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APPLICATION NOTE

KINETIC DYE DEGRADATION MEASURING ABSORBANCE & TRANSMITTANCE

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METHOD

APPLICATION BACKGROUND

Allura Red is a red powdered azo dye that is used in food, cosmetic, drug, textile, and paper industries. Allura Red is known by many names, but the most familiar probably being Red 40 or E129. Azo dyes are synthetic coloring agents that contain an azo group (-N=N-) in their chemical structure (Figure 1). Kinetics is the study of how quickly, or slowly, a chemical reaction occurs. This experiment applies kinetics to dye degradation. For the oxidizing agent, we used bleach to degrade the color of Allura Red. The active ingredient in bleach is sodium hypochlorite (NaClO). The sodium hypochlorite changes the chemical compound of Allura Red causing the red color to disappear (Figure 2).

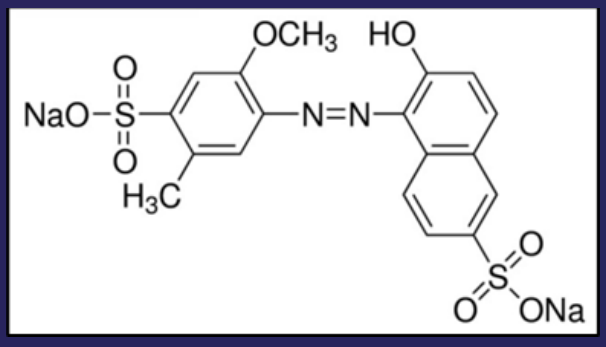

FIGURE 1: Chemical Structure of Allura Red

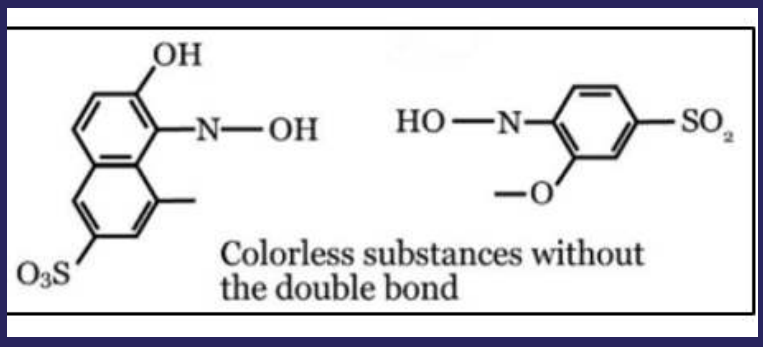

FIGURE 2: Chemical Structure after mixed with bleach

In this experiment, we measured the absorbance and transmittance of an Allura Red solution mixed with a bleach solution. Allura Red absorbs green coloring (480- 560nm) and transmits red coloring (640-700 nm). Using Beer-Lambert's Law, we used the absorption measurement conversions to solve for the unknown changing concentrations of Allura Red.

DESCRIPTION OF SYSTEM

The spectrometer used in this experiment was the AvaSpec-ULS4096CL-EVO (Figure 1). This spectrometer uses CMOS (complementary metal–oxide–semiconductor) technology instead of conventional CCD technology. The AvaSpec-ULS4096CL-EVO has the latest AS-7010 electronics which supports USB3 and gigabit ethernet communication protocols. The AS-7010 (AKA EVO) electronics offers a fast microprocessor and 50 times more on-board memory which can help to store more spectra facilitated by Avantes' proprietary store-to-ram capability. The spectrometer detector supports integration times as low as 9 microseconds and up to 40 seconds. The spectrometer used for this experiment was configured with our 300 groove/mm UA grating with a range from 200- 1100 nm, and for this experiment, we measured data in the visible range which is 380 to 780nm.

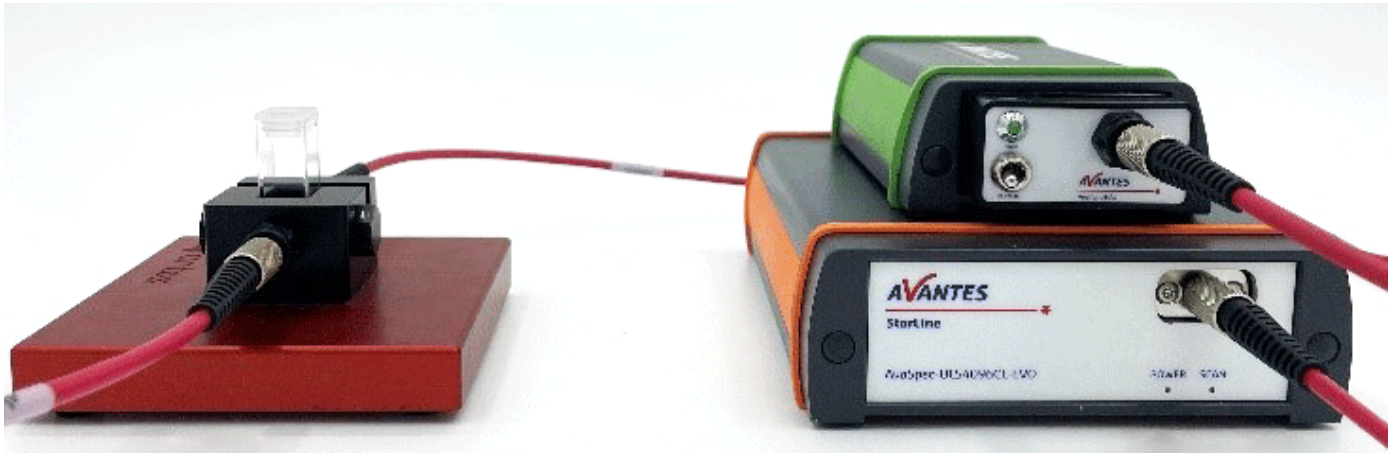

FIGURE 3: Absorbance/Transmission Setup

The light source used was the AvaLight-HAL-S-Mini which is a Tungsten-Halogen light source that is commonly used for spectral measurements in the visible to near-infrared wavelength ranges. It's a compact, stabilized halogen light source, with adjustable focusing of the fiber connection, maximizing output power at the desired wavelength. The light source also has adjustable output power to provide extra power or longer lamp life depending on the application needs. This light source was chosen for the experiment because the AvaLight-HAL is ideal for absorption/transmittance measurements in the visible spectrum. The wavelength range of this light source is 360- 2500 nm. The AvaLight-HAL-S-Mini also features an internal TTL-shutter, controllable from an AvaSpec Spectrometer. This gives the ability to periodically shutter the light source to take a dark during an experiment or process. This shuttering can be done manually or via software control.

The other two components of the system were a cuvette sample holder and two 400-micron UVIR fiber optic cables. The cuvette sample holder was designed for absorption and fluorescence measurements and should be used with a standard 10x10mm cuvette. A quartz cuvette was used inside the sample holder. Two 400-micron UVIR broadband fiber optic cables were used to connect the light source & cuvette and spectrometer & cuvette.

DESCRIPTION OF METHODOLOGY

Two solutions were made for this degradation experiment. The first being the Allura Red solution, and the second being the bleach solution. Both the Allura Red and bleach were diluted with deionized water (DI). The Allura Red solution had a concentration of 5.03×10^{-4} M (mol/L). The bleach solution had a sodium hypochlorite concentration of 0.163 M. When these two solutions were mixed, the added bleach solution diluted the Allura Red to a new concentration at time zero. The mixed solutions' new concentration of Allura Red was 3.36×10^{-4} M. The concentration of Allura Red will decrease with respect to time, while the sodium hypochlorite concentration will remain the same with respect to time. All calculations are provided below.

$$\frac{5.00 \text{ mg Allura Red}}{20.0 \text{ mL DI}} \left| \frac{1 \text{ g}}{1000 \text{ mg}} \right| \frac{1 \text{ mol Allura Red}}{496.42 \text{ g}} \left| \frac{1 \text{ mL of DI}}{10^{-3} \text{ L DI}} \right| = 5.03 \times 10^{-4} \text{ M (mol/L)}$$

Bleach solution NaClO concentration

The bleach used had 4.5 % NaClO and a density of 1.08 g/mL. For this calculation, we assumed 100 g of solution. The first step was to find the moles solute, and the second step was to find the liter of solution.

$$\text{Mass solute} = \frac{4.5\ \%}{100\ \%} \text{ X } 100 \text{ g} = 4.5 \text{ g NaClO} \qquad 4.5 \text{ g NaClO X } \frac{4.5\ \%}{100\ \%} = 0.0605 \text{ mol NaClO}$$

$$\frac{100 \text{ g solution}}{} \left| \frac{1 \text{ mL solution}}{1.08 \text{ g solution}} \right| \frac{1 \text{ L solution}}{1000 \text{ mL solution}} = 0.0926 \text{ L sol}$$

$$\text{M} = \text{Moles solute/ L solution} = \frac{0.0605 \text{ mol NaClO}}{0.0926 \text{ L solution}} = 0.653 \text{ M NaClO (mol/L)}$$

The bleach solution had a dilution ratio of 1 to 4. So, there were three parts of deionized water to one part bleach. So after the dilution, the NaCLO concentration became 0.163 M NaClO.

$$M_1V_1 = M_2V_2$$

$$0.653 \text{ M } (1 \text{ mL}) = M_2 \ (4 \text{ mL})$$

$$M_2 = 0.163 \text{ M NaClO}$$

Mixed solution Allura Red concentration at t = 0

The mixture had 2 mL of the Allura Red solution and 1 mL of the bleach solution.

$$M_1V_1 = M_2V_2$$

$$5.03 \times 10^{-4}\ M\ (2\ mL) = M_2\ (3\ mL)$$

$$M_2 = 3.36 \times 10^{-4}\ M\ \text{Allura Red}$$

Before samples were tested in the cuvette holder, we ran the experiment three times to verify the chemical reaction time. The dye degradation took about a minute to complete. In that time span, the red color faded until it became clear (Figure 4).

FIGURE 4: Dye Degradation of Allura Red

TEST DATA AND RESULTS

For this kinetics experiment, the spectra were saved periodically through a feature in AvaSoft. There was a five-second time delay, and then the spectra were saved every second for 90 seconds. The chemical reaction rate was around 60 seconds, but we added 30 seconds for the readings as a buffer. Light and dark reference of air was taken before any sample was added to the cuvette holder. Once it was ready to test, the Allura Red solution and bleach solution were quickly mixed and shaken in a cuvette and placed in the cuvette holder. The readings began and saved 90 scans. This test was run three times to ensure similar results. The testing was done in transmittance mode (Figures 5, 6, & 7). Originally, we were planning on using absorbance measurements, but the absorbance spectra were noisy and difficult to use. So instead, we used the transmittance data and then converted the data to absorbance later in the experiment for analysis. Transmittance and absorbance are inversely related.

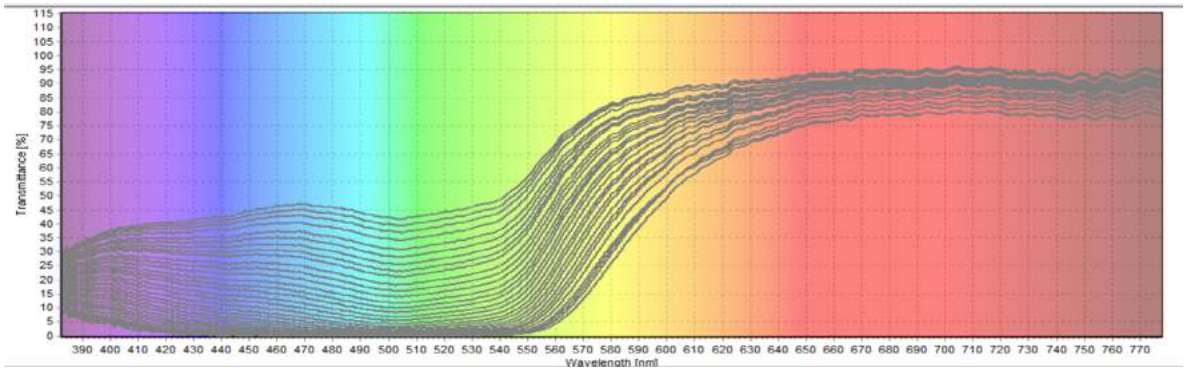

FIGURE 5: First Transmittance Test on Allura Red Degradation

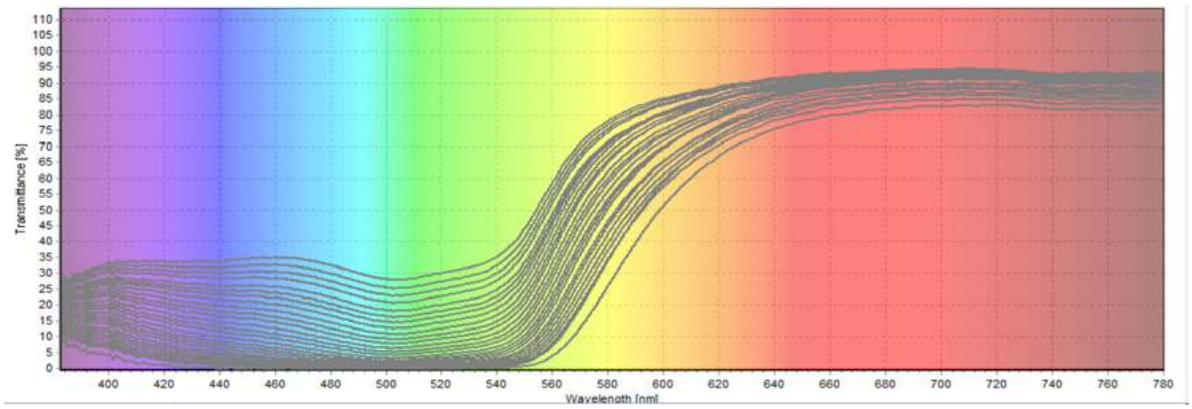

FIGURE 6: Second Transmittance Test on Allura Red Degradation

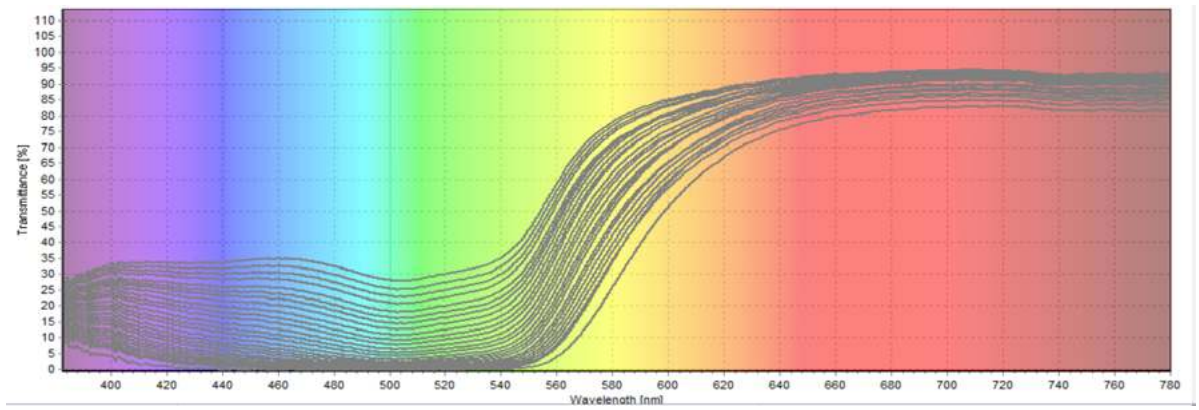

FIGURE 7: Third Transmittance Test on Allura Red Degradation

ANALYSIS

In all three of the transmittance tests, the readings show that the transmission percentage of green coloring steadily increases. As shown in the figures, the green coloring spans from the wavelength of 480-560 nm. The less Allura Red there is, the greater the transmission of green light there will be.

To find the unknown concentrations, we first converted the transmittance measurements into absorbance. The third transmittance test was used for this conversion. The wavelength that we used for these calculations was 520 nm (Figure 8). This wavelength was used because it was midway into the green wavelength range. The transmittance percentage was found by finding the max peak at the given wavelength. Out of the 90 scans, we used the 60 scans of the true start and end time of the chemical reaction for the calculations. The other thirty scans were added to buffer the first couple of scans and then the last round of scans. These extra scans were then excluded from the analysis.

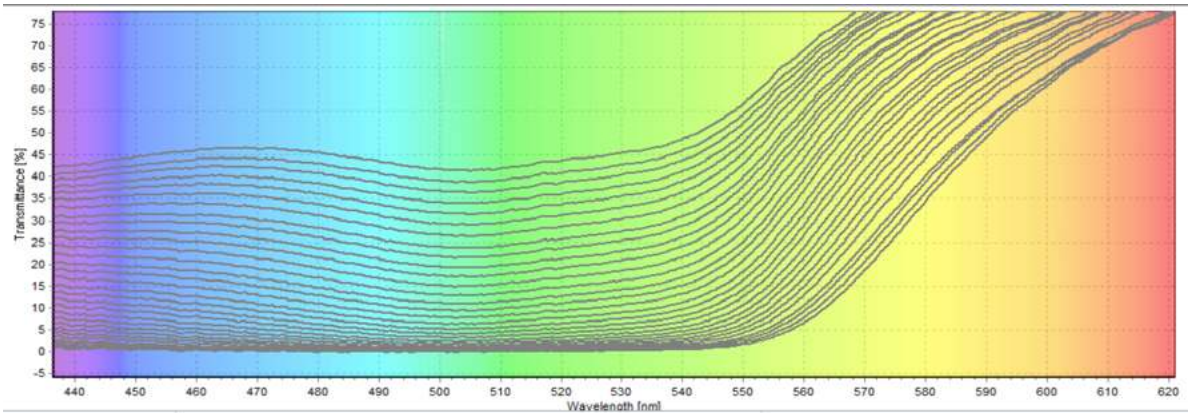

FIGURE 8: 520 nm wavelength window to use in the absorption conversion

Absorbance is calculated as a logarithmic function, and to convert the transmittance percentage we used the formula A = 2 – log10(% T). The 60 transmittance percentages were converted to absorbance and then a t = 0 data point was added. Since it is a logarithmic function, transmission cannot equal zero. So, we set the t = 0 transmittance percentage to 0.001. With these data points, we then plotted kinetic graphs of transmittance and absorption for the 520 green wavelength of the Allura Red dye degradation solution (Figures 9 & 10). Figure 9 shows the steady increase of transmission for green light, and figure 10 shows the rapid decrease of absorption of green light. These verify the logarithmic relationship

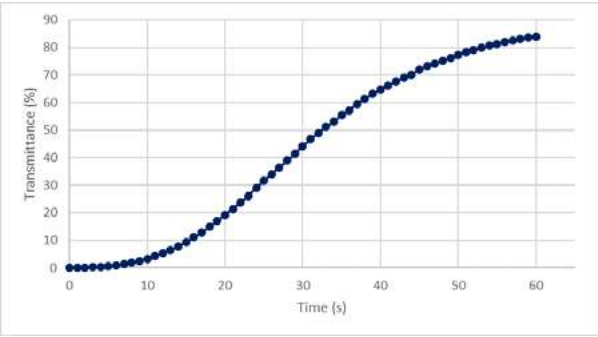

FIGURE 9: Transmittance % vs Time at 520nm green

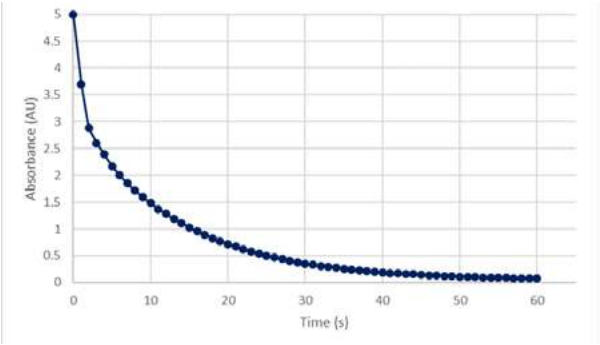

FIGURE 10: Absorbance vs Time at 520nm green

ANALYSIS

After calculating the absorbance, we then used Beer's Law to find the approximate concentrations of Allura Red with respect to time. Beer-Lambert's Law is A = εbC, where ε is molar absorptivity, b is the length of the light path, and C is concentration. The path length (b) was 1 cm since the cuvette we used was 10mmx10mm. To find the molar absorptivity, we used the known 3.36 x 10-4 M Allura Red concentration at t = 0. The absorbance was found by using the transmission percentage of 0.01 in the equation A = 2 – log10(% T). So, the absorbance from that transmission percentage was approximately 5. Solving for molar absorptivity gave us the value of 14893 M-1cm-1. Using that for ε, we then solved for the concentrations (Appendix A). Once the approximate concentrations were calculated, we plotted the data points against time to make another kinetic graph (Figure 11).

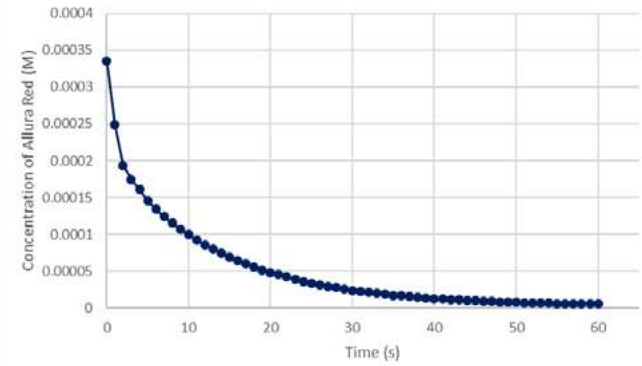

FIGURE 11: Absorbance vs Concentration of Allura Red at 520nm green

The relationship between absorptivity and concentration is linear (Figure 12). The slope of the line is the molar absorptivity (ε), and the y-intercept of the line should be zero. The equation plotted below has a y-intercept of 2 x 10^{-16}, which is essentially zero. However, this linear equation shows that the relationship is not perfect. This is expected, especially since the t = 0 transmission percentage was approximate.

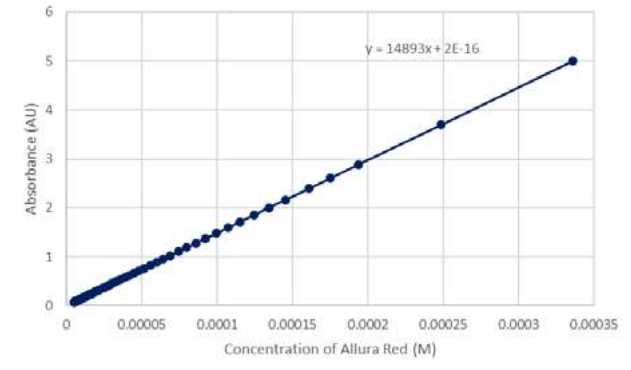

FIGURE 12: Absorbance vs Concentration of Allura Red at 520nm green

CONCLUSION

We could determine approximate Allura Red concentrations during dye degradation using the transmittance spectra from the cuvette holder spectroscopic system. Bleach was used as the oxidizer, with NaClO (sodium hypochlorite) being the active ingredient. The NaClO altered the chemical structure of Allura Red, causing the double bond (-N=N-) to split. This change turned the solution colorless. By saving the spectra periodically through AvaSoft, we were able to run analytical tests at a certain wavelength. We chose the wavelength of 520nm because that was where we could see the transmission of green light. The greater the transmission of green light, the less Allura Red remains in the sample. We then converted the transmittance percentage into absorbance measurements and then solved for the concentration of Allura Red using Beer-Lambert's Law. Using the received data, we were then able to create kinetic models. Absorption, transmittance, and concentration were all respectively measured against time.

Avantes AvaSoft software also offers a chemometric module that can facilitate Beer-Lambert concentration experiments. For more information, contact one of our support engineers. We are happy to help you!

APPENDIX A: CONCENTRATION CALCULATIONS

Scan	Time (s)	Transmittance %	Absorbance	Concentration (M)
	0	0.001	5.00	3.36E-04
9	1	0.020	3.70	2.48E-04
10	2	0.130	2.89	1.94E-04
11	3	0.250	2.60	1.75E-04
12	4	0.400	2.40	1.61E-04
13	5	0.680	2.17	1.46E-04
14	6	1.00	2.00	1.34E-04
15	7	1.41	1.85	1.24E-04
16	8	1.92	1.72	1.15E-04
17	9	2.54	1.60	1.07E-04
18	10	3.28	1.48	9.97E-05
19	11	4.25	1.37	9.21E-05
20	12	5.27	1.28	8.58E-05
21	13	6.47	1.19	7.98E-05
22	14	7.75	1.11	7.46E-05
23	15	9.40	1.03	6.90E-05
24	16	11.1	0.954	6.41E-05
25	17	12.8	0.893	6.00E-05
26	18	14.8	0.829	5.57E-05
27	19	17.0	0.770	5.17E-05
28	20	19.3	0.716	4.80E-05
29	21	21.3	0.671	4.51E-05
30	22	23.8	0.624	4.19E-05
31	23	25.9	0.586	3.94E-05
32	24	29.0	0.538	3.61E-05
33	25	31.6	0.501	3.36E-05
34	26	34.1	0.468	3.14E-05
35	27	36.5	0.438	2.94E-05
36	28	39.0	0.409	2.75E-05
37	29	41.4	0.383	2.57E-05
38	30	44.1	0.356	2.39E-05
39	31	46.7	0.330	2.22E-05
40	32	49.0	0.310	2.08E-05
41	33	51.2	0.291	1.95E-05
42	34	53.1	0.275	1.85E-05
43	35	55.4	0.256	1.72E-05
44	36	57.2	0.243	1.63E-05
45	37	59.6	0.225	1.51E-05
46	38	61.4	0.212	1.42E-05
47	39	63.1	0.200	1.34E-05
48	40	64.7	0.189	1.27E-05
49	41	66.2	0.179	1.20E-05
50	42	67.6	0.170	1.14E-05
51	43	69.0	0.161	1.08E-05
52	44	70.1	0.154	1.04E-05
53	45	71.9	0.143	9.62E-06
54	46	73.1	0.136	9.13E-06
55	47	74.2	0.130	8.71E-06
56	48	75.1	0.125	8.36E-06
57	49	76.0	0.119	7.99E-06
58	50	77.3	0.112	7.52E-06
59	51	78.4	0.106	7.10E-06
60	52	79.1	0.102	6.82E-06
61	53	79.8	0.098	6.59E-06
62	54	80.7	0.0933	6.27E-06
63	55	81.2	0.0902	6.06E-06
64	56	81.9	0.0868	5.83E-06
65	57	82.6	0.0831	5.58E-06
66	58	83.1	0.0804	5.40E-06
67	59	83.5	0.0782	5.25E-06
68	60	83.9	0.0761	5.11E-06

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