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Thiol Quantification Using Colorimetric Thiol−Disulfide Exchange in Nonaqueous Solvents

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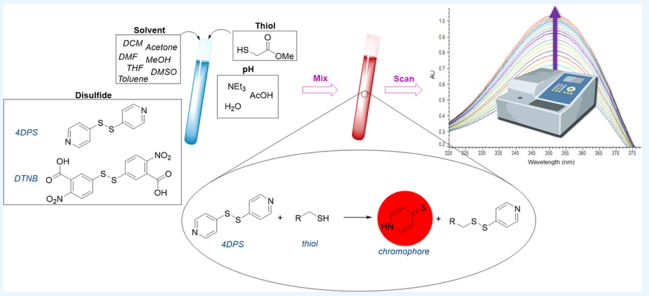
ABSTRACT: A careful analysis of two (thiol−disulfide exchange) thiol quantification chromophores' behavior (Ellman's reagent and Aldrithiol-4) in nonaqueous solvents is presented. A wide range of kinetic profiles and response factors were measured to exhibit a large variance for nonaqueous systems. We report several robust benchtop and room-temperature methods using different organic solvents compared to aqueous conditions. Validation of analytical analyses in nonaqueous systems and quantification of the cysteine content of ovalbumin are also presented. This work serves as a treatise on the utilization of thiol−disulfide exchange chromophores under nonaqueous conditions for the quantification of thiols.



INTRODUCTION

The analytical quantification of thiols (also known as sulfhydryls) is an essential technique in the fields of biochemistry,[3] physiology,[4] and materials science.[5] The thiol functional group is difficult to quantify because it lacks a unique/strong spectroscopic signature with standard analyses (i.e., UV−visible absorption, infrared absorption, nuclear magnetic resonance, etc.) and is thus easily overshadowed in diverse chemical environments. Over the years, several techniques to quantify the concentration of thiols have been developed, such as electrochemical,[6] nanomaterial-mediated detection,[7] photo-redox probes,[8] colorimetric chemical assays,[9] fluorescent chemical assays,[10] biochemical methods,[11] mass spectrometry,[12] and HPLC methods.[13,14]

The most widely used thiol quantification assay is 5,5′-dithiobis(2-nitrobenzoic acid), or as it is commonly known, Ellman's reagent (referred to herein as DTNB). The assay was developed by George Ellman at Dow Chemical in the 1950s for the purpose of measuring thiol concentration in biological tissue.[2] He based this work on previous observations that electron-deficient aromatic disulfides undergo fast exchange with free thiols to yield a strongly colored aromatic thiol and a heterodisulfide (Figure 1a,c).[1] Since then, DTNB has become a cornerstone assay for biochemists. The assay is not without its shortcomings. For example, factors like pH, salt concentration, co-solvents, reaction time, and oxygen exposure all affect the accuracy of thiol quantification and have led to the regular publication of scientific reports with clarifications and caveats years after the original publication.[15]

Other colorimetric chemical assays for thiols have been developed in the intervening years, giving predominance to 4,4′-dipyridyl disulfide (sometimes referred to as Aldrithiol-4; herein referred to as 4DPS). While 4DPS does not replace the use of DTNB, it nicely complements the spectrochemical and pH compatibility shortcomings of DTNB.[14]

The mechanisms of DTNB and 4DPS rely on thiol−disulfide exchange[16] to release a chromophore that can be measured spectrophotometrically (Figure 1). This feature makes thiol−disulfide exchange chromophores exceptionally simple assays to measure spectrophotometrically using a UV−vis instrument. Unfortunately, the list of caveats that accompany experimental methods with the preparation, handling, and storage of these reagents leaves the method itself prone to substantial error. For example: maximum absorption (λ_{max}) shifts in different ion concentrations and solvent blends, the observed extinction coefficient ε_{eff} is sensitive and highly variable, and oxidation from air is a pernicious factor that adds a timing variable unless the analytical method is performed air-free.

We were interested in finding nonaqueous data on the characteristics of these compounds for materials science-related research (non-water-soluble analytes). Most research

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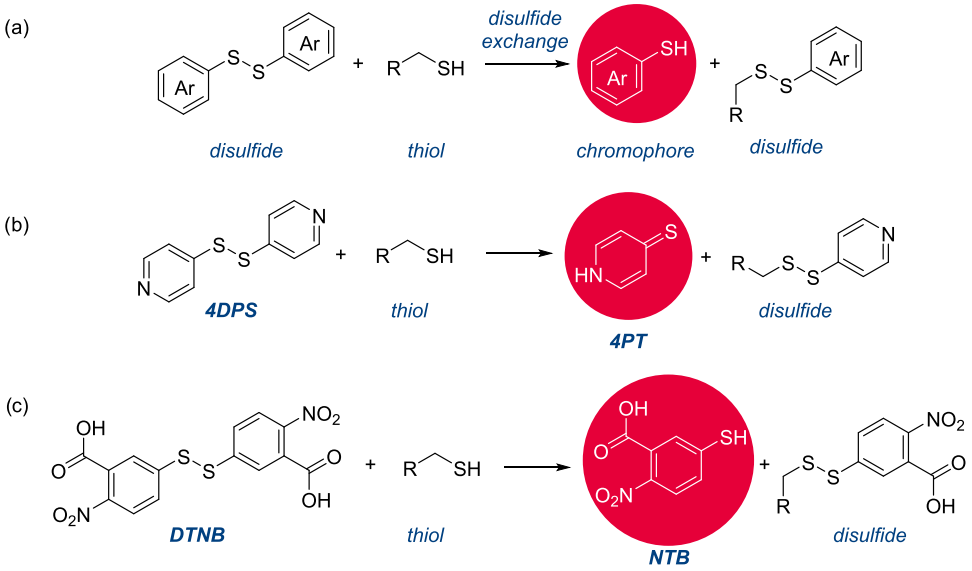
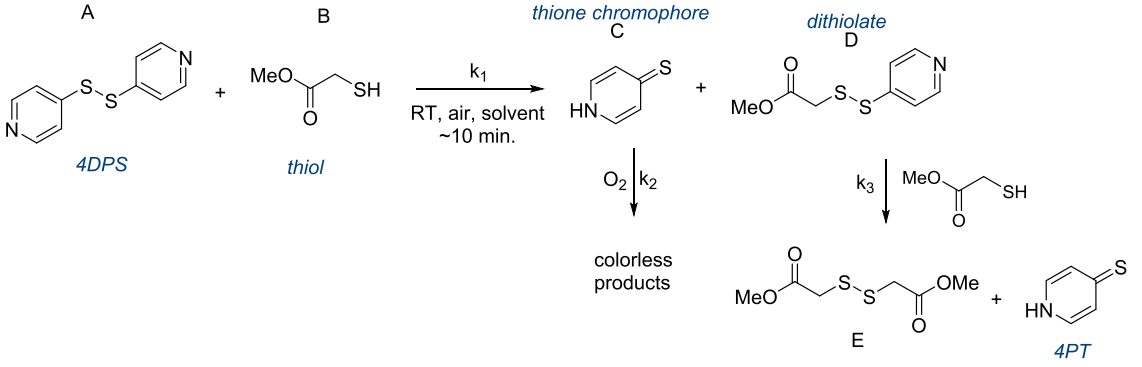


Figure 1. Thiol−disulfide exchange chromophores for spectrophotometric quantification of thiols: (a) general scheme; (b) 4DPS (4,4′-dipyridyl disulfide) → 4PT (4(1H)-pyridinethione); (c) DTNB (5,5-dithio-bis-(2-nitrobenzoic acid) → NTB (2-nitro-5-thiobenzoic acid).

Scheme 1. Chemical Kinetic Model for Thiol−Disulfide Exchange between Methyl Thioglycolate and 4DPS under Aerobic Conditions



using DTNB and 4DPS to quantify thiols are described in aqueous media and tend to be used in a biological context.[17] To our surprise, there was meager data outside of alcohol solvents from which to draw (even though Ellman's original paper[1] had organic co-solvents for many of his analyses). It was largely unknown how nonaqueous environments would affect the thiol−disulfide exchange assay, though it has been shown that polar aprotic co-solvents like dimethylsulfoxide (DMSO) accelerate thiol−disulfide exchange.[18] Because of the sensitivity and complexity of the DTNB and 4DPS assays along with a lack of precedent for nonaqueous systems, we determined that a thorough exploration and validation of a general methodology was needed. Understanding what specific constraints exist in nonaqueous solvents had to be independently determined and verified. The focus and purpose of this study is to present a thorough, empirical, and applicable method for thiol quantification outside aqueous environments that balances robustness, ease of use, and need for specialized chemistry equipment.

RESULTS AND DISCUSSION

Peak Shapes and Reaction Kinetics. The complications of using disulfide exchange chromophores such as DTNB and 4DPS arise from numerous factors. Before running experiments, we wanted to determine which variables can be attenuated or eliminated based on theory, i.e., how can we design the experiment to minimize variance. Reducing the number of steps involved in the kinetics of the thiol−disulfide reaction minimizes the dependence of the kinetics on nonthiol factors. Consider the simple kinetic model (Scheme 1):

Based on the above chemical kinetic model for the thiol−disulfide reaction under aerobic conditions, we find that the change in concentration of the chromophore at any given time is described kinetically by eq 1

$$\frac{d[C]}{dt} = k_1[A][B] + k_3[B][D] - k_2[O_2][C] \quad (1)$$

If we assume that $[A] \gg [B]$ and $k_1 \gg k_3$, then the differential simplifies to eq 2

$$\frac{d[C]}{dt} \approx k_1[A][B] - k_2[O_2][C] \quad (2)$$

Further, under pseudo-first-order conditions (based on $[A] \gg [B]$ and $[O_2]$ being approximately constant), we can further simplify the equation to eq 3

$$\frac{d[C]}{dt} \approx k_1'[B] - k_2'[C] \quad (3)$$

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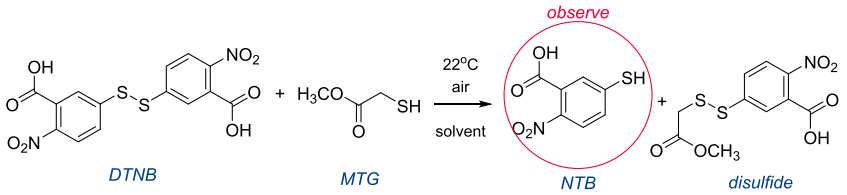
Table 1. Reaction of DTNB with MTG under Different Solvent Conditions[a,b]



line	solvent	λ_{max} (nm)	time (min)	$\varepsilon_{eff}\left(\frac{L}{mol \cdot cm}\right)$
1	DMSO	500	45	5960
2	DMSO[a]	‡	‡	‡
3	DMSO[b]	489	40	66,900
4	DMSO[c]	482	<1	9980
5	DMF	502	60	10,700
6	DMF[a]	501	41	8140
7	DMF[b]	498	46	64,600
8	DMF[c]	485	<1	838
9	DCM	§	§	§
10	DCM[a]	§	§	§
11	DCM[b]	§	§	§
12	DCM[c]	§	§	§
13	toluene	§	§	§
14	toluene[a]	§	§	§
15	toluene[b]	§	§	§
16	toluene[c]	§	§	§
17	acetone	‡	‡	‡
18	acetone[a]	‡	‡	‡
19	acetone[b]	479	<1	16,200
20	acetone[c]	500	65	135
21	THF	‡	‡	‡
22	THF[a]	‡	‡	‡
23	THF[b]	500	<1	81
24	THF[c]	‡	‡	‡
25	MeOH[b]	412	270	*
26	EtOH[b]	422	720	*
27	H_2O**	409	30	14,100
range		409−502	1−720	135−66,900

[a]Note that ε is an effective extinction coefficient since NTB is transient in some cases. [b]*Inconsistent values, **literature values, ‡UV−vis change not measurable, §insoluble, [a]0.5% AcOH, [b]0.25% NEt_3, [c]10% H_2O.

where $k_1' = [A]_0 \cdot k_1$, $k_2' = [O_2]_0 \cdot k_2$, $[A]_0$ = initial concentration of A, and $[O_2]_0$ = initial concentration of oxygen. The integrated rate law[2] for this system becomes (4)

$$[C] = [B]_0\left(\frac{k_1'}{k_2' - k_1'}\right)(e^{-k_1'} - e^{-k_2'}) \quad (4)$$

Using the chemical kinetics described above to design a robust methodology lends insight into two advantageous experimental conditions: (1) elimination of exogenous oxidants (e.g., O_2), and (2) [A] ≫ [B] (i.e., [disulfide] ≫ [thiol]). As to the first point, it is strongly encouraged to work under air-free conditions when possible because the exclusion of exogenous oxidants (e.g., O_2) would eliminate the second term in eqs 2 and 3. However, for other practical reasons, we pursued the development of an *aerobic* method for thiol quantification. Second, it is significantly advantageous, kinetically speaking, to ensure that an excess of disulfide is used in the analysis. Practically, we use 10-fold excess disulfide per thiol to speed the chromophore production and simplify the kinetic profile.

Even with the simple kinetic model achieved in eq 3, a complex curve emerges for the concentration of chromophore over time (eq 4), which is validated by Ellman's original article and our own observations (see SI Figure S6).

Solvent Screen. To determine the general compatibility and variability of organic solvents on the thiol−disulfide exchange for DTNB and 4DPS, a model reaction between a disulfide and methyl thioglycolate (MTG) using a 10:1 molar ratio was observed in various organic solvents at room temperature in air. Reactions were spectrophotometrically observed over 250−700 nm at 5 min intervals over 6 h to determine λ_{max}, reaction time, and profile, and calculate the maximum observed effective extinction coefficient, ε_{eff}. (Note that ε_{eff} is a function of *reaction time* and *wavelength* due to the transient intermediate from aerobic oxidation such that $\varepsilon_{eff} \leq \varepsilon$). Additionally, the method was tested for sensitivity to 10% (v/v) DI water, 0.5% (v/v) acetic acid (AcOH), or 0.25% (v/v) trimethylamine (NEt_3). The results are displayed in Tables 1 and 2.

Several conclusions can be drawn from the data in Tables 1 and 2. First, the observable extinction coefficients for both NTB and 4PT were considerably larger in organic solvents compared with aqueous conditions (with a few exceptions). In practice, this translates to higher sensitivity (larger ε_{eff}) for

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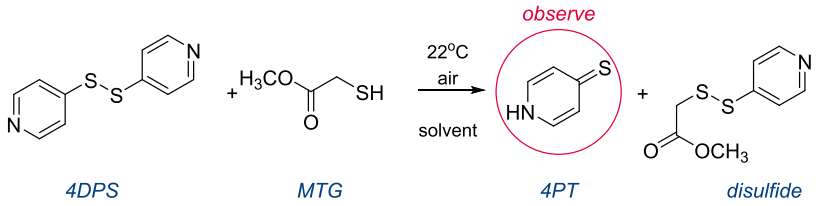
Table 2. Reaction of 4DPS with MTG under Different Solvent Conditions[a,b]



line	solvent	λ_{max} (nm)	time (min)	$\varepsilon_{eff}\left(\frac{L}{mol \cdot cm}\right)$
1	DMSO	350	100	37,800
2	DMSO[a]	352	30	33,600
3	DMSO[b]	352	25	38,700
4	DMSO[c]	346	10	41,400
5	DMF	352	25	36,600
6	DMF[a]	352	30	33,600
7	DMF[b]	346	15	56,500
8	DMF[c]	347	100	40,600
9	DCM	350	>200	900
10	DCM[a]	‡	‡	‡
11	DCM[b]	345	300	15,700
12	DCM[c]	350	>200	2100
13	toluene	‡	‡	‡
14	toluene[a]	‡	‡	‡
15	toluene[b]	350	370	2520
16	toluene[c]	‡	‡	‡
17	acetone	‡	‡	‡
18	acetone[a]	‡	‡	‡
19	acetone[b]	‡	‡	‡
20	acetone[c]	‡	‡	‡
21	THF	‡	‡	‡
22	THF[a]	‡	‡	‡
23	THF[b]	‡	‡	‡
24	THF[c]	344	<1	15,000
25	MeOH	337	70	*
26	EtOH	341	>700	*
27	H_2O**	324	30	20,000
range		324−352	1−700	2100−41,400

[a]Note that ε is an effective extinction coefficient since 4PT is transient in some cases. [b]*Inconsistent values, **literature values, ‡UV−vis change not measurable, [a]0.5% AcOH, [b]0.25% NEt_3, [c]10% H_2O.

Table 3. Summary of Highest Sensitivity Conditions for Thiol−Disulfide Exchange for Analytical Quantification of Thiols

	reagent	solvent[a]	λ_{max} (nm)	time (min.)	ε_{eff} (M^{-1} cm^{-1})
acidic	4DPS	0.5% AcOH (v/v) in DMSO	352	30	33,600
neutral	4DPS	DMSO	350	100	37,800
basic	DTNB	0.25% NEt_3 (v/v) in DMSO	489	40	66,900

[a]For details on methods, see the Experimental Section and Figures S8−S10.

detecting thiols. In the case of the reagent DTNB, DMSO (Table 1, Line 3) and DMF (Table 1, Line 7) under basic conditions are approximately 4.5 times more sensitive than DTNB in water (Table 1, Line 27) with a comparable reaction development time. In the case of the reagent 4DPS, all DMSO and DMF trials had increased ε_{eff} by a factor of 2−3 (Table 2, Lines 1−8) compared with water (Table 2, Line 27). Second, the significant variance in the results reinforces the unpredictability of outcomes from simple variables—for example, the range of measured λ_{max} for DTNB spans 90 nm (Table 1, Range) merely by changing solvents. Overall, these results show not only the viability for certain organic solvents but also an advantage of thiol−disulfide exchange using DTNB and 4DPS in those organic solvents.

The robustness of these results was also probed across additives such as NEt_3, AcOH, and H_2O to determine acid, base, and water effects. Generally, the presence of NEt_3 led to higher ε_{eff} and shorter reaction times. The presence of AcOH was tolerable in many cases, though typically resulted in attenuated ε_{eff}. Water was also tolerable to a high degree (10% in many cases), suggesting that dry solvents are unnecessary. It was observed that higher concentrations of water caused the chromophore to develop slowly in the *absence of a thiol*—suggesting a disproportionation mechanism of the disulfide under some conditions (Supporting Information (SI), Figure S7). This observation was made for DTNB in the presence of NEt_3.These results emphasize the requirement of running

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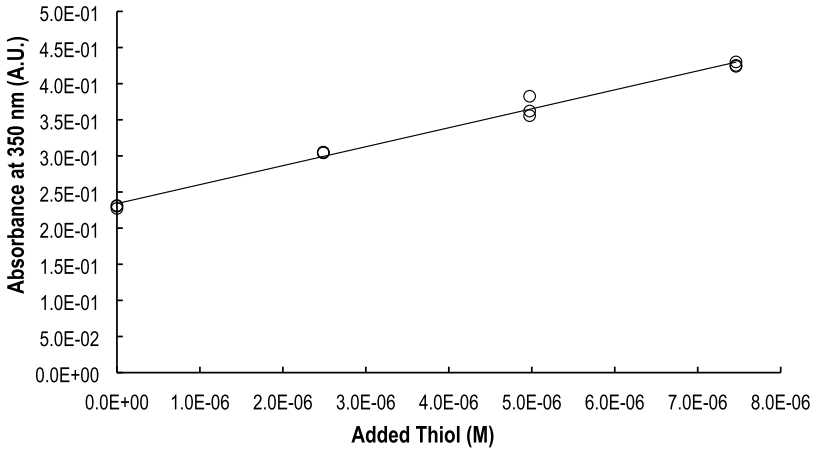

Figure 2. Ovalbumin (2.8 μM) spiked with methyl thioglycolate (MTG).

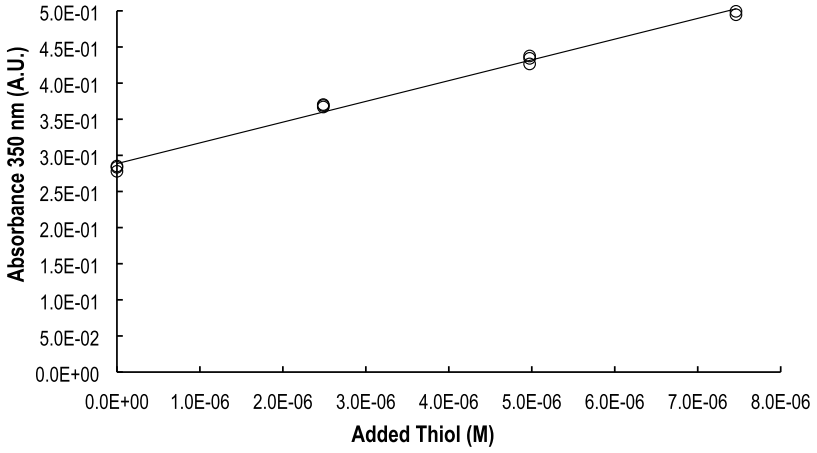

Figure 3. NaH reduced ovalbumin (2.1 μM) spike with methyl thioglycolate (MTG).

control experiments for analyses in wet solvents and with nucleophiles present.

Calibration curves of ε_{eff} ($L{\cdot}mol^{-1}$ cm^{-1}) were determined using five concentration sets of triplicate data points with a linear regression fitting of R^2 of $\geq$ 0.999 (see SI Figures S8–S10) for acidic, basic, and neutral conditions (summarized in Table 3).

Protein Assay. To test our method, we sought to measure the thiol content of a protein. Specifically, the ovalbumin protein in its native and reduced states in DMSO. Ovalbumin (CAS: 9006-59-1, uniprot.org Ascension number: A0A2H4Y842) has four free cysteine residues and one disulfide bond, is readily commercially available with a high purity, and is well studied. Note that the concentration of protein was determined using absorbance at 280 nm in DMSO (A_{280};ε_{DMSO} = 31,400 M^{-1} cm^{-1}, see the SI for details).

To address the unknown complications that having a protein (along with salts, metal ions, residual biomolecules, etc.) in DMSO would have on the assay, an *in situ* extinction coefficient was determined by analytically spiking the sample with exogenous thiol. A denatured solution of 2.8 μM ovalbumin in DMSO was assayed for thiol content, and parallel samples were analytically spiked with MTG (change in thiol concentrations: +2.5, +5.0, and +7. 5 μM) to yield a response curve (Figure 2).

Data from Figure 2 were linearly fitted to yield a slope (*in situ* extinction coefficient of 26,200 M^{-1} cm^{-1}) using the Beer–Lambert law and can be solved for the abscissa intercept (as an absolute value) to determine the original thiol concentration (8.9 μM). Normalizing this value to the concentration of protein in solution (by UV–vis absorption at 280 nm) yields the value of 3.97 $\frac{\text{mol Cys}}{\text{mol ovalbumin}}$. These data agree with literature values for free cysteine residues in ovalbumin.[19]

Another denatured solution of 2.1 μM ovalbumin in DMSO was reduced using NaH,[20] then assayed for thiol content, and parallel samples were analytically spiked with MTG (change in thiol concentrations: +2.5, +5.0, and +7.5 μM) to yield a response curve (Figure 3).

Again, data from Figure 3 were linearly fitted to yield a slope (*in situ* extinction coefficient of 28,700 $M^{-1}cm^{-1}$) and can be solved for the abscissa intercept (as an absolute value) to determine the un-spiked thiol concentration (13.5 μM). Normalizing this value to the concentration of protein in solution (by UV–vis absorption at 280 nm pre-reduction) yields the value of 5.99 $\frac{\text{mol Cys}}{\text{mol reduced ovalbumin}}$. These data match expectations for ovalbumin containing four cysteine thiols and one disulfide bridge.[19]

Thiourethane Oligomer (TUO) Assay. The generality of the method described herein was further tested using a pre-polymer thiourethane oligomer (TUO) used by our laboratory.[21] The TUO samples were synthesized by combining 4,4′-diisocyanato dicyclohexylmethane with pen-

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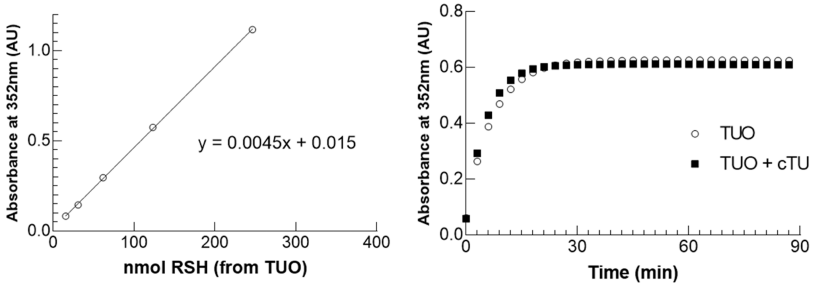

Figure 4. (Left) Spiking experiment of TUO in the presence of 123 nmol of a cyclic thiourethane, 2-thiazolidinone (cTU), The presence of an additional thiourethane moiety without any pendant thiol groups resulted in no additional signal. (Right) Kinetic profiles of TUO alone and with cTU. The presence of an additional thiourethane moiety had no influence on the kinetics of the assay.

taerythritol tetrakis(3-mercaptopropionate) in solution (used as received, 97% purity), at 1:2 diisocyanate:tetrathiol, resulting in a viscous liquid mixture of oligomers decorated with pendant thiols and intramolecularly connected through thiourethane bonds.

First, a model small-molecule cyclic thiourethane (2-thiazolidinone; cTU) was spiked into analysis samples to determine if the presence of a thiourethane moiety would interfere with the quantification (Figure 4, left). Results from these studies showed no interference. Next, the kinetics of TUO samples were determined over time to determine if it matched the kinetic profile of a standard thiol quantification (Figure 4, right). Results from these experiments show nearly identical kinetic curves. The *in situ* extinction coefficient was measured (in the same manner as the ovalbumin experiment) by spiking 4DPS and TUO samples with MTG and plotting the absorbance vs change in concentration of thiol and using the slope to determine the *in situ* extinction coefficient (Figure 5). This was found to be 41,100 M^{-1} cm^{-1} under neutral

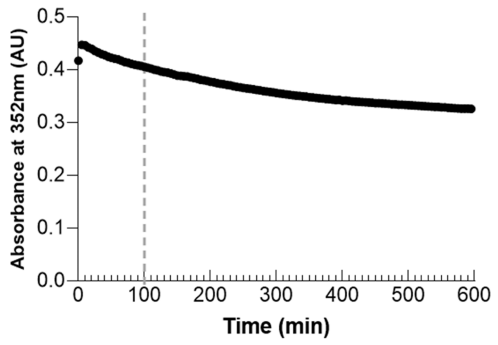

Figure 5. Kinetic profile of TUO with 4DPS under neutral conditions. The 100 min timepoint was used to calculate an *in situ* extinction coefficient of 41,100 M^{-1} cm^{-1}.

conditions, very close to the value of 37,800 M^{-1} cm^{-1} reported in Table 3. Finally, to validate the method development, results for molality of thiols in TUO were compared against an iodometric titration (SI, Figure S4). (Note that iodometric titration is only useful when several grams of sample are available due to many orders of magnitude lower sensitivity to spectrophotometric techniques.) These experiments consistently yielded 2.4 mmol/g thiol while iodometric titrations of the same samples yielded 3.7 mmol/g after calibrating both methods for MTG.

The unique TUO discrepancy in observed absorbance was probed further; however, after an exhaustive study of TUO response to 4DPS, the conclusion was drawn that it is highly likely that the TUO sample precipitates out of solution during the 4DPS assay, leading to artificially low observed absorbance at 352 nm. This conclusion is suggested by several experimental observations: (1) Kinetics for TUO reacting with 4DPS are well behaved, (2) TUO was calibrated with *in situ* spiking of MTG to yield an extinction coefficient at unity with MTG calibrations, (3) doping in a "model-compound" with the thiocarbamate structure (1,3-thiazolidin-2-one) did not affect the UV−vis measurements (or iodometric titrations), (4) attempted substoichiometric oxidation of TUO using I_2 before exposure to 4DPS resulted in a disproportional reduction in observable 4PT signal, not attributable to the extinction coefficient or I_2 oxidation mechanism (Figures 6), and (5) 4DPS experiments consistently yielded 2.3−2.6 mmol/g thiol while iodometric titrations on the same samples yielded 3.6−3.7 mmol/g after calibrating *both methods* for MTG. To summarize, in the context of an oligomer sample, the 4DPS method in acidic, neutral, or basic conditions was unable to reproduce (tending to underestimate by a factor of ∼2) the thiol molality values determined by iodometric titration. Great care should be taken if using 4DPS or DTNB to analyze polymer samples, as this underestimation is likely impacted by LogP, PDI, average MW, and more. Each polymer system should be validated with an additional method, such as iodometric titration, to ensure that a colorimetric disulfide exchange assay is compatible.

SUMMARY AND CONCLUSIONS

The quantification of thiols using DTNB and 4DPS in nonaqueous solvents has produced several important findings. Foremost is the severe sensitivity of the assay to the environment. The high degree of variance across solvents, additives, etc. suggests that independent validation is required for every independent system to ensure accurate analyses. The large variance in our results (namely, ε_{eff}) carefully catalogued in this study cast serious doubts on colorimetric disulfide−thiol exchange thiol quantifications that use arbitrary literature values for time, ε_{eff}, and λ_{max}. We strongly encourage measuring these variables *in situ*, especially in complex mixtures such as organic resins and biological samples. For simple systems (i.e., a molecularly discrete thiol) in DMSO, we have found high reproducibility with our described method. Some thiol-containing oligomeric materials may not be amenable to this method if they do not remain in solution.

There are advantages to using polar aprotic solvents for thiol analysis—namely that it affords access to an entirely different suite of soluble compounds. pH also mostly falls out as a factor, though the presence of acids, bases, and water do have

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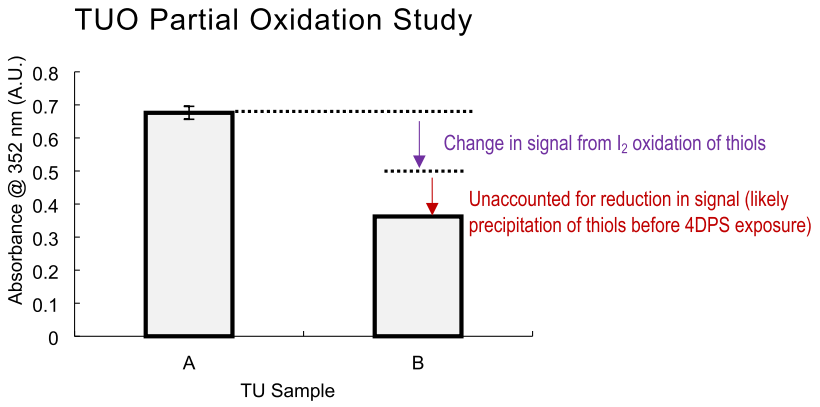

Figure 6. UV−vis absorbance of 4PT for (A) TUO sample in acidic DMSO (control) and (B) TUO sample partly oxidized by introduction of substoichiometric I_2 to the TUO sample prior to the introduction of 4DPS. Note that the calculated molar amount of I_2 did not account for the reduction in signal.

effects on the results (Tables 1 and 2). It was serendipitously discovered that even with the handful of nonaqueous solvents studied, many granted access to higher-sensitivity conditions for quantifying thiols. The major disadvantages of nonaqueous solvents observed were the need to wait for chromophore development, with reaction times ranging from 30 to 100 min, and the insolubility of low-molecular-weight zwitterionic compounds in DMSO (i.e., cysteine, cystine, etc.).

There are also obvious improvements that could be pursued to the described method that lay outside the scope of this study. For example, application of a high-throughput device (i.e., a 96-well plate) would make it possible to run *in situ* calibration standards in line with the analysis of many samples in parallel. The sensitivity of the assay could also be increased using an HPLC with inline detection.[14] It is also likely that other polar aprotic solvents could improve the sensitivity of thiol quantification and may be more useful than DMSO for select applications (e.g., *N*-methyl-2-pyrrolidone, ethylene carbonate, sulfolane, etc.). Also, air-free conditions would simplify the assay kinetics and produce maximum sensitivity.

Overall, this study provides a cautionary tale of thiol quantification using thiol−disulfide exchange chromophores. The kinetics and physiochemical photophysics of chromophore development have large variances across seemingly innocuous variables. A simple methodology for analysis and validation is presented here to be used as a threshold for analytical confidence. It is our hope that this work provides a blueprint to reliably quantify thiols using thiol−disulfide exchange chromophores across a diverse class of chemicals and materials. This can be particularly useful for a whole host of applications, including the ones presented here, and may expand to applications in drug design, where sulfur−sulfur motifs are common and difficult to achieve.[22]

EXPERIMENTAL SECTION

Validation Experiments. Always run blanks and check for compatibility of reagents to ensure absorptivity is (1) from the chromophore and (2) proportional to the thiol concentration, not a background reaction.

λ_{max}, ε_{eff}, and reaction time all need to be validated. To accomplish this, we recommend combining 10:1 disulfide reagent:thiol in a solvent and observe the full spectrum UV−vis change over time (e.g., every 3 min for 2 h). These data will provide λ_{max} and reaction time and give a close approximation for ε_{eff}. Plotting $A_{\lambda max}$ vs time will instruct as to whether the kinetics for the chromophore development are well behaved as described in eq 4. ε_{eff} can then be analytically determined *in situ* with thiol spiking experiments as in the case of ovalbumin.

Discrete Thiol Quantification in DMSO. *Acidic Conditions.* 0.35 mM 4DPS with 0.035 mM thiol, and 0.5% glacial acetic acid (v/v) in dry DMSO

Transfer to a cuvette and scan at 352 nm at 30 min, ε = 33,600 M^{-1} cm^{-1}.

Blank against 0.35 mM 4DPS with 0.5% glacial acetic acid (v/v) in dry DMSO.

Basic Conditions. 0.150 mM DTNB with 0.0150 mM thiol, and 0.25% triethylamine (v/v) in dry DMSO

Transfer to a cuvette and scan at 489 nm at 40 min, ε = 66,900 M^{-1} cm^{-1}.

Blank against 0.150 mM DTNB with 0.25% triethylamine (v/v) in dry DMSO.

Neutral Conditions. 0.30 mM 4DPS 0.035 mM thiol in dry DMSO

Transfer to a cuvette and scan at 350 nm at 100 min, ε = 37,800 M^{-1} cm^{-1}.

Blank against 0.30 mM 4DPS in dry DMSO.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07792.

Detailed experimental protocol description; raw data for calibration curves; raw UV−vis data for all experiments; curves for kinetics experiments with absorbance at 500 nm as a function of time; and example calculation from which the results presented were derived (PDF)

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Notes

The authors declare no competing financial interest.

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