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ASSESSMENT OF HOMOEOPATHIC PREPARATION OF SULPHUR WITH THE HELP OF U.V SPECTROSCOPY

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ABSTRACT

Spectroscopy is a technique that uses the interaction of energy with a sample to perform an analysis. The data that is obtained from spectroscopy is called as a spectrum. A spectrum is nothing but a plot of the intensity of energy detected versus the wavelength of the energy. Often, spectra are used to identify the components of a sample (Qualitative analysis). Spectra may also be used to measure the amount of material in a sample (Quantitative analysis). The main aim of this study was to judge the efficacy of U.V. Spectroscopy in differentiating different potencies of the same medicine. [1]. 3 centesimal scale of potencies of sulphur were selected namely 30, 200 and 1M/1000. It was observed that the UV trans-mission for homeopathic preparations of Sulphur was significantly different for different potencies of Sulphur that were tested which was evident from ƛmax.

Keywords: Interaction of energy with sample, spectrum, wavelength of energy, Qualitative & Quantitative analysis.

INTRODUCTION

A variety of different analytical techniques have been used in standardization of Homoeopathic drugs. U.V. Spectroscopy, NMR Spectroscopy, Infra-red Spectroscopy are few such examples. They are not only useful in measuring the amount of material in the sample (Quantitative analysis) but are also often used to identify the components of a sample (Qualitative analysis). Some like NMR Spectroscopy have been used successfully in assessment of differences of the physico- chemical properties of homoeopathic substances. [1] Ives (2000) has stated that she believes the most promising line of research has to come with the use of U.V. & NMR Spectroscopy. This is due to the fact that NMR Spectroscopy can record energy transitions of protons, which are reliant on their precision rates and electronic environments. NMR Spectroscopy is also a powerful tool used to determine the structure of molecules. The process of NMR entails placing the sample in a simple magnetic field and irradiating it with radio waves. [1] Earlier, research was conducted by Smith & Boricke (1966) on Sulphur potencies to evaluate the homoeopathic drug structure. Distinct changes were noted in the hydroxyl part of the spectrum. They concluded that the solvent structure in unsuccussed serial dilutions as compared to undiluted solvent.

They established further differences in succussed serial dilutions and the changes became more extreme as the potencies passed Avogadro's limit. This led them to believe that there is a physical rearrangement in the solvent most likely in the form of self-replicating polymers. [1] A further study by Smith & Boricke (1968) with higher potency levels up to 60X level compounded the evidence that the act of succussion increased the area of the hydroxyl spectrum as opposed to identical unsuccussed dilutions. [1] The employment of spectroscopy as an experimental technique on homoeopathic potencies has proven very useful (Schulte,1999). [1] Sukul et al (2001) conducted an experiment comparing the effects of Nux Vomica 30c succussed and unsuccussed on adult toads as well as the NMR

Spectra of the above mentioned compared to a range of variations. In the preparation of their samples they used 10 succussions except with the samples that were unsuccussed. They concluded that the ethanol-water mixture has the capability to imbibe some specific properties of drug molecules or particles during the dynamisation process. Using U.V. Vis Spectroscopy Roy et al distinguished 2 different homoeopathic medicines (Nux Vomica & Natrum Mur) and differentiated their 6c, 12c & 30c potencies. He concluded that Natrum Mur and Nux Vomica are distinctly different while the same potencies with different succussions also show a clear evidence of the difference in the structure of individual samples. [1]

Thus, the employment of these spectroscopic techniques as methods for the analysis of structure within homoeopathic potencies and as a method for the analysis of the differences between the respective homoeopathic potencies and lactose-based control and differences between parallel potencies and a control has been well substantiated (Ross, 1997). [1]
Ultraviolet-visible spectrophotometer

The instrument used in ultraviolet-visible spectroscopy is called a UV/Vis **spectrophotometer**. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (Io). The ratio is called the *transmittance*, and is usually expressed as a percentage (%T). The absorbance, A , is based on the transmittance. The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light, and a detector. [2]

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument, all of the light passes through the sample cell must be measured by removing the sample. In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission (or 0 Absorbance), and the measurement displayed is the ratio of the two beam intensities. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam in synchronism with the chopper. There may also be one or more dark intervals in the chopper cycle. In this case, the measured beam intensities may be corrected by subtracting the intensity measured in the dark interval before the ratio is taken. [2] A complete spectrum of the absorption at all wavelengths of interest can often be produced directly by a more sophisticated spectrophotometer. In simpler instruments the absorption is determined one wavelength at a time and then compiled into a spectrum by the operator. By removing the concentration dependence, the extinction coefficient (ε) can be determined as a function of wavelength. [2]

UV-Visible Spectrum shows a simple UV-visible absorption spectrum. Absorbance (on the vertical axis) is just a measure of the amount of light absorbed. One can readily see what wavelengths of light are absorbed (peaks), and what wavelengths of light are transmitted (troughs). The higher the value, the more of a particular wavelength is being absorbed. [2]

The wavelength at which measurement is to be made may be in the visible or in the U.V region and is specified in main text of respective monographs. [3]
The wavelength that corresponds to the highest absorption is usually referred to as **"lambda-max" (/\max).** [3]

If the absorbance of a series of sample solutions of known concentrations are measured and plotted against their corresponding concentrations, the plot of absorbance versus concentration should be linear if the Beer-Lambert Law is obeyed. This graph is known as a calibration graph. A calibration graph can be used to determine the concentration of unknown sample solution by measuring its absorbance. [2]

To conclude, the characteristic spectrum of a molecule is produced by changes in the electronic levels with various chromophoric groups within the molecules. These changes involve the absorption of relatively high amount of energy accompanied by changes in the vibrational and rotational energy within the molecule. These are different at different potency levels. Thus, homoeopathic remedies are spectroscopically distinct from the original solvent (water/ ethanol). It should be noted that different homoeopathic potencies & different dilutions of the same remedy can be distinctly distinguished by U.V. Spectroscopy. Also U.V. Spectroscopy has proved useful to investigate the subtle but significant changes in the structural parameters in both water and alcohol based remedies. [4]

PURPOSE OF SELECTION OF TOPIC-

It is often argued that the effects of homoeopathic dilutions are either unspecific or placebo, since common scientific theories and models cannot account for any specific effects of homoeopathic dilutions. Within the last years, several working hypotheses have been developed to reveal the mode of action of homoeopathic preparations but none of them has been validated so far. Therefore, knowledge of the nature and action of homoeopathic preparations is yet insufficient. In addition, question regarding standardization of Homoeopathic medicines arises. Also it is very difficult to differentiate same potencies of 2 different homoeopathic medicines as well as different potencies of the same homoeopathic drug. Given these uncertainties, there clearly is need for further research. More so, U.V. Spectroscopy distinguishes 2 different homoeopathic medicines and also differentiates between different potencies of the same medicine as well as 2 different medicines.

AIMS AND OBJECTIVES

Aim: To assess the homoeopathic preparation of Sulphur with the help of U.V. Spectroscopy.

Objectives: The objective of the study was to differentiate different potencies of Sulphur like 30C, 200C, 1M etc. from one another with the help of U.V. Spectroscopy

MATERIALS AND METHODS

Laboratories and Clean Room: The experiments were be carried out at the fully equipped research laboratory of Quality Assurance Department of Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune.

Instrument: UV-Visible double beam spectrophotometer with 1cm matched quartz cells, Micropipette of Variable volume 10-1000 L and Digital balance (Citizen Co.) from the Quality Assurance Lab of Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune was used.

Chemicals and Reagents: All chemicals and reagents used like Ethanol, Potassium dihydrogen phosphate, Hydrochloric acid, Sodium hydroxide etc. were of analytical grade and purchased form a standard laboratory.

Preparation of standard stock solution: 100 mg of Sulphur was weighed accurately and transferred to 100 ml of calibrated volumetric flask. 50 ml of previously prepared and standardized 0.1 N HCl was transferred to same flask and swirled for solublization . Volume was made upto 100 ml mark by 0.1 N to obtain solution of 1mg/ml concentration. This solution was used as standard stock solution. From this solution 10 ml was withdrawn and diluted to 100 ml with 0.1 N HCl, (100 µg/ml). This solution was used as working standard solution.

Preparation of sample solution: The average weight of Sulphur taken was determined by weighing 20 tablets and powdered. Tablet powder weight equivalent to 20mg of Sulphur was weighed and transferred to 100 ml volumetric flask. About 50 ml of 0.1 N HCl was added and sonicated for 15-20 min for complete dissolution of drug. Volume was made upto 100 ml with 0.1 N HCl and mixed. Above solution was filtered through Whatmann filter paper No. 41 and further diluted to obtain solution of 10µg/ml.

Method validation:
Various method of analysis of Sulphur in bulk and pharmaceutical formulations (marketed and developed) were carried out as per the guidelines.

Measurement of absorbance and calibration curve:
The absorbance of solutions containing 10µg/ml was determined in UV range 200-800 nm using 0.1 N HCl as blank. The λmax was found. At this wavelength maximum, calibration curve was drawn by plotting graph between absorbance and concentration.

U.V. Spectroscopy Measurements:
The light transmission of all samples was measured from 190 to 290 nm. Each measurement was repeated three times, respectively. The reference was air and a cuvette filled with distilled water. Both UV spectrometers were turned on 1 h before the measurement to allow a warm-up of the instruments. In pilot studies, wavelength calibration and handling were tested to optimize reproducibility. Both instruments scanned with a speed set at 120 nm/min. Every 4^{th} measurement, respectively, was without any sample inserted in the UV spectrometer, followed by one with a sample of the cleaning water. After measuring a sample, the cuvette was cleaned twice with distilled water and shaken out before filling it with the next sample. When filling the cuvettes, care was given to avoid bubbles and cuvettes were visually inspected for bubbles. The

cuvettes were filled using pipettes with a standardized volume.

RESULTS

3 centesimal scale of potencies of sulphur were selected namely 30, 200 and 1M/1000. To make 1000 ml of tincture, 0.2mg of Sulphur is added to 1000ml of Strong alcohol and allowed to remain in well stoppered bottle for at least 48hrs. the bottle being shaken 2 times a day. It is then filtered. All further higher potencies are prepared by using dispensing alcohol. [5,6]

Findings:

1. Sulphur 30- Transmission- Started at 230 nm
   Ceased at 200nm
   Absorbance- 2.819
   /\ max(peak)- 196.9

2. Sulphur 200- Transmission- Started at 214 nm
   Ceased at 200nm
   Absorbance- 2.847
   /\ max(peak)- 196

3. Sulphur 1000- Transmission- Started at 212 nm
   Ceased at 200nm
   Absorbance- 3.134
   /\ max(peak)- 193

Hence, we can see that as we go from 30C to 1M, i.e, as we increase the potency, absorbance increases, i.e. amount of light absorbed by each potency particle increases as compared to transmittance. Also /\max, i.e. wavelength at which maximum absorption takes place also decreases as we go to higher potencies.

Thus, from the above readings we can conclude that the 3 potencies, i.e. 30C, 200C and 1M of the same drug sulphur are different in their behaviour which is evident from the absorbance and /\ max and can be differentiated with the help of U.V. Spectroscopy.

DISCUSSION

The UV trans-mission for homeopathic preparations of Sulphur was significantly different for different potencies of Sulphur that were tested.

We can see that as we go from 30C to 1M, i.e, as we increase the potency, absorbance increases, i.e. amount of light absorbed by each potency particle increases as compared to transmittance. Also /\max, i.e. wavelength at which maximum absorption takes place also decreases as we go to higher potencies.

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SUMMARY & CONCLUSION

As we go from 30C to 1M, i.e, as we increase the potency, absorbance increases, i.e. amount of light absorbed by each potency particle increases as compared to transmittance. Also /\max, i.e. wavelength at which maximum absorption takes place also decreases as we go to higher potencies.

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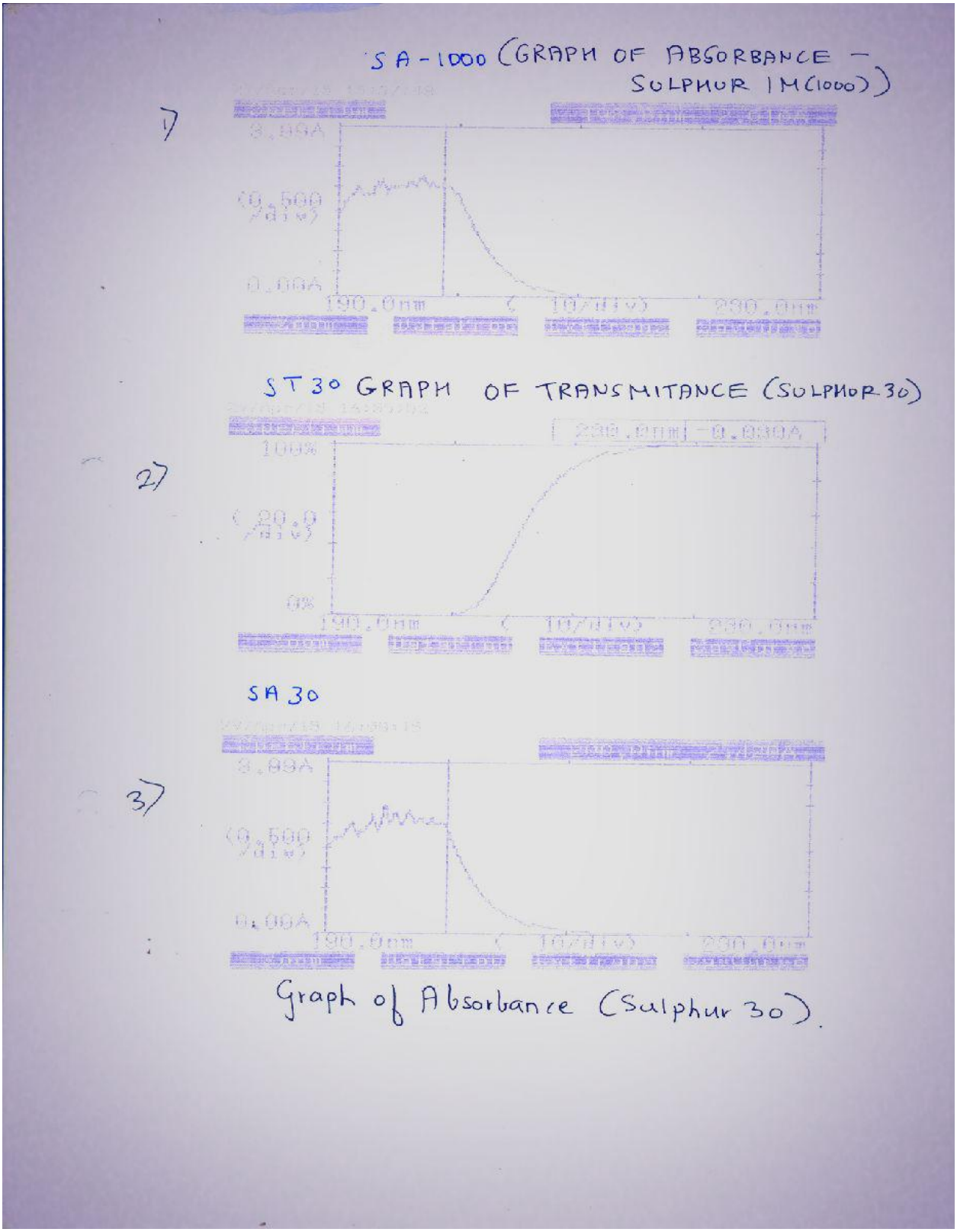

Figure 1: UV spectrums

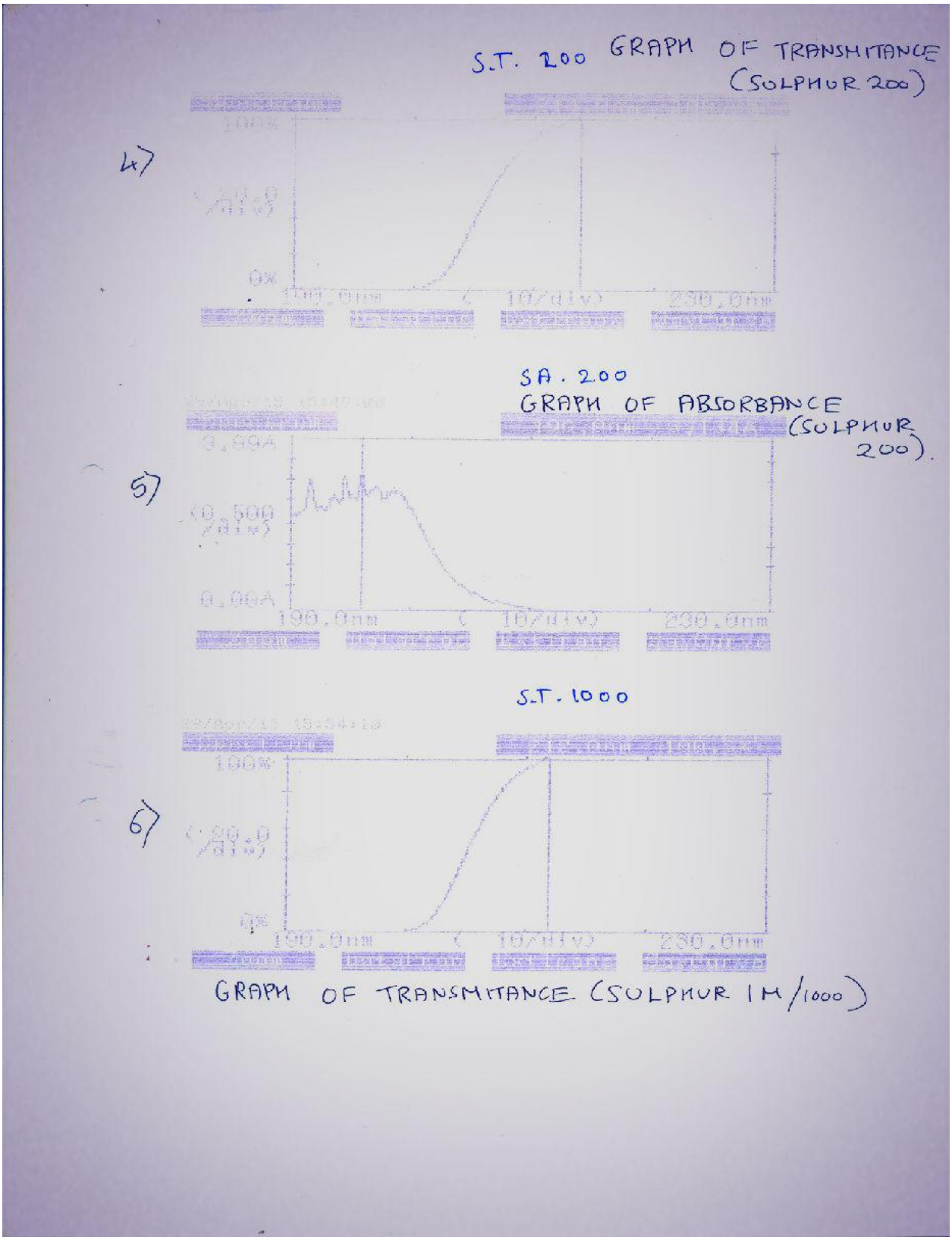

Figure 1: UV spectrums (Continued)

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