Introduction

The Video Spectral Comparator 6000 (VSC) is a machine by Foster + Freeman. The machine utilizes many functions and different light sources to examine documents, and is usually used as a forensic tool to check the validity of valuable documents. Machine is a comparator, and it is also used to compare one set of images or spectra up against another. The VSC has also had a variety of uses in the cultural heritage sector, like analyzing ink and looking for deterioration in documents. As the name suggests the VSC is a comparator, and works by enhancing a document from different angles and in different light as well as taking spectral measurements. The results obtained by using the VSC are only indicative and not definite. The reason for this is that the machine is made to compare one set of data to another, and is not an analyzing instrument. But perhaps its results are good enough. The VSC in itself is costly, but the cost of use is not huge. The main item of expenditure is the time and cost of the user. Other than that it is mainly electricity and maintenance of the machine which decide its cost. The VSC also has another advantage compared to some of its competitors. It is a machine with more than one function. This can make it a good investment, since one gets a multifunctional tool, which can replace other instruments used in a conservation laboratory. The VSC uses different light sources UV light, Visible light, Infrared light. VSC instrument is based on spectroscopic Methods [1-16]. Spectrophotometric methods for blood identification are the measurement of interactions between electromagnetic radiation and matter. Electromagnetic radiation, which includes light, has characteristics of waves and particles. The absorption of radiation by matter is a quantized process, in that a molecule will only absorb certain discrete frequencies. These frequencies are determined by well-defined spacing’s of energy levels in the molecule under investigation. The absorption of photons of high energy cause large changes in the molecules and correlate to moving electrons to higher energies [11]. One of the most important components of blood is hemoglobin. This pigment transports 65 times more oxygen in the erythrocytes than plasma does, and it can be found in two forms, deoxygenated and oxygenated [13]. In UV visible

1) To use VSC as a non-destructive technique for the presumptive identification of blood.

2) To utilize VSC (Visual Spectral Comparator) as a source of visible source of light in the spectroscopic analysis of blood.

3) To postulate that the canopy of blood spectrum remains “similar” with the passage of time.

4) To compare porous and nonporous substrates for better spectroscopic analysis of the unknown blood samples.

Materials and Methods

The study involved the collection of waste animal blood and 25 healthy individual blood samples under highly hygienic and controlled conditions with individual informed consent on two different substrates, porous (Whatman) and nonporous (slide) surfaces. Before this prior approval from Institutional Ethics Committee of Institute of Forensic Science, Mumbai (M.H.) was obtained (IEC/ 031/ 2014) for conducting the study. Separate sterile lancets were used to collect blood from individuals whose fingers were sterilized prior to pricking with absolute alcohol and 1-2 drops of blood was collected each on a sequentially numbered Whatman paper and a glass slide respectively. The used lancets and cottons were safely disposed off. The collected blood specimens were stored at room temperature in a safe plastic box away from moisture and direct sunlight. A
constant storage procedure was followed throughout the study. The analysis of the blood specimens, both Whatman and slide, was done using VSC 6000/HS Foster + Freeman. The procedure entailed the collection of spectral data of blood from both, Whatman and slide on a same graphical plane so as to enable clear comparison of their absorption characteristics on different platforms. In cases of both the types of samples, firstly, the scanning was initiated from UV light to visible light followed by measuring of the “white reference” i.e., the absorption spectra of the platform viz. the Whatman paper or the glass slides. Secondly, the absorption spectra of the blood specimen were collected, from which the previously recorded “white reference” was subtracted. This gave the absorption spectra of the actual blood sample at visible wavelength of light and graphical representation were developed using the inbuilt VSC 6000/ HS PC Suite software of the instrument. The graphical data and with their peaks were recorded and the graphs were saved as a bitmap file format on the computer drive. The very same procedures were strictly followed for all the 25 samples on different days. The samples were observed for the variability in the spectra at the interval of 24 hours, 48 hours, 4th day, 6th day, 8th day, 15th day. The spectral data was observed, recorded and analyzed for the spectral variations with the passage of age.

Results and Discussion

The study which aimed at the spectroscopic analysis of blood showed that VSC 6000/HS can be effectively used as a potential source for visible light. The (Figure 1-6) shows visible range of light from VSC can successfully be used for non-destructive and presumptive identification of blood. The spectra were found to be unique and characteristic to blood and dissimilar from the negative controls included in the experiment (Table 1 and 2). All of the 25 blood samples under study yielded a similar spectrum which entailed peaks characteristic to the constituents of blood (Figure 1-6). The samples continued to give similar spectrums up to a period of twelve months (Figure 7) after which, slight variations were witnessed with the gross appearances and characteristics remaining the same (Figure 7). These variations seen were probably due to ageing and decomposition of blood samples which were, although, stored under controlled conditions. A few extra peaks were witnessed in case of blood preserved using EDTA. This was due to the fact that EDTA itself has absorption peaks in the UV-Vis range (Figure 7). The spectrum
Figure 3: Showing results of Day 4.

Figure 4: Showing results of Day 5.

Figure 5: Showing results of Day 8.
of animal blood was found to be very similar to that of human blood and thus the technique can be of no use in determination of origin of species (Figure 7). It was also evident from this pilot study that porous substrates used in the experiment gives distinct and better results as compared to non-porous substrates. (Table 1 and 2). The final negative control, i.e. spectrum of substrate Slide and Whatman filter paper produced no peaks, thus confirming the validity of the study (Figure 7).

Conclusion

Blood is one of the most crucial evidence available at the scene of crime. Its accurate identification and analysis is a must for proving guilt or innocence. If very trace amounts of blood is available, then it becomes difficult for the analysis team to utilize destructive methodologies for trial and error analysis. This pilot study successfully concluded that a questioned stain at a scene of crime can be subjected to non-destructive spectral analysis using VSC (Visual Spectral Comparator) and can be ascertained to be blood or not based on the very same spectral characteristics of blood. Thus, the non-destructively presumed stain to be blood can now be directed for a routine confirmatory assay which would not have been possible with a destructive presumptive technique. Moreover, the study also

### Table 1: Range of wavelength observed for blood sample on porous surface.

| DAY | SAMPLE (WHATMANN FILTER PAPER) | WAVELENGTH(NM) |
|-----|--------------------------------|----------------|
| 1   |                                | 432 TO 575     |
| 2   |                                | 432 TO 577     |
| 4   |                                | 431 TO 571     |
| 5   |                                | 431 TO 573     |
| 8   |                                | 431 TO 571     |
| 15  |                                | 431 TO 571     |

![Figure 6: Showing results of Day 15.](image)

![Figure 7: Showing results of control sample of both Slide and Whatman filter paper.](image)
revealed that the overall canopy of the spectroscopic graphs were “identical” in one year old samples. In addition to this, the study also revealed that a porous substrate for retrieval of blood evidence tends to give comparatively better outputs for analysis as compared to non-porous techniques. The advantage of the study is that does not involve preparation of reagents and less time consuming as other techniques to analyze the blood sample and disadvantage is that it's high cost and not possible to carry the machine at the crime scene but once employed does not cause as much as the cost required to prepare reagents which could be easily transported. In a nut shell, the study suggests a new, quick and easy, non-destructive, presumptive instrumental technique for analysis of blood which would help in aiding forensic investigations involving insufficient and aged blood samples.

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