1.1

The object

The object is to study ion exchange resin-cinchona alkaloid system and the usefulness towards practical application.

1.2

Synthetic ion exchange resins : (1 - 16)

Ion exchange materials are of a wide variety. These may be inorganic or organic and of different shape and size. The common general structural principle is a framework with electric surplus charge and mobile counter ions. However, the various types of materials behave markedly differently. Ion exchange resins are the most significant of the ion exchange materials. These are gels and the matrix consists of an irregular, macromolecular, three dimensional network of hydrocarbon chains. The ionic groups attached to the matrix may be of various types such as

$$-SO_3^-\ ,\quad -COO^-\ ,\quad -PO_3^{-2}\ ,\quad -AsO_3^{-2}\ ,$$

for cation exchangers and

$$-NH_3^+\ ,\quad >NH_2^+\ ,\quad >N^+<\ ,\quad \gtrless S^+$$

for anion exchangers. Hence, ion exchange resins are crosslinked polyelectrolytes. They are insoluble, but have a limited swelling in water, depending on the crosslinking. The ion exchange behaviour of the resins is mainly dependent on the nature of the fixed ionic groups.

Commercial ion exchangers are insoluble solids. Liquid ion exchangers, such as long chain aliphatic amines and fatty acids or alkyl phosphates have also become of great interest.

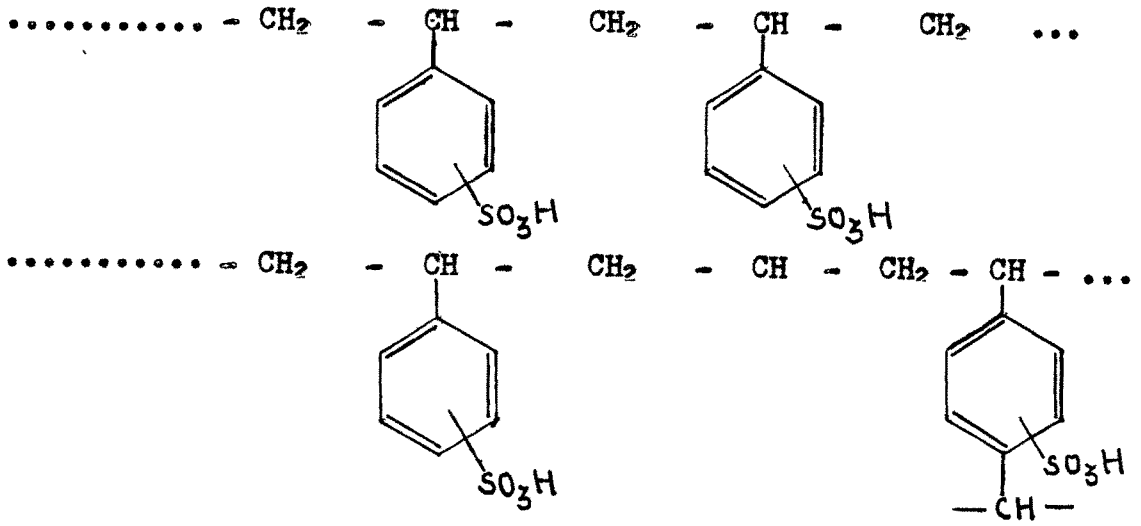
Synthetic resins in general, have superior chemical and mechanical stability, exchange capacity, exchange rates and versatility. Inorganic materials possess better thermal stability and resistance to radiation.

A wide variety of ion exchange resins have been prepared and some are available commercially under various trade names.

The synthesis of ion exchange resins should yield a three dimensional, crosslinked matrix of hydrocarbon chains carrying ionic groups. The resins can be prepared by condensation polymerisation or addition polymerisation and the ionogenic groups can be introduced, before, during or after the polymerisation. These groups may be of one or more types giving monofunctional or multifunctional cation exchange or anion exchange or amphoteric resins. Most of the earlier cation exchange resins were condensation products of phenol derivatives and aldehydes. Most of the present commercial resins are addition polymers prepared from vinyl monomers. These resins have a better chemical and thermal stability than the condensation polymers and their degree of crosslinking and particle size can be more easily controlled.

The monofunctional sulfonic acid cation exchange resins available are crosslinked polystyrenes with sulfonic acid groups, introduced by sulfonation of the polymer. The crosslinking agent used is divinylbenzene. Amberlite IR-120, Dowex 50, Nalcite HCR, Permutit Q, Duolite C-20 and C-25 and Lewatit

S-100 are resins of this type. The structure may be imagined as



Pure divinylbenzene is not readily available, hence the resins are prepared with a commercial product consisting of a mixture of the different divinylbenzene isomers (about 40 to 55 %) and ethylstyrene (about 60 to 45 %). Ethylstyrene is also incorporated into the matrix.

By varying the divinylbenzene content, the degree of crosslinking can be adjusted. The nominal DVB content is used to indicate the degree of crosslinking. It refers to the mole per cent of pure divinylbenzene (not of the commercial product) in the polymerisation mixture. Resins with low degree of crosslinking swell strongly and resins with high DVB content swell much less.

The copolymer beads are prepared by the pearl polymerisation technique. The monomers, from which stabilisers have been removed, are mixed and a polymerisation catalyst,

such as benzoyl peroxide is added. The mixture is then added to a thoroughly agitated aqueous solution at a required temperature (usually 85° to 100°C.). The mixture forms small droplets, which remain suspended. A suspension stabiliser (gelatin, polyvinylalcohol etc.) in the aqueous phase prevents agglomeration of the droplets. The size of the droplets depends mainly on the nature of the stabiliser, the viscosity of the solution and the agitation and can be varied within wide limits. The polymer is obtained in the form of fairly uniform beads.

The sulfonation of the beads is simple, if proper precautions are taken. The cracking of beads may be avoided by first swelling the beads in an organic solvent such as toluene, nitrobenzene etc. It is advisable to transfer the sulfonated beads first to a highly concentrated electrolyte solution, which causes less swelling and then to dilute the solution stepwise. Sulfonation with concentrated sulfuric acid or chlorosulfonic acid results in practically complete monosulfonation of all the benzene rings.

Highly porous, macromolecular ion exchange resins are prepared by a variation in the conventional pearl polymerisation technique. An organic solvent, which is a good solvent for the monomers, but a poor solvent for the polymer is added to the polymerisation mixture. As polymerisation progresses, the solvent is squeezed out by the growing copolymer regions. In this way, spherical beads are obtained with wide pores which permit access to the

interior of the beads even when nonpolar solvents are used. The recent Amberlyst ion exchange resins are of this type.

Cation exchangers with specific preference for certain cations can be made by introducing groups which form strong complexes, preferably chelates with these cations. Resins with chelating iminodiacetic acid groups are now commercially available.

Most of the earlier anion exchange resins were condensation products of aromatic or aliphatic amines and aldehydes, dihaloparaffins or haloepoxides. Most of these contain weakly basic groups.

The more important anion exchangers are crosslinked polystyrenes, into which strongly or weakly basic groups are introduced by chloromethylation and subsequent amination. Reaction with tertiary alkylamines gives strong base quaternary ammonium groups and reaction with primary or secondary alkylamines or ammonia gives weak base amino groups. Anion exchangers with strong base quaternary phosphonium and tertiary sulfonium groups have also been prepared.

Amphoteric ion exchangers contain both acidic and basic groups. Snake cage polyelectrolytes are a novel variety of amphoteric resins. These are prepared from conventional ion exchangers by polymerisation of monomeric counter ions within the resin.

For specific purposes ion exchangers in the form of pellets, rods, belts etc. have been prepared by cementing ion

exchange particles together with an inert binder or by impregnating suitable supporting carriers.

Ion exchange membranes have been prepared by various methods. The membranes may be homogeneous or heterogeneous and have become of significant interest, particularly for desalination of water.

1.3

Cinchona alkaloids : (17)

Introduction :

Quinine is the most important of the cinchona alkaloids. In addition about 20 other alkaloids have been isolated from cinchona of which cinchonidine, quinidine and cinchonine are important. The alkaloids chiefly exist as salts of quinic and cinchotannic acids and their relative concentrations vary in different species. The bark which is known to the trade as druggist's bark has a quinine content of 1.8 to 2.0 %.

In the early years of planting, the total alkaloids were used for medicinal purposes under the name of quinetum. In India quinetum was gradually replaced by cinchona febrifuge consisting of the residual alkaloids left after the removal of quinine. The Malaria Commission of the League of Nations redefined quinetum as a mixture of equal parts of quinine, cinchonidine and cinchonine and introduced a new product called totaquine or totaquina which is defined in the B.P. as containing not less than 70 % of crystallisable cinchona alkaloids-quinine, cinchonidine, cinchonine and quinidine

of which not less than one fifth is quinine. Cinchona febrifuge varies greatly in physical character and composition, for use as an antimalarial drug. It should be of the same standard as totaquine.

Extraction :

The greater part of the world's production of cinchona bark is employed in the manufacture of quinine. For this purpose finely powdered bark is mixed with about one third of its weight of sifted slaked lime and a 5 % aqueous solution of caustic soda. The mixture is extracted under stirring in steam jacketed vessels, with high boiling kerosene. Three successive extractions are made. The mixed extracts are shaken with sufficient hot, dilute sulfuric acid to convert the alkaloids into sulfates. The oil is separated while hot and then neutral aqueous solution cooled when quinine sulfate separates out and is subsequently purified by recrystallisation from aqueous solutions after decolorising with animal charcoal. The mother liquor containing the other alkaloids is treated with caustic soda and the precipitate of quinidine, cinchonidine and cinchonine extracted with dilute alcohol which dissolves the first two, leaving cinchonine behind ; the former two can then be separated by means of their neutral tartarates, that of quinidine being considerably more soluble.

The method adopted by the Bureau of Science, Philippines, is to percolate to exhaustion with alcohol, a mixture of finely powdered bark, lime and water. The percolate

is distilled to recover the alcohol, and the gummy residue treated with sulfuric acid to dissolve the alkaloids. The solution is decolorised with charcoal, filtered and the mixed alkaloids precipitated by the addition of sodium hydroxide.

An ion exchange process for the separation of alkaloids from cinchona barks poor in alkaloids had been developed and was successfully employed in the U.S.A. during the war period. This process was suggested for the recovery of alkaloids from the waste material left after the separation of the barks in India. The analysis of the alkaloids is based on processes such as polarimetry, colorimetry, turbidimetry, fluorometry and chromatography.

Uses :

The oldest and the most important use of quinine is for the treatment of malarial fevers. Quinine continues to be effective inspite of its prolonged use. Quinine possesses marked bactericidal action and until the advent of sulfanilamide derivatives, quinine and certain of its derivatives were being employed in the treatment of bacterial infections. Quinine has been used as a sclerosing agent in the treatment of internal haemorrhoids and vericose veins. Quinine added to aquaphor, protects the skin against the sun burn. Quinine sulfate is the most important salt of quinine used in therapy. Quinine ethyl carbonate and tannate are almost tasteless and are specially useful for children. Quinine with urea hydrochloride is used as a local anaesthetic. Practically tasteless compounds are

obtained by combining quinine with an acid mixture derived from camphoric acid and an aromatic alcohol or a terpene alcohol or a phenol. In addition to their use in pharmacy, quinine and quinidine and their derivatives are utilised in insecticide compositions for the preservation of fur, feathers, wool felts and textiles. They are also ingredients of moth repelling preparations. Quinine sterate is used in hair lotions and pomades. The residual bark of quinine factories after the extraction of the alkaloids is a tanning material. Debarked cinchona poles are durable and resistant to termites. New and effective antimalarial drugs, specially, paludrine, chloroquin (aralen) and camoquine have certain advantages over quinine in the treatment of malaria. These new developments have no doubt affected cinchona expansion schemes in India. However, from a strategic point of view, cinchona alkaloids are still of importance as indigenous materials particularly in war time, when imports may not be feasible.

The major portion of the quinine salts, mostly quinine sulfate, quinine hydrochloride and quinine dihydrochloride are being exported and there appears to be a greater demand both for export and internal use.

There also appears to be a substantial demand from abroad for quinidine, cinchonine and cinchonidine products ; although the production of later two is at present, little. Recently Government of Tamilnadu(Madras) has considered to plan for cinchona plantation to increase the production (166).

1.4

Ion exchange studies with cinchona alkaloids :

Ungerer (18) first examined the uptake of salts of quinine, cinchonine and strychnine on the calcium form of synthetic zeolites. Fink (19) took a patent for isolating cinchonine, strychnine and adrenaline from their aqueous extracts by a filtering material having adsorptive properties, such as asbestos and kaolin, cotton and asbestos or asbestos and kieselguhr. The pH of the solution was suitably adjusted to facilitate the separation. Applezweig (20) studied the removal of cinchona alkaloids by a cation exchanger of sulfonic acid type. Three possibilities were explored : (a) recovery of alkaloids from the mother liquor of the acid extracts of the bark after the major portion had been removed by alkaline precipitation, (b) purification of the crude totaquine obtained from alkaline precipitation and (c) application of ion exchange directly to the acid extracts of the bark in a cyclic system. Capacity determinations were carried out on a 200 cc. Zeo-karb column using quinine concentrations of 0.033 and 0.0033 M and flow rates of approximately 5 and 50 cc./min. The capacity of a 200 cc. bed of Zeo-karb for quinine,from acid solution (1 % H_2SO_4) was found to be between 7 and 8 grams, before break through (Mayer's Reagent). To liberate the alkaloids from the column ammonical alcohol was used. Purification of totaquine prepared by alkaline precipitation of acid extracts of the bark was attempted by ion exchange. From

20 grams of totaquine precipitate, 2.5 grams of white crystalline material was obtained. Recoveries of totaquine from cinchona bark and scopolamine from datura plants were also effected by Sussman and others (21). The extract containing the alkaloids was brought into contact with a cation exchanger and then the cation exchanger was treated with aqueous alkali and a solvent. In a subsequent paper, Applezweig and Ronzone(22) described a portable unit for extracting usable antimalarial from freshly stripped cinchona bark in the field. Commercially dried cinchona bark was macerated with 0.1 N H_2SO_4. The acid was repeatedly cycled through a sulfonated coal cation exchanger and back into the maceration tank. The exchanger was regenerated with 0.5 N NaOH and stripped with alcohol, the crude alkaloid being recovered by evaporation. Rectified totaquine was obtained by precipitation from aqueous solution. An overall yield of 81.2 % within 82 hours was obtained. Mukherjee and Gupta (23) investigated the extraction of alkaloids from cinchona bark with hydrochloric acid and sulfuric acid over a range of acid concentrations and temperatures in the presence and absence of sodium chloride with Amberlite IR-100 and Ionac C-284. For elution, alkali was used and after an interval, alcohol was percolated. Ionac C-284 proved to be the best sorbent and showed highest elution efficiency but tended to soften and form a jelly in contact with alkali. Hence Zeo-karb, the next best and free from this defect was used. Applezweig (24) took a patent for the removal of quinine from a dilute solution in acid with

Zeo-karb cation exchanger. The juice of the fresh material was passed through a column of Zeo-karb or Amberlite IR-100 or Ionac C-284. The sorbed alkaloids were eluted with ammonical methanol. This method was used for the extraction of atropine, scopolamine and quinine alkaloids. Mukherjee and others (25) examined three cation exchange resins (Zeo-karb, Amberlite IR-100 and Ionac C-284) and two anion exchange resins (Deacidite and Ionac A-293) for the sorption of quinine sulfate, strychnine hydrochloride and other organic bases. The results showed that a resin having high sorption power for one alkaloid may not behave similarly with another alkaloid. The relative sorptive powers of the different resins, for each of the alkaloids studied were given.

Jindra (26) used an anion exchange resin of weakly basic type for the determination of several alkaloids. 0.1 to 0.2 grams of alkaloid salt was dissolved in 20 cc. of alcohol and passed through a prepared column of Amberlite IR-4B. The flask and the column were washed with 50 cc. of alcohol at 50°C and the alkaloidal solution in alcohol was titrated with 0.1 N hydrochloric acid using a mixture of 10 drops of methyl red and 2 drops of methylene blue as indicator. The method was applied to quinine and cinchonine hydrochlorides and to a number of other alkaloids. Jindra and Pohorsky (27) investigated the determination of various alkaloids including quinine hydrochloride and quinine sulfate using Amberlite IR-4B resin. The rapid change in pH value at the stage when process is about

to reach the equivalent point was utilised for such determinations. In a subsequent paper, they (28) have given detailed descriptions of the apparatus, reagents, preparation of the ion exchange columns and general micro and semi micro methods of assay, applied to cinchona bark and other alkaloids. Bjorling and Barbro William Johnson (29) found that alkaloids of molecular weight about 300 are retained quantitatively on a column of SE-sephadex (sulfoethyl dextran gel) ; but they may be eluted readily with 0.1 N aqueous HCl or in some cases with 0.1 N HCl in 70 % ethyl alcohol. The elution from SE-sephadex as compared with Dowex 50W X1 was studied for strychnine, quinine, quinidine, cinchonine and others organic bases. D.Malejka and H.Witkowski (30) showed how ethanolic extracts of cinchona were chromatographed on ion exchange paper containing a sulfonated phenol-formaldehyde resin. 0.02 N acetic acid was the developing agent. The alkaloids were located with Dragendroff reagent. Comparision of the spot area with a standard curve was utilised to calculate the concentration of alkaloids. H.Witkowski (31) has described a new method for the indirect determination of chromatographic zones developed by means of Dragendroff reagent. A cation exchange paper was used. The ion exchange affinities of the alkaloids to the ion exchangers of the cation exchange paper were given for many bases, in which quinine showed greater affinity than cinchonine. Buchi and Furrer (32) have described in detail the determination of quinine and total alkaloids in the cinchona bark extracts by the use of ion

exchange resins. In a subsequent paper, they (33) found that sorption from cinchona bark was best with sulfuric acid extracts and the elution was best done with ethanol, with or without addition of sodium hydroxide. A quantitative sorption occured within 14 hours by shaking the powdered cinchona bark and Duolite in dilute sulfuric acid but the subsequent separation of alkaloids from the resin was found to be difficult. Sanders and others (34) described an assay process using strongly basic anion exchange columns to separate quinine salts and ephedrine hydrochloride, which was capable of giving results within 0.5 %. One and two column procedures are described.

Yoshino and Sugihara (35) described that caffeine can be separated from strychnine, brucine and quinine by passing through a resin IRC-50 at a pH 6.2, in which case, all the alkaloids except caffeine are adsorbed. In a subsequent paper, they (36) classified various alkaloids in two different category : (a) low molecular weight alkaloids (nicotine, caffeine and ephedrine) and (b) high molecular weight alkaloids (strychnine, brucine and berberine). The alkaloids adsorbed by a weakly acidic (PhOH) cation exchange resin Amberlite IRC-50 were eluted less with higher concentration of HCl because of the shrinkage of the resin, retarding the diffusion of alkaloids through the net-work structure of the resin. Alkaloids having low molecular weight were easily eluted by 6 N HCl, while those having high molecular weight eluted only slightly. The intermediate molecular weight alkaloids

(cinchonine, cinchonidine and quinine) were similarly eluted to about 50-80 %. Yoshino and Sugihara (37) separated quinine and strychnine chromatographically by sorption on weakly acidic cation exchange (NH_4-R) resins such as Duolite CS-101 or Amberlite IRC-50 and the subsequent elution with 0.1-0.3 M ammonium chloride respectively. H-R exchanger could also be used but then a large amount of eluting solution was required to separate strychnine. Yoshino and others (38) classified some organic bases (a) quinine, cinchonine, ephedrine and berberine (b) nicotine and yohimbine (c) amino pyridine (d) antipyrine (e) acetanilide, caffeine, theobromine and theophylline, according to the facilities of being eluted with water from the cation exchange columns (sulfonic acid type and carboxylic acid type in the H and NH_4 form), on to which they had been sorbed. Yoshino and Sugihara (39) described a method of separating 5 common antipyretics. In this, 30-50 cc. of aqueous solution containing 10-90 milli grams of each was passed through 3 columns packed with ion exchange resins connected in a series. Column first containing Amberlite IRC-50, NH_4-R type, adsorbed quinine hydrochloride, which was then eluted with ammonical methanol. Column second containing Amberlite IRC-50, H-R type, adsorbed aminopyrine, which was then eluted with 7 % HCl. Column third adsorbed caffeine, antipyrine and phenacetin ; the last was eluted with water, caffeine with 0.1 M tartaric acid and antipyrine with 7 % HCl. Yoshino and others (40) studied the behaviour of $R-SO_3$ M type exchange

resins against strychnine or caffeine with regard to its sorption-desorption characteristics. The weak acid type (RCOOM) exchangers on the other hand, take up strychnine nitrate, depending upon the kind of M in the order $Li > Na > NH_4 > K > Mg > Ca > Ag > Cu$. The ease with which the organic bases are eluted from the cation exchange resins in water was not proportionate to their exchange capacity but is probably governed by pH of the effluent and the degree of dissociation of the base at the particular pH. The specificity of the resin is a determining factor. Yoshino and others (41) investigated the distribution coefficient of various alkaloids with anion exchange column. Along with other alkaloids, cinchonidine-H_2SO_4, quinidine-H_2SO_4 and quinine-HCl showed different Kd values. The result showed that substances having different Kd values were separated easily by ion exclusion using water as an eluant. Low molecular weight crosslinked resins gave better results than high molecular weight resins. Yoshino and Sugihara(42) described a method of determining and separating organic ions with ion exchange paper. Alkaloids like caffeine, strychnine and quinine and others can be determined in presence of 100 fold amounts of sodium chloride and potassium chloride. For separation of quinine hydrochloride and antipyrine, Amberlite SB-2 (OH^- form) and Amberlite SA-2 (H-form) exchangers are used. Antipyrine adsorbed on SA-2 is eluted with tartaric acid solution. Quinine is sorbed on SB-2 exchanger. Various other acids and bases are also studied.

Street and Niyogi (43) separated a mixture of acetophenetidine, sulphacetamide, promacyine and quinine by a combination of chromatography and ionophoresis on cellulosic ion exchange sheets. Detection was accomplished by examination in ultraviolet light. Similarly (44) separation of a mixture of tablet fragments containing amobarbital, acetylsalicyǿlic acid, acetophenetidine, caffeine, codeine and quinine into its constituent parts was accomplished by chromatography on modified cellulose ion exchange papers using both horizontal circular and ascending cylindrical paper chromatography. Street (45) has described a rapid method using ion exchange paper for the preliminary separation and detection of a mixture of quinine, strychnine and nicotine in whole blood. Proteins were precipitated and the acid filtrate was extracted with ether. The aqueous phase was made alkaline with ammonium hydroxide and shaken with ether to extract basic compounds. The ether extract was evaporated to dryness and the residue was taken up in chloroform. This solution was spotted on a cellulose cation exchange paper and subjected to chromatography in an aqueous solvent at pH 4.5. The separated compounds were detected by their fluorescence or absorbance in ultraviolet light at 254 mμ. Quinine showed a bright blue fluorescent spot and strychnine and nicotine as dark purple absorbing areas. A.Bonati (46) has used ion exchange resins and electrodialysis in the isolation and purification of mixtures of plant alkaloids. The advantages of these methods over traditionally extractive and

purification methods were cited. Quinine, belladonna, ipceac, sabidilla and rauwolfia were some of the drugs obtained by electrodialysis.

Saunders and Srivastava (47) studied the rates of sorption on, and elution from a carboxylic acid cation exchange resin for quinine. The factors which influenced the rate of sorption of quinine on Amberlite IRC-50 were found to be (a) the initial concentration of the solute ('a' millimoles/100 cc.) (b) the nature of solvent, sorption from 50 % ethanol solution being more rapid than that from pure ethanol for a given value of 'a' (c) the method by which the resin was converted to the hydrogen form, aqueous 2 N acid producing a less effective absorbent than alcoholic 2 N acid (d) the amount of base already sorbed on the resin (x millimoles) (e) the stirring condition (f) the particle size of the resin and (g) the initial pH of the solution. An empirical relation $dx / dt = K.a (a-x) / x^2$ (where K = constant, a = initial solution concentration in millimoles / 100 cc. ; x = millimoles sorbed by 5 grams of resin and t = time in hours) ; represented the rate of sorption quite closely for values upto 24 hours. The interaction of quinine with the resin was considered to be mainly a molecular sorption process. The sorption process has been visualised as a diffusion of base into the resin particle under chemical potential difference enhanced by the acid-base interaction with the resin and by the van der Waal's forces between the base molecule and gel structure of the resin ; the effect of viscous flow into spherical particles

and the swelling of the resin caused the rate of sorption to fall off rapidly as the resin became saturated with base. Saunders and Srivastava (48) also examined the sorption of a number of organic bases from aqueous ethanol or ethanolic solutions by various carboxylic acid ion exchange resins and described the results of studies of the equilibrium distributions of some bases between the solutions and the resins. This, in the case of quinine has been demonstrated by showing that the distribution was independent of resin particle size and also that it was reversible. The systems studied, have been classified into two groups. The first consisted of very weak bases which followed a simple distribution law, concentration of the base in resin / concentration in equilibrium solution = constant. The second group consisted of strong bases, the distribution mostly followed a logarithmic law, $Y = A \log c + B$ where c = concentration of base in the equilibrium solution in moles per litre of total phase volume and Y = corresponding base concentration in the resin phase in moles of base per litre of total resin phase volume. Observations have also been made of the swelling of the resins caused by saturating them with the different bases and it was found that swelling was a function of base sorbed.

Segal, Miller and Morton (49) have described the quinine form of a weak cation exchanger as an indicator for the determination of the presence of free hydrochloric acid in gastric juice without intubation. If a special cation (quininium) is combined with a cation exchange resin

(Amberlite IRC-50 or XE-96) and the cation is displaceable only or mainly by hydrogen ion, is readily sorbed from the stomach and detectable in the urine or blood ; the presence of free hydrochloric acid in gastric juice can be detected if quinine appears in the urine within two hours, after introduction of the complex, without subjecting the patient to intubation. Shay and others (50) found that the results of studies in patients after subtotal gastric resection indicated that the tubeless method for the detection of the presence of free hydrochloric acid in the remaining gastric pouch was not suitable in these patients because of the rapid emptying of the quininium resin from the pouch. In such patients thedëtermination of pH of gastric contents during fractional gastric analysis was the best method for studying gastric acidity. W.Kamp and J.A.Fresen (51) have described the separation of quinine from other alkaloids on a column of Dowex 1 in the chromate form. A neutral, alcoholic mixture of the alkaloid sulfates was poured on the column and left standing for 12 hours. Elution with water removed all alkaloids except quinine which was subsequently eluted with N NH_4OH or N formic acid in 96 % ethanol. Kamp (52) determined quinine together with caffeine and strychnine in cola syrup by ion exchange resin. In subsequent paper, he (53) described that when mixture containing thalleioquin in 6 N NH_4OH - H_2O - 90 % ethyl alcohol (40 : 15 : 45) was layered on a cation exchange resin Dowex 50W X2 (50/100 mesh) and was chromatographed through the column, a stationary green colour developed

when quinine, quinidine and hydroquinine were present. If the same column was prepared with 5 cc. of 0.3 % bromine water and 5 cc. of N hydrochloric acid, followed by a mixture of 0.5 cc. of 2.65 % $K_3[Fe(CN)_6]$ and 0.5 cc. of N hydrochloric acid and then a mixture of chloroform saturated with 25 cc. ammonium hydroxide containing erythroquin was layered, a stationary red colour appeared at the top of the column when quinine, quinidine and hydroquinine were present.

Einar Brochmann Hanssen (54) described a method of extracting the alkaloids by shaking the powdered drug with an aqueous suspension of strongly acidic cationic exchange resin, removing the crude drug residue by a backwash and eluting the alkaloids with methanolic ammonia. The same alkaloids can be purified by passing through a highly porous, strongly basic anionic exchange resin. Ultraviolet spectrophotometry is used to determine them. Eero, Sjostrom and Walter Rittner (55) described a method for the determination of quinine hydrochloride, quinine sulfate, atropine sulfate and other alkaloid salts. In this, the solution of milligram quantities of alkaloid salt was passed through a column of Dowex 50 in Mg^{++} form ; and released magnesium was washed out with 50 cc. water. It was titrated with 0.01 or 0.001 M disodiumtetraacetate. Error was 0.5 % or less. James M.Bobbitt (56) suggested the use of strongly basic resin Amberlite IRA-400 in bicarbonate form for the decomposition of alkaloids by a " columnar technique ". The picrate (0.5 gm.) in 100 cc.

of 10 % aqueous acetone was passed through the above said resin at the rate of 30 - 60 cc. per minute. Then the column was washed with 100 cc. solvent, the washings and eluate combined and acetone removed in vacuo ; the aqueous phase filtered, salted out and filtered to give pure alkaloids. Cinchonidine, cinchonine, morphine and other alkaloids were obtained in this manner. On strongly basic resins, acetone condensed with itself. E.Haberli and E.Beguin (57) gave method for the quantitative determination of drugs by means of ion exchange and spectrophotometry. The method is based on the adsorption of acidic and basic substances on ion exchange resins, followed by the elimination of interfering products by washing or backwash procedures and the elution of pure drugs. The resins used were Dowex 50W X2 and 1X1, with only few eluants being used. Procedures are given for the determination of tropa, cinchona, strychnine, brucine and ipecac alkaloids and various other substances. Spectrophotometry was used to determine the amount of various alkaloids. In a subsequent paper, they (58) described the separation of dimethylaminophenazone and / or antipyrine from an alkaloid such as quinine, codeine and other bases by means of ion exchange. The bases were first adsorbed on Dowex 50W X2 and the pyrazolones are then eluted by means of a buffer solution (pH = 7.6), whereby the alkaloid is recovered by subsequent elution with methanolic ammonia and thus can be separated. Separated products were determined spectrophotometrically. The process is suitable for milligram

amounts of alkaloids in the presence of neutral or acidic substances. J.S.Foster and J.W.Murfin (59) used alginic acid after suitable treatment with formaldehyde as a carboxylic cation exchange medium for the separation of organic bases from solutions. The adsorption from aqueous solution and subsequent elution with ammonium sulfate and spectrophotometric determination of strychnine hydrochloride, quinine hydrochloride and various other alkaloid salts is described. Robert Kunin (60) has described a method of purifying the alkaloids and antibiotics. An antibiotic salt (4 gm./ litre) was passed through Amberlite IRC-50 (Na^+ form) ; until the effluent does not give any precipitate with phosphotungstic acid. The resin was washed with ion free water and water containing CO_2 was passed through the resin. 4 % HCl was passed through the column to elute out the antibiotic. Streptomycin sulfate, neomycin sulfate and quinine sulfate were eluted in the same manner.

Chen - Yeh Tu and Feng - Ho Tsang (61) determined 10 - 20 miligram samples of the hydrochlorides of procaine, thiamine and the sulfates of atropine, strychnine and quinine by passing their salt solution through a column of cation exchange resin in copper form. The Cu^{++} liberated is washed out with 40 cc. of water and titrated with 0.005 or 0.0025 M disodium - EDTA with murexide as the indicator. H.Auterhoff and K.Kalpathy (62) have described the paper and column chromatographic procedures for the examination and separation of quinoidine. Also epiquinine and epiquinidine were prepared and their properties were compared with those of

quinine and quinidine. J.Bosly (63) outlined the use of cobalt-thiocynate organic base complexes and spectrophotometry in an anhydrous media. Organic bases gave stable blue colours in hydrous medium containing $H_2\left[Co(SCN)_4\right]$, methanol and a nonoxygenated solvent (chloroform). A solution of ammonium thiocynate in methanol was passed through a column of Amberlite IR-120, to give HSCN. Organic bases like caffeine, acetanilide failed to give a blue colour, while strychnine behaved as a univalent base, quinine and sparteine as bivalent bases. The colour showed an adsorption maximum at 625 mμ. H.Sion (64) has described a method for the determination of mixtures of analgesics. A mixture of phenacetin, caffeine, antipyrine and euquinine in 40 % aqueous methanol was passed through a Dowex 50W X2 column, which retains basic components. Phenacetin and caffeine, which were collected in the eluate, were measured spectrophotometrically at 247 mμ. (maximum for phenacetin) and 273 mμ. (maximum for caffeine). Antipyrine was measured after elution with 0.05 N NaOH in 70 % methanol, at 230 mμ. and euquinine as sulfate by fluorimetry. H.W.Dibbern and G.Scholz (65) showed that by selective elution of a cationic and anionic exchange columns, mixtures of analgesic and antipyretic pharmaceuticals can be divided into 5 fractions. Fractions were of neutral compounds, weak acids, strong acids, weak bases and strong bases. The fractions were further separated by adsorption chromatography and compounds were determined by ultraviolet spectrophotometry. Frank J.Wolf and others (66) have described ion exchange process for the recovery of ionic

organic substances. Quinine, morphine, K-penicillin G and other organic substances, adsorbed on exchangers and difficult to eluate in high yield or easily changed during elution, are rapidly eluted by a mixed solution system. The solution contains atleast 50 % by volume of water-miscible organic compound such as an alcohol, ketone, amide, ether or the like, and 0.1 to 15 % of an ionizable salt with enough water to dissolve it. The medium should also dissolve the adsorbed organic species.

Jozef Dadlez and others (67) determined alkaloids in animal material by the use of a cationitized paper. The paper contained cationate K28LW in hydrogen form which is a phenolic formaldehyde cationate with SO_3H as an active group. Quantitative determination of quinine and sparteine on such paper was conducted in a thermostate at a constant temperature ; and solution of potassium permanganate was used to make the alkaloids visible. A.P.Kreshkov and others (68) determined organic salts by titration with ion exchange in non aqueous medium. Sodium-salicylate, quinine dihydrochloride and mixtures of organic acids and salts were dissolved in either methanol, ethanol or iso-propanol. The solution of the sample was passed through a column of a cation exchange resin SDV-3 (acid form). The acids so obtained were titrated potentiometrically. An anion exchange resin AB-17 (OH form) was used for anion exchange and bases obtained were titrated. K.D.G.Edwards and others (69) studied the binding properties of acidic, basic and neutral drugs to anion and cation exchange resins

and charcoal in vitro. Strongly basic anion exchange resins removed acidic drugs like barbiturates, salicylates, aspirin etc. from aqueous solution. Cholestyramine resin being most efficient. Cation exchange resins removed basic drugs like quinine, quinidine, strychnine, morphine etc. from aqueous solution. Resonium-A resin being most efficient. The mixed cation and anion exchangers, carbo-resin and activated charcoal, removed most of drugs from solution to some extent ; carbo-resin was the most effective agent against chlorpromazine. Activated charcoal was effective against the neutral drugs like gutethimide, carbromal etc. The presence of bicarbonate or taurochlorate ions reduced the removal of anionic drugs from solution by anion exchange resins, indicating competition amongs the anions for ionic bonding sites on the resin. It appeared that hydrogen bonding, dipole-dipole interactions might also be involved in resin binding. The approximate amounts of resins or charcoal which would have been needed as an antidote for fatal cases of poisoning in man were calculated from the in vitro-relationships and seemed in most instances to involve practicable amounts. Thus, ion exchange resins may be of value in the treatment of a wide range of poisonings. Upjohm Co. (70) described a method for separation of various organic substances including quinidine from their aqueous solutions by extracting with a water - insoluble liquid cation exchanger containing a water-insoluble organic solvent and an oil-soluble aromatic sulfonate, followed by extraction of the organic solvent with water

containing amines or their quarternary salts. L.Nałęzgodzki and others (71) used a paper with an incorporated phenol sulfonic cation exchanger for the determination of hydrochlorides of quinine, pilo-carpine, papaverine etc. and strychnine nitrate in ampuled solutions.

1.5

Ultraviolet absorption spectra of cinchona alkaloids :

Earlier (72-109), the ultraviolet absorption of the four major cinchona alkaloids (quinine, quinidine, cinchonine, and cinchonidine) and their salts has been studied by a number of workers.

Stimson and Reuter (80) studied the ultraviolet absorption spectra of the four common naturally occurring alkaloids in alcoholic solutions or alcoholic/hydrochloric acid solutions and some related compounds over a pH range 1 - 10 to facilitate the estimation of methoxy cinchona alkaloids.

H.S.Grant and J.H.Jones (84) studied the ultraviolet absorption spectra of quinine, quinidine, cinchonine and cinchonidine in 0.1 N hydrochloric acid solution. It was observed that cinchonine and cinchonidine have identical ultraviolet absorption with maxima at 235 mμ. and 316 mμ. Similarly quinine and quinidine also have identical ultraviolet absorption with maxima at 318 mμ.and 348 mμ.

S.P.Popli and M.M.Dhar (92) studied the ultraviolet absorption of cinchona alkaloids in ethanolic solution and in 0.1 N hydrochloric acid solution. In the study, it was

found that quinine and quinidine have identical ultraviolet absorption with a maximum at 348 mμ. and cinchonine and cinchonidine also have identical ultraviolet absorption with maxima at 315 or 318 mμ. in hydrochloric acid solution. In ethanolic solution, shift in maxima and lowering of extinction coefficient for the four alkaloid bases were observed.

T.E.Gulimova (104) estimated quinine or its sulfate by dissolving it in 0.1 N hydrochloric acid solution and by reading the absorbance of the solution at 318 mμ. or 347 mμ. The method was applied to the determination of quinine in tablets and the error was found to be ± 4.2 %.

Henning Sattler (106) studied the ultraviolet absorption of many organic substances. He gave a procedure for the determination of quinine and / or quinidine in the presence of cinchonine and / or cinchonidine ; and of quinine in ajmatines.

Kanhere, Shah and Bafna (114) studied the ultraviolet absorption spectra of aqueous solution of four cinchona alkaloid sulfates of different pH (adjusted by addition of sulfuric acid or sodium hydroxide) and the ultraviolet absorption at invarient wavelengths was used for the estimation of the alkaloid sulfates in dilute aqueous solutions.

Kamath, Bhat and Bafna (115) studied the ultraviolet absorption spectra of four cinchona alkaloid bases in various aliphatic alcohols. At 332 mμ. the value of extinction coefficient, ε , for cinchonine or cinchonidine is quite small, about 2 to 4 % of the value of ε for quinine or quinidine. Hence, the absorption at 332 mμ. enables the

estimation of quinine and / or quinidine in the presence of cinchonine and / or cinchonidine.

1.6

Summary of the earlier work done in this laboratory : (110-119)

Ultraviolet absorption spectra : (114 - 115)

Ultraviolet absorption studies were made with aqueous solutions of cinchona alkaloid (quinine, quinidine, cinchonine and cinchonidine) sulfates of different pH (adjusted by the addition of sulfuric acid or sodium hydroxide solution) and the ultraviolet absorption at the invarient wavelengths was used for the estimation of the alkaloid sulfates in dilute aqueous solution. The ultraviolet absorption spectra of the four cinchona alkaloid bases in twelve aliphatic alcohols were also studied. It was found that the spectra of quinine and quinidine are similar, so also were those of cinchonine and cinchonidine ; but the spectra of quinine and quinidine are distinctly different from those of cinchonine and cinchonidine. The maxima and minima are not significantly affected by change of the alcohol, but the value of the extinction coefficient, ϵ , is, in some cases, affected to some extent. At 332 mμ., the value of ϵ for cinchonine or cinchonidine is quite small, about 2 to 4 % of the value of ϵ for quinine or quinidine. Hence, the absorption at 332 mμ. enables the estimation of quinine and/or quinidine in the presence of cinchonine and/or cinchonidine.

Exchange equilibria : (116 - 117)

The exchange equilibria with cinchona alkaloid (quinine, quinidine, cinchonine and cinchonidine) sulfates in dilute aqueous solutions with various cation exchange resins were studied. The variables studied were the relative degree of crosslinking of the resin, the particle size of the resin and the ratio of resin concentration to the alkaloid sulfate concentration. The behaviour noted with alkaloid sulfates is of different type as compared to that of simple alkali cations. For resins of higher degree of crosslinking, the same fraction of the resin capacity is exchanged irrespective of the ratio of the resin concentration to the alkaloid sulfate concentration. This value varies with the relative degree of crosslinking of the resin. For low crosslinked resins, the effective exchange capacity is to a small extent dependent on the ratio of the resin concentration to alkaloid sulfate conscentration. The equilibrium exchange between the aqueous sulfate solutions of the alkaloids and cation exchange resins in different ionic forms was also studied. The variables studied included the relative degree of crosslinking of the resin, the structure of the resin matrix and the ratio of the resin concentration to the alkaloid sulfate concentration. It was found that for resins X2, X4 and X8 in the alkali metal form, lithium, sodium and potassium, P_R, the fraction of the resin capacity exchanged at equilibrium, is in the order $Li \geqslant Na \geqslant K$ for all the four alkaloid sulfates. For resins in bivalent ionic forms, Mg and Zn, the order is $Mg \geqslant Zn$. For the resins in ionic form of ions of different valence, the order is

monovalent > bivalent > trivalent. The value of P_R for the alkaloid sulfates decreases as the degree of crosslinking increases for each ionic form of the resins. Exchange equlibria of quinine, quinidine, cinchonine and cinchonidine sulfates in the presence of added sulfuric acid of different concentrations were studied with Amberlite [illegible]-200, Amberlyst - 15, Dowex 50W X4 and 50W X8 resins in hydrogen form.

Exchange rates : (118)

The rates of exchange for dilute aqueous sulfate solutions of the four alkaloids with various resins were studied at 35° and 45° C. A simplified procedure was adopted by applying the second order law to the exchange process. For low crosslinked resins the rate of exchange, over a good part of the exchange reaction, was uniform and the rate constants were evaluated. For higher crosslinked resins, the exchange was by two exchange rates ; one relatively fast and of shorter duration, and the other relatively slow and of longer duration. The second order law was applied to the slower rate and the relative rate constants were evaluated. The rate constants were a function of X and of particle size of the resin. The temperature coefficients and the apparent energies of activation were evaluated for the four alkaloid sulfates.

Uptake of the alkaloid bases from alcoholic solutions :

The uptake of quinine and cinchonine from alcoholic and aqueous alcoholic solutions by sulfonic acid cation exchange resins in hydrogen form was studied. The variables studied included the extent of uptake with time by different resins

and the effect of solution medium on the amount of uptake. The equilibrium uptake of the four cinchona alkaloid bases on the sulfonic acid resins in six aliphatic alcohols was also studied. The values of equilibrium uptakes of quinine and quinidine on a resin in a particular alcohol were similar, so also those of cinchonine and cinchonidine. The value of the equilibrium uptake of quinine or quinidine in methyl alcohol was less than that for cinchonine or cinchonidine for the same resin ; for other solvents, the values for quinine or quinidine were not significantly different from those of cinchonine or cinchonidine. With increase in the crosslinking of the resin, the value of the equilibrium uptake of each base decreases in each alcohol. The value of P_R decreases or remains almost constant with increase in the size of the counter ions and first decreases and then again either increases or remains almost unchanged with increase in the chain length of the solvent molecule.

Column studies : (119)

The exchange behaviour of aqueous quinine sulfate and cinchonine sulfate solutions with different resins and their elution with sulfuric acid of different concentration and by liberation of the base with caustic soda and then elution with distilled ethyl alcohol was studied. The exchange behaviour of aqueous quinine sulfate solution with resins of different crosslinking, matrix structure and particle size, at different flow rates and elution with sulfuric acid of different strength at different flow rates was also studied.

The exchange of the four alkaloid sulfates in aqueous and N/100 H_2SO_4 solution was studied with the resin IR-200 in hydrogen, sodium and ammonium forms and the elution of the exchanged alkaloids from these columns with sodium hydroxide followed by distilled ethyl alcohol, N/25 ammonical ethyl alcohol and N/10 ammonical ethyl alcohol was studied. For the resin IR-200, the equilibrium conditions are attained faster than those for resin X4. Also for resin IR-200, N/100 and for resin X4, N/10 sulfuric acid may be used as extraction solvent with the alkaloid containing material without considerable decrease in amount exchanged. With N sodium hydroxide, the liberation of quinine from resin X4 is slow and if the contact time of the exchanged resin with thealkali is increased, the resin undergoes some disintegration. The resin IR-200 is quite stable under the conditions encountered and the column may be used in the ammonium form, N/100 sulfuric acid may be used as the extraction solvent for the alkaloid containing material and N/10 ammonical alcohol as the eluant for the recovery of the alkaloids.