Hindawi
Journal of Spectroscopy
Volume 2022, Article ID 9491360, 8 pages
https://doi.org/10.1155/2022/9491360

*Research Article*

# Correlation between the NMR Chemical Shifts and Thiolate Protonation Constants of Cysteamine, Homocysteine, and Penicillamine

**Juliana Ferreira de Santana**,[1] **Arash Mirzahosseini**,[1,2] **and Béla Noszál**[1,2]

[1]*Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary*
[2]*Research Group of Drugs of Abuse and Doping Agents, Hungarian Academy of Sciences, Budapest, Hungary*

Correspondence should be addressed to Béla Noszál; noszal.bela@pharma.semmelweis-univ.hu

Received 24 March 2022; Revised 7 June 2022; Accepted 11 July 2022; Published 4 August 2022

Academic Editor: Davidson Sajan



$^{1}$H and $^{13}$C NMR measurements were carried out to explore anticipated correlations between chemical shifts versus thiolate basicities and redox potentials of cysteamine, homocysteine, penicillamine, and their homodisulfides. All correlations were analyzed and statistically evaluated. The closest correlation was observed for the $\alpha$CH nuclei concerning $^{1}$H and $^{13}$C NMR data. Since neither site-specific basicities nor site-specific redox potentials can be directly measured by any means in peptides and proteins containing several thiol and/or disulfide units, these data provide a simple method and predictive power to estimate the aforementioned site-specific physicochemical parameters for analogous sulfur-containing moieties in related biopolymers.

## 1. Introduction

Oxidants in biological systems, commonly known as reactive oxygen species (ROS) or reactive nitrogen species (RNS), are produced chiefly in the mitochondria during the normal cellular metabolism. In the cytosol and plasma membrane, certain enzymes such as NADPH oxidase and cytochrome P450 oxidase are able to produce ROS/RNS as well [1]. ROS have an important role against infectious agents and in cellular signaling systems, although their effects seem to be beneficial only at low and highly regulated concentration [2]. At higher concentrations, these species evolve oxidative stress and can become toxic. During evolution, cells have adapted to counter the detrimental effects of ROS using small antioxidant molecules and detoxifying enzymes [3]. However, when the antioxidant processes are not sufficient, free radicals in various tissues will lead to organ damage and in the long term will act as risk factors of serious illnesses, such as cancer, arthritis, and various neurodegenerative diseases [4].

In biological systems, the major defensive process against oxidative stress is the transition of the thiol (-SH) groups into disulfides (-S-S-), ensuring thus the redox homeostasis. The thiol-containing cysteine (CysSH or Cys) is a principal chemical entity targeted by oxidizing species in the redox signaling routes [5]. The two main low molecular weight redox couples in human plasma are cysteine/cystine (CysSSCys) and glutathione (GSH)/glutathione disulfide (GSSG) [6]. Redox transitions are known to actually take place via the thiolate ($-S^{-}$) form, which has not only reducing, but also, proton-binding propensities, and the involvement of the perturbing acid-base processes is therefore inevitably necessary. Apart from perturbing redox and NMR phenomena, protonation states within a molecule are known to have an effect on other spectroscopic properties as well [7–11]. This work is focused on extending the co-dependent relationship observed between the NMR chemical shifts of the aforementioned thiols and their acid-base characteristics to the following compounds: cysteamine (CysASH)/cystamine (CysASSCysA); homocysteine (hCysSH)/homocystine (hCysSShCys); penicillamine (PenSH)/penicillamine disulfide (PenSSPen). The studied compounds are presented in Figure 1, where the reduced form is always in the

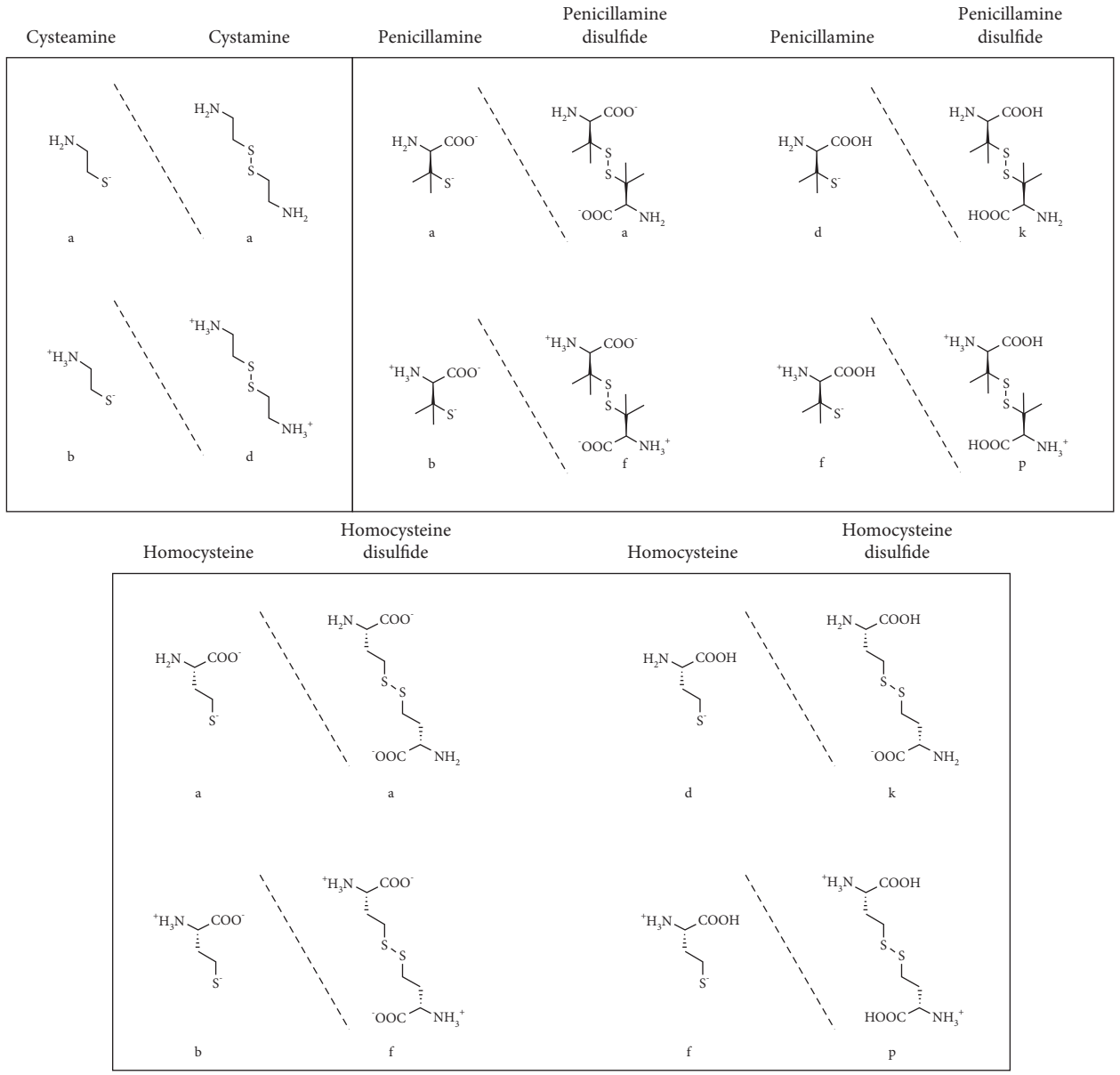


Figure 1: *Structural formulae (and their one letter symbol) of the various protonation states of the studied compounds in thiolate form and their related disulfide derivatives.*

redox-active thiolate form. The neighboring basic moieties are in all possible combinations of protonation state. The oxidized forms are also depicted.

Cysteamine, the decarboxylated derivative of cysteine, can be oxidized in the presence of oxygen or transition metals, producing its disulfide form, cystamine [12]. However, without these factors and in a reducing condition, cysteamine can behave as an antioxidant [13]. When this biogenic amine is present in low concentrations, it may impact the cellular redox homeostasis, as it can help to transport cysteine into cells, which is a substrate for glutathione [14]. The other studied compound, homocysteine, is an amino acid that acts as an intermediate product in the metabolism of cysteine and methionine [15]. Its oxidation generates homocystine leading to the production of hydrogen peroxide that may cause damage of endothelium [16]. Penicillamine is an analog of cysteine, with two extra methyl groups instead of the methylene protons; its thiolate site is therefore more sterically hindered than the one in cysteine [17]. Nevertheless, when exposed to oxidizing agents, the oxidation of penicillamine leads to the formation of penicillamine disulfide [18].

In 2016, our group reported an indirect method through species-specific standard potential to describe thiolate-disulfide equilibria with pH-independent parameters [19]. For better comprehension about the biological role of cysteine oxidation and also to establish an appropriate antioxidant therapy, which could eliminate the currently unmet medical need of oxidative stress [1, 20], it is essential to elaborate new methods to reveal the potential relationship between the co-dependent, subtle redox, acid-base, and spectroscopic features.

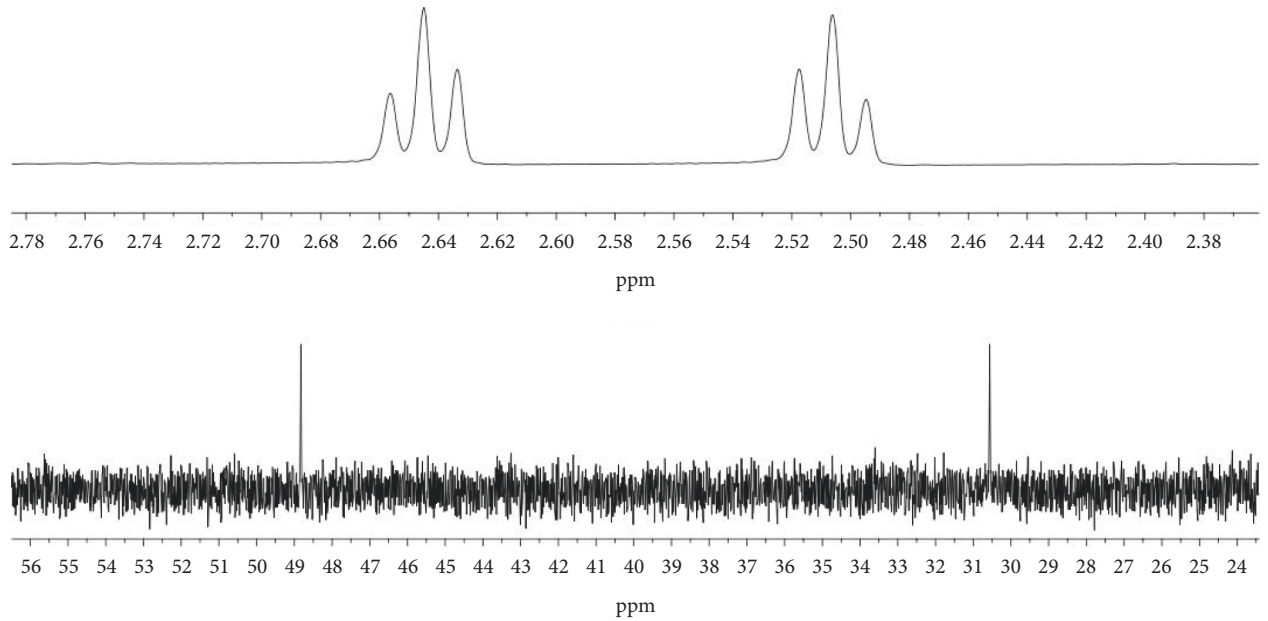


Figure 2: *Sample 1D NMR spectra (top $^{1}H$ and bottom $^{13}C$) recorded for cysteamine at* pH *13.*

Table 1: The species-specific chemical shifts (on the ppm scale) determined for cysteamine, homocysteine, and penicillamine microspecies (the thiolate protonation constants were determined previously in [21]; the N, S, and O symbols represent the amino, thiolate, and carboxylate basic moieties, respectively).

| Log*k* | | $1_H$ | | | | $13_C$ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *α* | *β* | *γ* | $CH_3$ | *α* | *β* | *γ* | $CH_3$ |
| *Cysteamine* | | | | | | | | | |
| CysA a | 9.67 | 2.646 | 2.506 | | | 48.83 | 30.57 | | |
| CysA b | 8.37 | 3.076 | 2.720 | | | 46.95 | 25.43 | | |
| *Δδ N* | | *0.431* | *0.213* | | | *−1.88* | *−5.14* | | |
| *Δδ S* | | *0.129* | *0.118* | | | *−2.08* | *−1.30* | | |
| *Homocysteine* | | | | | | | | | |
| hCys a | 9.94 | 3.288 | 1.726 | 1.805 | 2.424 | | 58.97 | 23.71 | 45.22 |
| hCys b | 8.99 | 3.807 | 2.020 | 2.068 | 2.503 | | 55.68 | 23.40 | 43.58 |
| hCys d | 9.35 | 3.653 | 1.793 | 1.927 | 2.492 | | 56.95 | 23.48 | 44.41 |
| hCys f | 8.4 | 4.172 | 2.087 | 2.190 | 2.571 | | 51.18 | 23.05 | 42.85 |
| *Δδ N* | | *0.519* | *0.294* | *0.263* | *0.079* | | *−3.29* | *−0.31* | *−1.64* |
| *Δδ S* | | *0.074* | *0.096* | *0.108* | *0.155* | | *0.77* | *−0.81* | *−6.22* |
| *Δδ O* | | *0.365* | *0.067* | *0.122* | *0.069* | | *−2.02* | *−0.23* | *−0.81* |
| *Penicillamine* | | | | | | | | | |
| Pen a | 9.34 | 3.049 | | 1.191 | 1.437 | 71.83 | 49.42 | 30.91 | 37.10 |
| Pen b | 8.09 | 3.298 | | 1.294 | 1.535 | 69.31 | 47.81 | 35.42 | 31.69 |
| Pen d | 8.24 | 3.464 | | 1.250 | 1.470 | 69.94 | 49.16 | 30.85 | 36.66 |
| Pen f | 6.99 | 3.713 | | 1.353 | 1.568 | 67.42 | 47.55 | 35.36 | 31.25 |
| *Δδ N* | | *0.249* | | *0.103* | *0.098* | *−2.52* | *−1.61* | *4.51* | *−5.41* |
| *Δδ S* | | *0.404* | | *0.187* | *0.031* | *−1.77* | *−1.07* | *−4.95* | *1.32* |
| *Δδ O* | | *0.415* | | *0.059* | *0.033* | *−1.89* | *−0.26* | *−0.06* | *−0.44* |

*Δδ* (Delta delta) shows how the protonation shift observed on a nucleus, caused by the protonation of the various basic moieties can be quantified in ppm. Note that *Δδ* is nucleus-dependent; its value is different and distinctive not only for all types of nuclei but also for every atom in the molecule.

The acid-base protonation microconstants of cysteine, homocysteine, penicillamine, and their respective homodisulfides have been determined, using $^{1}H$ NMR-pH titrations and appropriate evaluations [21, 22]. In this work, we are extending the correlation between standard redox potentials and thiolate logK values [19] to chemical shifts as well, to emphasize the predictive ability of NMR parameters obtainable from fairly simple, single spectroscopic measurements. A recent work from our group [23] reported such a correlation between the NMR chemical shift and thiolate protonation constants for cysteine and cysteine-containing peptides. Here cysteamine, homocysteine, penicillamine, and their homodisulfides are investigated.

Table 2: The species-specific chemical shifts (on the ppm scale) determined for cystamine, homocystine, and penicillamine disulfide microspecies (the thiolate protonation constants were determined previously in [21]).

| Log*k* | | $^1$H | | | |
|---|---|---|---|---|---|
| | | $\alpha$ | $\beta$ | $\gamma$ | $CH_3$ |
| *Cystamine* | | | | | |
| $CysA_2^a$ | 9.67 | 2.928 | 2.807 | | |
| $CysA_2^d$ | 8.37 | 3.409 | 3.025 | | |
| *Δδ NN′* | | *0.480* | *0.219* | | |
| *Homocystine* | | | | | |
| $hCys_2a$ | 9.94 | 3.347 | 2.052 | 1.942 | 2.783 |
| $hCys_2f$ | 8.99 | 3.860 | 2.315 | 2.247 | 2.826 |
| $hCys_2k$ | 9.35 | 3.727 | 2.075 | 2.123 | 2.859 |
| $hCys_2p$ | 8.4 | 4.240 | 2.338 | 2.428 | 2.902 |
| *Δδ NN′* | | *0.512* | *0.263* | *0.305* | *0.043* |
| *Δδ OO′* | | *0.380* | *0.023* | *0.181* | *0.076* |
| *Panicillamine disulfide* | | | | | |
| $Pen_2a$ | 9.34 | 3.691 | | 1.296 | 1.321 |
| $Pen_2f$ | 8.09 | 3.982 | | 1.454 | 1.398 |
| $Pen_2k$ | 8.24 | 3.989 | | 1.340 | 1.416 |
| $Pen_2p$ | 6.99 | 4.280 | | 1.498 | 1.493 |
| *Δδ NN′* | | *0.291* | | *0.158* | *0.077* |
| *Δδ OO′* | | *0.298* | | *0.044* | *0.095* |

*Δδ* (Delta delta) shows how the protonation shift observed on a nucleus, caused by the protonation of the various basic moieties can be quantified in ppm. Note that *Δδ* is nucleus-dependent; its value is different and distinctive not only for all types of nuclei but also for every atom in the molecule.

## 2. Materials and Methods

*2.1. Materials.* All the compounds, cysteamine, cystamine, homocysteine, homocystine, penicillamine, and penicillamine disulfide were purchased from Sigma (Merck) and were used without further purification. Deionized water was prepared with a Milli-Q Direct 8 Millipore system.

*2.2. NMR Spectroscopy Measurements.* A Varian Unity Inova DDR spectrometer (599.9 MHz for 1H) with a 5 mm $^1$H $\{^{13}C/^{31}P\text{-}^{15}N\}$ pulse field gradient triple resonance probehead at 298.15 ± 0.1K was used to record all the NMR spectra. As a solvent, $H_2O$: $D_2O$ 95:5 (V/V) was used with ionic strength settled to 0.15 mol/L. The pH evaluation was stated in situ through internal indicator molecules (at ca. 1 mmol/L) optimized for $^1$H NMR [24, 25]. Regarding the sample, the volume was 550 $\mu$L and all of them contained ca. 1 mmol/L DSS (3-(trimethylsilyl) propane-1-sulfonate) as chemical shift reference. It was used a presaturation sequence to suppress the $H_2O$ 1H signal; the mean acquisition parameters for $^1$H measurements are number of transients = 16, number of points = 65536, acquisition time = 3.33 s, and relaxation delay = 1.5 s. $^1$H decoupled $^{13}$C measurements were recorded with number of transients = 32768, number of points = 262144, and relaxation delay = 1 s.

*2.3. Statistical Analysis.* To analyze the titration data, nonlinear regression was performed using R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) [26] with the function

$$\delta_{obs(pH)} = \frac{\delta_L + \delta_{HL} \times 10^{\log K - pH}}{1 + 10^{\log K - pH}}, \quad (1)$$

where $\delta_L$ is the chemical shift of an unprotonated moiety, $\delta_{HL}$ is the chemical shift of the protonated moiety, and logK is the base 10 logarithm of the protonation constant. Linear regressions were carried out using R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) [26] to analyze the chemical shift-log*K* data.

## 3. Results

The species-specific protonation schemes of cysteamine, homocysteine, penicillamine, and their respective homodisulfides were elaborated and shown in two previous studies [19, 21]; the thiolate-specific protonation constants of these compounds are imported from the aforementioned literature. The species-specific NMR chemical shifts of the $\alpha$CH and $\beta CH_2$ nuclei were determined by measuring 1D NMR spectra at pH values corresponding to the plateaus on the titration curves of the compounds. In Figure 2, sample NMR spectra are presented that were obtained for cysteamine at basic pH. The limiting chemical shifts of all studied compounds were determined analogously followed by the calculation of species-specific chemical shifts using Sudmeier–Reilley equations [27]; this determination method was recently formulated for the similar selenocysteine/selenocystine pair [28]. The species-specific chemical shifts are assembled in Tables 1 and 2, for the reduced and oxidized forms of the compounds, respectively. The species-specific chemical shifts also afford the protonation shifts ($^{\Delta}\delta$) associated with the various basic moieties. The protonation shift is the chemical shift change a nucleus undergoes when a certain basic moiety protonates.

A multiple linear regression analysis was performed on the grouped data from both Tables 1 and 2 using the NMR chemical shifts and log*K* values as independent and dependent variables, respectively. The result of the linear regression is shown in Figure 3, where a good fit can be observed for the chemical shift data of both $^1$H and $^{13}$C. Table 3 presents the parameters of the regression analysis, expanding the results from our last work [23].

## 4. Discussion

The sulfur atom of biomolecules can be found in many different functional groups, in some metabolites (coenzyme A, glutathione—GSH, and mycothiol) and amino acids such as cysteine, or its derivatives cysteamine, homocysteine, and penicillamine. Among its versatile reactivities, sulfur has an important role in the redox biochemistry [29, 30]. As shown in our previous work [23], the analysis of the chemical shift data demonstrated a direct and inverse relationship between the thiolate log*K* and chemical shifts of the nearby $^{13}C/^1H$ nuclei. The linear relationship found for the studied compounds is presented in Table 3 and Figure 3; it is noteworthy to highlight the strong correlation between almost all the

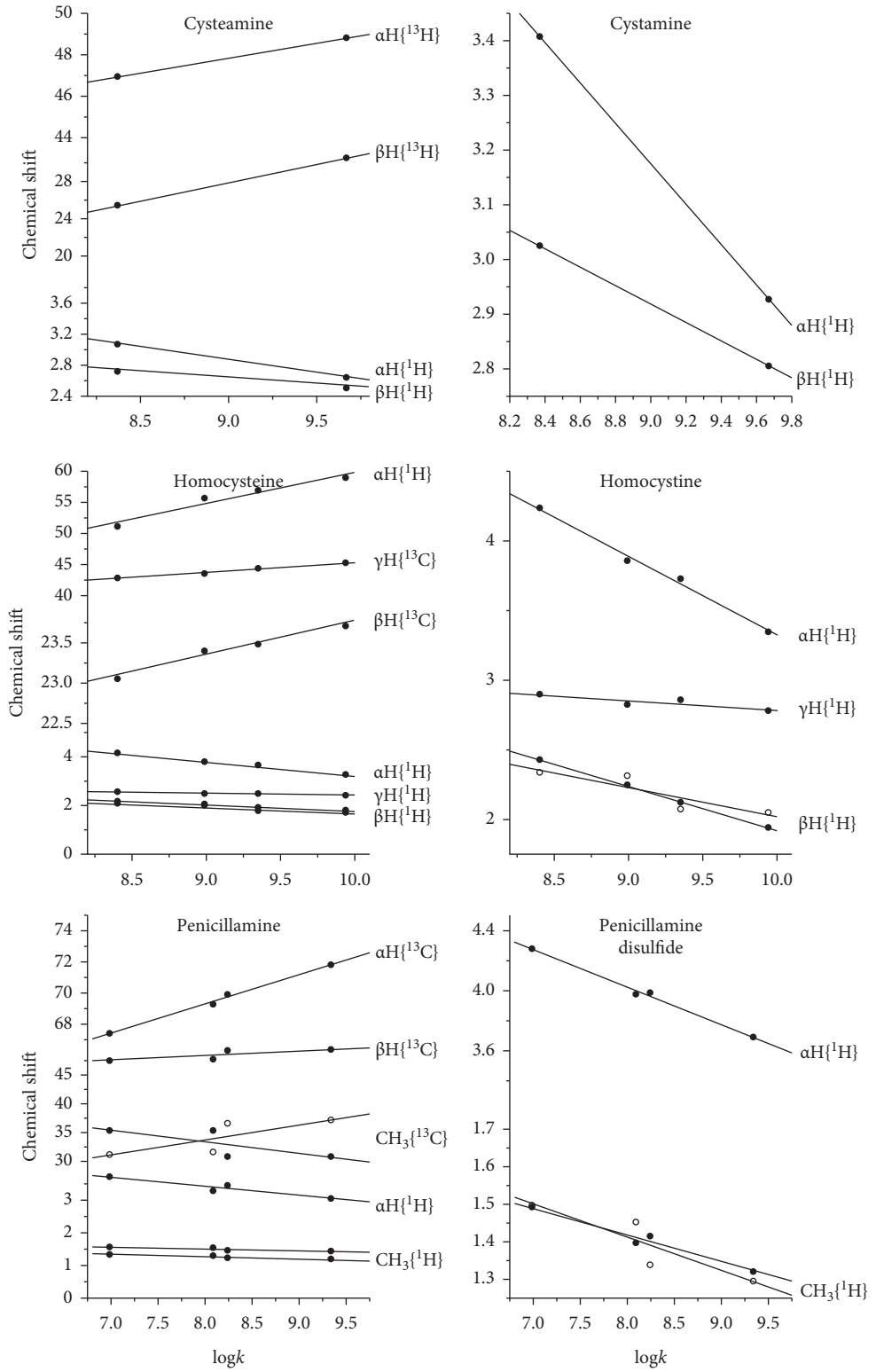


Figure 3: *The multivariate linear regression fits of the chemical shift data versus thiolate basicities. Note that the horizontal axes are not on the same position and scale.*

studied nuclei and thiolate basicities. Similar to the case of cysteine and cysteine-containing peptides, the $\alpha$CH nuclei showed the best correlation, for both $^{1}$H and $^{13}$C chemical shifts. This finding further supports the claim that the $\alpha$CH nuclei are the best choice as an estimator of thiolate basicity from NMR data. This statement could also be stated purely based on the fact that the covalent distance of the sulfur atom to these nuclei is the optimal one. Among the studied compounds, the thiolate in penicillamine has lower basicity when comparing equivalent protonation states, due to the

Table 3: *The regression statistics of the cysteamine, cystamine, homocysteine, homocystine, penicillamine, and penicillamine disulfide chemical shift data.*

| | | | Slope | Std. err | Intercept | Std. err | Adj $R^2$ | $p$ value |
|---|---|---|---|---|---|---|---|---|
| Cysteamine | $^1$H | $\alpha$ | −0.33 | — | 5.85 | — | — | — |
| | | $\beta$ | −0.16 | — | 4.09 | — | — | — |
| | $^{13}$C | $\alpha$ | 1.44 | — | 34.88 | — | — | — |
| | | $\beta$ | 3.95 | — | −7.66 | — | — | — |
| Cystamine | $^1$H | $\alpha$ | −0.37 | — | 6.50 | — | — | — |
| | | $\beta$ | −0.17 | — | 4.43 | — | — | — |
| | $^1$H | $\alpha$ | **−0.57** | ***0.02*** | **8.92** | ***0.21*** | **0.9951** | **0.0016** |
| | | $\beta_a$ | −0.26 | *0.06* | 4.24 | *0.57* | 0.8415 | 0.0543 |
| | | $\beta_b$ | **−0.26** | ***0.02*** | **4.36** | ***0.20*** | **0.9783** | **0.0073** |
| Homocysteine | $^{13}$C | **γ** | **−0.09** | ***0.01*** | **3.34** | ***0.10*** | **0.9619** | **0.0128** |
| | | **α** | **4.98** | ***0.82*** | **10.0** | ***7.5*** | **0.9228** | **0.0261** |
| | | **β** | **0.42** | ***0.05*** | **19.58** | ***0.46*** | **0.9585** | **0.0139** |
| | | **γ** | **1.58** | ***0.12*** | **29.5** | ***1.1*** | **0.9821** | **0.0060** |
| | | **α** | **−0.57** | ***0.03*** | **9.01** | ***0.31*** | **0.9898** | **0.0034** |
| Homocystine | $^1$H | $\beta_a$ | −0.21 | *0.08* | 4.13 | *0.69* | 0.6941 | 0.1078 |
| | | $\beta_b$ | **−0.32** | ***0.00*** | **5.09** | ***0.04*** | **0.9994** | **0.0002** |
| Panicillamine | $^1$H | γ | −0.07 | *0.03* | 3.47 | *0.24* | 0.6550 | 0.1225 |
| | | **α** | **−0.28** | ***0.06*** | **5.64** | ***0.52*** | **0.8613** | **0.0473** |
| | | $CH_{3a}$ | **−0.07** | ***0.01*** | **1.84** | ***0.08*** | **0.9382** | **0.0208** |
| | | $CH_{3b}$ | −0.06 | *0.02* | 1.97 | *0.13* | 0.7782 | 0.0769 |
| | $^{13}$C | **α** | **1.89** | ***0.10*** | **54.23** | ***0.86*** | **0.9909** | **0.0031** |
| | | β | 0.83 | *0.37* | 41.7 | *3.0* | 0.5747 | 0.1536 |
| | | $CH_{3a}$ | −2.01 | *1.28* | 49.5 | *10.5* | 0.3255 | 0.2581 |
| | | $CH_{3b}$ | 2.61 | *1.38* | 12.8 | *11.3* | 0.4643 | 0.1982 |
| Penicillamine disulfide | $^1$H | **α** | **−0.25** | ***0.01*** | **6.02** | ***0.11*** | **0.9915** | **0.0028** |
| | | $CH_{3a}$ | −0.09 | *0.03* | 2.12 | *0.25* | 0.7183 | 0.0988 |
| | | $CH_{3b}$ | **−0.07** | ***0.01*** | **2.00** | ***0.07*** | **0.9574** | **0.0143** |

shielding effect of the two methyl groups that restrain steric freedom near the thiolate [31]; however, it is important to observe that the correlation is still maintained. Further expansion of the correlation window to lower log$K$ values is indispensable to expand this regression model for better utility; therefore, larger peptides are also in search for the determination of species-specific chemical shifts and protonation constants.

It is usually assumed that the chemical shift of an NMR active nucleus is sensitive to changes in the electron density of geminal and vicinal atoms. Furthermore, the correlations between cysteamine, homocysteine, and penicillamine chemical shifts, log$K$, and redox potentials could lead to better comprehension regarding the acid-base and redox chemistry and the biological functions of these oxidations [32].

## 5. Conclusion

Besides the already known strong linear relationship within the cysteine microspecies for the chemical shift data versus thiolate basicities, it is hereby shown that this correlation holds true for the cysteine derivatives cysteamine, cystamine, homocysteine, homocystine, penicillamine, and penicillamine disulfide. The highest degree of correlation was observed for the $\alpha$CH nuclei concerning both $^1$H and $^{13}$C NMR data of all studied compounds. The next step is expanding this model to study cysteine-containing peptides with lower thiolate basicities and extend the correlation model to be used on larger proteins to estimate the otherwise unmeasurable site-specific acid-base and redox properties of their cysteine residues using only the NMR chemical shifts.

## Data Availability

The NMR spectroscopy data used to support the findings of this study are included within the article.

## Disclosure

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

This study was funded by the Thematic Excellence Program of the Hungarian National Research, Development and Innovation Office (TKP2021-EGA-24) and the ÚNKP-21-5-SE-4 New National Excellence Program of the Hungarian Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

Juliana Ferreira de Santana is grateful for the Stipendium Hungaricum Scholarship. Arash Mirzahosseini is grateful for the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

## References


[1] G. N. Sharma, G. Gupta, and P. Sharma, "A comprehensive review of free radicals, antioxidants, and their relationship with human ailments," *Critical Reviews in Eukaryotic Gene Expression*, vol. 28, no. 2, pp. 139–154, 2018.

[2] M. Valko, D. Leibfritz, J. Moncol, M. T. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease," *The International Journal of Biochemistry & Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.

[3] R. Mittler, S. Vanderauwera, N. Suzuki et al., "ROS signaling: the new wave?" *Trends in Plant Science*, vol. 16, no. 6, pp. 300–309, 2011.

[4] J. H. Qi and F. X. Dong, "The relevant targets of anti-oxidative stress: a review," *Journal of Drug Targeting*, vol. 29, no. 7, pp. 677–686, 2021.

[5] G. Roos and J. Messens, "Protein sulfenic acid formation: from cellular damage to redox regulation," *Free Radical Biology and Medicine*, vol. 51, no. 2, pp. 314–326, 2011.

[6] Y. M. Go and D. P. Jones, "Cysteine/cystine redox signaling in cardiovascular disease," *Free Radical Biology and Medicine*, vol. 50, no. 4, pp. 495–509, 2011.

[7] N. Noureddine, N. Issaoui, and O. Al-Dossary, "DFT and molecular docking study chloroquine derivatives as antiviral to coronavirus COVID-19," *Journal of King Saud University—Science*, vol. 33, 2020.

[8] A. S. Kazachenko, F. Akman, H. Abdelmoulahi et al., "Intermolecular hydrogen bonds interactions in water clusters of ammonium sulfamate: FTIR, X-ray diffraction, AIM, DFT, RDG, ELF, NBO analysis," *Journal of Molecular Liquids*, vol. 342, Article ID 117475, 2021.

[9] S. Gatfaoui, N. Issaoui, T. Roisnel, and H. Marouani, "A proton transfer compound template phenylethylamine: synthesis, a collective experimental and theoretical investigations," *Journal of Molecular Structure*, vol. 1191, pp. 183–196, 2019.

[10] O. Noureddine, N. Issaoui, M. Medimagh, O. Al-Dossary, and H. Marouani, "Quantum chemical studies on molecular structure, AIM, ELF, RDG and antiviral activities of hybrid hydroxychloroquine in the treatment of COVID-19: molecular docking and DFT calculations," *Journal of King Saud University Science*, vol. 33, no. 2, p. 101334, 2021.

[11] A. Sagaama, S. A. Brandan, T. Ben Issa, and N. Issaouia, "Searching potential antiviral candidates for the treatment of the 2019 novel coronavirus based on DFT calculations and molecular docking," *Heliyon*, vol. 6, 2020.

[12] B. D. Paul and S. H. Snyder, "Therapeutic applications of cysteamine and cystamine in neurodegenerative and neuropsychiatric diseases," *Frontiers in Neurology*, vol. 10, p. 1315, 2019.

[13] D. J. Fraser-Pitt, D. K. Mercer, D. Smith et al., "Cysteamine, an endogenous aminothiol, and cystamine, the disulfide product of oxidation, increase *Pseudomonas aeruginosa* sensitivity to reactive oxygen and nitrogen species and potentiate therapeutic antibiotics against bacterial infection," *Infection and Immunity*, vol. 86, no. 6, Article ID e00947-17, 2018.

[14] M. Besouw, R. Masereeuw, L. van den Heuvel, and E. Levtchenko, "Cysteamine: an old drug with new potential," *Drug Discovery Today*, vol. 18, no. 15-16, pp. 785–792, 2013.

[15] D. Djuric, V. Jakovljevic, V. Zivkovic, and I. Srejovic, "Homocysteine and homocysteine-related compounds: an overview of the roles in the pathology of the cardiovascular and nervous systems," *Canadian Journal of Physiology and Pharmacology*, vol. 96, no. 10, pp. 991–1003, 2018.

[16] L. Debreceni, "Homocysteine-a risk factor for atherosclerosis," *Orvosi Hetilap*, vol. 142, no. 27, pp. 1439–1444, 2001.

[17] R. Bhushan and R. Kumar, "Enantioresolution of dl-penicillamine," *Biomedical Chromatography*, vol. 24, no. 1, pp. 66–82, 2010.

[18] K. Hirte, B. Seiwert, G. Schuurmann, and T. Reemtsma, "New hydrolysis products of the beta-lactam antibiotic amoxicillin, their pH-dependent formation and search in municipal wastewater," *Water Research*, vol. 88, pp. 880–888, 2016.

[19] A. Mirzahosseini and B. Noszal, "Species-specific standard redox potential of thiol- disulfide systems: a key parameter to develop agents against oxidative stress," *Scientific Reports*, vol. 6, no. 1, Article ID 37596, 2016.

[20] L. J. Alcock, M. V. Perkins, and J. M. Chalker, "Chemical methods for mapping cysteine oxidation," *Chemical Society Reviews*, vol. 47, no. 1, pp. 231–268, 2018.

[21] A. Mirzahosseini and B. Noszal, "The species- and site-specific acid-base properties of biological thiols and their homodisulfides," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 95, pp. 184–192, 2014.

[22] A. Mirzahosseini, M. Somlyay, and B. Noszal, "Species-specific thiol-disulfide equilibrium constant: a tool to characterize redox transitions of biological importance," *Journal of Physical Chemistry B*, vol. 119, no. 32, pp. 10191–10197, 2015.

[23] J. Ferreira de Santana, A. Mirzahosseini, B. Mándity, D. Bogdán, I. Mándity, and B. Noszál, "Close correlation between thiolate basicity and certain NMR parameters in cysteine and cystine microspecies," *PLoS One*, vol. 17, no. 3, Article ID e0264866, 2022.

[24] Z. Szakacs, G. Hagele, and R. Tyka, "1H/31P NMR pH indicator series to eliminate the glass electrode in NMR spectroscopic pKa determinations," *Analytica Chimica Acta*, vol. 522, no. 2, pp. 247–258, 2004.

[25] G. Orgovan and B. Noszal, "Electrodeless, accurate pH determination in highly basic media using a new set of (1)H NMR pH indicators," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 54, no. 5, pp. 958–964, 2011.

[26] R. R Core Team, "A language and environment for statistical computing," *R Foundation for Statistical Computing*, Vienna, Austria, 2021, https://www.R-project.org/.

[27] J. L. Submeier and C. N. Reilley, "Nuclear magnetic resonance studies of protonation of polyamine and aminocarboxylate compounds in aqueous solution," *Analytical Chemistry*, vol. 36, no. 9, pp. 1698–1706, 1964.

[28] T. Pálla, A. Mirzahosseini, and B. Noszál, "The species-specific acid-base and multinuclear magnetic resonance properties of selenocysteamine, selenocysteine, and their homodiselenides," *Chemical Physics Letters*, vol. 741, Article ID 137076, 2020.

[29] M. A. Comini, "Measurement and meaning of cellular thiol: disufhide redox status," *Free Radical Research*, vol. 50, no. 2, pp. 246–271, 2016.

[30] H. Mutlu, E. B. Ceper, X. Li et al., "Sulfur chemistry in polymer and materials science," *Macromolecular Rapid Communications*, vol. 40, no. 1, Article ID e1800650, 2019.

[31] A. Mirzahosseini and B. Noszál, "Species-specific thiol-disulfide equilibrium constants of ovothiol A and penicillamine with glutathione," *RSC Advances*, vol. 6, no. 32, pp. 26757–26764, 2016.

[32] D. Sharma and K. Rajarathnam, "13C NMR chemical shifts can predict disulfide bond formation," *Journal of Biomolecular NMR*, vol. 18, no. 2, pp. 165–171, 2000.