

On the Absorption Spectrum of Oxyhæmoglobin in the Ultra-violet and Extreme Ultra-violet Region.

By

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In order to investigate the molecular constitution of a substance, its physical and chemical properties are utilized. Among physical properties, the spectrum affords us a convenient and sensitive means for attacking the subject. For a liquid substance its absorption spectrum is used, if it has any selective absorption. Sometimes it is possible to ascertain the nature and the structure of the molecule of the substance by its absorption spectrum.

Hæmoglobin in the red blood corpuscles, which plays a great rôle in human metabolism, has been investigated physically and chemically by many; and its absorption spectrum has been exhaustively examined. By adding oxygen gas, hæmoglobin can be changed into oxyhæmoglobin; and the latter can be reversed to the former by means of ammonium sulphide. Oxyhæmoglobin easily decomposes to its derivatives. As each of those derivatives gives its characteristic absorption band, this change can be easily traced by the spectroscopic method. Oxyhæmoglobin and some of its derivatives in very dilute solutions have each an absorption band at $\lambda_{410\mu\mu}$; and this absorption band is recommended in forensic medicine and internal medicine for the proof of the existence of blood.

With regard to the ultra-violet absorption, many investigations have been made. J. L. Soret¹ first discovered three absorption bands of oxyhæmoglobin in this region, and determined their positions as

¹ J. L. Soret: Arch. sci. phys. et natur. (Geneve), **61**, 322 (1878), **66**, 429 (1883).

follows: the first begins from the Fraunhofer line G and extends beyond the line H, the second at Cd-line $\lambda 3247 \text{ \AA.U.}$ and the third at Cd-line $\lambda 2743 \text{ \AA.U.}$ A. Gamgee¹ examined for these bands, but the band at Cd-line $\lambda 3247 \text{ \AA.U.}$ was not observed; while Dhéré² found two at $\lambda 4156 \text{ \AA.U.}$ and $\lambda 2801\text{--}2665 \text{ \AA.U.}$, but could not observe any near Cd-line $\lambda 3247 \text{ \AA.U.}$

R. Hiller³ and H. Grabe⁴ also investigated this subject. Recently E. Peyrega and F. Vlès⁵ have noticed that the band which had been found by Soret at Cd-line $\lambda 3247 \text{ \AA.U.}$ really exists, though it is not at Cd-line $\lambda 3247 \text{ \AA.U.}$, but at $\lambda 333 \mu\mu$. Before reading the Peyrega and Vlès' paper the writer examined the absorption spectrum of oxyhæmoglobin and found a band at $\lambda 350 \mu\mu$, but not at $\lambda 333 \mu\mu$.

Method of Experiment.

(A) The ultra-violet region. The spectrograph used was a quartz-spectrograph of the type C, made by Hilger. By making use of a Baly's absorption tube with quartz windows, the thickness of the solution of oxyhæmoglobin through which the rays of light is to pass was changed. It was assumed that hydration of oxyhæmoglobin did not occur during the process of the experiment and that Beer's law relating to the thickness of absorbing layer and its concentration holds true. For the source of light the positive crater of a 90° carbon arc was used. In the investigation of absorption spectra, the iron arc is commonly recommended, for the reason that this arc emits many lines of nearly equal intensity and also is convenient for determining the wave lengths of absorption bands. But a merely slight inequality in the intensity of the lines renders the detection of a weak absorption band impossible; and for this reason a carbon arc, instead of an iron arc, was employed in the present investigation. The constancy of the electric current through the arc was checked by an amperemeter. The photographic plates employed were "Wratten M plate." The existence of a trace of methæmoglobin, found during the spectroscopic treatment,

¹ Zs. Biol, **34**, 505 (1896).

² Compt. Rend. Soc. Biol., **61**, 718 (1906), Jahresber. Fortschr. Tierchem., **40**, 128 (1910).

³ Diss. Rostock., (1904), Jahresber. Fortschr. Tierchem., **35**, 140 (1905).

⁴ H. Grabe: Diss. Dorpat., (1892).

⁵ C. R., **154**, 133 (1912).

might be detected by its characteristic strong absorption band in the visible part of the spectrum. But methæmoglobin does not give an absorption band in the region of the present investigation. The oxyhæmoglobin was prepared by the Hoppe Seyler's method from arterial blood which was taken by canule from a carotis of a dog; it was recrystallized three times, and then, without drying, the crystal was dissolved in distilled water. By this manipulation the absolute quantity of the oxyhæmoglobin could not be determined; but for the purpose this was not necessary. Many photographs were taken with the solution of the same degree of dilution which was estimated by comparison with the standard colour of Gower's hæmoglobinometer.

(B) The Schumann region. To obtain the spectrum of the extreme ultra-violet i.e. of the Schumann region, a quartz spectrograph of the type A of Hilger was used. As the source of light, a condensed spark of Co, Fe, or Wo in the air was used. In order to avoid the absorption due to the gelatin of the photographic plate; Schumann plates were taken. And also in order to get rid of the absorption of the water layer in this region of the spectrum as much as possible, 1 mm thick cuvette with quartz windows was used for the absorption vessel. The solutions of the different concentrations were examined.

Result.

In the annexed photograph, the white lines represent the emission lines. The thickness of the liquid layer was changed in the ratio of the numbers 1 : 2 : 3 etc. up to 42. The 43rd spectrum is that of the source only. The band seen at $\lambda 350 \mu\mu$ is probably a controverted absorption band, and is of course the one which Peyrega and Vlès found at $\lambda 333 \mu\mu$. By tracing the absorption curve for this band in the photograph and from the curvature of this curve, the centre of this band was estimated to be at $\lambda 350 \mu\mu$.

Any noticeable absorption band was not detected in the Schumann region.

Conclusion.

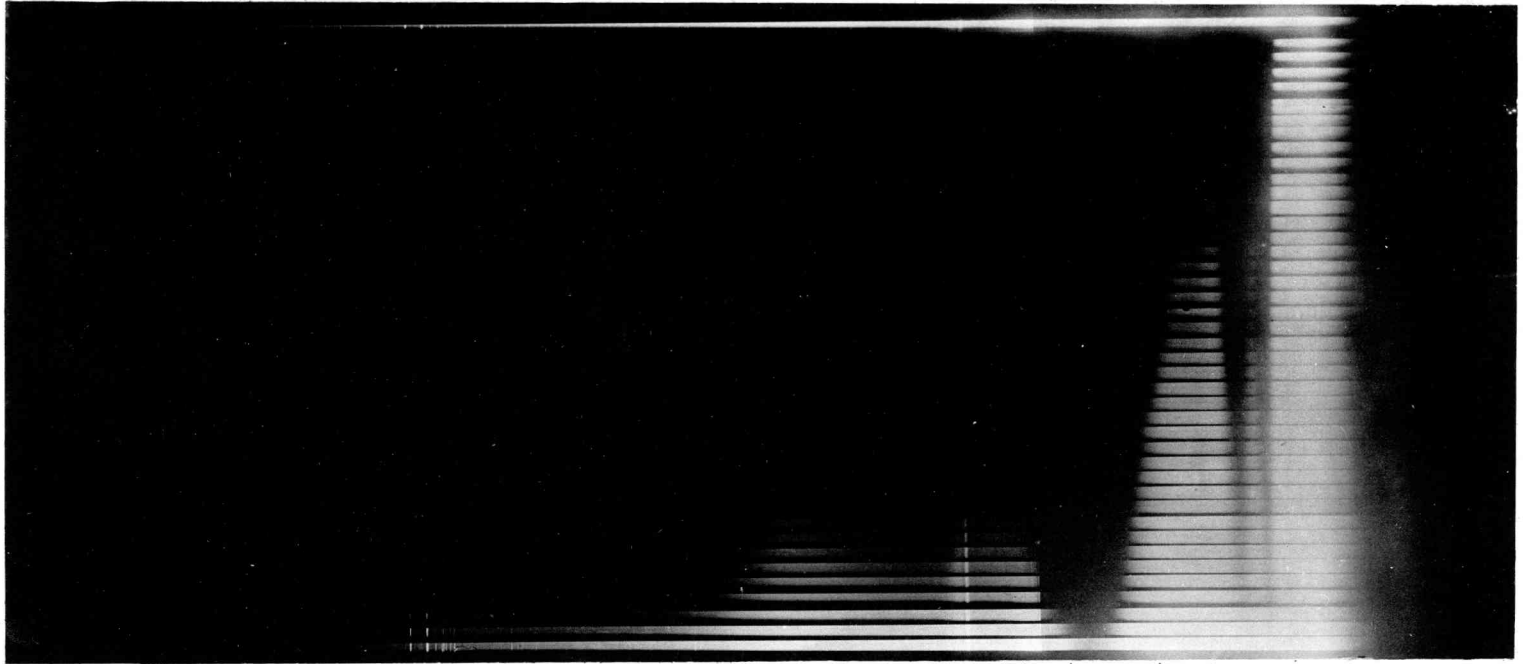
The disagreement among the observations on the absorption band of oxyhæmoglobin by many authors seems to be due to the fact that the concentration employed were not appropriate, as was pointed out by Peyrega and Vlès in the paper before cited. The writer took 42 degrees of concentration, and determined the position of the centre of the absorption band from the absorption curve thus obtained, and found

an absorption band at λ 350 $\mu\mu$, which may perhaps be the one observed by Peyrega and Vlès at λ 333 $\mu\mu$.

In the Schumann region no absorption band was observed.

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Absorption Spectrum of Oxyhæmoglobin
in Ultra-violet Region.



Wave-length scale
(in $\mu\mu$)

λ for Na-lines
(in $\mu\mu$)

249.8	250	259.4	260	268.1	270	280	288.2	290	300	310	320	330.3	330	340	350	359.5	360	370	380	386.2	400	421.6	450	500
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