Determination of the Magnesium/8-Hydroxyquinoline-5-sulfonic acid equilibrium constant using UV Spectroscopy

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ABSTRACT

Research was performed to derive the HQS-Mg equilibrium constant K_{eq} at a pH of 8, using spectroscopic methods. Scans of HQS and HQS-Mg were performed 450 to 275 to obtain extinction coefficients for the HQS (ε_{HQS}), and for the HQS-Mg ($\varepsilon_{HQS\text{-}Mg}$) isosbestic point. The HQS peak was at 313nm (3.96 eV or 31,949 cm^{-1}) with a ε_{HQS} value of 3162.5. The HQS-Mg isosbestic point was at 331nm with the scans yielding a $\varepsilon_{HQS\text{-}Mg}$ value of 2436.3. A value of 8000 for the K_{eq} fits the data the best. The shape of the X vs Absorbance plot showed it to be abnormal, especially in the low-end range of HQS_{init}/Mg_{init} (X) values, where the Mg levels were much higher than HQS. Bound fraction values of 97.4% at an X of 0.05, 93.4 at an X of 0.125, 86.3 at an X of 0.25, 71.9% at an X of 0.5, 50.0% at an X of 1, and 29.3% at an X of 2.0 were established. Given a higher number of trials and more careful laboratory technique, the predicted vs experimental differences should minimize, yielding a higher confidence in the K_{eq} value identified.

1.0 INTRODUCTION

In the 18th century, Pierre Bouguer and Heinrick Lambert published a paper showing that loss of light intensity was directly proportional to the length of the path through a medium.[1] In the 19th century, this relationship between light transmittance and the medium was furthered by August Beers. This formed the basis for what is currently referred to as the Beers-Lambert Law, or Beers Law, for short. It states the absorbance (the negative $logarithm_{10}$ of [transmitted light intensity divided by the incident light intensity]) is equal to the path length times the molar concentration of an absorbing material (solute) dissolved in the transmitting medium (solvent) times a coefficient. The coefficient is called the extinction coefficient (ε). It is specific to an absorbing material and the wavelength of the incident light.[2] This helped form the basis of spectroscopic analysis.

Spectroscopy has been used for the quantitative determination of solute concentrations by chemists since. The applications of this have expanded into areas not initially envisioned. In this paper spectroscopy was used to determine the equilibrium constant (K_{eq}) for the reaction between magnesium ions with the acid 8-Hydroxyquinoline-5-sulfonic acid (HQS), at a pH of 8. The acid has a different UV absorption spectrum than that of the magnesium-HQS salt. This research took advantage of the isosbestic point of the two absorption scans. An isosbestic point is the wavelength where the extinction coefficient remains constant for a given reaction, though the reactants and product(s) may have varied.[3] In different words, it's the wavelength where all absorptive entities have the same extinction coefficient.[4] This isosbestic point, along with the extinction coefficient of an HQS solution was used in the determination of the K_{eq} of the reaction (Mg + HQS <--> MgHQS).

2.0 EXPERIMENTAL

2.1 Chemicals

The chemicals used include 8-Hydroxyquinoline-5-sulfonic acid monohydrate: no purity listed on bottle, Sigma-Aldrich, Riedstrasse 2, Steinheim, Germany, D-89555, CAS# 84-88-8; EPPS: 99%, Janssen-Pharmaceuticalaan 3A, 2440 Geel, Belgium, CAS# 16052-06-5; Magnesium sulfate heptahydrate: Certified ACS grade, Fisher Chemical, 300 Industry Drive, Pittsburgh, PA, United States, 15275, CAS# 10034-99-8; and Deionized water: >99.9% pure, Georgia State University, Science Annex, Atlanta, GA, CAS# 7732-18-5.

2.2 Equipment

The equipment used included PerkinElmer Lamda35 spectrometer, PerkinElmer, 710 Bridgeport Avenue Shelton, CT 06484-4794; (SW) PerkinElmer UV Winlab 6.3.1.0748/Labmda35 1.27, 710 Bridgeport Avenue Shelton, CT 06484-4794.

2.3 Experimental Conditions

All spectroscopic scans were run with a slit width of 1.0 nm, a scan rate of 120 nm/min, at 20° C, with a scan range from wavelengths of 450 nm to 275 nm, using a Deuterium lamp as the light source, at a pH of 8.

2.4 Procedures

For measurements of 8-Hydroxyquinoline-5-sulfonic acid monohydrate (HQS) concentration absorbance, the UV scan parameters were set with a slit width of 1.0 nm, a scan rate at 120 nm/minute, and a scan range from 450 nm to 275 nm. The light source was set to UV.

The dilutions started with a stock solution of 2.5 mM solution of HQS and an HEPPS (EPPS) buffer. The dilutions are shown in table 1. The first dilution was 1:5, by putting 2 mL in a 10 mL volumetric then diluted to the mark with EPPS buffer. The EPPS buffer is to maintain a pH of 8.0. Afterward there was a serial, 1:1 dilution, made by placing 5 mL of each previous dilution in a 10 mL volumetric flask and diluting it with EPPS buffer to the 10 mL mark. This yielded HQS concentrations from 0.5000 mM to 0.01563 mM. Six samples were prepared giving the following concentrations: 0.050, 0.125, 0.250, 0.500, 1.00, and 2.00 mM. Absorbances of these concentrations were obtained from the spectrometer, using an EPPS buffer baseline. The peaks were plotted and used to derive the extinction coefficient for HQS via the LSQ method in Excel.

Solutions of HQS and $MgSO_4$, from stock concentrations of 2.5 mM HQS and 10.0 and 2.5 mM $MgSO_4$ were used to prepare six samples with a consistent 0.25 mM HQS concentration and $MgSO_4$ concentrations of 5.00, 2.00, 1.00, 0.50, 0.250, and 0.125 mM. These correspond to the X values ($[HQS]_0/[Mg]_0$) of 0.05, 0.125, 0.25, 0.50, 1.00, and 2.00, as they are referred to later in this paper. Absorbance scans of these were taken. Overlaying the plots of these scans gave the general range in which the isosbestic point of each pair of plots. Examination of the raw data

produced the isosbestic point for each pair of plots. The most common isosbestic point was used to derive the [Mg-HQS] extinction coefficient. Once the [Mg-HQS] K_{eq} was obtained, putting it into an Excel table allowed for trying equilibrium constants (Eq 1, below) to determine where the X vs absorbance$_{predicted}$ intersect the X vs absorbance$_{experimental}$ when plotted – which helped assess the K_{eq} with the best fit.

Equation 1 $$K_{eq} = \frac{[HQS-Mg]}{[HQS][Mg]}$$

3.0 RESULTS AND DISCUSSION

3.1 HQS Scans

Scans of the HQS dilutions were from wavelengths of 275 to 450 nm. This misses the most pronounced UV peak of HQS, at 243 nm (3.95 eV, 31,847 cm^{-1}), but does include a good peak at about 314 nm (5.10 eV, 41,152 cm^{-1}).[5] The initial scans of HQS dilutions produced absorbance peaks at 314. These were processed via a least square regression (LSQ) producing an extinction coefficient (ε) for HQS, in the form of the slope of the line. Table 1, in section 6.4, shows the dilutions used and the amounts used to make them. Figure 1 shows an overlay of the scans of the different concentration samples. The peak absorbances of these scans were plotted in Figure 2, adjusting the concentration to molar from millimolar. Excel did the LSQ regression, producing the line and equation so the slope was in the proper units. The R^2 value showed a good fit for the line, but with only 6 samples, the R^2 result was of limited value.

The equation of the regression line is shown as Equation 2, below

Equation 2 $$y = 3162.5x - 0.0161$$

Given the x values were in absorbance units and the y are HQS concentration values in Molar units, with the scan run on a cuvette of 1.0 cm, the slope of the regression is the extinction coefficient, with units of M^{-1} cm^{-1}. The absorbance values plotted are found in Table 2, with the

plot found in Figure 2 of the Conclusions section. The LQS regression computed a value of 3162.5 as the slope of the line. This was the extinction coefficient value for HQS at pH 8.

3.2 Mg/HQS Scans

Once the HQS extinction coefficient was determined, a series of seven dilutions of $MgSO_4$/HQS solutions were prepared and buffered to a pH of 8. The dilution information and concentrations of $MgSO_4$ and HQS in each solution can be found in table 3 after the Conclusions section. They were prepared in solutions with a fixed HQS molarity and a varying $MgSO_4$, referred to by an 'X' value, such that $X = (H_0/M_0)$, where H_0 is the initial HQS molarity, and M_0 is the initial $MgSO_4$ concentration.

Scans were run on the prepared solutions. An overlay of the scans was created and can be found as Figure 3 after the Conclusion section. The most common isosbestic point was determined to occur at 331 nm (3.75 eV, 430,211 cm^{-1}). In Figure 3, it will be the point where the most scan crossings occur.

Once the absorbances at the isosbestic point have been determined, the extinction coefficient for (Mg-HQS) was computed from the absorbances, referred to as $\varepsilon_{HQS\text{-}Mg}$ was 2436.3.

and along with the initial concentrations of magnesium ions and HQS (H_0 and M_0), and the HQS extinction coefficient, the following equations will allow estimation of the equilibrium constant (K_{eq}).

Equation 1 $$K_{eq} = \frac{[HQS-Mg]}{[HQS][Mg]}$$

Equation 3 $$K_{eq} = \frac{\alpha}{[H_0-\alpha][M_0-\alpha]}$$

Equation 4 $$(H_0 - \alpha)(M_0 - \alpha)\, K_{eq} = \alpha$$

Equation 5 $$H_0M_0 - \alpha\left(M_0 + H_0 - \frac{1}{K_{eq}}\right) + \alpha^2 = 0$$

Equation 6 $$X = \frac{H_0}{M_0}$$

Equation 7 $$H_0\frac{[H_0]}{X} - \alpha\left(\frac{[H_0]}{X} + H_0 + \frac{1}{K_{eq}}\right) + \alpha^2 = 0$$

Equation 8 $$\alpha^2 + \alpha\{-H_0\left(\frac{X+1}{X}\right) + H_0 + \frac{1}{K_{eq}}\} = 0$$

Equation 9 $$\alpha = \frac{\left\{H_0\left(\frac{X+1}{X}\right)+\frac{1}{K_{eq}}\right\} - \sqrt{\{H_0+\frac{1}{K_{eq}}\}^2 - 4\frac{[H_0]^2}{X}}}{2}$$

Equation 10 Absorbance = $\{\varepsilon_{HQS}[H_0\text{-}\alpha] + \varepsilon_{HQS\text{-}Mg}[\alpha]\}(1\text{cm})$

The assumption that the fraction of Mg-HQS complex would be at 100%, with zero free HQS, was made to simplify the calculations. It was at an X = 0.05. The large Mg to HQS ratio supported this assumption due equilibrium reducing free HCS to a very low level. It should have introduced only a small error.

Using the isosbestic point, an extinction coefficient for [HQS-MG], or $\varepsilon_{HQS\text{-}Mg}$, was calculated. In conjunction with the above equations, it was loaded into an Excel spreadsheet along with experimental data and the ε_{HQS} obtained from the HQS-only scans. The produced table allowed experimenting with different values for K_{eq} to produce computed absorbances. Plotting the X vs computed and the X vs experimental absorbances, the closer the two plots were, the more accurate the K_{eq} value. The experimental data obtained in this research was not of high quality, most of the plots being far apart. The lowest K_{eq} value resulting in a small portion of the two plots intersecting was considered the optimal value. Values from 500 to well past 10,000 for the K_{eq} were tried. The lowest K_{eq} tried that appeared most reasonable was 8000. It produced fraction values from 97.44% at an X of 0.05 to 29.29% at an X of 2.0. The list of bound fraction values tried can be found in

table 4, after the Conclusion section. The plots of the concentration ratios (X) vs absorbance are shown in Figure 4. The distorted plot is evidence of both a low numbers of sample trials and possibly poor technique, as mentioned above.

4.0 CONCLUSIONS

Scans of HQS and HQS-Mg were performed in the UV spectrum from 450 to 275 to obtain extinction coefficients for the HQS (ε_{HQS}), and for the HQS-Mg ($\varepsilon_{HQS\text{-}Mg}$) isosbestic point. These were run buffered to a pH of 8. The HQS peak was at 313nm with a ε_{HQS} 3162.5. The HQS-Mg isosbestic point was at 331nm with a $\varepsilon_{HQS\text{-}Mg}$ value of 2436.3. A series of trial K_{eq} values were tried from 500 up through 10,000,000, and while the larger values did minimize the sum of difference and sum of squared differences between experimental and computed absorbances, the computed bound fractions (HQS-Mg) became absurdly high, and that method was dismissed. The shape of the X vs Absorbance plot showed it to be abnormal, especially in the low-end range of X values, where the Mg levels were much higher than HQS. A value of 8000 for the K_{eq}. appeared to be the best value for the data. Bound fraction values of 97.4% at an X of 0.05, 93.4 at an X of 0.125, 86.3 at an X of 0.25, 71.9% at an X of 0.5, 50.0% at an X of 1, and 29.3% at an X of 2.0. With a higher number of trials and more careful laboratory technique, the predicted vs experimental values should reduce, yielding a higher confidence in the K_{eq} value determined.

Figures

Figure 1 Shows absorbances of HQS vs concentrations, overlaid for all six samples scanned.

Figure 2 Shows a plot of the peaks of all six HQS absorbances, against the concentration values and producing an LQS regression with the equation of the line.

Figure 3 Shows an overlay of the absorbance scans of the Mg/HQS solutions, to be used to determine the equilibrium constant of Magnesium ions with EQS at a pH of 8. Aside from illuminating the peaks of each, it shows the isosbestic point between each.

Figure 4 Shows the experimental absorbance curve against concentration ratio (X) and the predicted absorbance plot against concentration ratio (X).

Figure 1

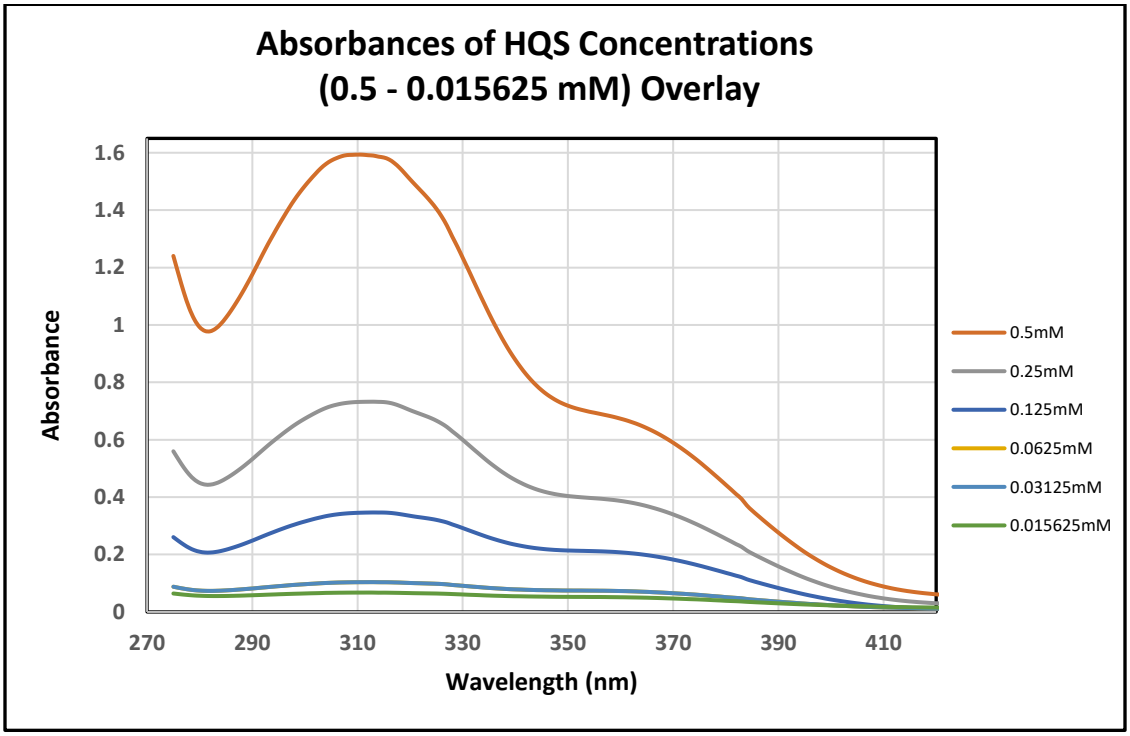

Figure 2

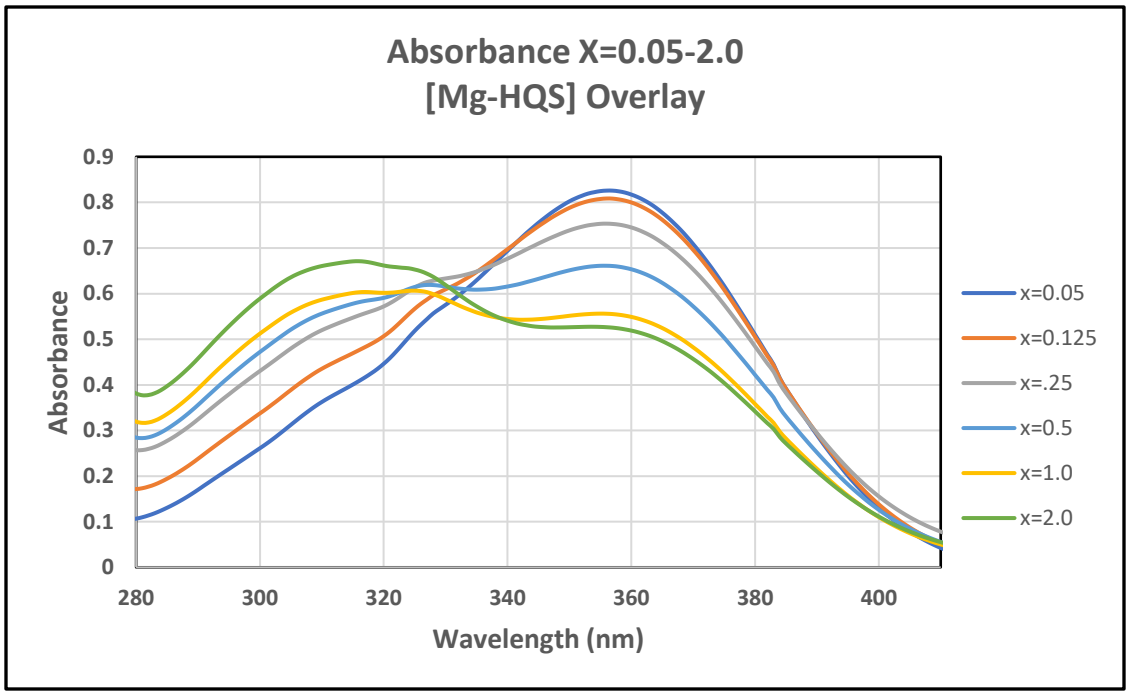

Figure 3

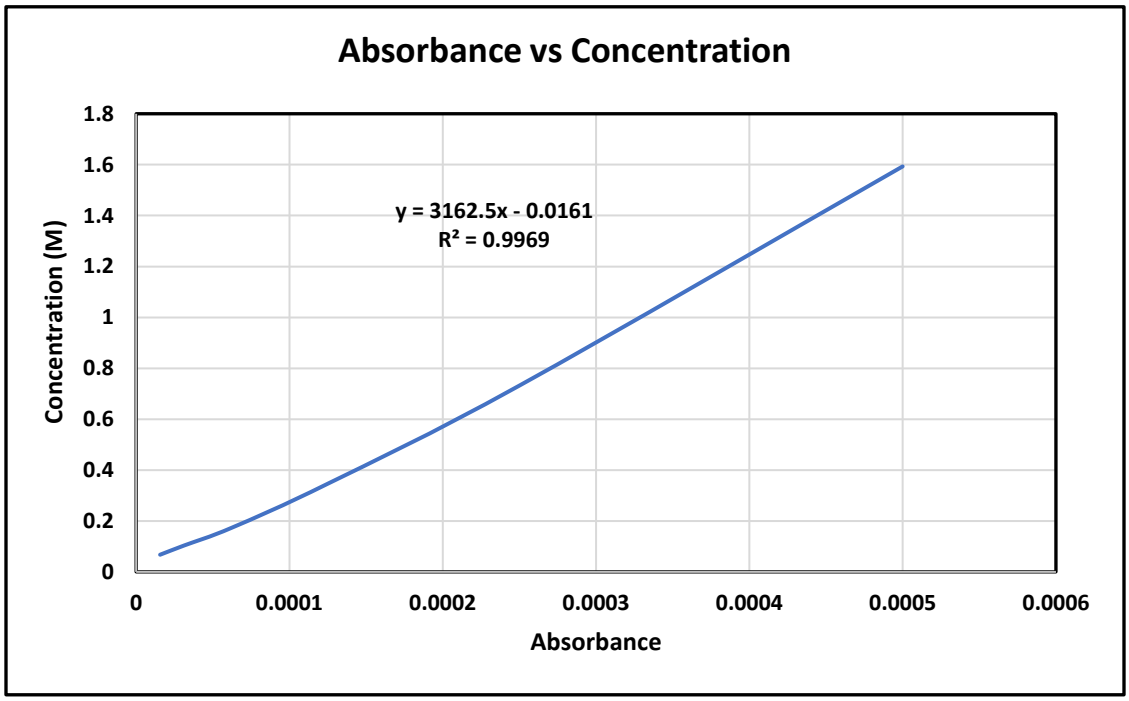

Figure 4

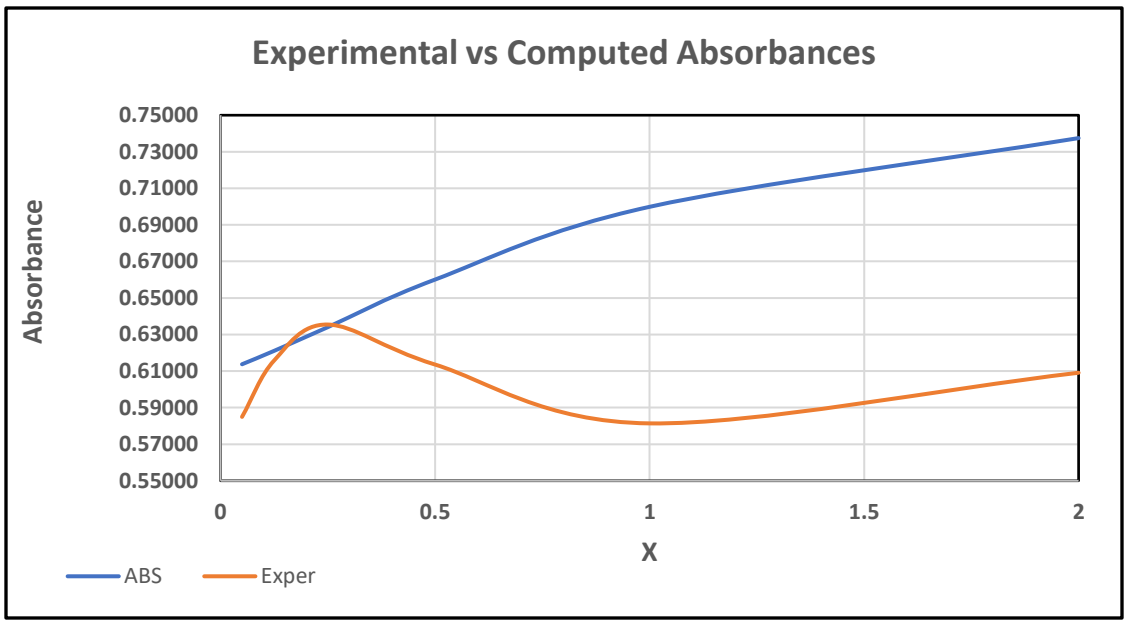

Table 1. Solution preparation of HQS dilutions

A breakdown of the preparation of the dilutions of HQS/EPPS from HQS and EPPS stock solutions.

Sample	Prev dilution vol(mL)	stock EPPS(mL)	HQS Conc (mM)
HQS stock		0	2.5
1	2	8	0.5
2	5	5	0.25
3	5	5	0.125
4	5	5	0.0625
5	5	5	0.03125
6	5	5	0.015625

Table 2. HQS Absorbances vs Concentrations

This table presents the absorbances of each dilution and it's corresponding concentration. See Table 1 for preparation details.

Scan	Absorbance	Conc (mM)	Conc(M)
6	0.067426	0.015625	0.000015625
5	0.103579	0.03125	0.00003125
4	0.173765	0.0625	0.0000625
3	0.346403	0.125	0.000125
2	0.731983	0.25	0.00025
1	1.59317	0.5	0.0005

Table 3. Solution preparation of $MgSO_4$/HQS dilutions

This table shows the preparation breakdown of $MgSO_4$/HQS solutions. X represents the ratio of HQS (H_0) to $MgSO_4$ (M_0).

Sample ndx	$MgSO_4$ Stock 10 mM(mL)	2.5 mM(mL)	Target Mg Conc mM(mL)	EPPS Stock (mL)	HQS Stock (mL)	Comp Mg Conc (mM)	H_0 mmol	M_0 mmol	X (H_0/M_0)
1	5.00		5.00	4.00	1.00	5.000	0.250	0.005000	0.050
2	2.00		2.00	7.00	1.00	2.000	0.250	0.002000	0.125
3	1.00		1.00	8.00	1.00	1.000	0.250	0.001000	0.250
4		2.00	0.50	7.00	1.00	0.500	0.250	0.000500	0.500
5		1.00	0.25	8.00	1.00	0.250	0.250	0.000250	1.000
6		0.50	0.13	8.50	1.00	0.125	0.250	0.000125	2.000

Table 4. Fraction HQS Complexed computed from a K_{eq} of 8000

This Table compares the X ratio with the fraction of HQS complexed % when computed with a K_{eq} of 8000.

X	Fract HQS Complexed (%)
0.05	97.44
0.125	93.39
0.25	86.25
0.5	71.92
1	50.00
2	29.29

5.0 REFERENCES

1 Lambert, J.H. Photometria sive de mensura et gradibus luminis, colorum et umbrae [Photometry, or, On the measure and gradations of light intensity, colors, and shade] **1760**, Augsburg, Germany

2 Ingle, J. D.; Crouch, S. R., *Spectrochemical Analysis*; New Jersey Prentice Hall, **1988**

3 *IUPAC Compendium of Chemical Terminology*, 3rd ed. International Union of Pure and Applied Chemistry; 2006. Online version 3.0.1, **2019**. https://doi.org/10.1351/goldbook.I03310

4 Moore, J. W.; Pearson, R. G.; Frost, A. A. *Kinetics and Mechanism*, 3rd ed.; John Wiley and Sons, **1981**, pp 49.

5 "Quest Graph™ Absorption [8-hydroxyquinoline-5-sulfonic Acid]." AAT Bioquest, Inc., 3 Oct. 2024, https://www.aatbio.com/absorbance-uv-visible-spectrum-graph-viewer/8_hydroxyquinoline_5_sulfonic_acid.

6.0 SUPPLEMENTAL INFORMATION

6.1 Formulas

Equation 1 $K_{eq} = \frac{[HQS-Mg]}{[HQS][Mg]}$

Equation 2 y = 3162.5x - 0.0161

Equation 3 $K_{eq} = \frac{\alpha}{[H_0-\alpha][M_0-\alpha]}$

Equation 4 $(H_0 - \alpha)(M_0 - \alpha)\, K_{eq} = \alpha$

Equation 5 $H_0M_0 - \alpha\left(M_0 + H_0 - \frac{1}{K_{eq}}\right) + \alpha^2 = 0$

Equation 6 $X = \frac{H_0}{M_0}$

Equation 7 $H_0\frac{[H_0]}{X} - \alpha\left(\frac{[H_0]}{X} + H_0 + \frac{1}{K_{eq}}\right) + \alpha^2 = 0$

Equation 8 $\alpha^2 + \alpha\{-H_0\left(\frac{X+1}{X}\right) + H_0 + \frac{1}{K_{eq}}\} = 0$

Equation 9 $\alpha = \frac{\left\{H_0\left(\frac{X+1}{X}\right)+\frac{1}{K_{eq}}\right\} - \sqrt{\{H_0+\frac{1}{K_{eq}}\}^2 - 4\frac{[H_0]^2}{X}}}{2}$

6.2 Significant Figures

Significant figures used were the significant figures of the raw data plus one, for all calculation results.

6.3 Raw Data

Available on request.