CHAPTER II

Information

Piperazine, a heterocyclic nitrogenous compound, was used in the early 1900 s for treating human gout and other rheumatic diseases on the theory that piperazine would dissolve urates thereby aiding the elimination of uric acid from the body. However, extensive research failed to substantiate this theory, and piperazine was gradually removed from the list of drugs used to treat gout. The discovery of the anthelmintic properties of piperazine was usually credited to Fayard (1949), but these were first observed by Boismare, a Rouen pharmacist, whose reciped quoted in Fayard's thesis(1). Since then numerous derivatives, principally salts of piperazine, have been developed. All of the derivatives have similar efficacy safe and highly effective against both Ascaris lumbricoides and Enterobius (Oxyuris) vermicularis but zero to variable effective against helminths in human and domestic animals and are used commonly today as the drugs of choice in treatment of pinworm (Enterobius vermicularis) and roundworm (Ascaris lumbricoides) infestation.

Pharmacological action

The anthelmintic activity of piperazine and its salts depend upon their anticholinergic action at the myoneural

junction in worms which produce a neuromuscular block. According to study by Norton and de Beer[12], piperazine has a curare like action; that is, it blocks the stimulatory effects of acetylcholine on worm muscle. Piperazine also decreases production of succinic acid which is a metabolized product of the worm[3]. Succinate production supplies the energy for the muscular contraction of the parasite. It is probable that this energy is provided in worm muscle mitochondria via a reduction of fumarate to succinate by reduced diphosphopyridine nucleotide coupled with phosphotylation. Both results cause a narcotizing or paralytic effect. Worms lose their motility and thus their ability to maintain their position in the intestinal tract. They are passively swept along by intestinal peristalsis and voided live in the feces. If the drug is quickly voided by the host, e.g., when a purgative accompanies drug administration, the narcotized worm may regain its motility and reestablish its position in the gut. Purgation is consequently not generally advised when piperazine is being used. Mature worms are more susceptible to the action of piperazine than are younger stages. Lumen-dwelling larvae and immature adults are sufficiently susceptible to be at least partially liminated. Larval stages in host tissues, especially larvae that are molting, are little affected by the drug, thus repeated treatments are generally indicated.

Absorption, Fate and Excretion

Piperazine and its simple salts are readily absorbed from the proximal region of gastrointestinal tract. Some of them are metabolized in the tissue and the remainder(approximately 30 - 40 %) is excreted in the urine[4]. Piperazine base is detectable in the urine as early as 30 minutes after the drug is administered, the excretion rate is maximal at 1 - 8 hours and urinary excretion is practically complete within 24 hours. Roger (1958) observed no significant difference between the rates of urinary excretion of various salts of piperazine. However, there was a wide variation in the rates at which piperazine was excreted by different individuals.

Toxicity

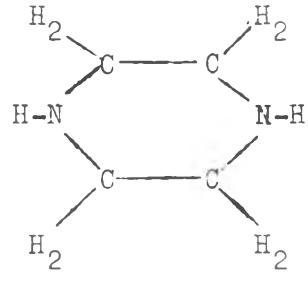
There is a wide range between effective therapeutic and overtly toxic doses of piperazine. Laboratory findings on mice[5] have shown that the oral LD_{50} of piperazine was 11.4 g/kg. of body weight , and on patients receiving treatment for several days have shown no abnormality , it also can be used without ill effect during pregnancy. Large oral doses occasionally produce gastrointestinal upset, transient neurological effects, and urticarial reactions. There are no known contraindications to the use of piperazine except in cases of long-standing renal or liver disease.

Preparations

Piperazine is marketed either as the free base or more frequently in the form of salts, such as the citrate, adipate, phosphate, dihydrochloride, tartrate and calcium edetate; all forming hexahydrate in solution.(6) The antiparasitic activity of the various salts of piperazine depends almost solely on the piperazine base. The amount of base varies, of course, among different salts as reflected in the difference in the dosage level of each. The hexahydrate (sometime calls hydrate) of piperazine contains 44 % of the base. The dosages of the salts of piperazine are customary expressed in terms of the hexahydrate equivalent, i.e., 100 mg of piperazine hexahydrate is approximately equivalent to 120 mg of piperazine adipate, to 125 mg of piperazine citrate, and to 104 mg of piperazine phosphate. A number of salts of piperazine are available by brandnames, usually in the form of syrup or elixir containing 100 mg or 125 mg/ml. and as tablet or wafer, each containing 250 or 500 mg. calculated as the hexahydrate. Other preparations are capsule and granule. For domestic animals, piperazine is incorporated into feeds. Of the various preparations, one is probably as good as another. The liquid preparations are more acceptable for children. Piperazine citrate U.S.P. and Piperazine citrate elixir B.P.C. are the official preparations.

Chemistry

Piperazine, a pyrazine derivative, chemically is diethylenediamine. Other names of piperazine are hexahydropyrazine or piperazidine and its chemical structure is



Piperazine is a typical cyclic secondary diamine, and can be prepared in the laboratory several ways as described here.

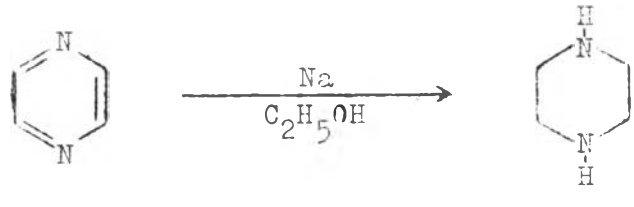
1) Cloez[7,46] synthesized piperazine as early as 1853 by heating ethylene dibromide with aqueous ammonia in a sealed tube.

$$2\ Br\text{-}CH_2\text{-}CH_2\text{-}Br + 6\ NH_3 \longrightarrow NH(CH_2\text{-}CH_2)_2\ NH + 4\ NH_4Br$$

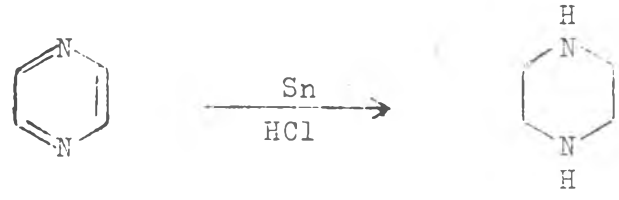
Piperazine also crystalizes as a hexahydrate from water by warming ethylene chloride with ammonia in alcoholic solution[8].

$$2\ Cl\text{-}CH_2\text{-}CH_2\text{-}Cl + 6\ NH_3 \longrightarrow NH(CH_2\text{-}CH_2)NH + 4\ NH_4Cl$$

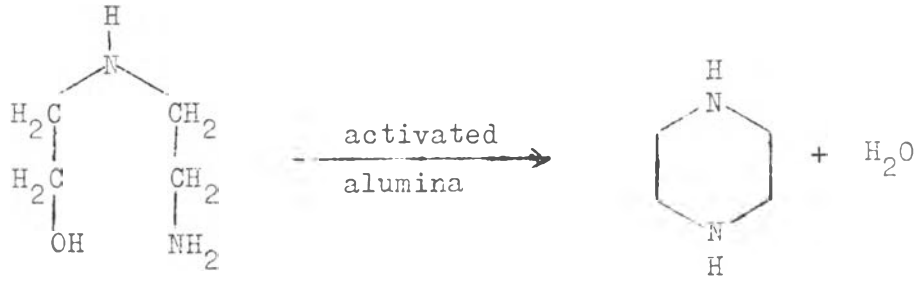
2). Piperazine can be prepared by reduction of pyrazine, using sodium and ethanol as catalyst[9].



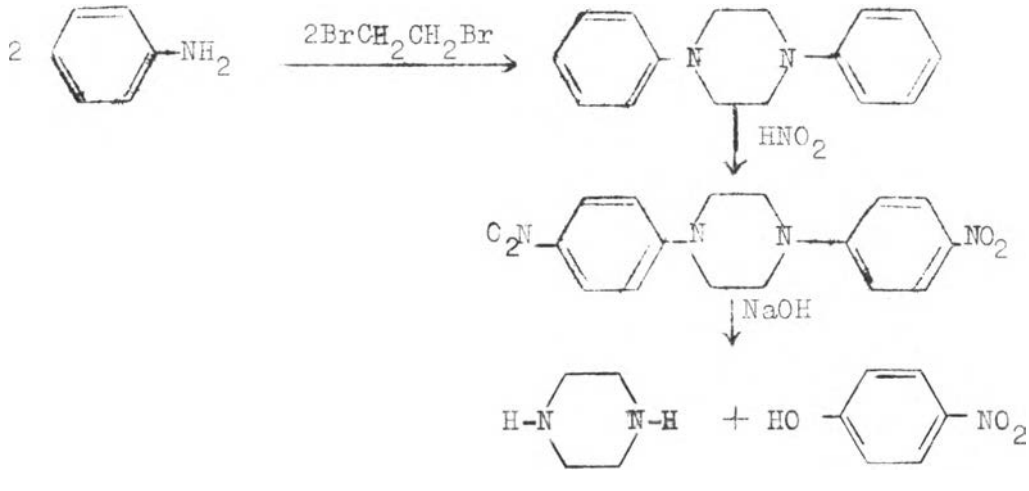
Pyrazine are also reduced by tin and hydrochloric acid or with a variety of other reducing agents to gain piperazine[10].



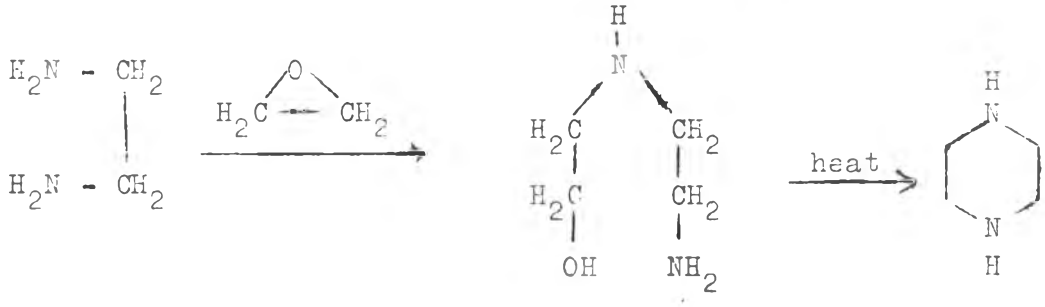
3) Piperazine can be prepared by the high temperature cyclodehydration of a suitable hydroxylamine, such as N-(2-hydroxyethyl) ethylenediamine. The catalyst is activated alumina on which some metallic nickel has been deposited[11].



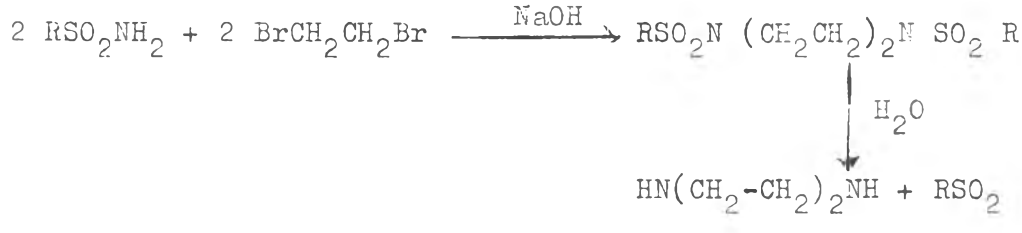
4) Piperazine can be prepared from aniline by the general secondary aliphatic amine synthesis[12].



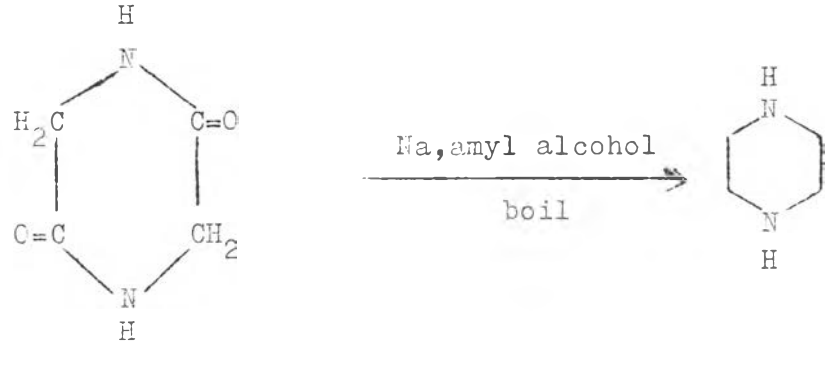
5). Piperazine can be prepared by catalytic deamination of diethylenetriamine or ethylenediamine[13].



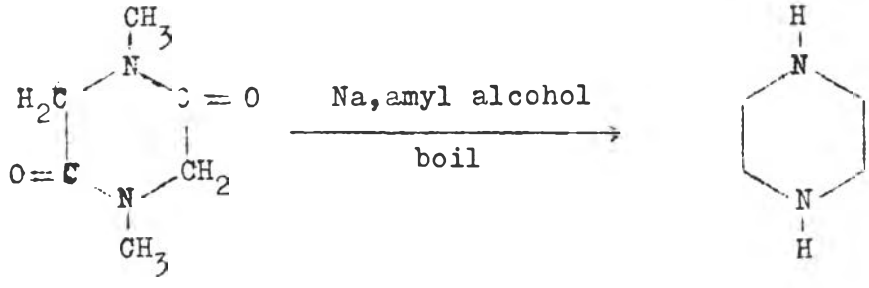
6) Arylsulfonamides react with ethylene dibromide in the presence of alkali to give a sulfonylpiperazine compound which by hydrolysis gives piperazine[14].



7) Reduction of amino acid anhydrides, such as occur in protein, with sodium and boiling amyl alcohol or electrolytic reduction of diketopiperazine in general gives piperazine[15].



Glycine anhydride



Sarcosine anhydride

Cloez's synthesis method[7,46] gives a poor result because of the many possible side reactions. At present there are a number of ways to synthesize piperazine but the deamination of diethylenetriamine or ethylenediamine, catalytically, gives satisfactory yields of piperazine.

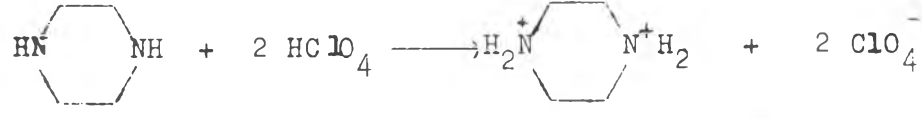
Piperazine is freely soluble in water and glycerol, less soluble in alcohol, and insoluble in ether. It is a strong base, having pK_a value 9.82 and 5.68.[16] Piperazine consequently easily absorbs water and carbon dioxide, thus containers should be tightly closed. In the presence of moisture, the hexahydrate of piperazine is formed, occuring as small colorless crystals which are very unstable and are soluble in water. Stability of piperazine can be accomplished by used of its simple salts; piperazine adipate, citrate, calcium edetate, phophate, dihydrochloride and tartrate, all of which are formed by treatment of an equimolar quantity of acid ,e.g. adipic acid, citric acid, ethylenediaminetetraacetic acid on calcium carbonate, phosphoric acid, hydrochloric acid and tartaric acid, with

piperazine in aqueous solution, the resulting salts are caused to crystallize. Chemical structure of piperazine salts are shown in Table 1.

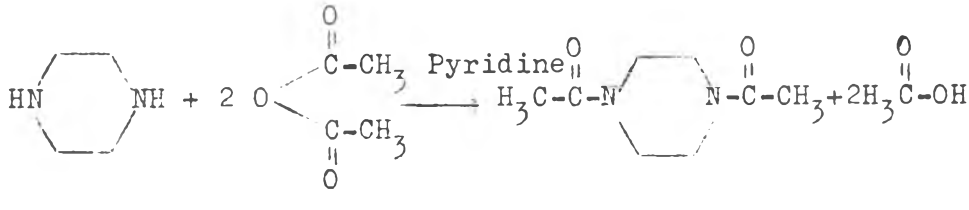
Salts of piperazine are stable than the piperazine base. All of the salts are white crystalline powders with a saline taste and are readily soluble in water except the insoluble phosphate and the adipate which dissolve slowly to only a maximum of 5 % in water.

Piperazine posses chemical properties as general secondary amines, its main properties are

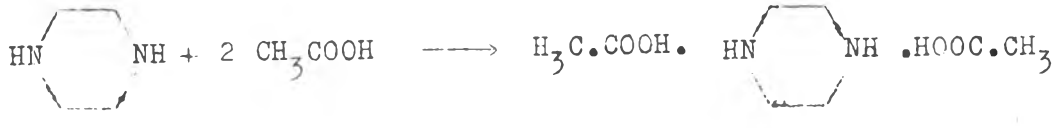
1) Reacts with perchloric acid in glacial acetic acid[18].



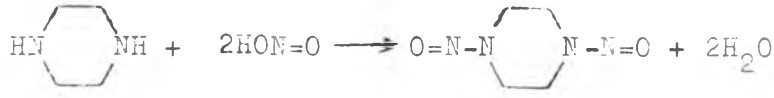
2) Reacts with acetic anhydride[19,46] or acetylchloride to form diacetyl piperazine, pyridine or another tertiary amine is generally employed as catalyst.



3) Reacts with glacial acetic acid in acetone cause a white crystalline precipitate of piperazine diacetate[20].

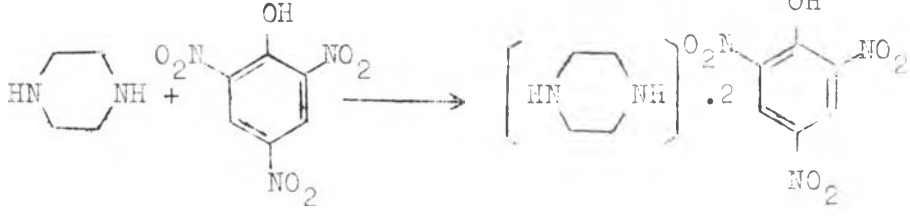


4) Reacts with nitrous acid to form N-nitroso derivative[21].



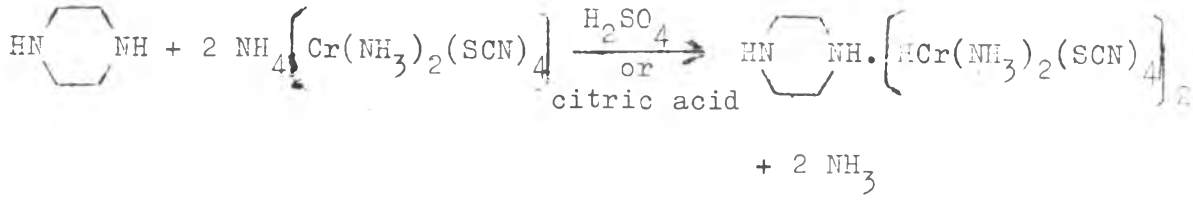
5) Reacts with carbon disulfide to produce a polymer call 1-piperazinecarbodithioic acid betaine or Picadex[5] which is also used as anthelmintic .

6) Reacts with picric acid, chloroplatinic acid mercuric salts and other metal salts to form precipitate[14,22].

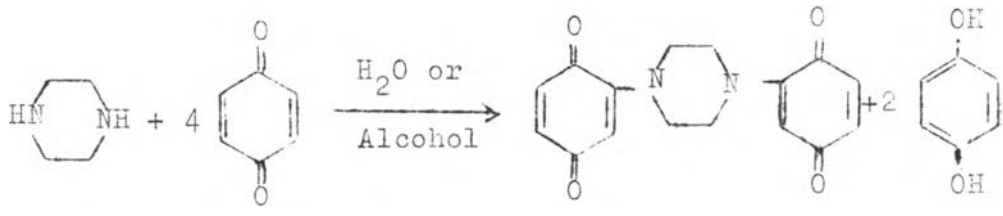


Picric acid piperazine dipicrate

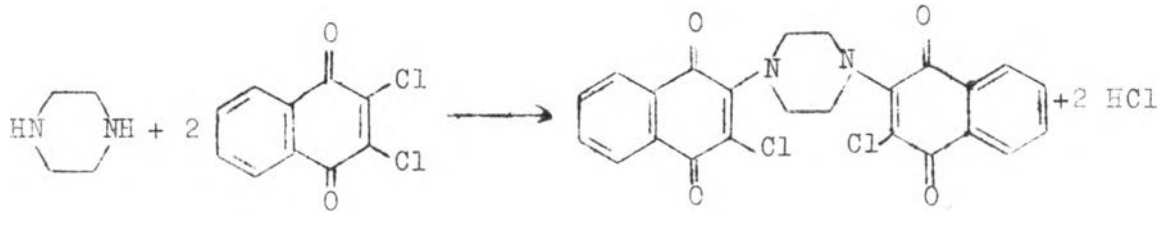
7) Reacts with ammonium reinechate in acid solution to form a pink precipitate[23].



8) Reacts with quinone reagent, e.g. p-benzoquinone[24]



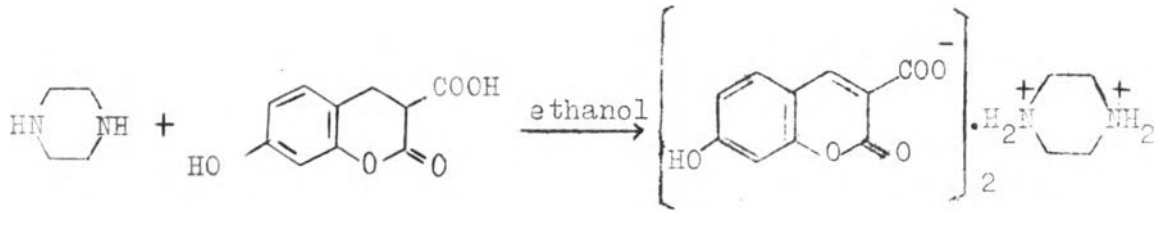
with dichlone or 2,3-dichloro-1,4-naphthoquinone to produce a rose-colored product[25].



9) Reacts with bromine to give an addition compound[14]

and also reacts with iodine to yield N,N -diiodopiperazine[26].

10) Reacts with 3-carboxy-7-hydroxycoumarin in ethanolic media[27].



11) Reacts with various aldehyde to yield condensate and polymers depending on the aldehyde and mole ratio used, e.g. with formaldehyde to form the insoluble polymer call polymethylenepiperazine[28] but when increase formaldehyde to two fold or more at 15-85° C yield 1,4-methylalpiperazine .

Analytical methods involved in quantitative determination of piperazine and its salts

The widespread use of piperazine and its salts as an anthelmintic agent have created a demand for a rapid, reliable method for their determination. Various methods have been employed for the quantitative determination of drugs which containing piperazine salts, but the accuracy of these methods have always been in question. These method are described below.

1) Titrimetric method

(1.1.) Complexometric titration

This method is based on the reaction of piperazine with $HgCl_2$ solution, the precipitated is filtered off. Add an excess of 0.05 M EDTA to the filtrate, then add NH_3-NH_4Cl buffer solution (pH 10) and back titrate with 0.05 M $MgSO_4$, with Eriochrome Black T as indicator[29]. Another method is used

for the determination of piperazine in an elixir and in effervescent granules[30]. An aqueous solution is treated with carbon disulfide and aqueous ammonia to form the 1,4-di-(carbodithioic acid), then a known excess of Cu^{II} acetate is added, the precipitate formed is removed by filtration and the excess of Cu^{II} in the filtrate is titrated with EDTA.

(1.2) Precipitation titration

This method is based on the precipitation of piperazine hydrate as tetraphenylboron salt[31], followed by argentometric titration or by precipitation of the reineckate, followed by hydrolysis and Volhard titration. Other method is titration of the excess of NH_4SCN after precipitation of the complex $Cd(C_4H_{10}N_2)(SCN^-)_2$[32]. An aqueous solution is treated with a known excess of 0.1 N NH_4SCN and an excess of 10 % cadmium acetate or sulfate solution. The mixture is diluted and well shaken and the precipitate is allowed to settle, then filtered off, the first portion of filtrate is rejected. The remainder of the filtrate is titrated with 0.1 N $AgNO_3$.

(1.3) Oxidation-reduction titration

An ethanolic solution of piperazine are treated with ether and ethanolic hexamine and then add HIO_4. After being set aside for 10 minutes at 10°C, the precipitate is filtered off on a Jena (J 3) sintered-glass crucible, washed with ethanol-ether and dissolved the precipitate in 5 % H_2SO_4, KI is added and the liberated iodine is titrated with $Na_2S_2O_3$[33]

(1.4) Acid-base titration

The method is based on the Sorensen method for amino acid. Piperazine is dissolved in water and treated with formaldehyde solution, previously neutralized to phenolphthalein. Add few drops of an ethanolic solution of thymolphthalein and the mixture is titrated with NaOH solution, compare the end point with a standard color prepared in the same manner(34).

Another reaction is carried out in a thermally isolated system by titration of piperazine in an alcoholic or aqueous solution with hydrochloric acid(35).

(1.5) Nonaqueous acid-base titration

By this method, the piperazine base first brought into aqueous solution, and the solution is made strongly alkaline and extracted with chloroform. The chloroform solution of piperazine is then titrated with acetous perchloric acid using alpha-naphthol benzoin as indicator(36).

Another method is an official method of piperazine citrate and phosphate by dissolving piperazine salt in glacial acetic acid TS, warming slightly to effect solution, titrated with 0.1 N perchloric acid, crystal violet TS is used as indicator(18).

Titrimetric methods offer an accurate, rapid, simple and inexpensive mean of assay but they can not be used for the determination of piperazine in colored pharmaceutical preparations as the color masks the end point, giving variable results. Nonaqueous acid- base titration[18] can not be used in effervescent granules because of the presence and interference of hexamine, citric and tartaric acid, sodium citrate, sodium hydrogen carbonate and other interfering ingredients that yield either higher or lower results. Although nonaqueous acid-base titration gives very satisfactory determination of piperazine in dry, powdered form, but it can tolerate practically no water without introducing serious interference. However, this can be circumvented in liquid preparations by evaporating a suitable aliquot of the solution being assayed to dryness, the residue is redissolved in glacial acetic acid and titrated with standard perchloric acid. This method can cause partial acetylation of the piperazine and in many instances produce erratically low results. The use of acetic anhydride to eliminate the water present is also not workable since piperazine readily acetylates in its presence.

2) Potentiometric method

Piperazine and its salts are estimated by potentiometric titration. Subert[37] used hydrochloric acid as titrant in quantitative determination of piperazine in medium of aqueous solution of $MgCl_2$ or NaCl. Polaczek et al[38] also used

hydrochloric acid as titrant but changed the medium to ethanediol -isopropyl alcohol(1:1). In the U.S.P. method[18], piperazine is quantitatively determined in medium of glacial acetic acid, using perchloric acid as titrant, in conjunction of silver-glass electrode system.

Potentiometric method gives a similar disadvantage to nonaqueous acid-base titration.

3) Chromatographic method

3.1) Thin-layer chromatography

This method was introduced in separation and quantitative determination of piperazine in 1971. Wang[39] separated piperazine and its salts from bephenium hydroxy naphthoate, viprynium embonate, santonin and oxyphenisatin diacetate on 0.25 mm layer of silica gel with anhydrous acetic acid-methanol-ethanol (3:1:1) as developing solvent and used iodine vapour or 5 % bromine solution in carbon tetrachloride or 0.25 % fluorescein solution in dimethylformamide for detection. Limits of detection ranged from 3 to 30 ug. Rao[40] used the same method but he separated piperazine from its 1-nitroso and 1,4-dinitroso-derivatives on silica gel GF_{254} by development (for ~ 50 minutes) with ethylacetate -methanol (1:1). All three compound appeared as brown spot on exposure of the plate to iodine vapour, whereas under 254 nm radiation, 1-nitroso and 1,4-dinitroso-derivatives appeared as blue spots but piperazine remained invisible. Detection limits were 0.5 µg for piperazine,

1.0 µg for 1-nitroso and 0.5 ug for 1,4-dinitroso-derivatives. Wiesner[41] determined piperazine separated from mixture of polyamine example ethylenediamine, triethylenetetramine and ethanolamine on Lucefol Quick (microcrystalline cellulose)plate.

3.2) Paper chromatography

Piperazine and its phosphate, tartrate, citrate and adipate, viprynium embonate, kainic acid, bephenium hydroxy-napthoate, santonin and oxyphenisatin diacetate can be separated on Whatman No.1 paper[42] by the ascending technique with propanol-ethanol-water (1:6:4) or benzene-chloroform-methanol (6:2:1) as developing solvent and iodine vapour, 5 % bromine solution in carbon tetrachloride or 0.25 % fluorescein sodium solution in dimethylformamide for detection. The limits of detection ranged from 5 to 60 ug. Wiesner[41] separated piperazine from mixture of polyamine by chromatography on Whatman No.3 paper at 20°C to 25°C which pyridine-4-methylpentan-11-2-ol aqueous 40 % dimethylamine (1:1:1) as mobile phase. Phenol red or sodium nitroprusside reagent was used for detection and quantitative work was carried out with an integrating densitometer.

3.3) Column chromatography

This procedure was used for determining piperazine adipate in pharmaceutical preparation by passing aqueous solution of sample through a column (15 cm x 1 cm) packed with amberlite IR-20(Zn^{2+} or Mg^{2+} form), elution was affected with water and

the Zn^{2+} or Mg^{2+} in the eluate was determined with 5 mM-EDTA in the presence of Eriochrome Black T in an ammonia buffer medium of pH 10.4[43].

3.4) Gas-liquid chromatography

Piperazine in a filtered aqueous extract of the feed is mixed with Celite and sodium carbamate and packed into a glass tube (300 mm x 40 mm); after addition of acetic anhydride, it is eluted as diacetylpiperazine with chloroform and subjected to gas-liquid chromatography. A glass column (6 ft x 4 mm) of QF-1 on Gas-chrom Q (80 to 100 mesh) at 200°C is used, with Ar or N (110 ml per minute) as carrier gas; detection is by Ar ionization (^{3}H or Ra source) or H-flame ionization. Phenothiazine is used as internal standard.[44] Recoveries of added piperazine adipate ranged from 97 to 100 %. Mikhailova[45] used gas-liquid chromatography to separate piperazine from sample containing ethylenediamine, diethylenetriamine by passing sample through 3-metre column of PTFE(0.25 to 0.50 mm) which immobile phase was 7 % silicone elastomer SKTE, using decanol as internal standard, operated at 180°C, with He (50 to 80 ml/minute) as carrier gas.

Chromatographic method provides an accurate and good result for separation and determination of piperazine and its salts from its degraded product and other ingredients in the formula. However, the method is time consuming and require special technique in preparing column and chromatographic plate

and the equipments are also still expensive.

4) Gravimetric method

Various reagents have been employed for the gravimetric determination of piperazine and its salts. Maynard[46] and Kondos[47] introduced a gravimetric method in which the piperazine salt was treated in a saturated bicarbonate solution with acetic anhydride, forming chloroform soluble piperazine diacetate,which coula be extracted and weighed. Leng[48] determined piperazine dihydrochloride in feed gravimetrically as the dipicrate after ion exchange and steam distillaticn separations, the precipitate was let stand at room temperature over night, filtered and weighed. Zawadzki[49] precipitated piperazine as the dipicrate by treating piperazine with saturated aqueous picric acid solution, followed by gravimetric determination. The U.S.P. XX and N.F. XV[18] method for the analysis of piperazine citrate in piperazine citrate syrup U.S.P. and piperazine phosphate in tablet requires reaction of piperazine with picric acid TS and dry the precipitate to constant weight at 105°C. In B.P.C. 1973[50] and B.P. 1980[51] use trinitrophencl solution (100 ml saturated solution of trinitrophenol + 0.5 ml 20 % NaOH) instead of picric acid TS in precipitating various salts of piperazine as the hexahydrate, adipate and phosphate; both raw material and sample preparation but there is something difference in step of washing piperazine dipicrate. In piperazine citrate elixir B.P.C. 1973, the washing solution is a mixture of equal volume of water and saturated solution of trinitrophenol before

continuing with dehydrated alcohol as well as in B.P.1980, U.S.P. XX and N.F.XV. Canescu[52] used $NH_4[Cr(SCN)_4(aniline)_2].H_2O$ or $NH_4[Cr(SCN)_4(benzylamine)_2].H_2O$ to form violet-rose crystalline salts in acidic solution of piperazine, particularly in pharmaceutical products that did not contain alkaloids or other organic bases with a heterocyclic structure. Romotowski[53] precipitated piperazine as either hexahydrate or adipate in several pharmaceutical products with sodium tetraphenylborate in acetate buffer solution pH 3.7. Grecu[54] determined piperazine contents on the formation of a complex $[Cd(C_4H_{10}N_2)(SCN)_2]$ with an excess of Cd^{2+} in the presence of NH_4SCN, after being washed with ethyl ether, the precipitate was air-dried and weighed.

Bandel[23] used ammonium reineckate to precipitate piperazine hexahydrate in citric acid solution and weighed the precipitate as $C_4H_{10}N_2[HCr(NH_3)_2(SCN)_4]_2$. Castiglioni[55] added 5 % ammonium molybdate to an aqueous mixture solution of piperazine and glacial acetic acid, heated the solution on a waterbath for two hours and set aside for six hours, $3\ C_4H_{10}N_2 \cdot 10\ MoO_3 \cdot 8\ H_2O$ was formed, filtered and dried the precipitate at 100°C.

According to Leng[48], however, the steam distillate from this separation was occasionally quite alkaline and would give distinctly high results. In fact, a steam distillation would not eliminate the interference due to various amines if they were present. The official method[18,50,51] is tedious

and time consuming as the precipitated dipicrate must be left undisturbed several hours before filtration and subsequent determination. It also can not be used in determination of effervescent granules because of the interference by hexamine, citric acid and other ingredients in granules that yield variable value. In general, the gravimetric method is accurate but requires considerable time and manipulation such as it requires filtration, drying and weighing and also experience to obtain consistent results.

5) Polarographic method

This method was applied in 1971 by Mc Lean[56] The basic principle based on the reaction between piperazine dihydrochloride with excess formaldehyde in Mc Ilvaine buffer solution pH 5 to form a polarographically reducible condensation product with a half wave potential of -0.98 V vs a saturated calomel reference electrode, and then gave an diffusion wave which was a linear function to the concentration.

This reaction can be carried out in the same flask, followed by an easy filtration and polarographic determination. Since amines can react with formaldehyde, several different amines are tested for possible interference.

6) Near infrared spectrophotometric method

The method[46] consisted of neutralizing the piperazine salt with sodium bicarbonate, treating the free piperazine with acetic anhydride in sodium bicarbonate solution, extracting

the diacetyl piperazine formed with chloroform, and taking the chloroform solution to dryness on a steambath, dissolved the residue with chloroform compared the absorbance of the sample with that of a standard at 10.03 μ with fixed slit opening of 220nm. Mohn[57] analyzed piperazine by using the property of secondary amines which had the first overtone N-H stretching absorption band, about 1538 mμ. He developed infrared method for analysis of piperazine in drug formulation by dissolving piperazine base or its salts in water and the solution was made strongly alkaline with NaOH. The base was then extracted with chloroform and diluted to a definite volume and the absorbance of the clear extract was measured by near infrared spectrophotometry. The net absorbance was determined by using baseline technique at minima points of about 1460 and 1565 mu. This method, at present, is official analytical method for determining piperazine in powder and syrup in AOAC 1980[58]. Spell[59] used $-NH_2$ absorption band at 1010 cm^{-1} in analysis of piperazine ring with the amino absorption at 1605 cm^{-1} as reference.

The infrared absorption procedure gives a good result. Although the method is more rapid, it is not suitable for the routine work because of the high cost of the instrument and its accessories. According to the wide spread of infrared technique in qualitative work but very few in quantitative analysis so it requires very more improvement to use in quantitative determination.

7) Spectrofluorometric method

According to Eisenbrand[60], aqueous solution of piperazine was mixed with hydrochloric acid to form the hydrochloride and dried to residue. Dichloroflucrescein was added to the mixture, followed by ethanol and 10 N sulfuric acid and set aside overnight. To an aliquot 10 N sulfuric acid was added, the fluorescence of the yellow-orange solution was measured at 605 to 610 nm. (excitation at 546 nm.). This method can detect 10 ng. of piperazine/ml. within ± 5 % after isolation from a syrup preparation. Another paper was reported by Stewart and Lotti[27], they formed reaction product between 3-carboxy-7-hydroxy coumarin with organic aliphatic and cyclic amines including piperazine in ethanolic media. The result showed an increase in fluorescence of the reaction mixture. This increase was accompanied by a change in the activation wavelength of the mixture and the increased in fluorescence, it was measured at 450 nm. (excitation at 420 nm.).

The advantage of this reaction[27] was that the reaction was not affected by various amides and aromatic amines but there were interference from inorganic and organic acids resulting in potentially lower fluorescence readings. The presence of an inorganic and organic bases resulted in increasing fluorescence reading owing to the salt formation between the base and the coumaric acid. Spectrofluorometric method gives a high sensitivity but great care is taken to prevent over exposure of the sample to the xenon source of the spectrofluorometer as well as

to prevent contamination of solution and cells use in the analysis otherwise mischief results are occured.

8) Spectrophotometric method

Sodium 1,2-naphthoquinone-4-sulfonate or Folin's amino acid is known to produce a red color with chemicals possessing an amino group. Piperazine and its salts have also been determined by use of this reagent. Hanna[61] used this reagent for quantitative determination of piperazine in human urine by dilute the sample of urine with water, then added 3 % aqueous borax, 95 % ethanol and 0.5 % sodium 1,2-napthoquinone -4-sulfonate solution, set the solution aside for 20 minutes and readings were carried out within 5 minutes at 490 nm. Dessouky[62] determined piperazine in effervescent granules and elixir containing hexamine, colchicine, atropine sulfate, etc by treating them with 0.6 % aqueous 1,2-naphthoquinone-4-sulfonate solution in the presence of acetate-citrate buffer at pH 7.5. The temperature of the reaction was between 10 and 15°C and the color produced was measured at 490 nm. Another quinone reagents using in quantitative analysis of piperazine were 1,4-benzoquinone[24,63], 2,3-dichloro-1,4-naphthoquinone[25], 2,6-dichloroquinone chlorimide[64], and chloranil[65]. Beckman[63] and Loucks[24] extracted piperazine from feeds with water or diluted acid, filtered through Super-Cel and then reacted with a buffered, hot alcoholic solution of p-benzoquinone to form the orange-red product. Absorbance was measured at 490 nm. Abou-Ouf[25] used dichlone or 2,3-dichloro -1,4-naphthoquinone to react with

piperazine (in pharmaceutical interest) in alcoholic solution, then added sodium bicarbonate solution. After acidification to pH 4, piperazine yielded a rose-colored product and gave maximum absorbance at 500 nm. Baggi et al[64] described a colorimetric method involved heating an aqueous solution of piperazine and its salts for 30 minutes with 2,6-dichloro-quinonechlorimide (chlorimide), measured the absorbance at 525 nm. Chloranil[65] or 2,3,5,6-tetrachloro-p-benzoquinone formed blue charged -transfer complex with piperazine and gave absorption maximum at 575 nm. The sample solution was treated with a standard solution of chloranil in chloroform, the mixture was diluted with chloroform and after ten minutes, the absorbance was measured against the reagent blank.

Ammonium reineckate was introduced to the estimation of piperazine in pharmaceutical preparations by Kamath[66] in 1957. Piperazine was quantitatively precipitated by a saturated aqueous solution of ammonium reineckate at pH 9 - 10. The pink precipitate of piperazine reineckate dissolved in pure acetone, giving a cherry red colored solution, measurment was done in a photo-electric colorimeter with a green filter. The papers introduced in 1958[67], 1961[68], 1975[69] used the same method but controlled pH of the solution between 2-4 by adding citric acid and cooled in ice bath 90 minutes after forming the precipitate, the red colored solution was measured at 525 nm. Pankratz[68] used dilute sulfuric acid instead of citric acid in adjusting pH of the solution.

Other papers were carried out by precipitating piperazine

with copper sulfate solution, the complex was dissolved and measured the absorbance[70]. Ganescu[52] formed violet-rose crystalline salt by adding piperazine to acid solution of $NH_4[Cr(SCN)_4^-.(aniline)_2].H_2O$ or $NH_4[Cr(SCN)_4^-(benzylamine)_2].H_2O$. After completing the precipitation, the precipitate was dissolved in acetone and measured the absorbance at 535 nm, good results were obtained on products that did not contain alkaloids or other organic bases with a heterocyclic structure. Abou-Ouf et al[21] developed identification test to quantitative determination for piperazine by interact with nitrous acid in solution pH 2.3 - 2.6 to give N-nitroso derivative. The chromophore developed by treating the reaction mixture at 80°C for 15 minutes. The reaction mixture exhibited an absorption maximum at 239 nm.

A colorimetric method bases on reaction of quinone reagents[24,25, 61-65] give comparable results for the most of the pharmaceutical products but they lack of reproducibility and in some preparations give a precipitation. However, since these methods have several variables which must be carefully controlled, example pH of the solution, time and temperature for the reaction. Also the quinone reagents may be troublesome, p-benzoquinone is a lacrimator and it should be store under nitrogen; 1,2-naphthoquinone-4-sulfonate sodium must be freshly prepared. In the reineckate method[66-69], control of pH is essential since slight difference in acidity will make

large difference in absorbance and the acid solution develops absorbance reading twice those of alkaline solution.

Various volumetric[18,29-36], potentiometric[18,37,38], chromatographic[39-45], gravimetric[18,23,46-55], polarographic[56], near infrared[46,57-59], spectrofluorometric[27,60], and spectrophotometric[21,24,25,52,61-70] methods for the determination of piperazine in raw materials and pharmaceutical preparations have been mentioned. Advantages and disadvantages of these methods are also discussed. Although some procedures could be employed in some instances for the analysis of piperazine but most of them are lack of precision, tedious procedure, interference from other components present in the formulation or require the expensive equipment which sometime may not be readily available to the analyst. The official methods[18,50,51] for the assay of piperazine salts in pharmaceutical preparations are also tedious and time consuming, require not less than four or five hours for determination which are not suitable in drug industry. Picric acid or trinitraphenol, the precipitating agent in official method, is explosive reagent. It must be kept under water and placed in a cool place and picric acid is also harmful to human body[17]. In present, there are troublesome in purchasing this reagent since it is the precursor of explosive substance so the chemical incorporations who are the representative agents of picric acid must report the total amount in each month to the Ministry of Defence. This stimulated the need for a satisfactory, simple, rapid, sensitive and stability-indicating method.

The purpose of this thesis is to develop a faster and simpler procedure which could be employed in quality control laboratories for analyzing piperazine and its salts both in pharmaceutical dosage forms and raw material, using equipment normally found in the laboratories instead of the official gravimetric method.[18,50,51]. The proposed method is based on the assumption that a cyclic secondary amine will be quantitatively determined by formation of complex or ion-paired with sulfonphthalein dyes[71,72] such as bromthymol blue, bromcresol green, bromcresol purple and bromphenol blue in chloroform and measure the color of the complex formed.

Sulfonphthalein dyes are very widespread used in the determination of compounds possesing amine[73-76] and quaternary ammonium group[77,78]. It has been reported that organic basic nitrogen and quaternary ammonium compounds can be assayed with high sensitivity by the formation of organic solvent soluble complexes with sulfonphthalein dyes. This is made possible by the fact that the salt or ion pair or complex which is formed between a positively charged nitrogen compound and a negatively charged dye has a lower solubility in aqueous and higher solubility in organic solvent[79]. The methods of analysis involving dyes base on two ways

1) A buffered aqueous solution containing the amine and a suitable dye is shaken with an organic solvent and the concentration of the resulting ion pair in the organic solvent is

determined spectrophotometrically. The pH of the aqueous phase is critical to the success of this method[80].

2. An alkaline solution containing the amine is shaken with an organic solvent, then add dye to form an ion pair in organic layer and measure spectrophotometrically.

Most amines and quaternary ammonium compounds exhibit a high sensitivity with sulfonphthalein dye while acidic, neutral, and weakly basic compounds as well as the commonly used excipients do not interfere[73]. Piperazine has been analyzed by Pfeiffer[81] and Gupta[82] by using indicator dye. Pfeiffer used erythrosine which is the member of fluorescein group, formed complex with piperazine in chloroform and measured the absorbance of the red complex at 525 nm. A recent paper was reported by Gupta (1976) was based on the interaction of piperazine with bromthymol blue in aqueous buffered solution pH 5.7 and extracted the yellow complex with chloroform, measured the absorbance at 420 nm. In Gupta's method, the amount of piperazine being used was 20.6 fold excess of bromthymol blue, showing that this method could not be used in the assay of piperazine because there were still excess of piperazine remained in aqueous phase while the dye were exhaustively used in forming the complex, so this method was suitable to analyze bromthymol blue quantitatively not piperazine. In preliminary work, a minor modification from Gupta's method has been done by

changing the concentration of bromthymol blue to 4 fold excess of piperazine. It was found that there was insignificant complex formed between piperazine and bromthymol blue at all pH value. In this thesis, the method used in quantitative determination of piperazine and its salts was done by using Pfeiffer's technique with four sulfonphthalein dyes; bromcresol green, bromthymol blue, bromcresol purple and bromphenol blue as the color forming agent. The outline of this thesis was based on the following statements.

1. Piperazine free base was liberated from its salts by treating with a suitable concentration of sodium hydroxide, followed by chloroform extraction. Color developed by the reaction with chloroformic solution of sulfonphthalein dyes were measured at wavelength which gave maximum absorption. Study the optimum reaction conditions and range of analytical utility, such as stability of complex on time and temperature, sensitivity and precision of this reaction and also the effect of variation in the concentration of each dye. These factors were compared and selected the best suitable sulfonphthalein dye for the assay of piperazine and its salts in drug formulations.

2. In order to measure the accuracy and precision of this method, piperazine citrate syrup U.S.P.[18] was prepared and the per cent recovery was determined in comparison with the

gravimetric official methods[18,50,51.]

Apply this procedure to several commercial preparations containing piperazine and its salts and the results obtained are compared with those obtained by the official method.

The usefulness of the proposed method

1. To find another new method which can be suitable used for routine control analysis of piperazine and its salts in drug industry. The new method is simple, sensitive, time-saving and using common equipment. The results are reliable and compare well with the official gravimetric method.

2. The expense of the new method is lower than the official method, using sulfonphthalein dye which is commercially available or easily synthesized and its prepared solution is stable to room temperature not less than one month[83]. It is also nontoxic and can use without special precautions.

3. To know the mechanism of reaction between piperazine and various sulfonphthalein dyes which may be introduced to those analytical methods probably useful in quality control work of another drugs.