aqueous solution with ether. The final statement in the assay that hydrastine is the alkaloid determined was not correct, the assay actually being an estimation of the ether-soluble alkaloids of hydrastis.

The assay requirement in the U.S.P. IX was changed to not less than 2.5 per cent of the ether-soluble alkaloids of hydrastis. The determination of the ether-soluble alkaloids did not differ essentially from the assay in the previous revision. The initial solvent was ether and the process was carried out as directed under the assay for belladonna root with certain modifications. After the alkaloids were extracted from the drug with ether, the ether solution was shaken out with successive portions of weak sulphuric acid, and the combined acid portion extracted

with ether after liberating the alkaloids with ammonia water. The alkaloids were determined gravimetrically.

The U.S.P. X requirement remained the same as that of the ninth revision. The assay process also remained essentially the same; however, it was included under those assays to be carried out according to Type Process A, type processes being included in this revision for the first time. Hydrastis is not official in the U.S.P. XI.

Ipecac

The first assay for ipecac was introduced in the U.S.P. VIII. The drug was assayed to contain not less than 2 per cent of ipecac alkaloids, these being determined volumetrically. The drug in No. 80 powder was first treated with an approximately 3:1 ether-chloroform mixture. An aliquot portion of the ethereal solution was poured off, and completely extracted with dilute sulphuric acid. The acid extracts were made alkaline and extracted repeatedly with ether, the combined ether extracts evaporated to dryness, the residue dissolved in standard acid, and titrated with standard alkali, using haemotoxylin, T. S. as the indicator.

The U.S.P. IX required that ipecac contain not less than 1.75 per cent of the ether-soluble alkaloids of ipecac. The initial solvent used was ether instead of an ether-chloroform mixture as in the previous revision.

Also, the final organic solvent was ether.

The U.S.P. X assay requirement remained the same as that in the ninth revision. The drug was assayed according to Type Process A, using ether as the initial and final solvent. The U.S.P. XI assay requirement for ipecac is not less than 2 per cent ether-soluble alkaloids. The solvents used are the same as in the assay of the previous revision; however, the ether used should be peroxide free.

Nux Vomica

The sixth revision of the Pharmacopoeia did not carry an assay for Nux Vomica or its preparations; however, upon examination of the literature it is found that several methods of assay were proposed for this drug prior to 1880. Likewise, the seventh revision did not carry an assay for the drug, but its official extract was assayed to contain 15 per cent of total alkaloids. The extract was assayed by dissolving in ammoniacal solution of alcohol consisting of two volumes of alcohol to one volume of water. The alkaloids were then extracted with several portions of chloroform and after proper treatment dissolved in standard acid and titrated with standard alkali, brazil wood T.S. as the indicator.

The eighth revision of the Pharmacopoeia included an assay for Nux Vomica and required that it contain not less than 1.25 per cent of strychnine. The drug was first treated with a mixture of ether, chloroform and al-

cohol, to which ammonia water had been added. The alkaloids were then extracted from the immiscible solvent with successive portions of sulphuric acid, the acid extracts made alkaline with ammonia and extracted with successive portions of chloroform. After the chloroform extracts were evaporated to dryness, the residue was taken up in weak sulphuric acid, treated with a fairly concentrated solution of nitric acid to destroy the brucine and finally extracted with several portions of chloroform to obtain the free strychnine. The per cent of strychnine was determined volumetrically.

The ninth revision required that Nux Vomica yield not less than 2.5 per cent of the alkaloids of nux vomica and the determination for strychnine alone was omitted. The assay underwent some radical changes from the previous one. The drug in No. 40 powder was first treated with a mixture of one volume of chloroform and two volumes of ether, and after allowing time for penetration ammonia was added. When the alkaloids were extracted, an aliquot portion was taken, extracted completely with weak acid and finally the acid solution made alkaline with ammonia and extracted completely with chloroform. The percentage of alkaloids were determined volumetrically, using cochineal T.S. as the indicator.

The tenth revision requirement for Nux Vomica is the same as that of the ninth, namely 2.5 per cent of total alkaloids. The initial solvent in this assay is a

3:1 mixture of ether and chloroform, and the final solvent chloroform. The method of assay is practically the same as that of the ninth revision, the only difference being in the solvents used to extract the alkaloids from the drug as mentioned above.

The eleventh revision states that Nux Vomica yields not less than 1.15 per cent of strychnine. The assay for the determination of strychnine has undergone many refinements as compared to previous assays. The initial solvent is a 3:1 mixture of ether-chloroform, the same as in the previous assay, however, a larger amount of the drug is used for the determination. The amount of dilute sulphuric acid used for extracting the alkaloids from the organic solvent is larger, the time of shaking is longer, and the amount of chloroform used to extract the alkaloids from alkaline aqueous solution is also much larger. The greatest difference, however, in the two assay procedures is the addition of a step, whereby the brucine is destroyed by exidation with nitric acid, etc., the strychnine recovered from the reaction mixture and determined as such. The solvent used for this recovery is chloroform.

Summary of the Pharmacopoeial History

Upon careful study it may be observed that since the introduction of alkaloidal assay methods in the Pharmacopoeia, very few have remained the same in succeeding revisions. These changes have been made as a result of care-

ful research in the field; however, it is quite evident that, while much progress has been made, there still remains much to be done before the problem of alkaloidal assaying will be satisfactorily solved.

IV

EXPERIMENTAL PART

SOLUBILITIES

The solubilities of strychnine, quinine, atropine and caffeine in isopropyl ether, methylene chloride, mixtures of isopropyl ether-methylene chloride, mixtures of ethyl ether-chloroform, mixtures of isopropyl ether-chloroform and mixtures of ethyl ether-methylene chloride were determined.

The method employed for these determinations was as follows: 25 cc. of the solvent was placed in a small bottle and enough of the alkaloid added to insure an excess after shaking in a mechanical shaker over night. The bottle was then placed in a thermostat bath, regulated at 25°C to±0.1°, and allowed to remain in the bath for at least twelve hours in order that equilibrium between the solute and solvent would be reached. A volume of about 5 cc. was then pipetted off, placed in a tared weighing bottle and its weight recorded. The solvent was allowed to evaporate spontaneously, the residue dried to constant weight at 100°C, cooled in a desiccator over sulphuric acid, and its weight recorded.

The bottle was again shaken for three hours in a mechanical shaker and a sample determined as before. This procedure was repeated until constant results were

obtained which was usually after the second shaking.

The calculations were made upon a basis of grams of alkaloid soluble in 100 grams of solvent at 25°C and in the cases of chloroform and ether the solubilities of the various alkaloids were calculated from solubility data given in the United States Pharmacopoeia, Tenth Revision.

The accompanying tables (Tables I, II, III & IV) and graphs (Graphs I-XVI inclusive) will show clearly the solubilities of the above mentioned alkaloids in the individual solvents and mixed solvents under consideration.

It will be observed upon examination of Table I that strychnine is more soluble in isopropyl ether than in ethyl ether, and a great deal more soluble in chloroform than in methylene chloride. The best solvent then for strychnine is chloroform and the best mixed solvent is a 3:1 mixture of isopropyl ether and chloroform. In the accompanying graphs (Graphs I-IV) one will observe that the solubility of strychnine in the mixed solvents falls between those in the individual solvents. This is to be expected.

Table II indicates that quinine is a great deal more soluble in ethyl ether than in isopropyl ether, while its solubility in methylene chloride is slightly less than in chloroform (Graphs V-VIII). Due to a gradual darkening and the formation of a viscid liquid in each case, it was very difficult to prepare saturated solutions of quinine in methylene chloride and in mixed solvents where

methylene chloride was one of the components. It would appear, however, that quinine is not nearly so soluble in mixtures of ethyl ether-methylene chloride and isopropyl ether-methylene chloride as might be expected when its solubility in the individual solvents is considered. It is also of interest to note that quinine in the presence of methylene chloride gradually undergoes decomposition. Such a decomposition, however, would probably not interfere with the use of this solvent in the assay of cinchona, since the assay could be completed before any appreciable change took place. Experiments will be recorded later to substantiate or disprove this conclusion.

Table III shows that caffeine is soluble to the extent of 0.18 grams in 100 grams of ethyl ether and 0.15 grams in 100 grams of isopropyl ether. It also shows that caffeine is soluble in chloroform to the extent of 12.2 grams per 100 grams of solvent and in methylene chloride to the extent of 8.67 grams in 100 grams of solvent. In other words, isopropyl ether is practically as good a solvent as ethyl ether for caffeine, while methylene chloride dissolves only about two-thirds as much as chloroform. (Graphs IX-XII).

In Table IV it may be seen that atropine is soluble in ethyl ether to the extent of 5.63 grams per 100 grams of solvent and in isopropyl ether to the extent of 1.03 grams per 100 grams of solvent. Its solubility in chloroform is 67.56 grams per 100 grams of solvent, and

in methylene chloride 65.23 grams per 100 grams of solvent. The accompanying graphs (Graphs XIII-XVI) indicate the number of grams of atropine soluble in 100 grams of solvent.

Table I.

Strychnine

Grams soluble in 100 grams solvent

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.021	1.62	3.26	6.27	13.5
Isopropyl ether	Isopropyl ether, 3 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 1 vol.	Isopropyl ether, 1 v. Chloroform, 3 vol.	Chloroform
0.058	1.73	3.38	7.39	13.5
Isopropyl ether	Isopropyl ether, 3 vol. Methylene chlor. 1 vol.	Isopropyl ether, 1 vol. Methylene chlor. 1 vol.	Isopropyl eth, 1 vol Methylene chl. 3 vol	Methylene Chloride
0.058	0.061	0.068	0.077	0.08
Ethyl ether	Ethyl ether, 3 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 3 vol	Methylene Chloride
0.021	0.029	0.032	0.047	0.08

Table II.

Quinine

Grams soluble in 100 grams solvent

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
74.0			44.31	61.7
Isopropyl eth.	Isopropyl eth. 3 vol. Chloroform, 1 vol.	Isopropyl eth. 1 vol. Chloroform, 1 vol.	Isopropyl eth. 1 vol. Chloroform, 3 vol.	Chloroform
0.312			37.9	61.7
Ethyl ether	Ethyl ether, 3 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 3 vol.	Methylene Chloride
74.00			41.80	58.2
Isopropyl eth.	Isopropyl eth. 3 vol. Methyl. chlor. 1 vol.	Isopropyl eth. 1 vol. Methyl. chlor. 1 vol.	Isopropyl eth. 1 vol. Methyl. chlor. 3 vol.	Methylene Chloride
0.312			40.84	58.2

Table III.

Caffeine

Grams soluble in 100 grams solvent

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.18	0.39	1.58	5.39	12.20
Isopropyl eth.	Isopropyl eth. 3 vol. Chloroform, 1 vol.	Isopropyl eth. 1 vol. Chloroform, 1 vol.	Isopropyl eth. 1 vol. Chloroform, 3 vol.	Chloroform
0.15	0.30	1.39	5.29	12.20
Isopropyl eth.	Isopropyl eth. 3 vol. Methyl. chlor. 1 vol.	Isopropyl eth. 1 vol. Methyl. chlor. 1 vol.	Isopropyl eth. 1 vol. Methyl. chlor. 3 vol.	Methylene Chloride
0.15	0.29	1.27	4.50	8.67
Ethyl ether	Ethyl ether, 3 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 3 vol.	Methylene Chloride
0.18	0.26	1.97	4.72	8.67

Table IV.

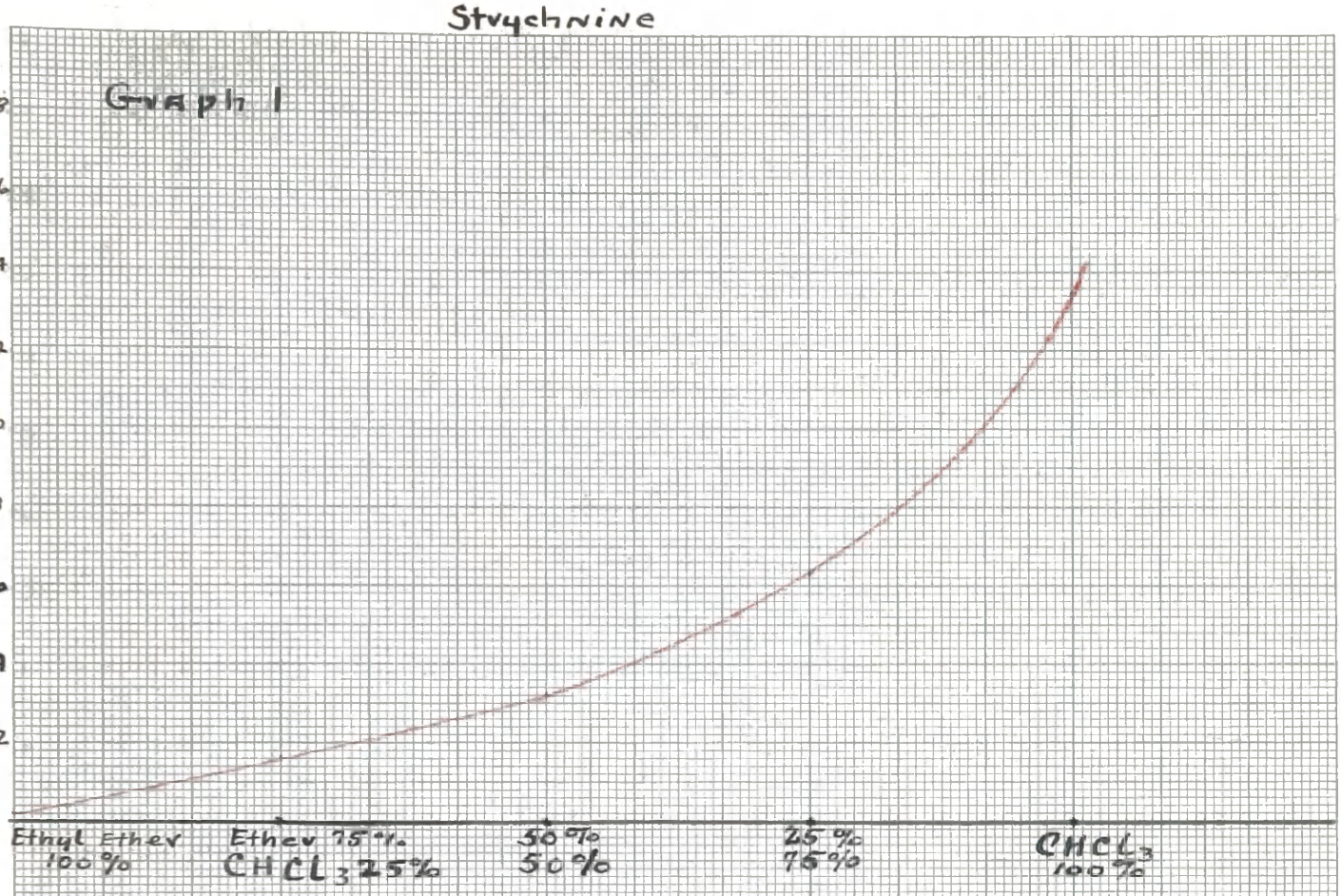
Atropine

Grams soluble in 100 grams solvent

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
5.63	12.14	28.50	52.50	67.56
Isopropyl eth.	Isopropyl eth. 3 vol. Chloroform, 1 vol.	Isopropyl eth. 1 vol. Chloroform, 1 vol.	Isopropyl eth. 1 vol. Chloroform, 3 vol.	Chloroform
1.03	11.22	24.9	47.20	67.56
Ethyl ether	Ethyl ether, 3 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 1 vol.	Ethyl ether, 1 vol. Methyl. chlor. 3 vol.	Methylene Chloride
5.63	17.69	32.23	45.55	65.23
Isopropyl eth.	Isopropyl eth. 3 vol. Methyl. chlor. 1 vol.	Isopropyl eth. 1 vol. Methyl. chlor. 1 vol.	Isopropyl eth. 1 vol. Methyl. chlor. 3 vol.	Methylene Chloride
1.03	10.08	22.9	43.7	65.23

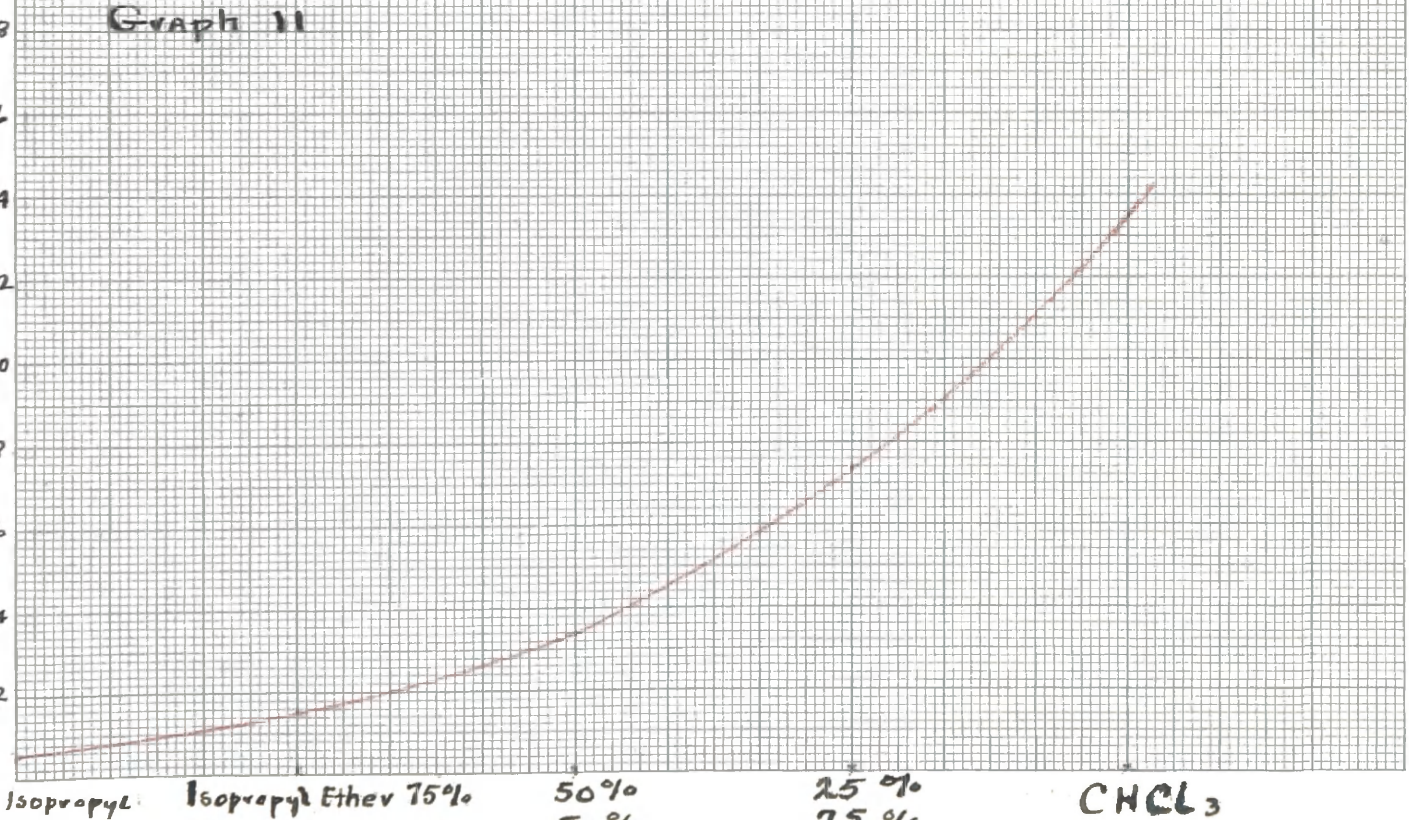
Strychnine

Graph I



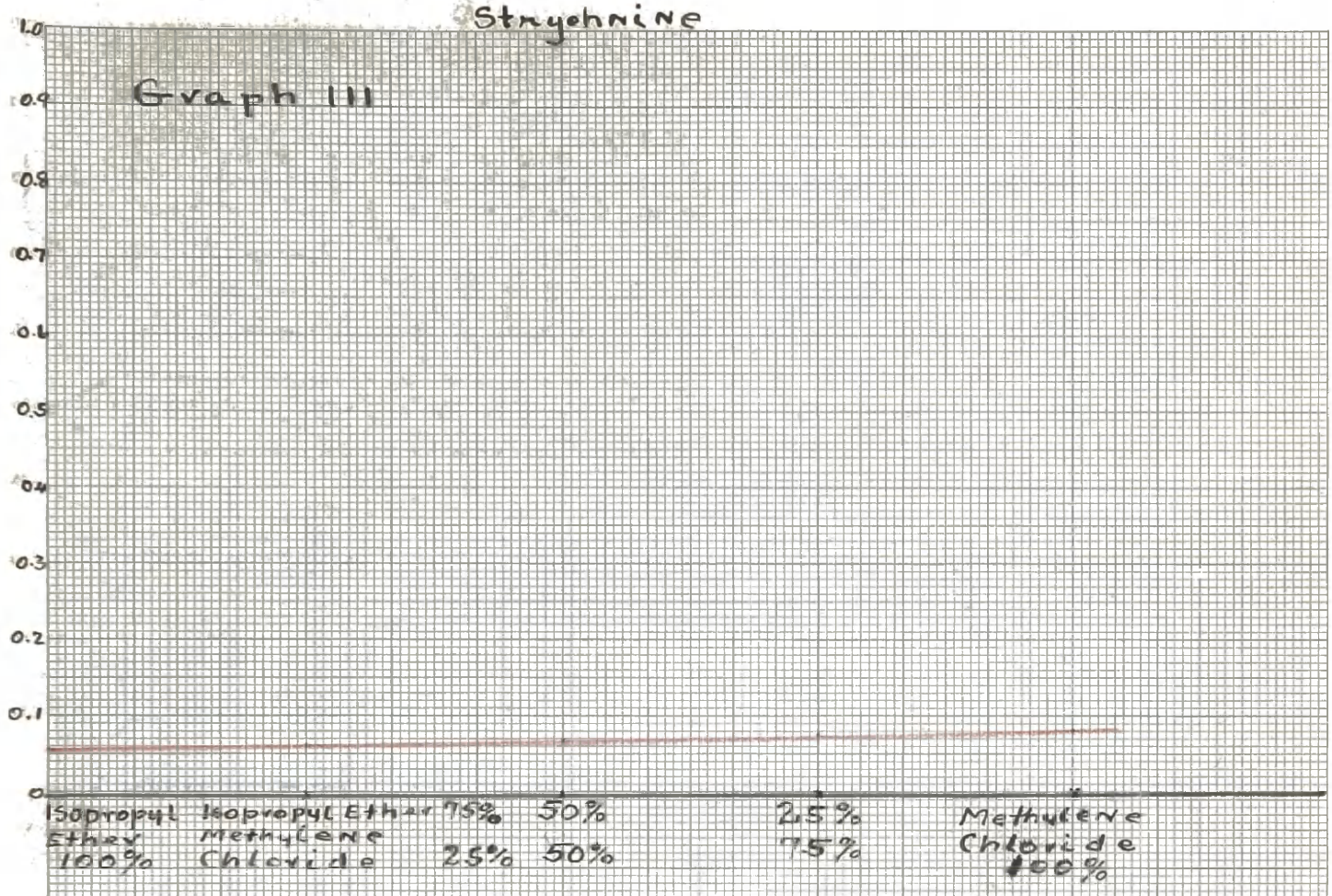
Strychnine

Graph II



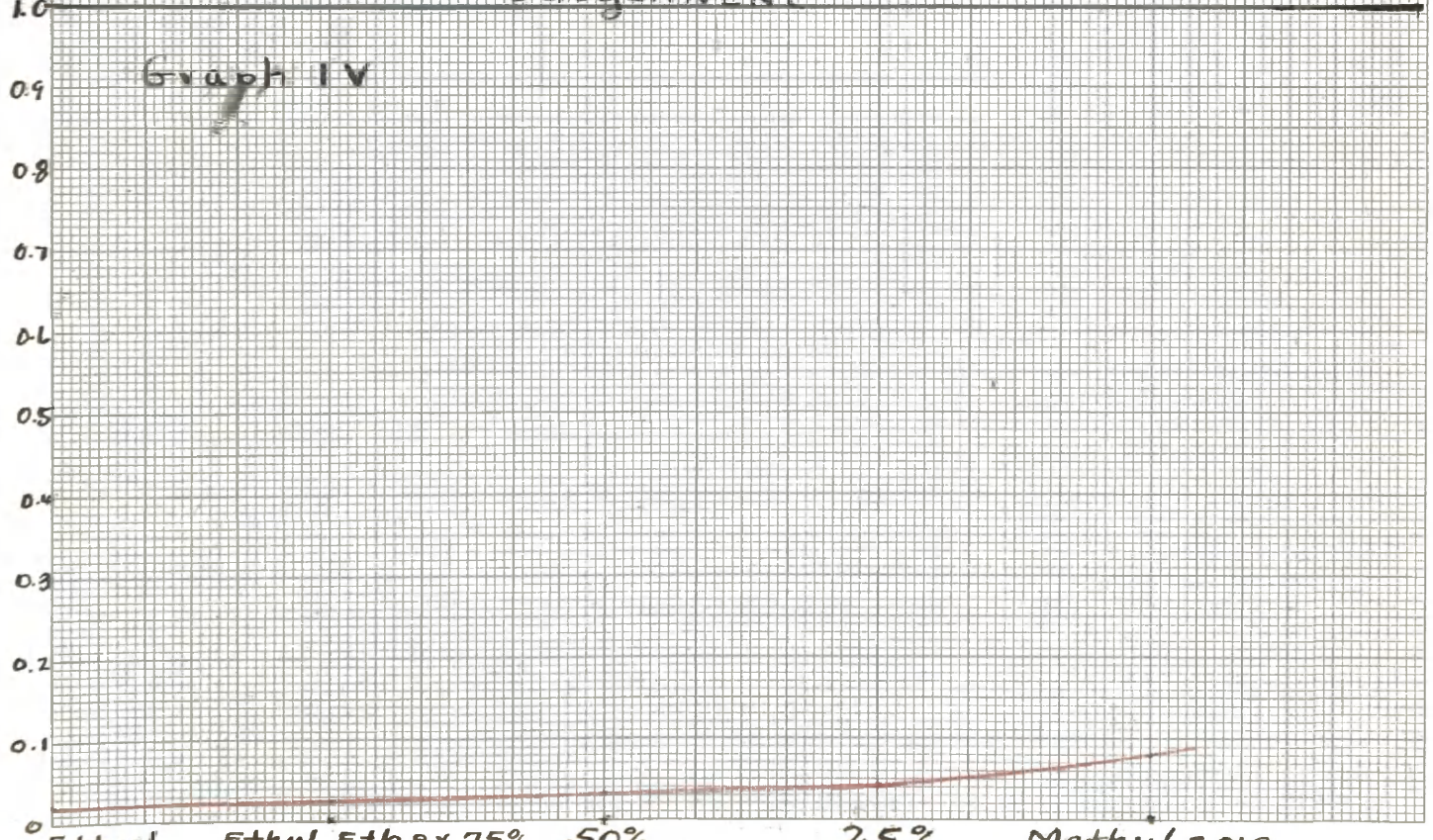
Strychnine

Graph III



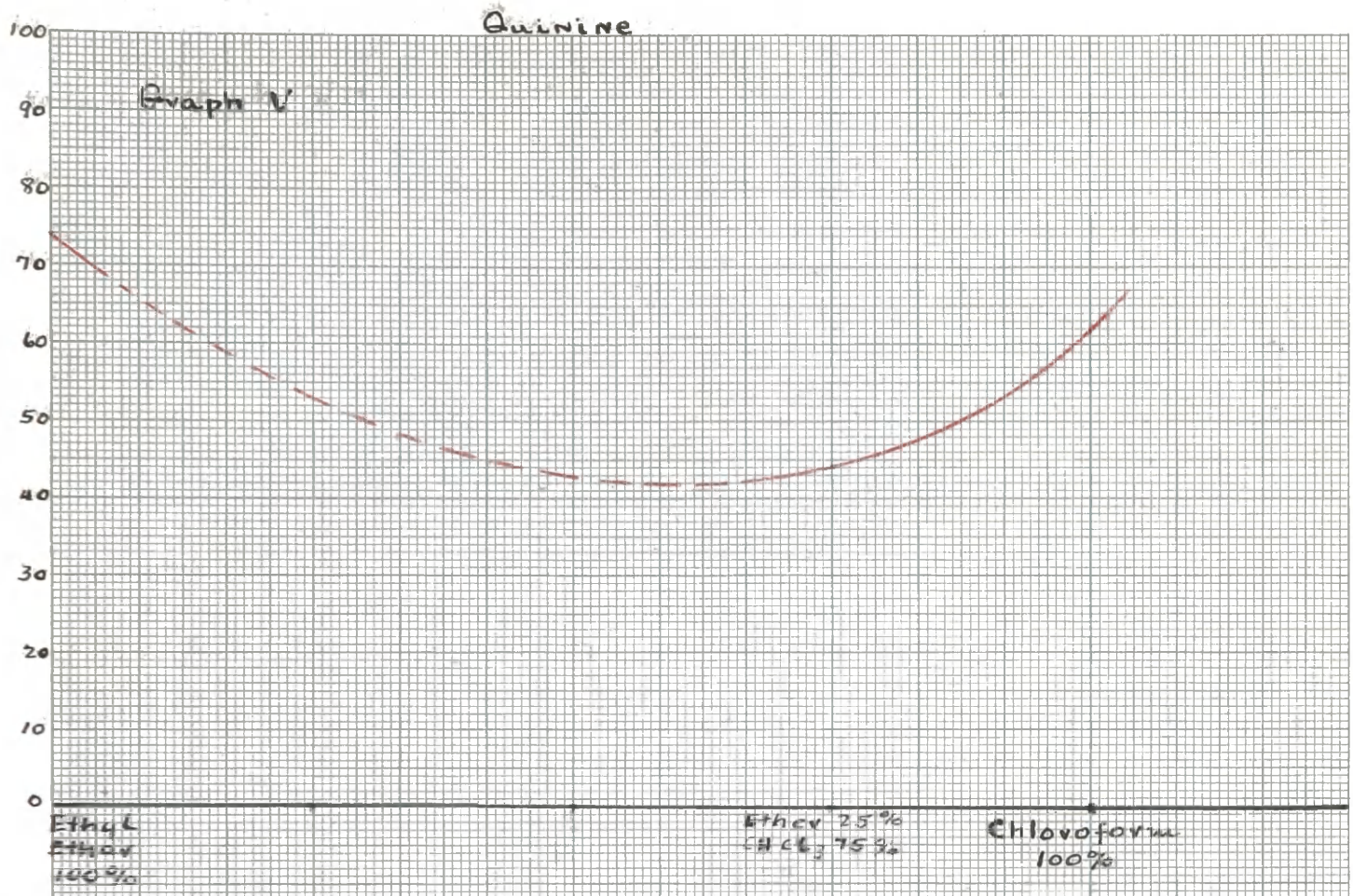
Strychnine

Graph IV



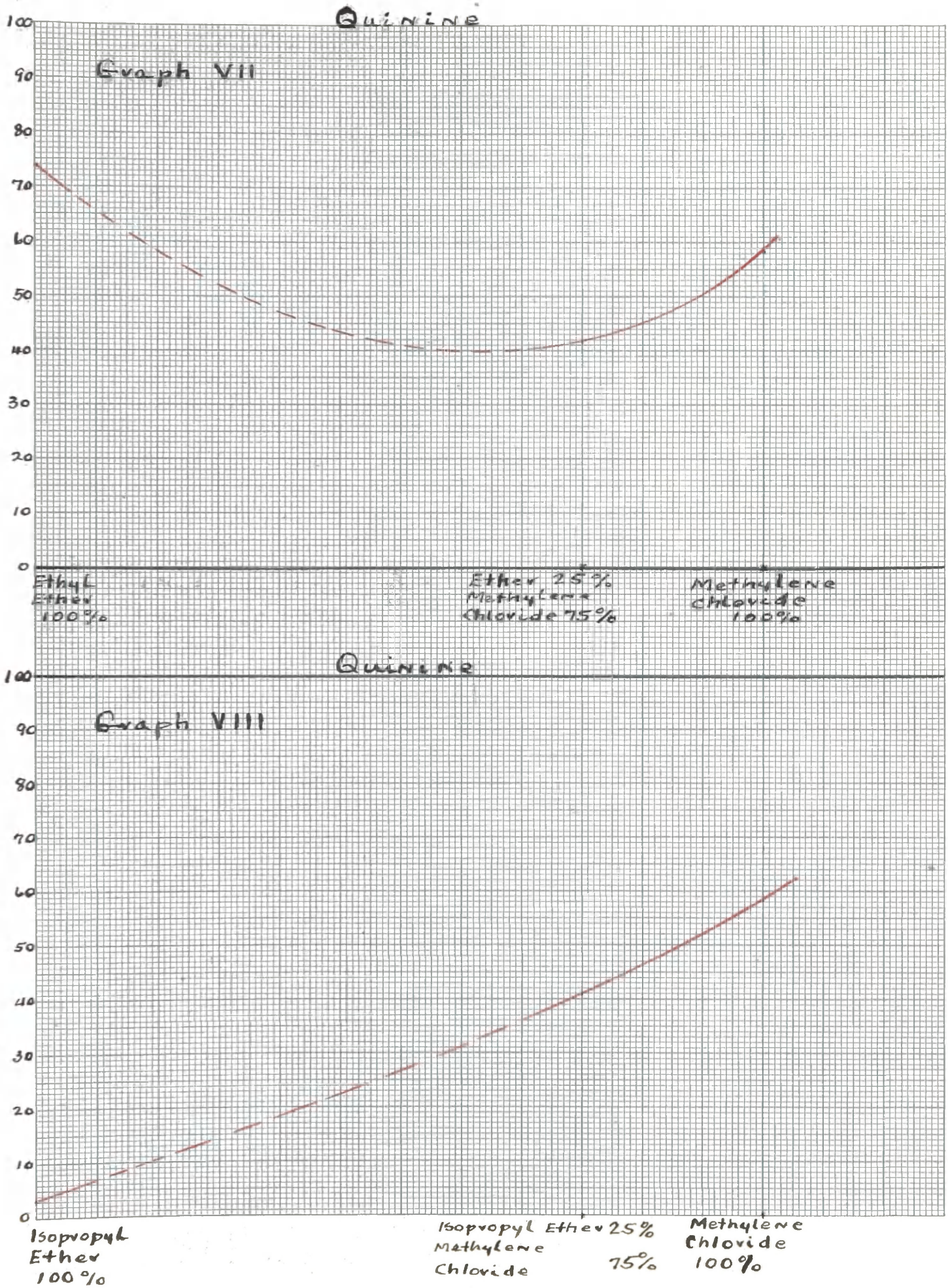
Quinine

Graph V



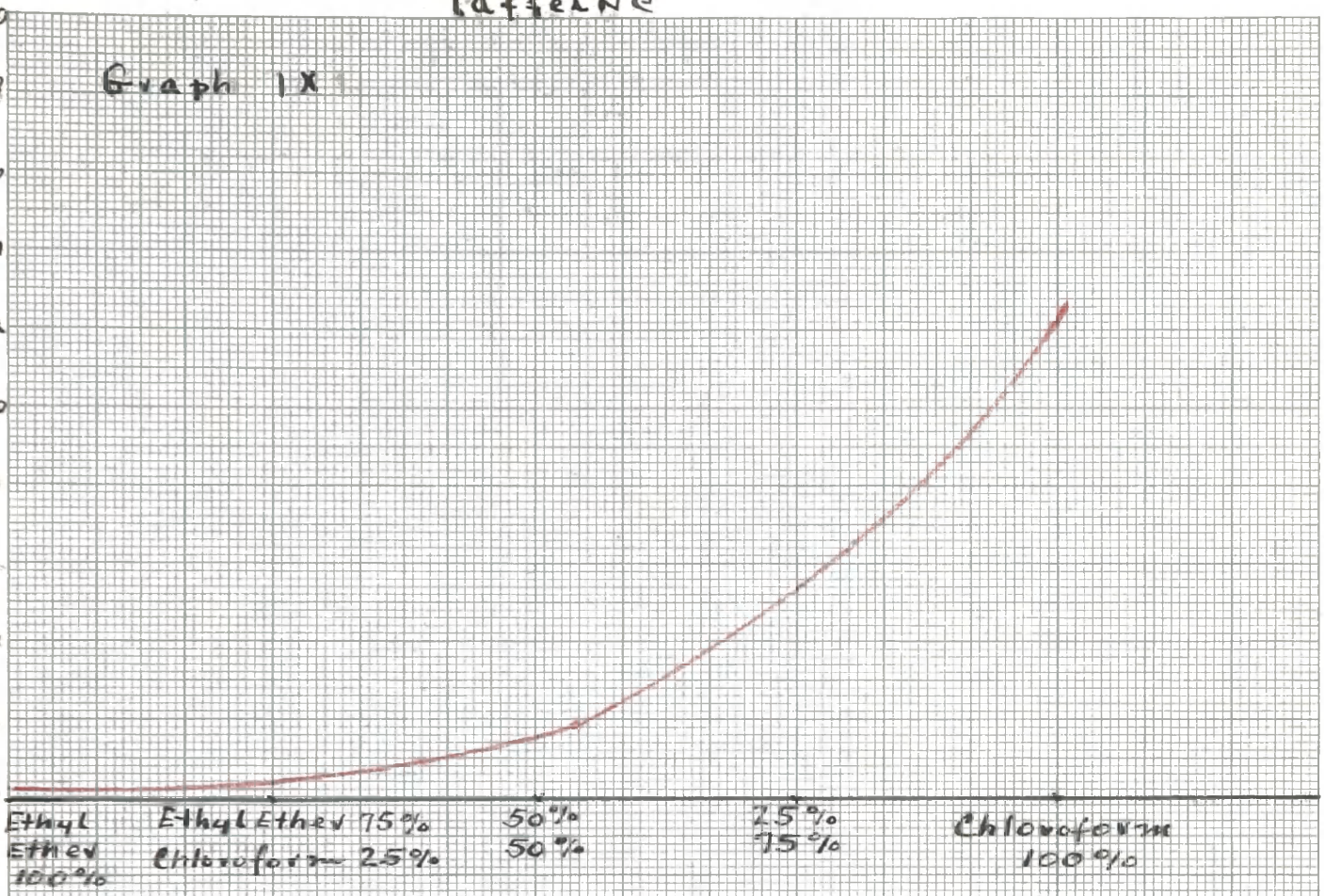
Quinine

Graph VI


Quinine
Graph VII
100
90
80
70
60
50
40
30
20
10
0
Ethyl Ether 100%
Ether 25% Methylene Chloride 75%
Methylene Chloride 100%
Quinine
Graph VIII
100
90
80
70
60
50
40
30
20
10
0
Isopropyl Ether 100%
Isopropyl Ether 25% Methylene Chloride 75%
Methylene Chloride 100%

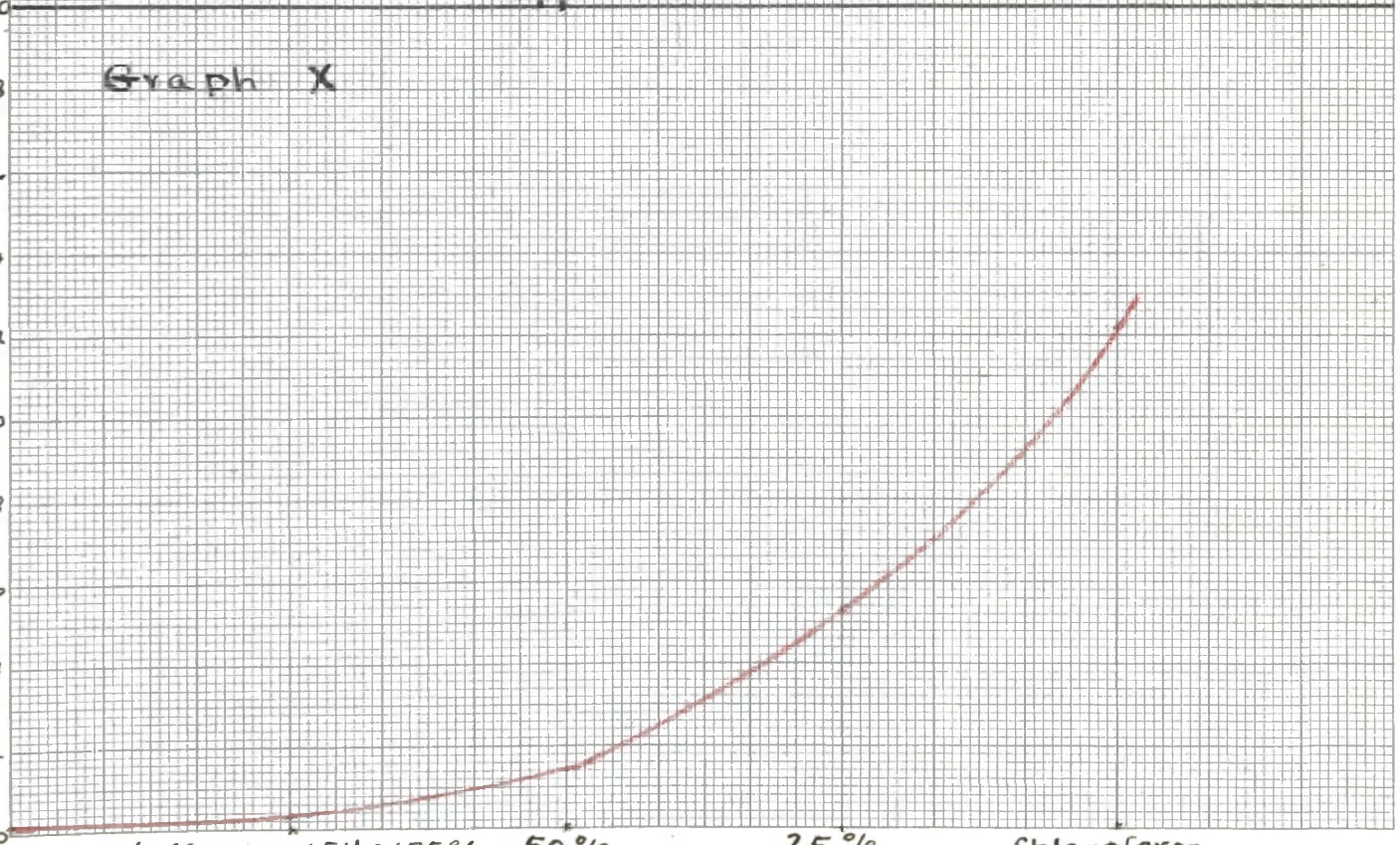
Caffeine

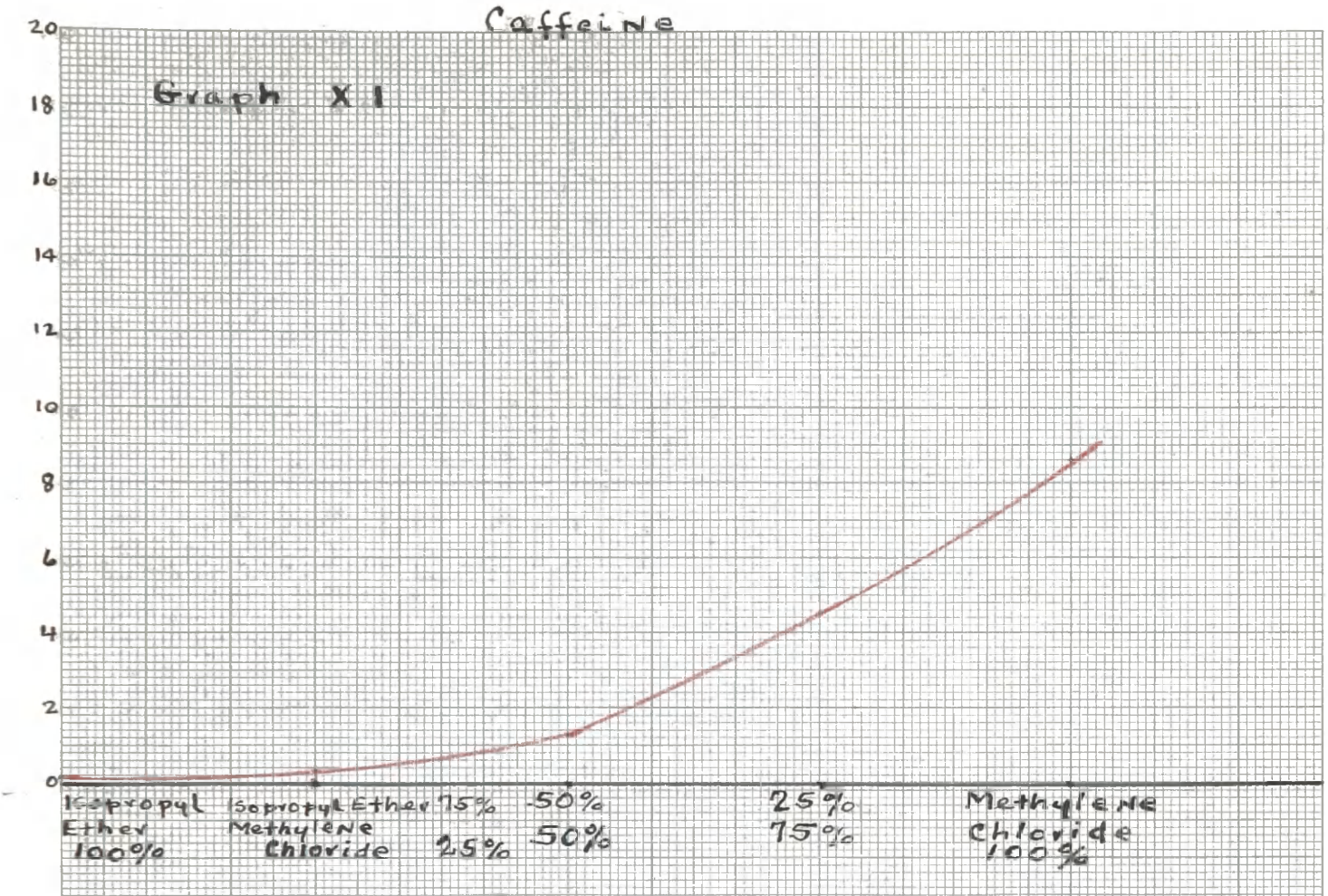
Graph IX

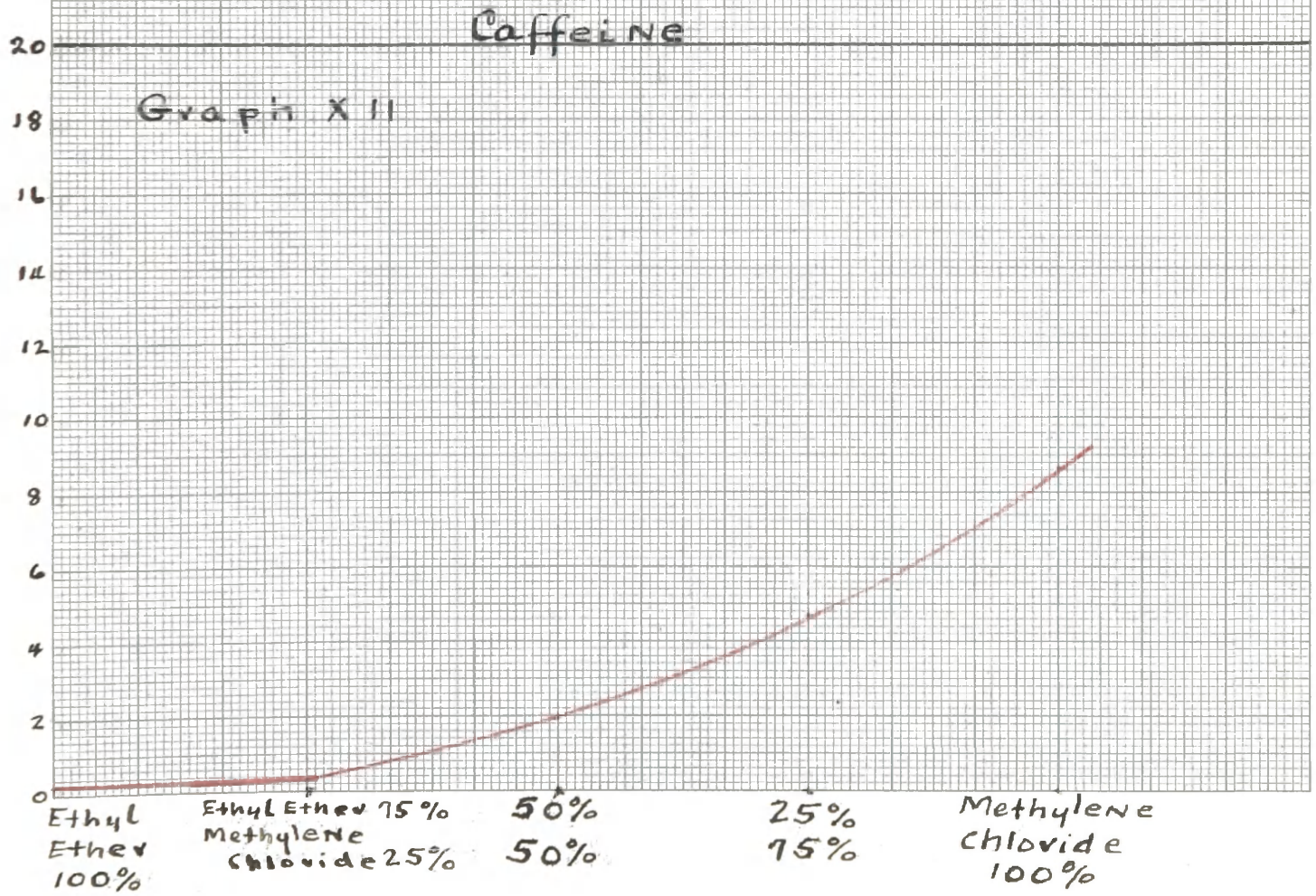


Caffeine

Graph X

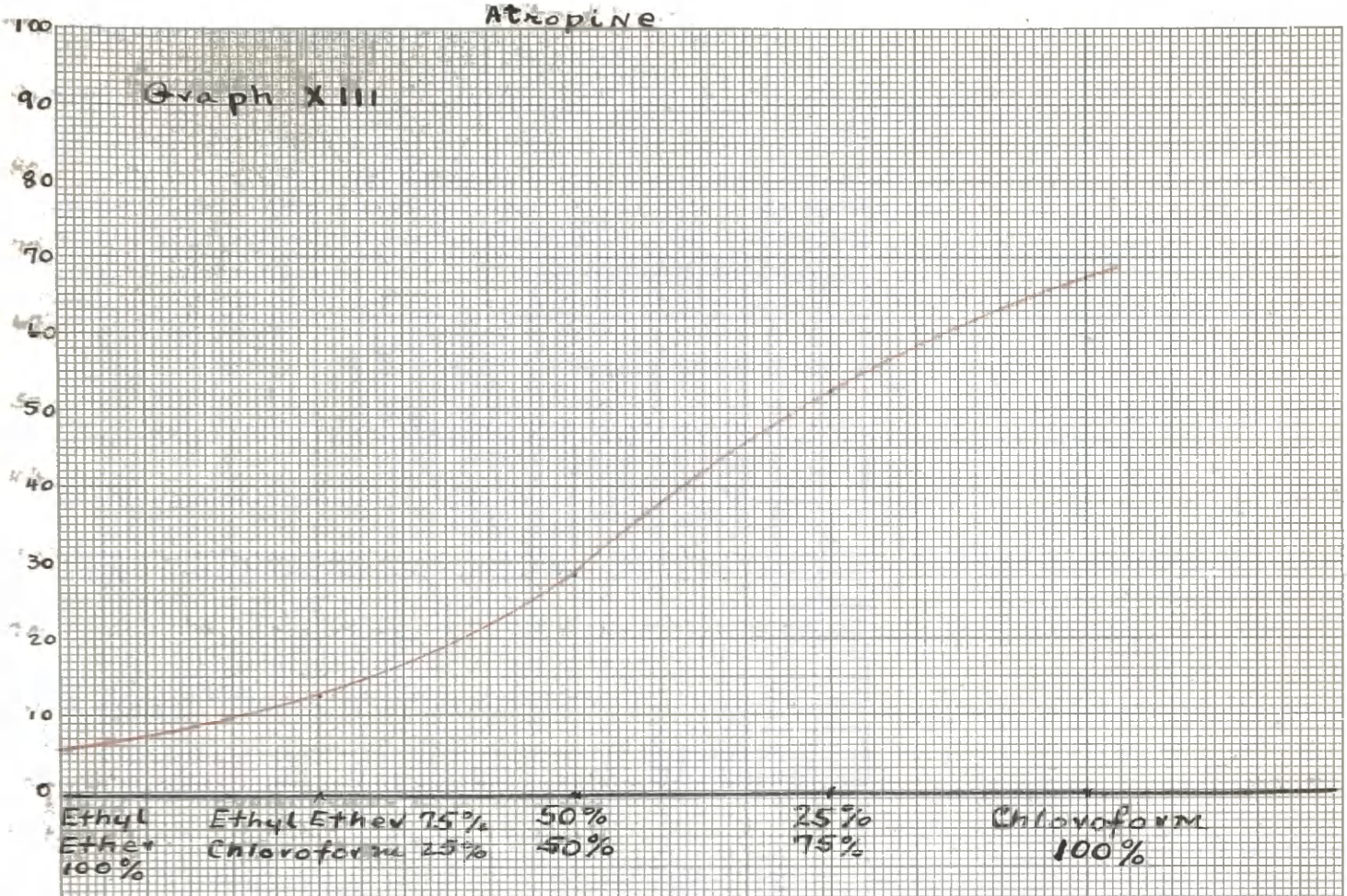



Caffeine
Graph XI
20
18
16
14
12
10
8
6
4
2
0
Isopropyl Ether 100%
Isopropyl Ether 75% Methylene Chloride 25%
50% 50%
25% 75%
Methylene Chloride 100%


Caffeine
Graph XII
20
18
16
14
12
10
8
6
4
2
0
Ethyl Ether 100%
Ethyl Ether 75% Methylene Chloride 25%
50% 50%
25% 75%
Methylene Chloride 100%

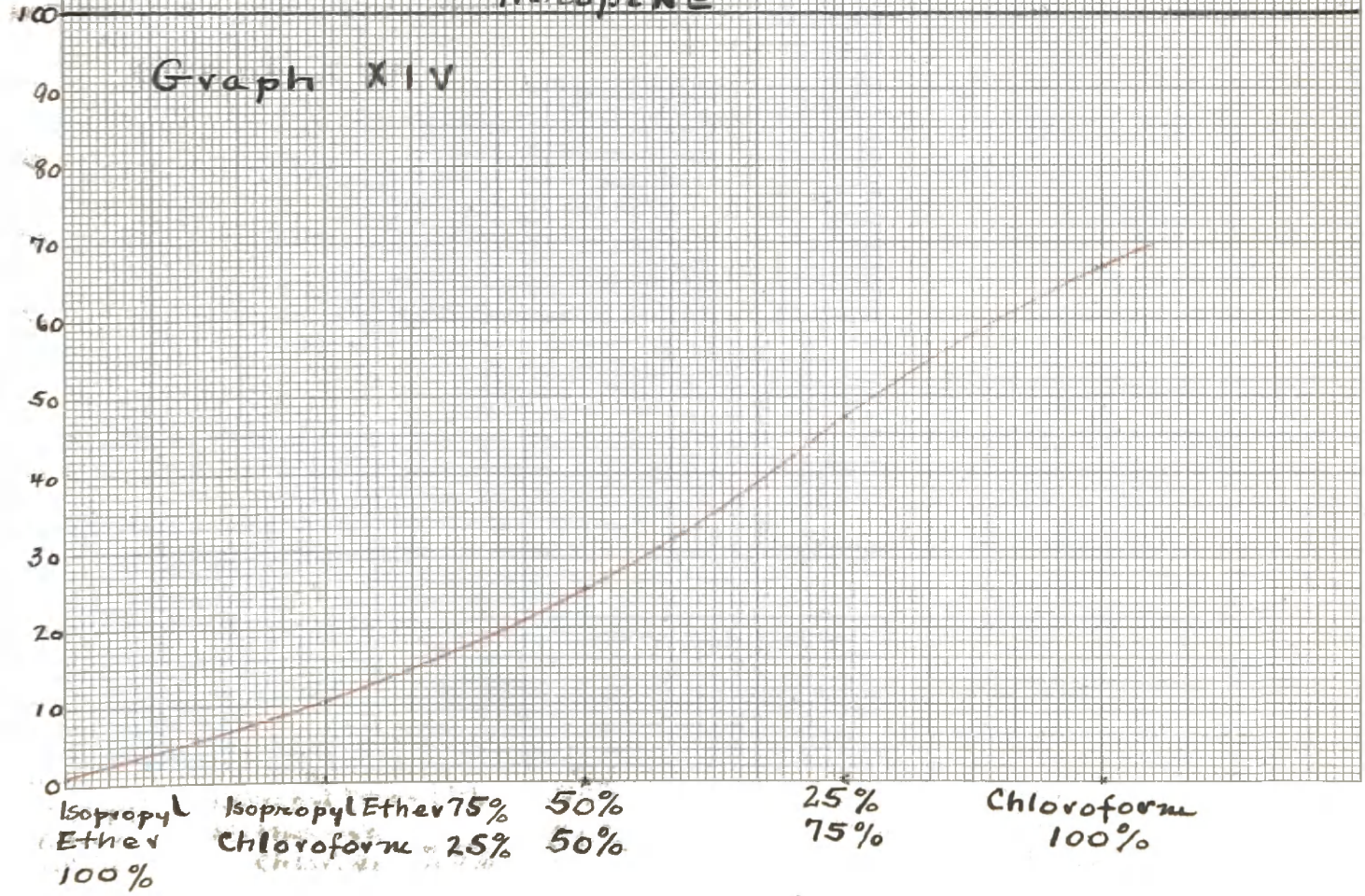
Atropine

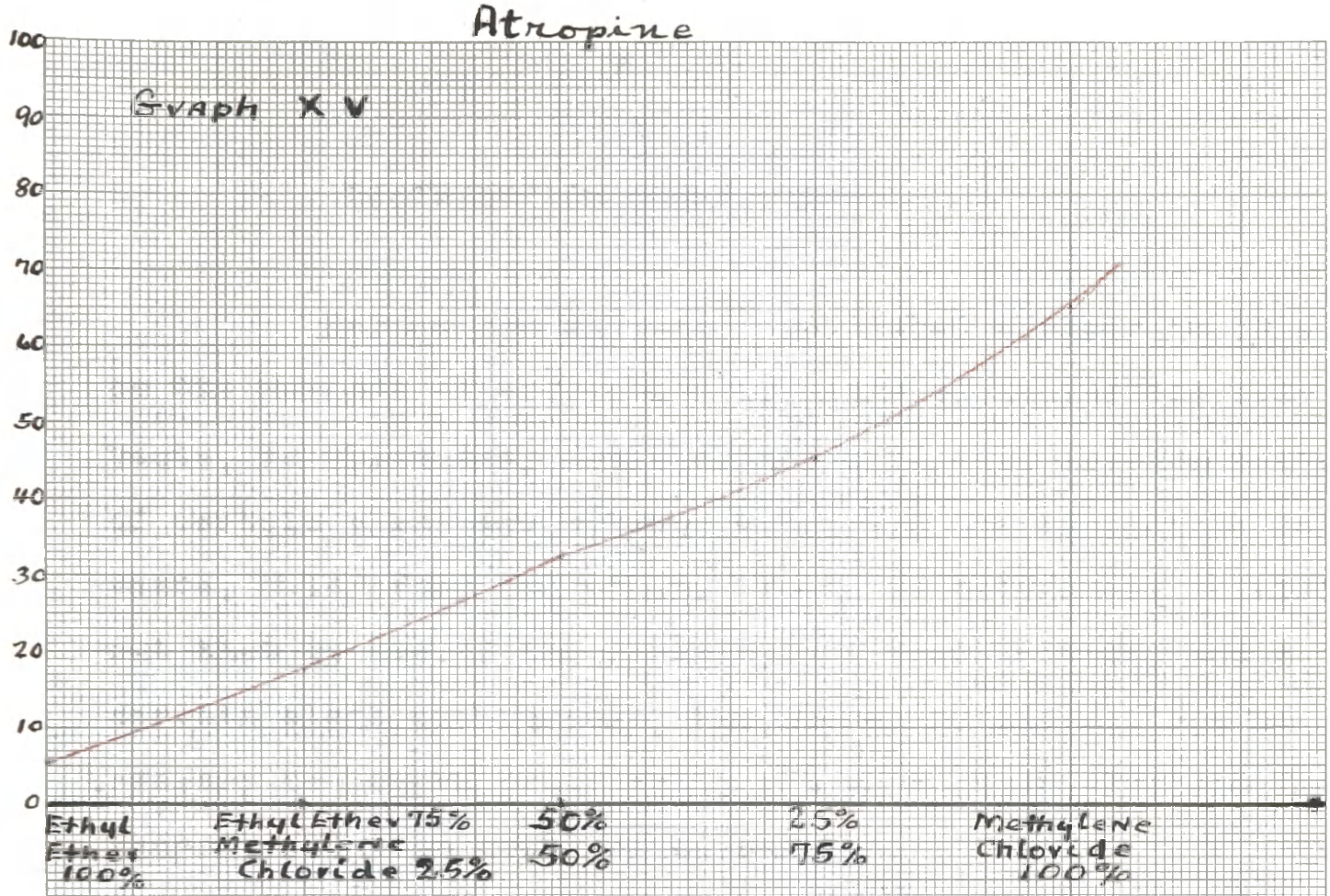
Graph XIII

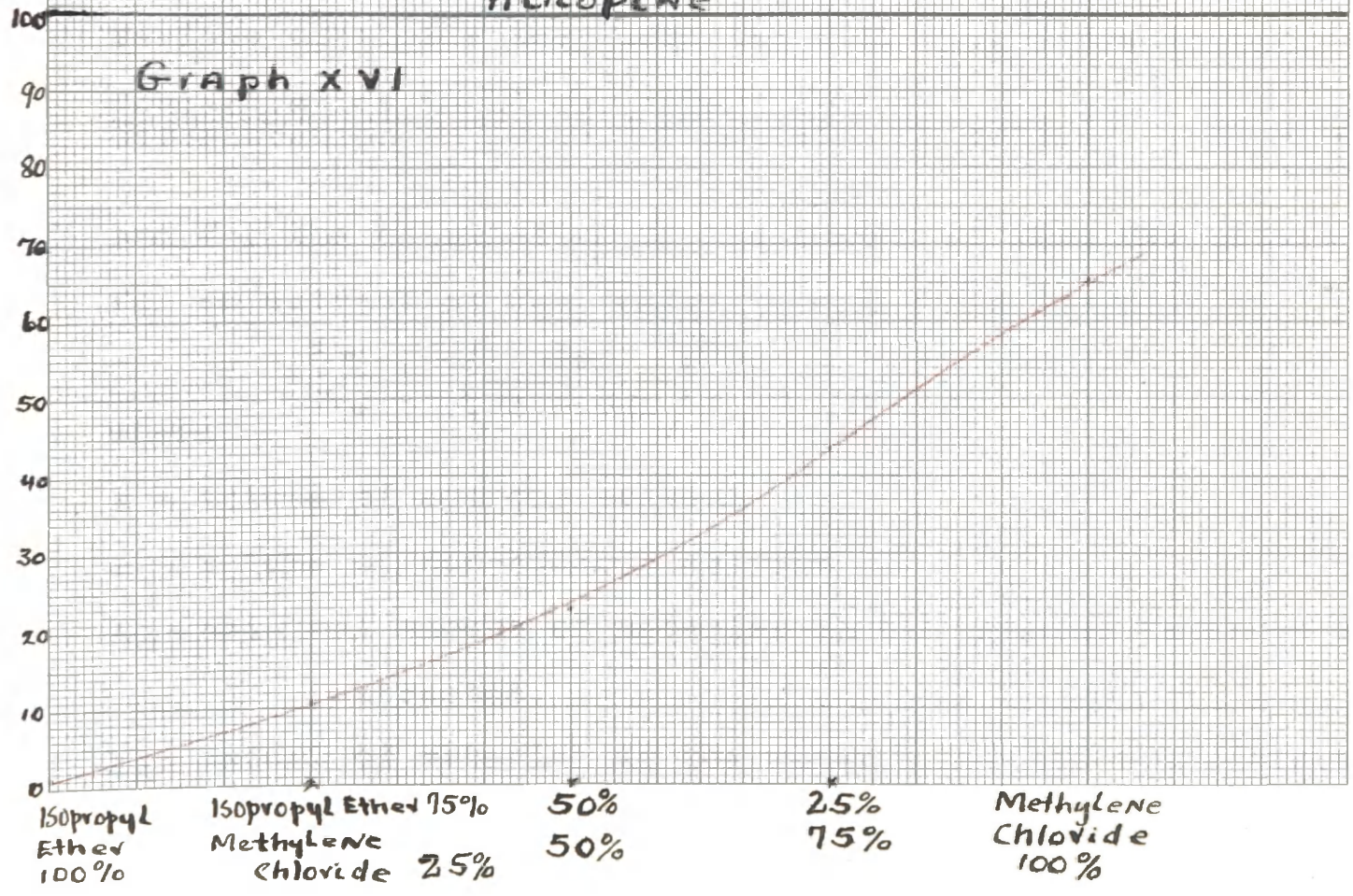


Atropine

Graph XIV




Atropine
Graph X V
100
90
80
70
60
50
40
30
20
10
0
Ethyl Ether 100%
Ethyl Ether 75% Methylene Chloride 25%
50% 50%
25% 75%
Methylene Chloride 100%


Atropine
Graph X VI
100
90
80
70
60
50
40
30
20
10
0
Isopropyl Ether 100%
Isopropyl Ether 75% Methylene Chloride 25%
50% 50%
25% 75%
Methylene Chloride 100%

V

DISTRIBUTION COEFFICIENTS

Marden and Elliott[59] published a paper in 1914 on the methods of extraction by means of immiscible solvents, in which they dealt mainly with distribution ratios of certain alkaloids between water and the immiscible solvents, chloroform and ether. These investigators pointed out that by use of the distribution coefficients, and a certain algebraic formula, the number of extractions necessary to remove practically all of a given alkaloid from aqueous solution could be calculated.

To calculate the distribution ratio (d) of the various alkaloids they used the following expression:

$$\frac{\text{Concentration in 10 cc. water}}{\text{Concentration in 10 cc. of non-aqueous solvent}} = \frac{C_1}{C_2} = (d)$$

and for the calculation of the number of shakings necessary for an extraction the following algebraic formula:

$$\frac{X_n}{X_o} = \left(\frac{da}{e + da}\right)^n$$

where

a = volume of aqueous solvent

e = volume of non-aqueous solvent

X_o = original amount of material in the aqueous layer to be extracted.

X_n = amount of material in the water layer after n extractions.

The expression X_n/X_o = the fraction of material in the water layer after n extractions. Thus, it is seen that the smaller the value of X_n/X_o, when calculated from the above formula, the fewer number of extractions necessary for complete removal of the alkaloid from aqueous solution.

In the aconitine, ether and aqueous ammoniacal system, using 100 cc. of water, 5 cc. of ammonia water and 50 cc. of ether, the result, (d) = 0.140 was obtained, and when 30 cc. of chloroform was used in place of the 50 cc. of ether the value of 0.017 (d = 0.017) was obtained. Thus, chloroform is shown to be a better immiscible solvent than ether for extracting aconitine from aqueous solution.

In the system atropine, water and chloroform the distribution ratio was found to be small, therefore, three extractions with 10 cc. portions of chloroform from 50 cc. of aqueous solution completely removed the atropine.

Codeine showed a nigh distribution ratio between water and ether (d = 0.939) and a low ratio between water and chloroform (d = 0.0067), indicating that chloroform is much the better immiscible solvent. Experiments showed this to be true.

The value of (d) for coniine between water and ether was shown to be about 0.05 and it was found that from three to five extractions, with 10 cc. portions of ether, would extract more than 99 per cent of this alkaloid. It was pointed out that due to the volatility of the coniine

the partition ratio was hard to obtain.

The distribution ratio of quinine between water and chloroform was found to be very small. Thus, three washings with 10 cc. portions of chloroform almost completely removed the quinine.

The value of knowing the distribution ratio between immiscible solvents in alkaloidal assaying is apparent when one considers that from such knowledge the number of extractions necessary in any given case may be calculated. Therefore, the tedious process of testing for complete extraction by means of some alkaloidal reagent is eliminated. With this thought in mind it was decided to determine the distribution ratios of certain alkaloids in the systems water-isopropyl ether, water-methylene chloride, and also, between water and various mixtures of these immiscible solvents. With this data at hand it was thought that a more intelligent study of the use of these solvents in alkaloidal assaying could be made.

Accordingly, the distribution ratios of atropine, caffeine, quinine and strychnine between water and isopropyl ether, water and methylene chloride and water and mixtures of isopropyl ether-methylene chloride were determined.

In calculating the distribution ratios (d) the solubility of methylene chloride in water and the solubility of water in methylene chloride was considered to be negligible, therefore, the volume of each upon saturation with the other was taken as the initial volume. In the

case of isopropyl ether and water the solubility of isopropyl ether in water was taken as 8 cc. in 100 cc. and of water in isopropyl ether as 2 cc. in 100 cc. at 25°C. These values are only approximately accurate, but are sufficiently close to the true values as not to make any appreciable difference.

The alkaloids used in the determinations were Merck and Company products. The isopropyl ether was obtained from the Carbide and Carbon Chemicals Corporation and conformed to the following specifications,

Boiling Range	90 per cent distilled between 66 and 69°C at 760 mm. pressure.
Initial Boiling Point	Not less than 60° at 760 mm. Pressure.
Dry Point	Not more than 70° at 760 mm. pressure.
Color	Not more than 2% yellow.
Specific Gravity. . .	0.723 to 0.729 at 20/20°C.
Residue	Not more than 0.1 per cent.
Acidity	A 50 cc. sample does not contain more than the equivalent of 0.1 cc. normal acid or alkali.
Suspended Matter. . .	Practically free from suspended matter.

The product was subjected to careful distillation before use in order to get rid of the non-volatile residue. Only that portion distilling between 66°C and 69°C was used.

The methylene chloride used was obtained from the Advance Solvents and Chemical Corporation and E. I. duPont de Nemours and Company. The two products were essentially the same and conformed to the following specifications:

Color	Water white
Odor	No foreign odor
Boiling Range	39.2°C to 40°C at 760 mm.
Specific Gravity	1.33 at 15°/4°C
Residue	None
Moisture	No cloud at - 24°C
Acidity	Less than 0.001 per cent calculated as HCl.

These products were also subjected to careful distillation in order to get rid of any non-volatile residue.

The experiments were carried out in narrow glass stoppered bottles in a constant temperature bath regulated at 25°C to $\pm$ 0.1°. The bottles were so arranged in the bath that they could be turned end over end at a rate of about 20 revolutions per minute. At the end of one hour the bottles were allowed to stand in the bath until the two layers were completely separated and clear, after which time 25 cc. of the non-aqueous solvent was pipetted off, the solvent allowed to evaporate spontaneously and the residue dried to constant weight in a desiccator over sulphuric acid. The alkaloids were determined gravimetrical-

ly.

The technic used is essentially the same as that used by Marden and Elliott[60], only differing in minor details, as to time, etc.

Strychnine

The distribution coefficients of strychnine in the systems (a) water and isopropyl ether (b) water and a 3:1 mixture of isopropyl ether-methylene chloride, and (c) water and methylene chloride were determined. For these determinations 100 cc. of aqueous alkaloidal solution, 40 cc. of immiscible solvent and 5 cc. of ammonia water were used in each case. The results obtained are given in the following table (Table V).

TABLE V

Distribution Ratio of Strychnine Between Water and Isopropyl Ether

Wt. strychnine in sample	Wt. found in 25 cc. isopropyl ether layer	Wt. in isopropyl ether 32 cc.	Wt. in aqueous layer. 113 cc.	(d)*
0.0103	0.0027	0.0035	0.0068	0.599
0.0123	0.0032	0.0041	0.0082	0.608
0.0166	0.0044	0.0056	0.0110	0.594
			Average	0.600

As may be seen from the above table, the value of (d) is 0.600. This means that 0.6 of the strychnine

*(d) = $\frac{\text{Concentration in 10 cc. water}}{\text{Concentration in 10 cc. of non-aqueous solvent}}$

remains in the aqueous layer after one extraction, and upon extracting 50 cc. of the aqueous solution with 10 cc. portions of isopropyl ether (using 14 cc. for the first portion to allow for saturation of the aqueous layer) the value of (d) would be

$$\frac{0.6 \times 50}{0.6 \times 50 + 10} = \frac{30}{40} = 0.75$$

and it may be calculated that twelve such extractions, $\left(\frac{1}{1.3}\right)^{12}$, would not remove much over 96.5 per cent of the alkaloid from aqueous solution, indicating that isopropyl ether is entirely unsatisfactory for this purpose. Calculations: $\left(\frac{1}{1.3}\right)^{12} = 30.00$; $\frac{1 \times 100}{30} = 3.33\%$; $100.00 - 3.33 = 96.67\%$

TABLE VI

Distribution Ratio of Strychnine Between Water and a 3:1 Mixture of Isopropyl ether--Methylene Chloride

Wt. strychnine in sample	Wt. found in 25 cc. isopropyl ether methylene chloride layer	Wt. in isopropyl ether-methylene chloride layer, (32 cc.)	Wt. in water layer (113 cc.)	(d)
0.0381	0.0214	0.0274	0.0107	0.110
0.0481	0.0231	0.0296	0.0111	0.107
0.0546	0.0263	0.0336	0.0125	0.106
			Average	0.107

It will be observed from the above table (d = 0.107) that about one-tenth of the strychnine remains in the aqueous solution when equal volumes of the two solvents are used, and therefore, if 50 cc. of the aqueous

solution of the alkaloid is extracted with 10 cc. portions of a 3:1 mixture of isopropyl ether-methylene chloride (14 cc. for the first portion to allow for saturation of the aqueous layer) the value of (d) becomes

$$\frac{0.107 \times 50}{0.107 \times 50 + 10} = \frac{5.35}{15.35} = 0.384$$

and it would require only four extractions, $\left(\frac{1}{2.8}\right)^4$, to remove over 99 per cent of the strychnine and six extractions to almost completely remove the alkaloid from aqueous solution.

When it is remembered that the specific gravity of the immiscible solvent is an important factor in alkaloidal assay procedures, especially in extracting the alkaloid from aqueous solution, and that the specific gravity of a 3:1 mixture of isopropyl ether-methylene chloride is less than water, such a mixture should prove satisfactory in the assay of nux vomica.

TABLE VII

Distribution Ratio of Strychnine Between Water and Methylene Chloride

Wt. strychnine	Wt. found in 25 cc. methylene chloride	Wt. in methylene chloride layer (40 cc.)	Wt. in aqueous layer (105 cc.)	(d)
0.0811	0.0493	0.0788	0.0023	0.010
0.1204	0.0733	0.1168	0.0036	0.011
			Average	0.0105

As may be seen in the case of water and methylene chloride the distribution ratio is 0.0105. Thus,

when equal mixtures of these liquids are used a large per cent of the strychnine passes into the methylene chloride layer and when 50 cc. of the aqueous solution is extracted with 10 cc. portions of methylene chloride the value of (d) becomes

$$\frac{0.010 \times 50}{0.010 \times 50 + 10} \simeq \frac{0.5}{10.5} \simeq 0.048$$

and two extractions with 10 cc. portions, $\left(\frac{1}{21}\right)^2$, will remove over 99.5 per cent of the strychnine from aqueous solution. This indicates very clearly that methylene chloride is satisfactory for this purpose.

To prove the conclusion that two 10 cc. portions of methylene chloride will extract practically all of the strychnine from aqueous solution, samples were prepared and extracted, using 1 cc. of ammonia water. The results are given in Table VIII

TABLE VIII

Wt. strychnine	Total weight found	Percentage found
0.1000	0.0994	99.4
0.1000	0.0995	99.5
	Average	99.45

The extractions were carried out by uniformly shaking the separatory funnels for two mimutes in each extraction and then allowing sufficient time for the separation of the two layers.

Brucine

The amount of brucine extracted from aqueous

solution with isopropyl ether is so small that it is difficult to obtain the correct distribution ratio. Check results were not obtained; however, it is clear from the results obtained that isopropyl ether would not be satisfactory for this purpose.

A 3:1 mixture of isopropyl ether-methylene chloride will extract more brucine from aqueous solution than isopropyl ether alone, but in this case also, the number of extractions required are too great for the solvent to have any practical value.

The distribution ratio of brucine between water and methylene chloride was found to be 0.098. This would indicate that brucine could be removed from aqueous solution by a relatively few extractions using methylene chloride; however, more extractions would be required for brucine than for strychnine.

Atropine

The distribution coefficients of atropine in the systems (a) water and isopropyl ether (b) water and a 3:1 mixture of isopropyl ether and methylene chloride, and (c) water and methylene chloride are given in Tables IX, X and XI.

TABLE IX

Using isopropyl ether:

Wt. atropine	Wt. in 25 cc. isopropyl ether	Wt. in iso-propyl ether layer (32 cc.)	Wt. in aqueous layer (113 cc.)	(d)
0.0284	0.0051	0.0062	0.0219	0.943

TABLE IX (Cont'd.)

0.0401	0.0073	0.0093	0.0307	0.935
			Average	0.939

Using equal volumes of the solvent and water about as much atropine remains in the aqueous solution after one extraction as is extracted. Obviously, it would require too many extractions with this solvent to be of practical value.

TABLE X

Using a 3:1 mixture of isopropyl ether-methylene chloride:

Wt. atropine	Wt. in 25 cc. immiscible solvent	Wt. in immiscible solvent layer (32 cc.)	Wt. in aqueous layer (133 cc.)	(d)
0.0821	0.0301	0.0409	0.0412	0.308
0.1004	0.0366	0.0498	0.0506	0.301
0.1164	0.0432	0.0587	0.0577	0.301
			Average	0.306

When a 3:1 mixture of isopropyl ether and methylene chloride is used the value of (d) is 0.306. It would require 10 extractions with 10 cc. portions of such a mixture to extract 96.5 per cent of the atropine from 50 cc. of aqueous solution. It is clear, therefore, that such a mixture would not be satisfactory for this purpose.

TABLE XI

Using methylene chloride:

Wt. atropine	Wt. in 25 cc. methylene chloride	Wt. in methlene chloride layer (40 cc.)	Wt. in aqueous layer (105 cc.)	(d)
0.0804	0.0397	0.0635	0.0169	0.101

TABLE XI (Cont'd)

0.1005	0.0484	0.0774	0.0220	0.109
0.1205	0.0584	0.0934	0.0261	0.107
			Average	0.106

As may be seen from Table XI, ($d \simeq 0.106$), methylene chloride is a much better immiscible solvent to extract atropine from aqueous solution than a 3:1 mixture of isopropyl ether-methylene chloride. Calculations show that five 10 cc. portions of methylene chloride will remove 99.5 per cent from aqueous solution, which is about the same amount as may be removed by five 10 cc. portions of chloroform. Thus, methylene chloride might be used as a satisfactory substitute for chloroform in extracting atropine from aqueous solution.

Quinine

The distribution ratio of quinine in the systems (s) water and isopropyl ether (b) water and methylene chloride and (c) water and a 3:1 mixture of isopropyl ether-methylene chloride is small in each case. Tables XII, XIII and XIV show the results obtained in the above cases.

TABLE XII

Distribution Ratio of Quinine Between Water and Isopropyl Ether

Wt. quinine	Wt. in 25 cc. isopropyl ether	Wt. in isopropyl ether layer (32 cc.)	Wt. in aqueous layer (113 cc.)	(d)
0.0662	0.0390	0.0624	0.0038	0.024
0.1036	0.0609	0.0974	0.0062	0.026
			Average	0.025

Using the Value ($d \simeq 0.025$) calculations show that three washings with isopropyl ether would remove over 99.9 per cent of q uinine from aqueous solution.

TABLE XIII

Distribution Ratio of Quinine Between Water and a 3:1 Mixture of Isopropyl Ether-Methylene Chloride.

Wt. quinine	Wt. in 25 cc. immiscible solvent	Wt. in immiscible solvent layer (32 cc.)	Wt. in aqueous layer, (113 cc.)	(d)
0.0801	0.0462	0.0739	0.0062	0.033
0.0951	0.0534	0.0854	0.0097	0.043
0.1002	0.0585	0.0936	0.0066	0.031
			Average	0.035

A 3:1 mixture of isopropyl ether-methylene chloride would be satisfactory for extracting quinine from aqueous solution; however, based on the distribution ratio ($d = 0.035$) it is not quite as good as isopropyl ether alone.

TABLE XIV

Distribution Ratio of Quinine Between Water and Methylene Chloride

Wt. quinine	Wt. in 25 cc. methylene chloride	Wt. in methylene chloride layer (40 cc.)	Wt. in aqueous layer (105 cc.)	(d)
0.1014	0.0628	0.1004	0.0010	0.0039
0.1200	0.0743	0.1188	0.0012	0.0041
			Average	0.004

Thus, it may be seen that methylene chloride is exceedingly efficient as an immiscible solvent for ex-

tracting quinine from aqueous solution. Two 10 cc. portions will almost completely extract the quinine from 50 cc. of aqueous solution. Where the value of (d) is so small as in the above case the solubility of methylene chloride in water and water in methylene chloride should be taken into consideration. These values could not be found in the literature, and therefore were not considered in calculating the distribution ratios.

Caffeine

The distribution ratios of caffeine in the systems (a) water and isopropyl ether (b) water and methylene chloride and (c) water and a 3:1 mixture of isopropyl ether-methylene chloride indicate that any of the three solvents could be used for extracting caffeine from aqueous solution. For some reason good results could not be obtained; however, sufficiently accurate data were obtained to justify the conclusion that a 3:1 mixture of isopropyl ether-methylene chloride or methylene chloride alone would be highly satisfactory for this purpose.

From a consideration of the physical properties, isopropyl ether and methylene chloride appear to be well suited for use in alkaloidal assay work. Isopropyl ether has a specific gravity of 0.723-0.729 at 20°C, and a boiling point of about 67°C. Methylene chloride has a specific gravity of 1.33 at 15°C and a boiling point of about 40°C. By using a 3:1 mixture of isopropyl ether and methylene chloride, therefore, a solvent is obtained which has a

specific gravity less than water. Such a condition is desirable when one wishes to extract an alkaloid from the immiscible solvent by use of acidulated water. On the other hand, if it is desired that the alkaloid be removed from aqueous solution methylene chloride alone is to be preferred, because it will constitute the bottom layer and thus may be drawn off from the aqueous layer in the separatory funnel.

VI

STUDY OF ISOPROPYL ETHER AND METHYLENE CHLORIDE AS SOLVENTS IN ALKALOIDAL ASSAYS

Upon consideration of the many factors involved in alkaloidal assaying, few generalizations in regard to the value of a solvent can be made with any degree of accuracy. The solubility of the alkaloid or alkaloids in a given solvent is not always a criterion of its usefulness in extracting the alkaloids from vegetable drugs. The physical properties of the powdered drug may be such as to make it difficult to extract the alkaloids in a given time, while on the other hand, the nature of the solvent may be such that it will easily penetrate the cell walls and thus prove to be highly efficient in dissolving out the alkaloids.

It is for these and other reasons that a comparative study of isopropyl ether and methylene chloride with those solvents now used in certain official assay processes has been undertaken.

The drugs selected for study are Belladonna, Cinchona, Nux Vomica and Guarana. The assay procedures as given in the United States Pharmacopoeia X have been strictly followed, with no attempt to alter the methods of procedure in any way.

Belladonna Leaves[61]

The assay procedure in the United States Pharmacopoeia X is carried out by taking 10 Gm. of Belladonna Leaves in No. 60 powder placing in a percolator of special design, and adding a sufficient amount of a 3:1 mixture of ether-chloroform to completely saturate the drug. The drug is allowed to macerate for a short period of time, and ammonia water added. After macerating for 1 hour the drug is packed firmly and a 3:1 mixture of ether-chloroform passed through the percolator slowly until the drug is extracted.

The ether-chloroform mixture is then extracted with dilute sulphuric acid, using successive 15 cc. portions of acid until the organic solvent is free from alkaloids.

The acid solution is made alkaline with ammonia and extracted with successive portions of chloroform until the aqueous layer is free from alkaloids.

Finally the chloroformic solution is evaporated to dryness, the residue taken up in a little ether, and again evaporated to dryness. The residue is finally dissolved in standard sulphuric acid and the excess acid determined with standard sodium hydroxide.

Thus, it is observed that the initial solvent used to extract the alkaloids in the assay of Belladonna is a 3:1 ether-chloroform mixture, and the final solvent

chloroform alone. The following tables (Tables XV, XVI, XVII, XVIII, XIX, & XX) will show the variations in solvents, the number of extractions required, and the percentage of alkaloids found.

TABLE XV

Assay of Belladonna Leaves

(U.S.P. X Method)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Ether Chloroform	H_2SO_4, 2%	4	Chloroform	4	0.308
"	"	4	"	4	0.318
"	"	5	"	4	0.312
"	"	4	"	4	0.310
			Average		0.312

TABLE XVI

Assay of Belladonna Leaves

(U.S.P. X Method--Modified)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Isopropyl Ether Methylene Cl.	H_2SO_4, 2%	4	Methylene Chloride	4	0.142
"	"	4	"	4	0.154
"	"	4	"	4	0.152
"	"	4	"	4	0.147
			Average		0.149

TABLE XVII

Assay of Belladonna Leaves

(U.S.P. X Method--Modified)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Ether Chloroform	H_2SO_4, 2%	4	Methylene Chloride	4	0.303
"	"	4	"	4	0.310
"	"	4	"	4	0.307
"	"	4	"	4	0.318
				Average	0.309

TABLE XVIII

Assay of Belladonna Leaves

(U.S.P. X Method--Modified)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Ether Methylene Cl.	H_2SO_4, 2%	4	Methylene Chloride	4	0.294
"	"	4	"	4	0.283
"	"	4	"	4	0.290
"	"	4	"	4	0.279
				Average	0.284

TABLE XIX

Assay of Belladonna Leaves
(U.S.P. XI Method)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
10 cc. Alcohol 20 cc. Ether	H_2SO_4, 0.1N	4	Chloroform	4	0.318
"	"	4	"	4	0.312
"	"	4	"	4	0.328
"	"	4	"	4	0.301
				Average	0.315

TABLE XX

Assay of Belladonna Leaves

(U.S.P. XI Method--Modified)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
10 cc. Alcohol 20 cc. Isopropyl Ether	H_2SO_4, 0.1N	4	Chloroform	4	0.292
"	"	4	"	4	0.268
"	"	4	"	4	0.272
"	"	4	"	4	0.284
			Average		0.279

The sample of Belladonna Leaves used for the determination in Tables XV-XX inclusive was in a No. 60 powder and was labeled U.S.P. The average of four determinations are recorded in each case. These determinations were run in duplicate, the first pair being numbered 1,2 and the second pair 3,4 in the tables. In some cases more than four determinations were made and the four in closest agreement selected.

Table XV will show the results obtained when assayed according to the U.S.P. X method. It will be observed that in three cases four extractions were required with dilute sulphuric acid and in one case five extractions. The number of extractions will depend somewhat upon the operator; however, it is safe to say that four extractions with 15 cc. portions of 2 per cent sulphuric acid are sufficient for complete extraction in most cases. It will

also be observed that four extractions with chloroform (25, 20, 15, 15 cc.) are sufficient for complete extraction of the alkaloids from the alkaline aqueous solution.

The results in Table XVI were obtained by extracting the drug with a 3:1 mixture of isopropyl ether-methylene chloride, and using methylene chloride as the final immiscible solvent. The low results obtained are no doubt due largely to the inability of the isopropyl ether to penetrate the powdered drug and allow the mixed solvent to completely extract the alkaloids. This conclusion is substantiated by an examination of the results in Table XVIII, where the initial solvent is a 3:1 mixture of ethyl ether-methylene chloride and the final solvent methylene chloride.

Table XVII shows the results obtained when an initial solvent of 3:1 ether-chloroform is used, and methylene chloride as the final immiscible solvent. Upon examination of these results it is clear that methylene chloride is equally as efficient as chloroform for extracting atropine from aqueous solution.

Table XVIII shows the results obtained when a 3:1 mixture of ethyl ether-methylene chloride and a final solvent of methylene chloride was used. It is observed that methylene chloride substituted for chloroform in the initial solvent is not quite as efficient as the latter when used in the same proportion.

Table XIX indicates the results obtained when the same sample of Belladonna was assayed by the continu-

ous extraction method as outlined in the U.S.P. XI. Table XX shows the results obtained when isopropyl ether was substituted for ethyl ether in the above procedure.

From an analysis of the results in Tables XV--XX inclusive, it is seen that isopropyl ether is not as efficient a solvent as ethyl ether in the assay of Belladonna Leaves, according to the U.S.P. X or U.S.P. XI assay methods. However, methylene chloride is shown to be as efficient as chloroform for removing the alkaloids from the alkaline aqueous solution in the final extraction, and might, therefore, be used instead of chloroform for this purpose.

Cinchona[62]

Cinchona was selected because it represents an official drug assayed gravimetrically by Type Process A of the U.S.P. X. The powdered drug used in the assays was in a No. 60 powder and labeled U.S.P.

The steps in the assay of cinchona for total alkaloids are as follows: The drug is heated for one hour on a water bath with a small amount of diluted hydrochloric acid and distilled water. A 3:1 ether-chloroform mixture is then added and followed by ammonia water to render the mixture alkaline. The alkaline liquid is then shaken intermittently during two hours, or in a mechanical shaker for one hour. After standing over night, the mixture is again shaken for one-half hour.

An aliquot portion of the liquid is decanted,

representing a definite weight of the drug, and transferred to a separator. The alkaloids are extracted from the organic solvent with successive 15 cc. portions of 2 per cent sulphuric acid.

The acid solution, containing the total alkaloids in the form of sulphates, is made alkaline with ammonia water and completely extracted with chloroform. Finally the chloroformic extract is evaporated to dryness, the residue dried to constant weight at 100°C and weighed.

The data recorded in Tables XXI--XXXI inclusive was obtained by following strictly the assay procedure in the U.S.P. X, the only change being in the nature of the solvents used, as recorded in the tables.

TABLE XXI

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Ether Chloroform	H_2SO_4, 2%	5	Chloroform	7	7.96
"	"	4	"	7	7.84
"	"	4	"	7	7.92
"	"	4	"	7	7.87
			Average		7.89

TABLE XXII

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Solution	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Isopropyl Ether Methylene Cl.	H_2SO_4, 2%	4	Chloroform	6	3.30
"	"	4	"	6	3.24
"	"	4	"	5	3.14
"	"	4	"	6	3.20
			Average		3.22

TABLE XXIII

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Isopropyl Ether Methylene Cl.	H_2SO_4, 2%	4	Methylene Chloride	6	3.37
"	"	4	"	6	3.28
"	"	4	"	6	3.19
"	"	4	"	6	3.31
			Average		3.28

TABLE XXIV

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Isopropyl Ether Chloroform	H_2SO_4, 2%	4	Methylene Chloride	6	3.47
"	"	4	"	6	3.55
"	"	4	"	6	3.44
"	"	4	"	6	3.18
			Average		3.51

TABLE XXV

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk.
3:1 Ethyl Ether Chloroform	H_2SO_4, 2%	4	Methylene Chloride	6	7.81
"	"	4	"	6	7.71
"	"	4	"	6	7.79
"	"	4	"	6	7.87
			Average		7.79

TABLE XXVI

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Eth. Methylene Cl.	H_2SO_4, 2%	4	Methylene Chloride	6	7.80
"	"	5	"	6	7.82
"	"	4	"	6	7.79
"	"	4	"	6	7.88
			Average		7.82

TABLE XXVII

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Eth. Chloroform 5 % Alcohol	H_2SO_4, 2%	4	Chloroform	6	8.22
"	"	4	"	6	8.31
"	"	4	"	6	8.28
"	"	4	"	6	8.14
			Average		8.23

TABLE XXVIII

Assay of Cinchona for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Eth. Methylene Cl. 5 % Alcohol	H_2SO_4, 2%	4	Methylene Chloride	6	8.12
"	"	4	"	6	8.02
"	"	4	"	6	8.09
"	"	4	"	6	8.04
			Average		8.06

Table XXI shows the results obtained when the cinchona was assayed according to the U.S.P. method. It required four extractions in three cases and five extractions in one case, with 15 cc. portions of sulphuric acid. Seven extractions were required with chloroform in every case to completely remove the alkaloids from the alkaline aqueous solution, using 25, 20, 15 - - - 15 cc.

Table XXII is a summary of the results obtained when a 3:1 mixture of isopropyl ether-methylene chloride is used to extract the drug and chloroform to remove the alkaloids from the alkaline aqueous solution. From the amount of alkaloids obtained it is clear that such a mixture of isopropyl ether-methylene chloride is not suited as a solvent to extract cinchona alkaloids from the crude drug. The low results obtained are due to the inefficiency of the isopropyl ether and not to the methylene chloride, as may be seen from an examination of Table XXVI. The same number of extractions with dilute sulphuric acid are required

to remove the alkaloids from the organic solvent as were used in the experiments recorded in Table XXI. From a consideration of the distribution ratio between these two liquids this is to be expected.

Table XXIII indicates the results obtained when the initial solvent was a 3:1 mixture of isopropyl ether-methylene chloride, and the final solvent methylene chloride. The only conclusion that can be drawn from the data is that methylene chloride seems to be about as efficient as chloroform for removing the cinchona alkaloids from alkaline aqueous solution.

Table XXIV shows the results obtained when a 3:1 mixture of isopropyl ether-methylene chloride is used to extract the alkaloids from the drug and methylene chloride to remove the alkaloids from alkaline aqueous solution. As in Table XXIII, it may be seen that isopropyl ether is not suitable for removing cinchona alkaloids from the drug quantitatively, whether it be mixed with methylene chloride or chloroform.

The experiments carried out and recorded in Table XXV show that methylene chloride is perhaps a little more efficient than chloroform for removing the cinchona alkaloids from alkaline aqueous solution. The number of extractions necessary for the complete removal of the alkaloids by methylene chloride is six, as compared to seven when chloroform is used. However, an average of 0.1 per cent less alkaloids recovered indicates that extraction is

not quite as complete with methylene chloride, even though the seventh extraction gave no test for alkaloids. Such a discrepancy in results is likely due to experimental error, because the same amounts of solvent were used in each case, namely 25, 20, 15 - - - 15 cc., and from a consideration of the distribution ratio of quinine between water and methylene chloride, six extractions should be sufficient for the complete removal of the alkaloids.

The results in Table XXVI show that methylene chloride may be substituted for chloroform in the initial solvent without changing the results of the assay to any extent. Thus, when a 3:1 mixture of ethyl ether-methylene chloride is used to extract the alkaloids from Cinchona, the amount of total alkaloids is found to be 7.82 per cent as compared to 7.79 per cent when an ethyl ether-chloroform mixture is used.

Experiments were run to show the effect of a small amount of alcohol when used with the ether-chloroform mixture and with the ether-methylene chloride mixture to extract the alkaloids from the drug. Tables XXVII and XXVIII show that 5 per cent of alcohol mixed with either of the above solvents will extract more total alkaloids than when the solvents are used alone. When added to the ether-chloroform mixture the amount of total alkaloids obtained is 8.23 per cent, and when mixed with the ether-methylene chloride mixture 8.06 per cent of total alkaloids is obtained.

Nux Vomica[63]

This drug is assayed by Type Process A of the U.S.P. X, and the alkaloids are determined volumetrically. The steps in the assay are as follows: The drug is extracted with a 3:1 ether-chloroform mixture, which has been made alkaline with ammonia, T.S., according to the general procedure under Type Process A. An aliquot portion of the liquid is collected and extracted with dilute sulphuric acid to remove the alkaloids as sulphates from the organic solvent.

The acid aqueous solution is then made alkaline with ammonia T.S., and the alkaloids completely extracted with successive portions of chloroform. Finally, the chloroform is evaporated to dryness, the residue dried at 100°C to remove traces of ammonia and the alkaloids determined volumetrically, using methyl red, or cochineal as the indicator.

The assay procedure in the U.S.P. X was followed strictly, using a No. 60 powder in all cases. The data recorded in Tables XXIX-XXXIII inclusive were obtained by following this procedure, the only changes being in the solvents used.

Table XXIX shows the results obtained by the U.S.P. X assay method. Five extractions with 15 cc. portions of 2 per cent sulphuric acid were required to completely remove the alkaloids from the mixed organic sol-

TABLE XXIX

Assay of Nux Vomica for Total Alkaloids

(U.S.P. X Method)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Eth. Chloroform	H_2SO_4, 2%	5	Chloroform	6	2.40
"	"	5	"	6	2.48
"	"	5	"	6	2.52
"	"	5	"	6	2.45
			Average		2.46

TABLE XXX

Assay of Nux Vomica for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Isopropyl Ether Methylene Cl.	H_2SO_4, 2%	5	Chloroform	5	1.02
"	"	5	"	5	1.04
"	"	5	"	5	1.10
"	"	5	"	5	1.07
			Average		1.05

TABLE XXXI

Assay of Nux Vomica for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Ethyl Eth. Methylene Cl.	H_2SO_4, 2%	5	Chloroform	5	1.11
"	"	5	"	5	1.07
"	"	5	"	6	1.16
"	"	5	"	5	1.08
			Average		1.10

TABLE XXXII

Assay of Nux Vomica for Total Alkaloids

(U.S.P. X Method--Modified)

1st Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
3:1 Isopropyl Ether Methylene Cl.	H_2SO_4, 2%	4	Methylene Chloride	6	0.102
"	"	4	"	6	0.092
"	"	4	"	6	0.090
"	"	4	"	6	0.084
			Average		0.092

TABLE XXXIII

Assay of Nux Vomica for Total Alkaloids

(U.S.P. X Method--Modified)

last Solvent	Acid Used	Number Ext'n's	Final Solvent	Number Ext'n's	% Alk. Found
45% Isopropyl Ether 50% Chloroform 5% Alcohol	H_2SO_4, 2%	4	Chloroform	6	1.45
"	"	4	"	6	1.52
"	"	4	"	6	1.58
"	"	4	"	6	1.48
			Average		1.51

vent, and six extractions to remove them from the alkaline aqueous solution. The sample of Nux Vomica determined showed an average alkaloid content of 2.46 per cent.

Table XXX indicates the efficiency of isopropyl ether-methylene chloride mixture when used to extract the alkaloids from Nux Vomica. The amount of alkaloids found is less than 50 per cent of the amount shown to be present by the U.S.P. method. It is clear, therefore, that a 3:1 mixture of isopropyl ether-methylene chloride is not suit-

able as the initial solvent in extracting nux vomica alkaloids from the crude drug.

Ethyl ether and methylene chloride in the ratio of 3:1 parts by volume is also shown not to be a good solvent for the alkaloids of nux vomica. (Table XXXI). From the results obtained, it may be seen that chloroform is to be preferred over methylene chloride for this purpose. From a consideration of solubility data a mixture of three parts of isopropyl ether and one part of chloroform should extract as much of the alkaloids of nux vomica as a similar mixture of ethyl ether-chloroform. However, I was unable to extract over seventy per cent of the alkaloids present with this solvent. The trouble, of course, is the inability of the isopropyl ether to penetrate and soften the powdered drug to the same extent as ethyl ether, and thereby enable the chloroform to dissolve out the alkaloids.

Table XXXII shows that methylene chloride is about as efficient as chloroform for extracting nux vomica alkaloids from aqueous solution. Here the initial solvent is isopropyl ether-methylene chloride and the final organic solvent methylene chloride. The per cent of alkaloids found is 0.092 as compared to 1.05 in Table XXX. The addition of 5 per cent alcohol to a mixture of three volumes of isopropyl ether and one volume of chloroform increases the extractive power of the mixture somewhat. But the amount of alkaloids extracted amounts to only about sixty per cent of that obtained when a mixture of ethyl ether-

chloroform is used. (Table XXXIII).

From a consideration of the data in Tables XXIX-XXXIII inclusive, it is clearly shown that isopropyl ether is not as efficient as ethyl ether when used with chloroform or methylene chloride to extract the alkaloids from Nux Vomica. It is also evident that methylene chloride may be used in place of chloroform in the assay, wether to extract the alkaloids from the crude drug, or to remove them from aqueous solution in a later step of the assay. Methylene chloride possesses the apparent additional advantage over chloroform of forming less troublesome emulsions when shaken with aqueous alkaline solutions. In many of the assays run it was noticeable that methylene chloride forms less permanent emulsions under approximately the same conditions. This observation, however, needs to be investigated further.

Guarana[64]

TABLE XXXIV

Assay of Guarana for Caffeine

(N.F.V. Method)

1st Solvent	Acid Used	Amt. Acid Used	Final Solvent	Number Ext'n's	% Alk. Found
Chloroform	H_2SO_4, 1%	10 cc.	Chloroform	6	4.12
"	"	10 cc.	"	6	4.19
"	"	10 cc.	"	6	4.14
"	"	10 cc.	"	6	4.21
			Average		4.16

TABLE XXXV

Assay of Guarana for Caffeine

(N.F.V. Method--Modified)

1st Solvent	Acid Used	Amt. Acid Used	Final Solvent	Number Ext'n's	% Alk. Found
Methylene Cl.	H_2SO_4, 1%	10 cc.	Methylene Chloride	6	4.15
"	"	10 cc.	"	6	4.08
"	"	10 cc.	"	6	4.27
"	"	10 cc.	"	6	4.34
			Average		4.21

The sample of Guarana used for analysis was labeled N.F.V. and was in a No. 60 powder. When assayed by the N.F.V procedure, 4.16 per cent of caffeine was found to be present (Table XXXIV). Since chloroform is the solvent used to extract the alkaloid from the drug according to the official procedure, and also, since caffeine is not soluble in isopropyl ether to any great extent, it was decided to substitute methylene chloride alone for chloroform in the assay. Table XXXV shows the results obtained when methylene chloride is used in place of chloroform as the initial solvent, and also as the immiscible solvent to extract the caffeine from the alkaline aqueous solution. An average of 4.21 per cent caffeine shows that methylene chloride may be substituted for chloroform in the assay. Six extractions with methylene chloride were required to completely remove the caffeine from the alkaline aqueous solution. This is the same as when chloroform is used, therefore, from the

standpoint of time required methylene chloride is equally efficient but no more so, than chloroform for this purpose.

SUMMARY AND CONCLUSIONS

1. A historical review of the literature dealing with alkaloidal assaying by the "immiscible solvent" method has been made. Also, the various other methods that have been proposed for the quantitative estimation of alkaloids have been included in the historical section, as well as a review of the solvents used in the assay procedures in the several revisions of the United States Pharmacopoeia.

2. The solubilities of atropine, caffeine, quinine and strychnine in isopropyl ether, methylene chloride, and mixtures of these solvents have been determined.

3. From a consideration of these solubilities, isopropyl ether and methylene chloride should prove to be valuable solvents in the quantitative estimation of certain alkaloids.

4. The distribution coefficients of atropine, quinine and strychnine in the systems (a) water and isopropyl ether, (b) water and methylene chloride, and (c) water and mixtures of isopropyl ether-methylene chloride have been determined.

5. The conclusion has been reached, from a study of the distribution coefficients, that these solvents possess definite possibilities as "immiscible solvents", to be used in the extraction of certain alkaloids from aqueous solution.

6. It has been shown that a 3:1 mixture of isopropyl ether-methylene chloride is not as efficient as a 3:1 mix-

ture of ethyl ether-chloroform for extracting the alkaloids from the drug in the assay of Belladonna Leaves. It has also been shown that, methylene chloride is not quite as good as chloroform for the same purpose; however, it has been established that, methylene chloride is about as efficient as chloroform for removing the alkaloids from aqueous solution in the final extraction with immiscible solvent (Tables I-VI).

7. In the assay of Cinchona, isopropyl ether can not be substituted for ethyl ether to extract the alkaloids from the drug; however, methylene chloride may be used in place of the chloroform. It has been found that methylene chloride is equally as efficient as chloroform, for removing the alkaloids from the aqueous layer, in the final extraction of cinchona alkaloids. Alcohol increases the amount of material extracted when used with ethyl ether-chloroform or ethyl ether-methylene chloride in extracting the drug. (Tables VII-XIV).

8. Isopropyl ether is not as efficient as ethyl ether when used with chloroform or methylene chloride to extract the alkaloids from Nux Vomica. Methylene chloride may be used as a substitute for chloroform in the assay, either to extract the alkaloids from the crude drug or to remove them from the aqueous layer in the final extraction step of the assay. Less troublesome emulsions were encountered when methylene chloride was used to extract nux vomica alkaloids from alkaline aqueous solution than when

chloroform was used. (Tables XV-XIX).

9. Methylene chloride may be used as a substitute for chloroform in the assay of Guarana (Tables XX-XXI).

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