

## Chapter IV

### Results and Discussion



#### Determination of Wavelength for Maximum Absorption of Complex between Piperazine and Four Sulfonphthalein Dyes

The interaction of piperazine and four sulfonphthalein dyes, e.g., bromcresol green (BCG), bromthymol blue (BTB), bromcresol purple (BCP), and bromphenol blue (BPP) in chloroform produced a yellow complex. The quantity of piperazine used could be determined by measuring the intensity of the color formed at wavelength of maximum absorption of each dye. The absorption spectra of the piperazine-dye complexes in chloroform were determined spectrophotometrically by scanning the developed color under visible light at a range from 350 to 680 nm. The maximum absorption of the complex formed showed an absorption peak at 420 nm. for BCG, 420 nm. for BTB, 410 nm. for BCP and 412 nm. for BPB as illustrated in Figure 1. These wavelengths were used with each dye in every experiments.

#### Effect of Various Concentrations of Sodium Hydroxide on Releasing Piperazine Free Base from Piperazine Citrate

A peculiar characteristic of piperazine base was that it was very soluble in aqueous solution but practically insoluble in organic extractive solvents so that it was difficult to isolate from aqueous solution. It was found that quantitative extraction into organic solvent was possible

as long as the aqueous phase remained strongly alkaline but alkalinity of the solution and number of extraction were always in question. Leng<sup>(48)</sup> extracted piperazine free base from feed and concentrate by using 0.1 N sodium hydroxide; Pfeiffer and Hasselmann<sup>(81)</sup> used 1 N sodium hydroxide to extract the free base from pharmaceutical dosage forms, in AOAC 1980<sup>(58)</sup> and Maybury's method<sup>(36)</sup>, 50% or 11.1 N sodium hydroxide was used. In this work the suitable concentration of alkali and the completion of extraction was checked by varying the normality of sodium hydroxide used in extraction and determined the fifth and sixth extracted chloroform separately to find the amount of piperazine left in the aqueous phase. The experimental data in Table 2 indicated that extraction of piperazine free base from its citrate salt with the sixth 10 ml. portion of chloroform showed absorbance values of  $1.236 \pm 0.004$  and  $0.152 \pm 0.003$  for 6 N and 8 N sodium hydroxide respectively, compared to  $0.013 \pm 0.001$  and  $0.004 \pm 0.002$  when using 9 N and 10 N sodium hydroxide. This seemed to indicate that, with the low alkalinity (8 N sodium hydroxide or less), piperazine free base was partially remained in the aqueous phase. The absorbance values of the fifth 10 ml. extract were compared, the results obtained were  $0.080 \pm 0.003$  for 9 N and  $0.017 \pm 0.001$  for 10 N sodium hydroxide. Sodium hydroxide 10 N with five extractions and 9 N sodium hydroxide with sixth extractions appeared to be the best since it could the

completely extracted the piperazine free base from aqueous solution, so 10 N sodium hydroxide with five extraction was chosen for all experiments in order to save the time of extraction .

The results obtained were agree with the previous paper (36,58) and proved that, especially strong alkaline could produce dissociation of piperazine free base from its salt and partitioned completely into chloroform. So it was impossible to determine piperazine in pharmaceutical preparation by using acid-dye method, owing to the high solubility of piperazine and its salts in aqueous phase. The extraction of the piperazine- dye complex, if formed, from the aqueous phase with the organic solvent was failed, thus extraction of piperazine free base with chloroform from strong alkaline solution overcame this problem .

In this work chloroform was used as the extractive solvent because of the property of a widely used solvent of the dye technique, its low toxicity, low price and also ease in extraction.

#### Preparation of Calibration Curve and Determination of Conformity to Beer's Law

The present investigations included studies pertinent to the nature of the complex, factors influencing its formation, and the stoichiometric balance were performed.

For the attainment of the ultimate in precision and accuracy, precise calibration curve was constructed to test whether a linear relationship existed between concentration and absorbance. The precision of the study was performed by running four replicate assays with equal weight of piperazine citrate used, the calibration curve of each dye was constructed from the average and Beer's law was obeyed for each dye as shown in Table 3, Table 12 and Figure 2.

The slope of the calibration curve of four sulfonphthalein dyes in Table 12 showed the difference. BCP showed the steepest of the curve, while BPB showed the least, however, the steeper of the curve, the higher of the sensitivity in quantitative determination.

BCG showed linear absorbance-concentration relationship from 77.8 to 466.9 mcg. per ml. of piperazine as hexahydrate, from 0 to 466.9 mcg. per ml. for BTB, 0 to 466.9 mcg. per ml. for BCP and 77.8 to 233.4 mcg per ml. for BPB. BCG and BPB showed the x-intercept while BTB and BCP, their calibration curve passed through the origin.

The coefficient of variation of four experiments in calibration curve were typically 0.47-9.54 % for BCG, 0.12-1.43 % for BTB, 0.54-7.44 % for BCP and 0.43-3.42 % for BPB. Inspection of the presented data indicated that BTB showed best reproducibility, followed by BPB, ECP and BCG respectively.

From the data obtained, BCP showed the highest sensitivity for quantitative determination of piperazine but its calibration curve showed poor reproducibility. BTB was less sensitive than BCP, its calibration curve showed a linear concentration range as BCP but with very good reproducibility. BCG showed less linear concentration than BCP and BTB, and its reproducibility was the least. BPB showed the least linear absorbance-concentration relationship with good reproducibility.

#### Effect of Dye Concentration of Absorbance of Piperazine-Dye Complex

To determine the amount of dye reagent required for maximum color development, various amount of the dye solution ( $4.00 \times 10^{-4} \text{M}$ ) were added to a fixed volume of piperazine as hexahydrate solution ( $1.00 \times 10^{-4} \text{M}$ ) until linearity in absorbance were obtained through out the desired concentration range. In Figure 3, the maximum color intensity were obtained with 3 ml.  $4.00 \times 10^{-4} \text{M}$  BCG, 3 ml.  $4.00 \times 10^{-4} \text{M}$  BTB, 4 ml.  $4.00 \times 10^{-4} \text{M}$  BCP, 4 ml.  $4.00 \times 10^{-4} \text{M}$  BPB, above this point, increasing the volume of dye, increased in absorbance slightly. In order to ensure an excess in quantitative determination, 5.0 ml. of  $4.00 \times 10^{-4} \text{M}$  dye was used when concentration of piperazine as hexahydrate was about 3.0 ml.  $1.00 \times 10^{-4} \text{M}$ .

The stoichiometric ratio between sulfonphthalein dyes and piperazine were studied by plotting the mole ratio of dye to

drug against the absorbance as shown in Figure 4. Two straight lines of different slope were obtained which the intersection showed a mole ratio corresponding to dye and drug complex<sup>(84)</sup>. The intersection occurred at the mole ratio of 2 :1 for all dyes, suggesting that the complex consisted of two moles of dye and one mole of piperazine. In the analytical procedure presented, sulfonphthalein dyes reacted with piperazine yield a yellow colored complex. Chemically, the colored product was most likely the complex formed by reaction between lidocaine with BCG and FTB<sup>(86)</sup> or between trifluoperazine with BCP<sup>(88)</sup>, etc. These were in agreement with published data<sup>(72,77,85)</sup> concerning the reaction of sulfonphthalein dyes with compounds containing nitrogen group. Further verification of this assumption was obtained by comparing the spectrum of a typical yellow products which were similar to each other. Since identical color was obtained when sulfonphthalein dyes were reacted with compound containing nitrogen group, however, it was difficult to reach a conclusion concerning the true nature of these colored products with the presented data done, but the structure might be postulated in the way similar to those amines and quaternary ammonium compound<sup>(72,77,85)</sup>.

Piperazine possessed two basic nitrogen groups, each exhibited basic characteristic in organic solvent. The two basic groups were susceptible to sulfonphthalein dyes. Sulfonphthalein dye itself, in dissociated form

was intensely yellow whereas the undissociated form as it existed in neutral or acidic solvent, e.g., chloroform was very slightly yellow. Since the nature of reaction appeared to be a simple acid base reaction, so the complex was formed between the amino group of piperazine and the sulfonic acid group of sulfonphthalein dye.

All other dyes except BPB produced yellow colored complex at all concentrations of the dye used. BPB gave a peculiar characteristic, its color of the complex was changed depend on the concentration of BPB used, precipitates were formed when using 0.5-1.5 ml.  $4.00 \times 10^{-4}$  M BPB but disappeared when the solution were shaken. The color of the solution which depended on the concentrations of the dye used were shown in Table 13.

This phenomenon might be explained that color of the complex depended on pH of the solution. BPB appeared yellow at pH 3.0 and purple at pH 4.6. According to the nature of reaction which appeared to be a simple acid-base reaction, the pH of the complex when using 0.5 ml.  $4.00 \times 10^{-4}$  M BPB might be exceeded 4.6 so the color of the complex formed was purple. Structure of four sulfonphthalein dyes and their transition ranges were shown in Table 14.

### Effect of Time on Stability of Piperazine-Dye Complex

The stability of color of the complex formed was investigated. The absorbance of the complexes were measured at selected intervals of time under six hours at room temperature. The results were shown in Table 5. BCG showed a mean absorbance of 0.427 with coefficient of variation of 0.65 %. The results indicated that BCG-piperazine complex was stable through out the period of not less than 6 hours. BTB showed a mean absorbance of 0.420 with coefficient of variation of 0.93%. The absorbance of BTB piperazine complex increased slightly in period of half an hour, then remained constant over a period of not less than 6 hours. BCP showed a mean absorbance of 0.500 with coefficient of variation of 0.90 %. The absorbance of BCP-piperazine complex increased slightly in a period of 20 minutes and remained nearly constant over a period of not less than 6 hours. BPB showed a mean absorbance of 0.475 with coefficient of variation of 1.74 %. The absorbance of the colored complex were increased significantly in period of 1 hour, then decreased in 2 hours and remained nearly constant in the period of 6 hours. From the presented data it was concluded that, in the period of 6 hours, the absorbance showed insignificant difference with BCG, BTB and BCP and significant difference for BPB. Owing to maximum yellow colored development, and hence yield a product, could be obtained immediatly upon shaking the solution at room temperature and was unchanged in the period of 6 hours



so the experiments with BCG, BTB and BCP could be done in any-time intervals but with BPB, the experiment should be done within the period between 2 to 6 hours.

#### Effect of Temperature on Stability of Piperazine-Dye Complex

The stability of the color of the complex due to changing in temperature was investigated. The complex between piperazine and four sulfonphthalein dyes were developed treated in constant temperature bath with temperature of  $40^{\circ}$ ,  $50^{\circ}$ ,  $60^{\circ}$ ,  $70^{\circ}\text{C}$  compared to room temperature ( $25^{\circ}\text{C}$ ). The results presented in Table 6 indicated that the absorbance the complexes were decreased when the temperature increased BCG showed stability of the color of the complex tend to  $50^{\circ}\text{C}$ , then absorbance of 0.412 at room temperature decreased to 0.403 at temperature of  $70^{\circ}\text{C}$ . BTB showed stability of the color of the complex tend to  $40^{\circ}\text{C}$ , then decreased significantly from 0.422 at room temperature to 0.385 at the temperature of  $70^{\circ}\text{C}$ . BCP showed stability of the color of the complex tend to  $50^{\circ}\text{C}$ , then decreased significantly from 0.503 at room temperature to 0.480 at the temperature of  $70^{\circ}\text{C}$ . BPB-piperazine complex was temperature sensitive the absorbance decreased rapidly from 0.478 at room temperature to 0.367 at temperature of  $70^{\circ}\text{C}$ . The absorbance of the complex decreased might be due to the dissociation of the complex at high temperature. FPB showed temperature sensitive while BCG, BTB and BCP did not. Owing to the maximum intensity of the complexes were attained at once in room temperature, no heating process was necessary.

Selection of Suitable Dye for Quantitative Determination of Piperazine and Its Salts by Compared Various Experimental Parameters

After studying the experimental parameters for the complete reaction and consequently maximum absorption between piperazine and four sulfonphthalein dyes. The most suitable dye was selected as the color forming agent. The conditions studied were summarized and shown in Table 7. The result shown indicated that BTB was selected as the dye of choice because of its high linear absorbance concentration relationship with very good reproducibility. The intensity of the complex colored was stable through out the period of time sufficient for quantitative work. Although the color of the complex was susceptible to temperature but its absorbance was slightly decrease at the temperature higher than  $40^{\circ}\text{C}$ , however, room temperature did not exceed  $40^{\circ}\text{C}$  so this effect was negligible. BCP showed high linear absorbance concentration relationship equal to BTB but its calibration curve showed higher sensitivity than BTB. Unfortunately, poor reproducibility was obtained with the higher concentration, however, it showed good stability to time the same as BTB but less stable than BCG. BCP was less susceptible to temperature than BTE, its absorbance appeared unchange until  $50^{\circ}\text{C}$  so BCP was selected as the second dye of choice.



BPB was not suitable as the color forming agent in quantitative work with piperazine due to its high susceptibility to time and temperature. The problems might be arising when concentration of dye was varied, causing erroneous results since the wavelength for maximum absorption might alter, otherwise incomplete reaction might occur. BPB also showed the shortest linear absorbance relationship between four sulfonphthalein dyes and its slope was less steep than other dyes.

BTB was used successfully in quantitative determination of piperazine and its salts in the concentration of  $4.00 \times 10^{-4}$  M. For routine work, a 150 mg. sample (as piperazine hexahydrate) was recommended for convenience in weighing, extracting and making up the solution. The BTB concentration used in each series of experiments was constant and was approximately six times of the maximum concentration of piperazine used. The absorbance of the complex was measured within 35 minutes after the complex was formed.

Determination of the Per Cent Labelled Amount of Piperazine as Hexahydrate in Piperazine Citrate Syrup USP XX Using Bromthymol Blue Method and Official Gravimetric Method

Piperazine citrate syrup USP XX was prepared and the content of piperazine in 5 ml. portion of the syrup calculated as hexahydrate was determined by bromthymol blue method and official gravimetric method<sup>(18)</sup>. The results obtained were

compared in Table 8. The mean value of the percentage from five determinations was 105.98 % with coefficient of variation of 0.23 for the BTB method and 106.76 % with coefficient of variation of 0.27 for the official gravimetric method<sup>(18)</sup>. The data presented were shown a good precision and a close relationship between the two methods. It was evident that the BTB method gave reproducible results compare well with the official gravimetric method<sup>(18)</sup>.

The high results obtained from developed formula were caused by the inaccuracy in process of preparation but the per cent labelled amounts were within U.S.P. limit(93.0-107.0 %), thus this preparation was continuously used for the purpose of testing accuracy in per cent recovery.

Determination of Percentage Recovery of Piperazine Citrate in Piperazine Citrate Syrup U.S.P. XX by Bromthymol Blue Method and Official Gravimetric Method

To check the accuracy of the method, recovery experiment was performed. Owing to other excipients in dosage form might interfere the absorbance of the sample these interferences could be tested by adding different accurately weighed amount of standard piperazine citrate 100, 125, 150 mg. to piperazine citrate syrup which the exact amounts of piperazine citrate presented were known. Piperazine free bases were extracted with chloroform and then treated with  $4.00 \times 10^{-4}$  M BTB , absorbance of the

resulting solution was measured. Amount of piperazine added was calculated as hexahydrate by subtracting the total absorbance of the resulting solution with the absorbance of the sample. Percent recoveries were calculated and the results were given in Table 9 .

The reproducibility study was also performed by running five replicate assays of each weight. The data obtained in Table 9 indicated that BTB method gave per cent recoveries of 101.98, 98.99 and 100.97 with coefficient of variation of 0.39, 0.40 and 0.47 for the weight of piperazine added : 100,125 and 150 mg., respectively. In official gravimetric method<sup>(18)</sup>, the per cent recoveries were 101.39, 100.69, 100.12 with coefficient of variation of 0.47, 0.39 and 0.78 for the weight of piperazine added : 100, 125 and 150 mg., respectively. The resulted data showed that both BTB method and official gravimetric method<sup>(18)</sup> were obtained good recoveries with high reproducibility for different weight of piperazine added. The presence of other excipients showed no significant difference on the development, intensity and stability of the complex formed .

Analysis of Piperazine and Its Salts in Commercial Pharmaceutical Dosage Forms, Using Bromthymol Blue and Official Gravimetric Method

To substantiate the validity of the method, ten commercial available formulations containing various salts

of piperazine, e.g., citrate, hydrate and adipate with different dosage forms and commercial sources were analyzed by the proposed BTB method, while official gravimetric methods<sup>(18,50,51)</sup> were used as a comparative means of analysis. The results obtained in Table 11 showed good agreement between the proposed BTB method and official gravimetric methods<sup>(18,50,51)</sup>.

The result obtained was the mean value of five replicate assays of each sample expressed as percentage of the amount declared. Eight in ten preparations were within pharmacopeial limits except sample B showed higher result, while sample I showed lower. Two types of syrup were studied; one was syrup U.S.P., consisted of 500 mg. of piperazine as hexahydrate per 5 ml., and the other was syrup BPC, contained 750 mg. of piperazine as hexahydrate per 5 ml. The results were in close value with coefficient of variation in the range of 0.22 to 0.48 for the proposed BTB method and 0.06 to 0.79 for the official gravimetric methods<sup>(18,50)</sup>. In elixir, each 5 ml, portion contained 750 mg. of piperazine as hexahydrate, the results obtained were shown coefficient of variation in the range of 0.26 to 0.88 for BTB method and 0.04 to 0.08 for official gravimetric method<sup>(50)</sup>. The mean value of percentage of plain and coated tablet were 93.41 and 87.96 with coefficient of variation of 0.70 and 0.76 for bromthymol blue method and = 92.04 (CV = 0.51) and 86.65 (CV = 0.99) for official gravimetric method<sup>(18)</sup>. In capsule, the results obtained

were 95.68 with coefficient of variation of 0.41 and 96.48 with coefficient of variation of 0.13 for bromthymol blue and official gravimetric<sup>(51)</sup>, respectively.

Other excipients in dosage forms might influence the absorbance of the sample such as coloring agent and any other water soluble compounds. They were eliminated in the step of extraction of piperazine as a free base since water soluble substances were maintained in the aqueous phase, then only chloroform soluble substances were participate with piperazine free base in extraction. In these extractions, there were no compounds containing nitrogen group presented in anthelmintic preparations of piperazine so there were no interferences in this method. In the official gravimetric methods<sup>(18,50,51)</sup> the syrup was weighed and precipitated directly with trinitrophenol solution, so some excipients might be formed precipitate with trinitrophenol, causing the higher results although in tablet or capsule, the step of filtration before precipitation was required but it could remove only water insoluble substances such as kaolin, starch, magnesium stearate, talc, etc. In soft gelatin capsule (Sample J), removing pasty content from the capsule must be carefully done otherwise lower result might be obtained.