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DETERMINATION OF THE AGE OF BLOOD STAINS BY NON-DESTRUCTIVE METHODS

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The purpose of this investigation was to determine the feasibility of Mössbauer spectroscopy and/or visible absorption spectroscopy as a tool for determining the age of blood stains by non-destructive methods.

Initial experiments were conducted to determine the optimum size of the blood samples to be used in obtaining the Mössbauer spectra. Pulse height spectra were obtained for dried blood samples ranging in size from 0.25cc to 1.00cc. By measuring the ratio of the area under the gamma ray peak (14.4 kev) corrected for background radiation to the total area (which includes both 14.4 kev. gamma radiation and background radiation), the optimum sample size was found to be that left by 0.25cc of whole blood after drying.

The Mössbauer spectra of whole blood samples dired in air for various periods of time were measured with a sample taken once a day. The samples were prepared from 0.5 ml aliquots of whole blood placed on china spot plates and exposed to air at room temperature. After the appropriate time, the sample of the dried blood was ground in a mullite mortar until it was finely powdered. The powder was placed in an aluminum cup and sealed with scotch tape. This was the cell used in obtaining the Mössbauer spectra. In spite of the results of the pulse height spectra, it was found that approximately 250 mg of powdered dried blood gave the best spectrum. All spectra were measured at 123°K in the usual transmission mode. The Mössbauer parameters obtained in this fashion are listed in Table I. An examination of the isomer shifts and quadrupole splittings reveals no correlation of either of these parameters with time. In addition to the two outer lines listed in Table I, a pair of inner lines of lesser intensity and greater scattering were noted in all of the spectra. In our third

Quarterly Report (April 1, 1972), we noted that the ratio of the areas of the left line to the total area of all the lines increases slightly from one to two days and remains constant for the remainder of the time. The ratio of the sum of the areas of the two inner lines to the total area of all the lines decreases in this same period whereas the ratio of the area of the right line to the total area of all the lines remains constant throughout the total time period. Since we now believe that some of the absorption at the positions of the two inner lines is due to iron in the aluminum sample holders, it is highly doubtful that the aforementioned area ratio changes are real.

The second approach to the problem was through the use of visible absorption spectroscopy. Again, 0.5 ml aliquots of whole blood were placed on china spot plates and exposed to air at room temperature. At various time intervals, the sample of dried blood was powdered in a mullite mortar. 20 mg of the dried powdered blood was then dissolved in 10 ml of 0.1M KCl. Visible absorption spectra were obtained on samples prepared in this fashion. The results are listed in Table II. The Soret peak occurs at 405 $\pm$ 001nm with an absorbance between .417 and .510. No correlation with time is noted. The band at 627nm, characteristic of methemoglobin (Ref.1), shows an increase in absorbance with time up to 174 hr. The bands at 575 and 538nm, characteristic of oxyhemoglobin (Ref. 2), show a decrease in absorbance with time up to 221 hr. The experimental error in absorbance was found to be .005. At almost every time interval, the observed increase or decrease is greater than this error and hence, the changes must be considered significant. We can account for the visible absorption data if we assume that upon exposing blood to air, oxyhemoglobin is oxidized to methemoglobin.

In conclusion, the prospects for using Mössbauer spectroscopy as a tool in the non-destructive determination of the age of blood stains do not look promising. On the other hand, visible absorption spectroscopy shows real promise in this area and in our opinion merits further investigation.

References

(1) D. Keilin and E. F. Hartree, Biochem. J., 49, 88 (1951).

(2) H. Sternberg and A. D. Virtanen, Acta.Chem. Scand., 6, 1342 (1952).

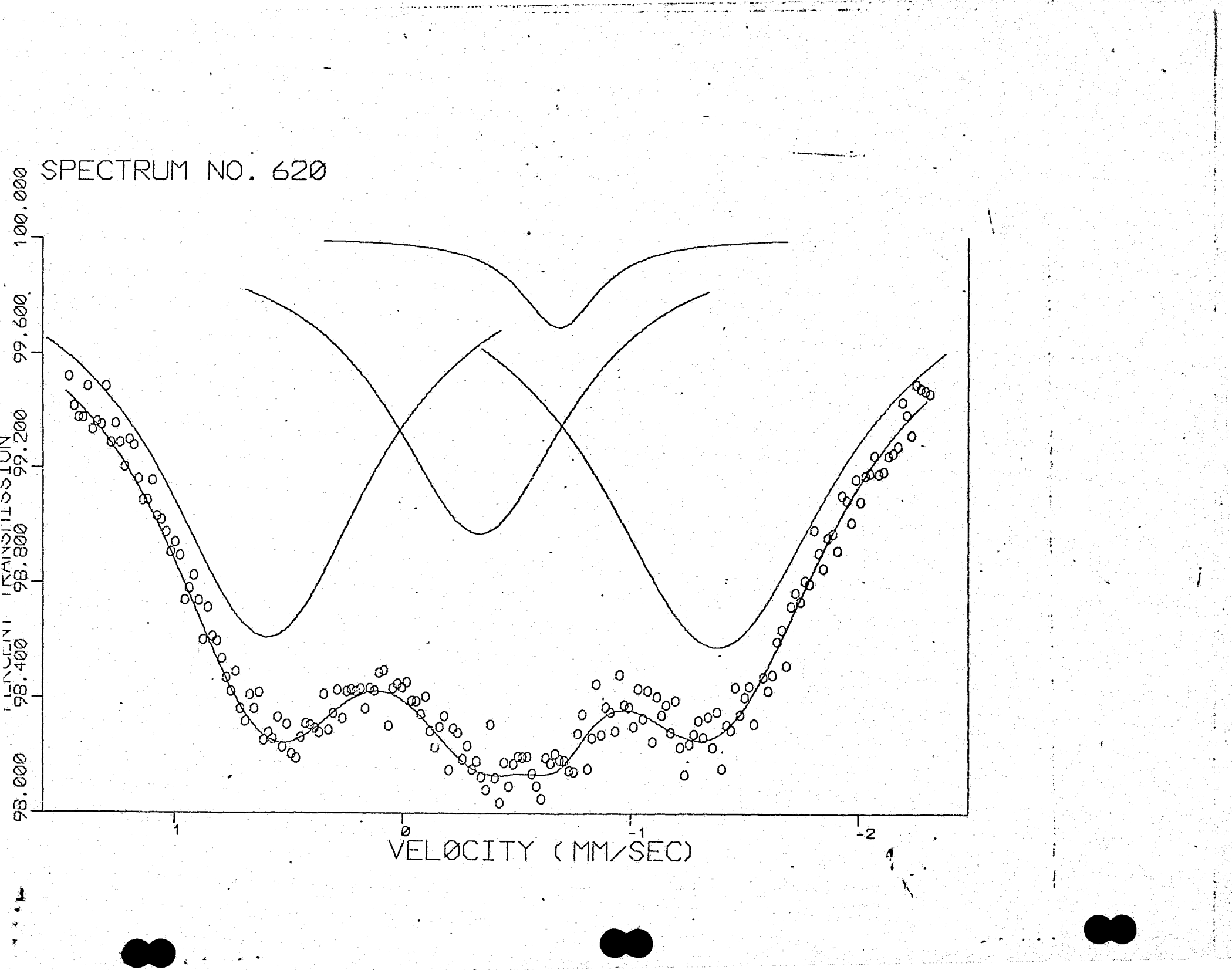
Table I. Mössbauer Parameters[a]

Run No.	t hrs.	t days	ΔE_Q	δ	τ_1	τ_2
633	0	0	2.16 ±0.03	0.45 ±0.01	0.266	0.134
634	2	0.083	1.98 ±0.03	0.42 ± .01	0.279	0.300
635	3	0.12	1.98 ± .06	0.44 ± .01	0.213	0.356
648	10	0.417	2.00 ± .02	0.46 ± .01	0.231	0.18
651	17	0.708	1.92 ± .01	0.42 ± .01	0.248	0.241
603	23.5	0.996	2.01 ± .03	0.45 ± .01	0.412	0.566
612	56	2.33	2.05 ± .03	0.44 ± .01	0.366	0.389
613	78	3.25	2.00 ± .03	0.43 ± .01	0.376	0.364
614	125	5.21	2.01 ± .02	0.43 ± .01	0.408	0.389
615	149	6.21	1.99 ± .02	0.41 ± .01	0.396	0.394
618	223	9.29	1.97 ± .03	0.41 ± .01	0.497	0.495
620	269	11.21	1.97 ± .03	0.39 ± .01	0.560	0.626
621	293	12.2	2.02 ± .02	0.41 ± .01	0.410	0.391

[a] All values in mm/sec relative to sodium nitroprusside

Table II. Visible Absorption Data

t hr.	625nm	575nm	538nm	Soret Peak Position (nm)	Soret A
29	.193	.568	.657	406	.457
53	.198	.557	.643	406	.444
77	.210	.544	.636	406	.438
101	.214	.544	.635	406	.443
174	.266	.452	.580	404	.510
197	.232	.387	.514	404	.458
221	.227	.355	.477	405	.417
244	.240	.341	.480	405	.487
268	.253	.354	.497	405	.470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TRANSMISSION
1
0
-1
-2
VELOCITY (MM/SEC)