# Molecular Diffusion Coefficients: Experimental Determination and Demonstration

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Diffusion is a phenomenon critical to all bimolecular chemical processes and places a formal upper limit on the rates of chemical and biochemical reactions. Yet there are no simple methods for measuring diffusion coefficients in solution or for *demonstrating* to the student the dramatic dependence on molar mass. While great precision is possible with the Gouy interference method (*1–3*), conductometric determinations (*4*), or by self-diffusion of isotopic solutions (*5*), these procedures require equipment that is not readily available. Further, the student is unable to observe the process of diffusion visually using these procedures. A simpler free-boundary method has been presented by Linder (*6*) and Clifford (*7*); however, constructing the diffusion cell requires considerable effort. The most straightforward method in terms of experimental design and data analysis is described by Irina (*8*). However, Irina's method is limited to crystalline compounds.

We required a method for measuring diffusion in the spatial definition of chemical reactions occurring in agar (*9*). In that regard, we have developed a method related to that of Irina; however, the reflection boundary is provided by the aqueous agar gel. Diffusion coefficients are determined with a standard UV/vis spectrophotometer requiring no specialized attachments. The results of this method compare favorably with data obtained in both agar and water by other methods, but, maybe most importantly, this approach provides a rapid and straightforward classroom or laboratory demonstration of the temperature and molecular size dependence of diffusion. In this paper we have applied the method to compounds ranging in size from oxygen to small proteins.

## Laboratory Preparations

### *Spectrometer*

The most intense point of the spectrophotometer light beam was found by constructing a light filter that could be placed in front of the cell compartment. A 1-mm slit was cut into an opaque plastic ruler, and the ruler was trimmed at one end until the slit was aligned with the spectrophotometer beam as it passed through the cell (Fig. 1). While in the spectrophotometer, the side of the UV cell was marked at the top edge of the cell compartment wall. Four graduations were penciled 3, 6, and 9 mm below this edge line (Fig. 2). Finally, the ruler mask was held along the frosted side of the UV cell, and a line was drawn 2 mm above the slit to mark the diffusion reflection boundary (rb, Fig. 2).

### *UV Cell*

A 1% solution of triterated agar (see below) was pipetted into two UV cells to the level of the reflection boundary mark on the cell. The agar was allowed to solidify (~10 min), and 500 $\mu$L of the test solution was added to the sample cell, and the same volume of distilled water was added to the reference. The agar gels approximate a planar reflection boundary perpendicular to the walls of the cuvette, and distortions due to meniscus formation appear insignificant in the 1-mm light beam used. During all experiments, the temperature of the UV compartment was maintained using a Haake D1 115-V circulating constant temperature bath. The experiments are run with initial concentrations, $c_0$, at the diffusion front that allow accurate detection of the absorbance to less than 5% of that value. The diffusants, $[Co(H_2O)_6]^{2+}$ at $10^{-4}$ M, the quinones (2,5-DHBQ and 2,6-DMBQ) at $10^{-2}$ M, myoglobin at $10^{-2}$ M, and tryptophan at $10^{-6}$ M were detected at $\lambda_{max}$ 510, 480, 420, 407, and 284 nm, respectively. The solidified agar is easily removed from the cuvette with hot water.

### *Triterated Agar*

Difco-Bacto agar (100 g, Sigma Chemical Co.) was added to distilled water (400 mL), and the resultant slurry was stirred and allowed to settle. The excess water was decanted, and the slurry was filtered under vacuum through a Buchner funnel. The process was repeated once again with water, once with 95% ethanol, and the resulting solid was either air dried in the funnel, dried in vacuo in a desiccator, or spread as a thin layer in a crystallizing dish and allowed to dry at room temperature overnight. The 1% agar solutions are made either by autoclaving the mixture for 15 min or by heating the mixture in a boiling water bath until homogeneous. The resulting solution is then pipetted warm into the UV cells to the reflection boundary mark. This procedure removed much of UV absorbing material in the agar and made the normally translucent 1% agar gels transparent.

### *$O_2$ Diffusion*

A $10^{-4}$ M solution of methylene blue in freshly melted agar was reduced by titration with $10^{-2}$ M sodium hydrosulfite. This clear, colorless agar solution was pipetted into the test tubes (or cuvettes), and the diffusion of atmospheric oxygen was monitored by measuring absorbance at 665 nm to determine the concentration of methylene blue in the oxidized state.

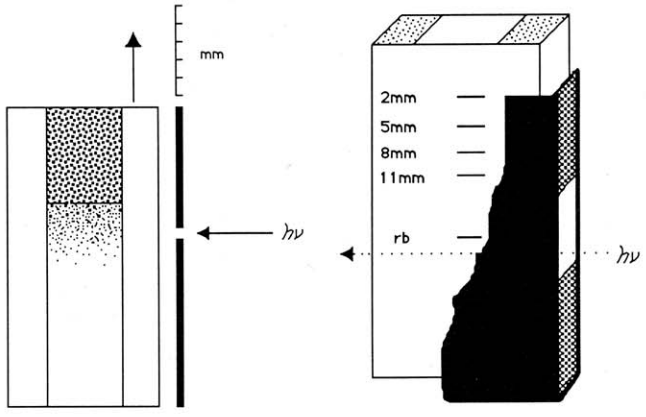


Figure 1. (left) The UV cell in the spectrophotometer with the 1-mm slit in place to determine the position of the light beam as it enters the cell.

Figure 2. (right) The reflection boundary is marked on the frosted side of the UV cell 2 mm above the point of entry of the spectrophotometer beam. A line is also drawn on the cell along the top of the spectrophotometer cell compartment wall (labeled 2 mm). When this line is aligned with the top of the compartment wall, the absorbance is measured 2 mm below the reflection boundary. Four additional lines were marked 3, 6, and 9 mm below the 2-mm line. When these lines are aligned with the cell compartment wall the absorbance is measured 5, 8, and 11 mm below the reflection boundary.

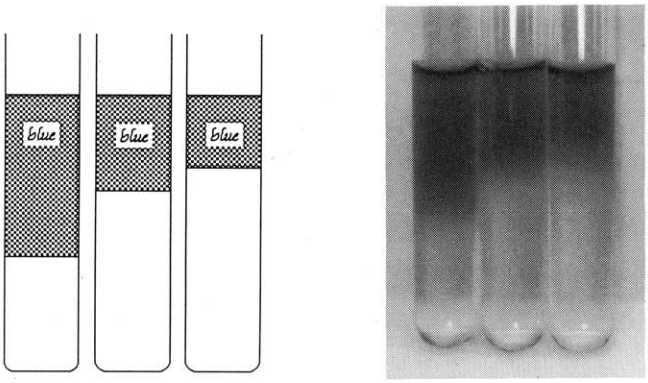


Figure 3. Oxygen diffusion into a solution of reduced methylene blue ($7 \times 10^{-5}$ M) in 1% agar (10 mL) at 27 °C (left), 23 °C (center), and 0 °C (right). The experiment was photographed after 6 h.

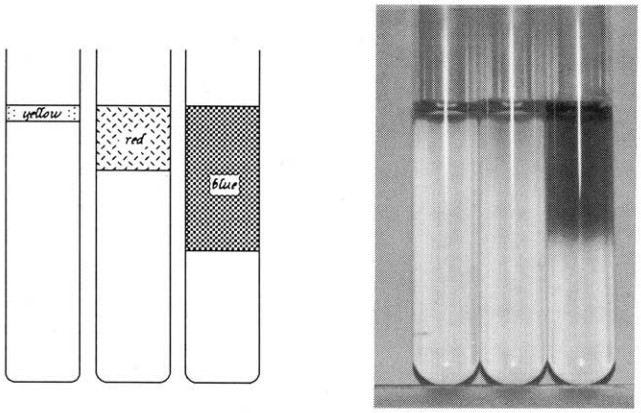


Figure 4. Diffusion demonstration run in test tubes (16 × 125 mm) containing 1% agar (10 mL, the agar is somewhat friable and must be handled carefully). After 6 h at room temp (25 °C), the diffusant solutions were removed for clarity and the tubes photographed. The test tubes contained myoglobin (left, $10^{-7}$ M), 2,5-dihydroxy-*p*-benzoquinone (center, $10^{-6}$ M), and oxygen (reduced methylene blue, right, $7 \times 10^{-5}$ M) as diffusant. Concentrations were chosen to give solutions of about the same color intensity to allow easy comparison between the diffusants.

## Results and Discussion

### *The Demonstration*

Simple demonstrations of molecular diffusion have been hampered not only by the equipment required but also by the time frame needed for suitable observation of the phenomenon. The diffusion of molecules in the gaseous state is typically detectable within a period of hours (*10*) and therefore suitable for such demonstrations. In this experiment, reduced methylene blue imbedded in an aqueous agar matrix provided a detection mechanism for the demonstration of the diffusion of $O_2$. For example, Figure 3 shows the $O_2$ diffusion into a set of three 16- × 125-mm test tubes, each held at a different temperature. As the atmospheric $O_2$ diffused into the agar, the reduced methylene blue was reoxidized to the easily detected blue form. Figure 3 is the result of a 6-h exposure; however, the difference is obvious even after a 3-h lab period.

The utility of the agar reflection boundary method is further demonstrated in Figure 4, where $O_2$ diffusion is compared with that of myoglobin (yellow) and 2,5-dihydroxybenzoquinone (red). The dependence of the diffusion coefficient on molecular size is clearly seen. These same experiments were done in a 10-mL graduated cylinder to provide a simple measure of the diffusion distance and the tubes could be handled with little fear of experimental distortions.

### *Quantification*

Slight modifications of the above demonstration were also used to measure the diffusion coefficients. The agar surface of a filled cuvette provided the reflection boundary. Solutions of the diffusate at $c_0$ were added at $t = 0$ to the surface of the agar, $x = 0$. It was possible to take four absorbance readings at defined distances from the reflection boundary before distortions were observed from the bottom of the cell. A concentration gradient had to be established across all four points before accurate diffusion measurements were made. The time frame for the establishment of this gradient varied with the diffusing species, i.e., $[Co(H_2O)_6]^{2+}$ required 16 h and myoglobin 20 h, but once the gradient was established, measurements were made repeatedly for 15 to 24 h. Over this time period, there was insignificant depletion of the solution on top of the agar, and $c$ still approached zero toward the lower part of the cuvette. Therefore Fick's second law could be rearranged (*11*) to eq 1 where a plot of ln $c$ versus $x^2$, or more conveniently ln $A$ versus $x^2$, gave a straight line with slope $(-4Dt)^{-1}$.

$$\frac{c}{c_0} = (Dt)^{1/2} \times e^{-x^2/4Dt} \qquad (1)$$

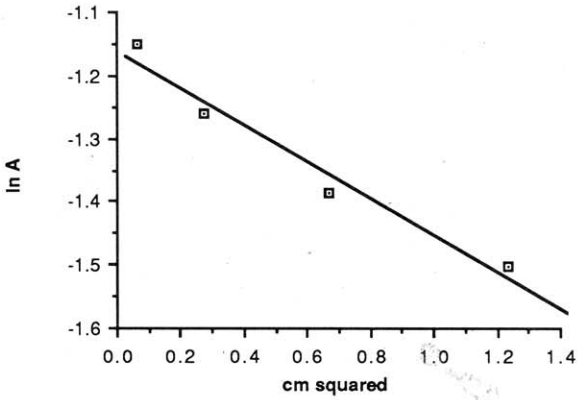


Figure 5. Graph of diffusion measurements for $[Co(H_2O)_6]^{2+}$ in agar at 31 °C. This measurement was made 18.3 h after the start of the experiment, slope = −0.29 cm², $R = 0.98$. For this diffusion rate, there is about an 18-h time period where the measurements can be made repeatedly and averaged.

Figure 5 shows a plot of one measurement for the diffusion of $[Co(H_2O)_6]^{2+}$ at 31 °C in agar. The linearity of this plot over the four distance points was the best indicator of the establishment of the concentration gradient. In this case, the measurements were made repeatedly over an 18-h period. Eight separate measurements were averaged to give a value of $13(\pm 1) \times 10^{-8}$ cm² s$^{-1}$, a value identical to that obtained previously in distilled water (*8*). The error arises primarily from the resolution of the spectrometer beam and the limited distance between the incident light and the bottom of the cuvette. These limitations could certainly be corrected with more elaborate equipment, but even with the simple design using the readily available equipment, the data proved sufficiently reliable for our uses. The table contains the measured diffusion coefficients for a wide range of molecular species. These values are consistent with expectations based on molecular size and are consistent with the values previously reported. The diffusion of $O_2$ is the least accurate (*12*) because of errors resulting from a low background level of oxidation, competing diffusion, and reaction of the indicator. Nevertheless, careful titration of the methylene blue with $Na_2S_2O_4$ gives a reproducible value of the diffusion constant of oxygen. The measured values for aqueous Co(II) ions in aqueous $CoCl_2$ and tryptophan are virtually identical to those previously reported (*12*), and the quinone measure-

**Observed Diffusion Coefficients at 20 °C**

| | $M^a$ g mol$^{-1}$ | $D_{obs}{}^b$ × $10^8$ cm$^2$/s, 1% agar |
|---|---|---|
| $O_2$ | 32 | 13 (±3) |
| 2,5-dihydroxy-*p*-benzoquinone | 140 | 6.4 (±.6) |
| 2,6-dimethoxy-*p*-benzoquinone | 168 | 7.4 (±1.3) |
| Tryptophan | 204 | 7.1 (±0.7) |
| $[Co(H_2O)_6]^{2+}$ | 168 | 4.8 (±0.5) |
| @ 31 °C | | 13 (±0.5) |
| methylene blue | 374 | 3.1 (±1) |
| Myoglobin (equine) | 18,800 | 0.60 (±0.02) |

[a] Molecular weights are included for reference; however, the size of the diffusing species determines the diffusion coefficient.

[b] Expressed as the experimental value ± the standard deviation of replicate determinations.

ments are consistent across a wide range of related structures (two are shown here).

The previously reported diffusion coefficients in agar are very similar to those found in distilled water. For example, the value for CsCl(aq) at 25 °C in water (*13*) is 19.5 × $10^{-8}$, while in agar (*14*) it is 19.2 × $10^{-8}$ cm$^2$/s. For molecules as large as hemoglobin, the coefficients in water (20 °C (*15*), $D$ = 0.69 × $10^{-8}$ cm$^2$/s) are generally larger than in agar (20 °C (*16*), $D$ = 0.58 × $10^{-8}$ cm$^2$/s) and this difference becomes more pronounced as the agar concentrations are increased (*16*). The values for myoglobin (18,800 daltons) are somewhat lower than expected. In general however, the value of this method is that it provides a good measure of the diffusion coefficients that fall within 10% of the measurements made in water.

## Conclusions

This demonstration has proven useful in both laboratory and classroom settings and highlights the dependence of molecular transport on molar mass and temperature. The real utility of the method lies in the ease of the setup, the availability of the instrumentation, and the dramatic demonstration of diffusion. While it should be possible to use the agar reflection boundary method to provide more accurate diffusion measurements, this method provides diffusion coefficients accurate enough to put spatial limits on reactions occurring over a three-dimensional agar matrix (*9*) and demonstrates the principles of diffusion to the student.

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