Check for updates

# The Interaction of Hemoglobin With Sodium Dodecyl Sulfate in Presence of Ascorbic Acid

Radnoosh Mirzajani[1], Ebrahim Mirzajani[2], Heshmatollah Ebrahimi-Najafabadi[3*]

[1]Department of Chemistry, Rasht Branch, Islamic Azad University, Rasht, Iran.
[2]Department of Biochemistry, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
[3]Department of Medicinal Chemistry, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.

*Corresponding author:
Heshmatollah Ebrahimi-Najafabadi,
School of Pharmacy, Guilan Academic Complex of Medical Sciences, Fouman-Saravan Road, Rasht, Guilan, Iran. P.O.Box: 41635-1655
Tel: +98 13 33486471;
Email: Ebrahimi.heshmat@gmail.com

## Abstract

**Background:** Hemoglobin (Hb), oxygen, carbon dioxide, and electron transporter of the body, may enter to an oxidation process that can convert oxyhemoglobin (oxyHb) to methemoglobin (metHb) and hemichrome. Surfactants can facilitate oxidation process that may accumulate hemichrome in red blood cells.

**Methods:** In the present study, the interaction of purified Hb with sodium dodecyl sulfate (SDS, 0-5 mM) and ascorbic acid (AA, 0-5 mM) was evaluated by UV-Vis spectroscopy and multivariate curve resolution techniques.

**Results:** Reconstructed spectral and concentration profiles showed three forms of Hb name as oxyHb, metHb, and hemichrome with lack of fit values less than 1.85%. AA hindered the oxidation process of Hb.

**Conclusion:** A decrease in critical micelle concentration of SDS in the presence of AA and interaction of AA with hydrogen peroxide, which is produced during the interaction of Hb with SDS, are two reasons for diminution in the oxidation process of Hb when accompanied with AA.

**Keywords:** Hemoglobin, Sodium dodecyl sulfate, Ascorbic acid, Peroxide, Multivariate curve resolution, Chemometrics

## Background

Hemoglobin (Hb) is the major hemeprotein of the red blood cells which is responsible for the transport of oxygen and carbon dioxide, hydrogen peroxide ($H_2O_2$) dispersion, and electron transportation to all organs, as well as regulation of blood pH (1). The structure and mechanism of Hb are completely understood. Previous studies have shown that interaction of Hb with some molecules such as surfactants (2,3), bacterial endotoxin (4), and trehalose (5) can alter oxyhemoglobin (oxyHb) to methemoglobin (metHb) and hemichrome, while some others may slow down or reverse the autooxidation process of Hb. Therefore, these species can modulate functional properties of the proteins. Accumulation of hemichrome in red blood cells is typical in some diseases, as well as the senescence of the erythrocytes (2).

Furthermore, surfactant-protein interactions are generally conducted by weak chemical forces such as electrostatic interactions, London forces, hydrogen bonding, and hydrophobic effects, which lead to conformational changes in protein. Therefore, the study of surfactant-protein interactions is of great importance, theoretically and practically (6).

Sodium dodecyl sulfate (SDS) is an anionic surfactant consumed in many detergents, cosmetic and cleansings, as well as pharmaceutical products. SDS could be straightly absorbed into the blood stream without filtering. There are two major pathways for heme degradation, viz enzymatic and non-enzymatic. In non-enzymatic process, oxyHb experiences redox reaction of heme iron with oxygen that produces reactive oxygen species (ROS) (7). Superoxide ($O_2^-$) and $H_2O_2$ can be produced upon the interaction of SDS with oxyHb. $H_2O_2$ is responsible for further degradation of oxyHb. Prior studies have suggested that SDS probably proceeds into the heme pocket and induces a conformational change due to the production of endogenous $H_2O_2$ and other ROS (8).

Ascorbic acid (AA), a water-soluble vitamin, is a potent antioxidant in vitro and in vivo (9). AA reacts with ROS, and hence, prevents the oxidation of a variety of biomolecules. AA can be readily oxidized under normal conditions, and therefore, act as a radical scavenging agent (10). AA can also reduce or prevent $H_2O_2$-induced lipid peroxidation, and the formation of OH-deoxyguanosine (11). Furthermore, AA protects aged dermal fibroblasts from $H_2O_2$- induced cytotoxicity (12) and protects thymocytes against oxidant-induced apoptosis (13).

Multivariate resolution techniques involve the description

of variations of measurements as an additive model of the contributions of their pure constituents (14). The aim of resolution techniques is the retrieval of chemical and/or physical information from the relevant and sufficiently informative experimental data. Such data include, for example, the number of intermediates present in a reaction, the rate or equilibrium constants, and the spectra for each one of those intermediates (15). Multivariate curve resolution alternative least squares (MCR-ALS) has become a popular chemometric method for the resolution of multiple component response in unknown unresolved mixture (16). Its popularity is due to the great variety of data sets that can be analyzed, ability to deal with multiple data matrices concurrently, and the diversity and flexible application of constraints to help and improve the resolution results. Regarding the application of MCR-ALS on the interaction of Hb with surfactants, Mojtahedi et al studied the interaction of *n*-dodecyl trimethylammonium bromide (DTAB) with oxyHb A and oxyHb S using UV–Vis absorption spectra in combination with MCR-ALS (15). The results showed that MCR-ALS was an adequate technique for the estimation of concentration and spectral profiles of Hb-denatured products upon interaction with DTAB. Salehi et al applied the combined MCR-ALS and two techniques of UV-Vis and fluorescence spectroscopy to investigate the effect of interaction of SDS with Hb. They found that endogenous $H_2O_2$ produced upon the interaction can alter oxyHb to metHb and hemichrome, while exogenous $H_2O_2$ lacks this capability (8). Fotouhi et al used augmented data from UV-Vis, fluorescence, and circular dichroism spectroscopy to test the interaction of tetradecyltrimethylammonium bromide as a surfactant denaturant of Hb. They utilized MCR-ALS to monitor different aspects of Hb species during the unfolding of Hb (17).

In the present study, we utilized MCR-ALS on UV-Vis spectroscopic data to study the interaction of Hb with SDS at different concentrations of AA. The resolved concentration and spectral profiles provided information about the effects of AA on the interaction of SDS with Hb.

## Materials and Methods
### Reagents and Solutions
SDS (Grade: Ph. Euro), sodium chloride (NaCl), methanol (MeOH), and ammonium sulfate ($NH_4SO_4$) were obtained from Merck Chemical Co., Germany. Ascorbic acid ($C_6H_8O_6$, 99%), disodium hydrogen phosphate ($Na_2HPO_4$, 98%), barbital buffer (B 5934, pH 8.6), acetic acid ($CH_3COOH$, 99.7%), ponceau S solution ($C_{22}H_{12}N_4Na_4O_{13}S_4$, 75%) cellulose acetate, and dialysis bag (50 kDa) were purchased from Sigma-Aldrich, Germany.

### Hemoglobin Purification
Blood was collected from a healthy, non-smoker donor of genotype HbA (17). Plasma components were removed by centrifugation at 1800 g for 5 minutes. The packed red cells were washed out by adding 10 volumes of an isotonic saline solution (NaCl 0.9%) and proceeded with 5600 g centrifugation for 15 minutes at 4ºC. Once the supernatants outpoured, 5 volumes of phosphate buffer (200 mM, pH 7.4) was added to the remaining and centrifuged at 2800 g for 15 minutes. To lyse packed red cells, 5 volumes of deionized water were added to them and centrifuged at 12 000 g for 20 minutes at 4ºC. Then stroma was removed. The Hb solution was then treated with 4 mL of ammonium sulfate solution (20% w/w) and proceeded with 12 000 g centrifugation for 60 minutes at 2ºC. The obtained supernatant was then placed in dialysis bag and held in phosphate buffer solution at 4ºC for 48 hours. The buffer solution was changed every 7 hours (17,18).

### Acetate-Cellulose Electrophoresis
The purity of Hb was tested using HELENA gel electrophoresis (TITAN plus). Barbital buffer and ponceau S solution were used for protein separation and staining, respectively. Once Hb samples were spotted on the gel, electrophoresis was carried out for 20 minutes at 180 mV. Afterward, gel was placed on ponceau S solution for 6 minutes. background color of the gel was removed using acetic acid solution (5% v/v) and methanol. Finally, the gel was dried using oven (50ºC for 10 minutes). The prepared gel revealed a single band of HbA indicating >98% purity (see Figure 1).
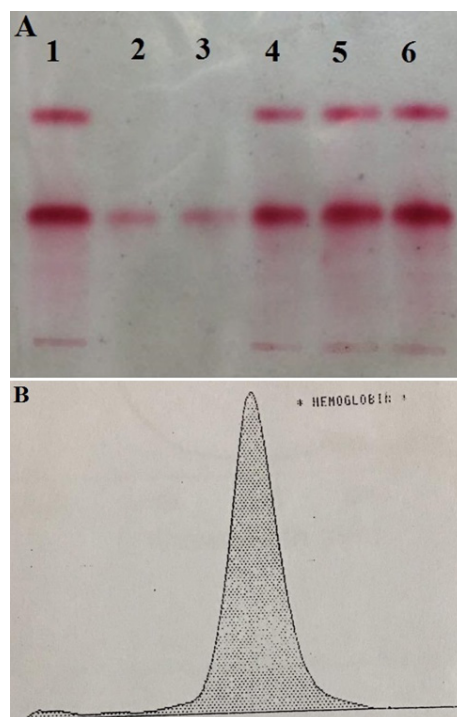


**Figure 1.** Acetate-Cellulose Electrophoresis, (A) Profile of Purified Hb Samples (Bands 2 and 3) and Total Blood Samples (Bands 1, 4, 5, 6), (B) Densitogram of Purified Hb.

## UV-Vis Spectroscopy

UV-Vis electronic absorption spectra were recorded in the ranges of 350 to 650 using PerkinElmer (LAMBDA 25) double beam instrument. As the Hb samples had intense color, the purified Hb was diluted 400 times using deionized water.

Stoke solutions of SDS (50 mM) and AA (50 mM) were used to investigate the interaction of HbA with SDS (1-5 mM) in the presence of AA (1-5 mM). Experiments were designed according to Table 1. Every experiment was replicated 3 times. The concentration range for SDS was selected based on the literature survey (8,19). However, AA concentration was chosen based on a preliminary study. Spectrophotometric measurements were carried out immediately after the solution was prepared.

## Data Analysis

All data were converted to ACSII to be compatible with MATLAB software (MATLAB 2014b, MathWorks Inc.; www.mathworks.com). The number of chemical components was estimated using principal component analysis (20), orthogonal projection approach (OPA) (21), and subspace comparison (22). The initial estimates of concentration and spectral profiles from four methods of evolving factor analysis (23), simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) (24), simplified Borgen method (25), and OPA were used as input for MCR-ALS algorithm. All programs were coded in MATLAB environment. The MCR-ALS program is

available on homepage of multivariate curve resolution coded by Tauler et al (https://www.cid.csic.es/homes/rtaqam)

In order to assess the number of chemical components and their spectral (identities) and concentration profiles during the oxidation process, MCR techniques were applied on the collected UV-Vis spectra. Before that, data matrix was split into 6 sub-matrices of D0, D1, D2, D3, D4, and D5, based on the concentration of AA. Sub-matrix D0 had zero concentration of AA, while D1, D2, D3, D4, and D5 were recorded from solutions that had 1, 2, 3, 4, and 5 mM of AA, respectively. Details of each matrix are presented in Table S1 (Supplementary file 1). MCR techniques were applied on each sub-matrix, separately.

## Results and Discussion

### UV-Vis Spectra

The spectroscopy is a well-adopted tool to study the changes in protein conformation upon binding with surfactants (26). The absorption coefficients of SDS and AA were negligible between 350 nm and 650 nm (spectra shown in Figure S1, Supplementary file 1), thus we could clearly study the interaction of SDS with oxyHb in the presence or absence of AA. The UV-Vis spectrum of purified Hb is shown in Figure 2. OxyHb shows two characteristic peaks at 575 nm and 540 nm due to Q band and ligand to metal charge transfer. Soret band of oxyHb at 408 is due to the heme group of protein which is embedded in the hydrophobic pocket formed by protein

**Table 1.** Design of the Experiment

| Run | HbA (mL) | SDS[a] µL (mM) | AA[b] µL (mM) | DW[c] (µL) | Run | HbA (mL) | SDS[a] µL (mM) | AA[b] µL (mM) | DW[c] (µL) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 0 (0) | 0 (0) | 1000 | 20 | 2 | 0 (0) | 0 (0) | 1000 |
| 2 | 2 | 60 (1) | 0 (0) | 940 | 21 | 2 | 240 (4) | 0 (0) | 760 |
| 3 | 2 | 60 (1) | 60 (1) | 880 | 22 | 2 | 240 (4) | 60 (1) | 700 |
| 4 | 2 | 60 (1) | 120 (2) | 820 | 23 | 2 | 240 (4) | 120 (2) | 640 |
| 5 | 2 | 60 (1) | 180 (3) | 760 | 24 | 2 | 240 (4) | 180 (3) | 580 |
| 6 | 2 | 60 (1) | 240 (4) | 700 | 25 | 2 | 240 (4) | 240 (4) | 520 |
| 7 | 2 | 60 (1) | 300 (5) | 640 | 26 | 2 | 240 (4) | 300 (5) | 460 |
| 8 | 2 | 120 (2) | 0 (0) | 880 | 27 | 2 | 300 (5) | 0 (0) | 700 |
| 9 | 2 | 120 (2) | 60 (1) | 820 | 28 | 2 | 300 (5) | 60 (1) | 640 |
| 10 | 2 | 120 (2) | 120 (2) | 760 | 29 | 2 | 300 (5) | 120 (2) | 580 |
| 11 | 2 | 120 (2) | 180 (3) | 700 | 30 | 2 | 300 (5) | 180 (3) | 520 |
| 12 | 2 | 120 (2) | 240 (4) | 640 | 31 | 2 | 300 (5) | 240 (4) | 460 |
| 13 | 2 | 120 (2) | 300 (5) | 580 | 32 | 2 | 300 (5) | 300 (5) | 400 |
| 14 | 2 | 180 (3) | 0 (0) | 820 | 33 | 2 | 0 (0) | 0 (0) | 1000 |
| 15 | 2 | 180 (3) | 60 (1) | 760 | 34 | 2 | 0 (0) | 60 (1) | 940 |
| 16 | 2 | 180 (3) | 120 (2) | 700 | 35 | 2 | 0 (0) | 120 (2) | 880 |
| 17 | 2 | 180 (3) | 180 (3) | 640 | 36 | 2 | 0 (0) | 180 (3) | 820 |
| 18 | 2 | 180 (3) | 240 (4) | 580 | 37 | 2 | 0 (0) | 240 (4) | 760 |
| 19 | 2 | 180 (3) | 300 (5) | 520 | 38 | 2 | 0 (0) | 300 (5) | 700 |

[a] Sodium dodecyl sulfate; [b] Ascorbic acid; [c] Distilled water.

backbone (27). Thus appearance of Soret band confirms the folded structure of oxyHb. Any disturbance or changes on the Soret band reveals the reduction in α-helix contents of Hb (28).

A glance at the overall UV-Vis spectra collected from all solutions (see Table 1 and Figure 3) revealed some shifts and changes in the intensities of Soret and Q bands of oxyHb. Previous studies have demonstrated that the intensity of Soret band will change or diminish if the protein is partially or completely denatured (17,29). In our experimental result, shown in Figure 3, the intensity of Soret band decreased, which was due to the unfolding of oxyHb and its oxidation to metHb and hemichrome. Furthermore, changes were observed for peaks at 575 and 540 nm due to the interaction.

### Multivariate Curve Resolution
A critical step of curve resolution is determination of the number of chemical components. In this study, eigenvalues and subspace comparison plots were applied for this purpose. Table 2 and Figure S2 (Supplementary file 1) demonstrate 3 significant components for D0.

It should be noted that D(k) is a measure of that part of subspace that is in the orthogonal complement of the other and $\sin^2(\theta)$ is the largest principal angle between subspaces and shows the degree of agreement between variables for the determination of the number of chemical components. The number of components is equal to the largest numbers that show D(k) and $\sin^2(\theta)$ close to zero (15,22). The same procedure has been implemented on the other data matrices.

The initial estimates of spectral profiles were made using the SIMPLISMA and OPA methods. In order to resolve the data using MCR-ALS, non-negativity constraint was applied on both concentration and spectral profiles and unimodality constraint was employed on the concentration profile. The concentration and spectral profiles for three components of D0 data matrix are presented in Figure 4.
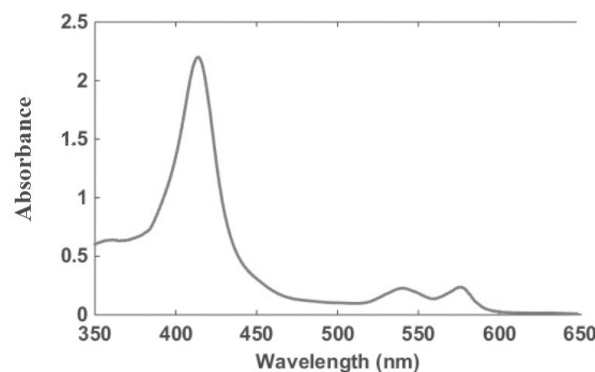
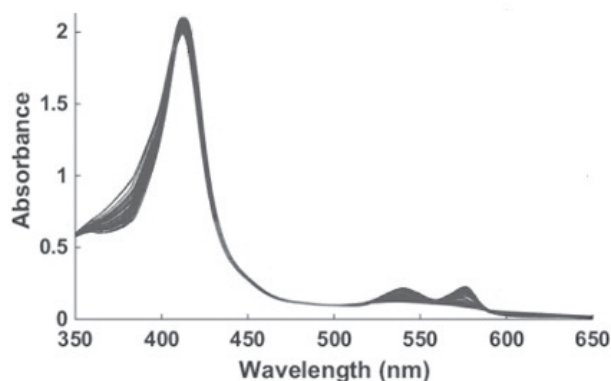The lack of fit (LOF%) and $R^2$ values of the MCR-ALS



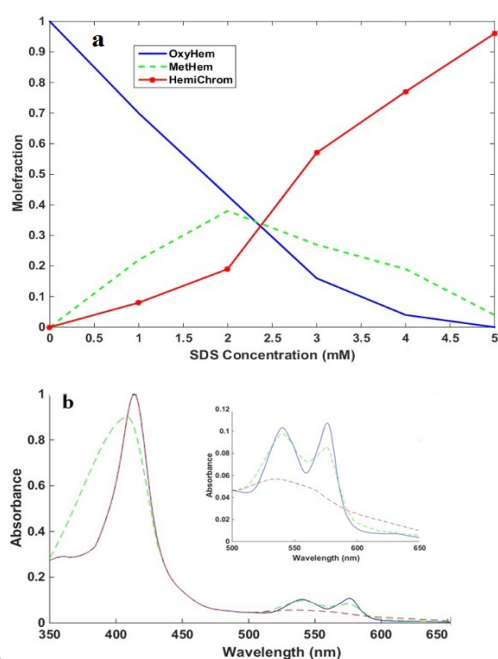**Figure 2.** UV-Vis Spectrum of Purified Oxy-Hb.



**Figure 3.** UV-Vis Spectra of Hemoglobin Interacting With Different Concentrations of SDS (0-5 mM) and AA (0-5 mM).

model are presented in Table 3.

All data matrices revealed the lack of fit values lower than 2%, which shows good agreement between the experimental data and resolved profiles.

The resolved concentration profiles of all data matrices are illustrated in Figure 5.

The mole fraction of oxyHb increased upon the presence of AA (Figure 5a-c). In other words, AA showed an inhibitory effect up to the concentration level of 3 mM. However, at a higher concentration of AA, a decrease in the concentration of oxyHb was observed. This could be due to the increase in the acidity of the mixture upon the presence of AA. A comparison between the concentration of oxyHb in the presence and absence of AA at zero concentration of SDS confirmed that the mole fraction of oxyHb reduced in the presence of AA. Therefore, AA demonstrated a dual effect on the interaction of Hb with SDS. On the one hand, AA disturbed the interaction of SDS with Hb and represented an antioxidative effect, while on the other hand, it caused a small decrease in the mole fraction of oxyHb. Salehi et al (8) proved that $H_2O_2$ produced during the interaction of Hb with SDS caused heme degradation, and subsequently, oxyHb denaturation and conversion to other components. Mass spectrometric studies (9,10) have reported that in the presence of $H_2O_2$, AA underwent oxidation reactions to form dehydroascorbic acid, tetrahydroxy-diketohexanoic

**Table 2.** Eigenvalues of Matrix D0

| Number of Components | Eigenvalue |
|---|---|
| 1 | $1.72 \times 10^2$ |
| 2 | 0.35 |
| 3 | 0.07 |
| 4 | $3.05 \times 10^{-8}$ |
| 5 | $1.92 \times 10^{-13}$ |

**Table 3.** Lack of Fit (LOF%) and R2 Values of MCR-ALS Models

| Data Matrix | LOF% | $R^2$ | Data Matrix | LOF% | $R^2$ |
|---|---|---|---|---|---|
| D0 | 0.458 | 99.9979 | D3 | 0.877 | 99.9923 |
| D1 | 0.787 | 99.9937 | D4 | 1.85 | 99.9656 |
| D2 | 0.332 | 99.9989 | D5 | 1.70 | 99.9712 |

**Figure 4.** (a) Concentration Profiles Resolved Using MCR-ALS Implemented on D0 Data Matrix, (b) Spectral Profiles of Components Resolved Using MCR-ALS Implemented on D0 Data Matrix .
*Note*. Inset is the Expanded Region of 500 to 650 nm. Blue: OxyHb, Green: MetHem, and Red: Hemichrome.
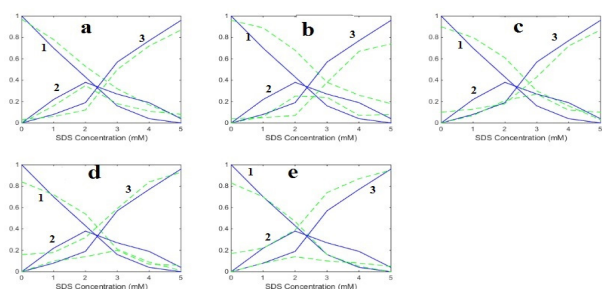


**Figure 5.** Concentration Profiles of Three Resolved Components Upon Interaction of Hemoglobin With SDS (0- 5 mM) at Different Concentrations of AA.
*Note*. Solid line (without AA) and dashed lines: (a) 1 mM, (b) 2 mM, (c) 3 mM, (d) 4 mM, (e) 5 mM. Curve 1: OxyHb, Curve 2: MetHb, Curve 3: Hemichrome.

acid, and threonic acid. Therefore, the concentration of $H_2O_2$ was reduced by AA and then the effective interaction of $H_2O_2$ with oxyHb was prevented.

Furthermore, as the Gibbs free energy for the SDS micelle formation became more negative in the presence of AA, the critical micelle concentration of SDS reduced (30). Therefore, in the presence of AA, SDS started to form micelles at concentrations lower than normal. This could reduce the available SDS to interact with oxyHb.

## Conclusion

The interaction of Hb with SDS in the presence of different concentrations of AA was examined in this study. As $H_2O_2$ was produced upon the interaction of oxyHb with SDS, AA effectively interacted with $H_2O_2$ and reduced

the destructive effects of $H_2O_2$ on oxyHb. In fact, AA underwent oxidation reactions with $H_2O_2$ and therefore reduced the concentration of $H_2O_2$. Furthermore, AA diminished the available SDS by reducing the critical micelle concentration of SDS. Therefore, AA protects oxyHb by reducing the available $H_2O_2$ and SDS. However, due to the acidic nature of AA, oxyHb undergoes denaturation at higher concentrations of AA.

## Authors' Contributions

RM: Data acquisition, data analysis and interpretation, drafting of the manuscript, obtaining funding. EM: Administrative, technical, or material support, supervision. HEN: Conception and design, data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, technical, or material support, supervision.

## Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

## Ethical issues

Not applicable.

## Funding

## Supplementary files

Supplementary file 1 contains Table S1, Figure S1 and Figure S2.

**References**
1. Wu Y, Cui W, Zhou S, Ma F. The binding behavior of itraconazole with hemoglobin: studies from multi-spectroscopic techniques. Spectrochim Acta A Mol Biomol Spectrosc. 2014;131:407-12. doi: 10.1016/j.saa.2014.04.092.
2. Liu W, Guo X, Guo R. The interaction of hemoglobin with hexadecyltrimethylammonium bromide. Int J Biol Macromol. 2005;37(5):232-8. doi: 10.1016/j.ijbiomac.2005.11.007.
3. Liu W, Guo X, Guo R. The interaction between hemoglobin and two surfactants with different charges. Int J Biol Macromol. 2007;41(5):548-57. doi: 10.1016/j.ijbiomac.2007.07.006.
4. Kaca W, Roth RI, Vandegriff KD, Chen GC, Kuypers FA, Winslow RM, et al. Effects of bacterial endotoxin on human cross-linked and native hemoglobins. Biochemistry. 1995;34(35):11176-85. doi: 10.1021/bi00035a024.
5. Ray A, Friedman BA, Friedman JM. Trehalose glass-facilitated thermal reduction of metmyoglobin and methemoglobin. J Am Chem Soc. 2002;124(25):7270-1. doi: 10.1021/ja0201348.
6. Akram M, Anwar S, Bhat IA, Kabir-Ud-Din. Unraveling the interaction of hemoglobin with a biocompatible and cleavable oxy-diester-functionalized gemini surfactant. Int J Biol Macromol. 2017;96:474-84. doi: 10.1016/j.ijbiomac.2016.11.112.
7. Nagababu E, Rifkind JM. Heme degradation by reactive oxygen species. Antioxid Redox Signal. 2004;6(6):967-78. doi: 10.1089/ars.2004.6.967.
8. Salehi N, Moosavi-Movahedi AA, Fotouhi L, Yousefinejad S, Shourian

M, Hosseinzadeh R, et al. Heme degradation upon production of endogenous hydrogen peroxide via interaction of hemoglobin with sodium dodecyl sulfate. J Photochem Photobiol B. 2014;133:11-7. doi: 10.1016/j.jphotobiol.2014.02.014.

9. Deutsch JC. Ascorbic acid oxidation by hydrogen peroxide. Anal Biochem. 1998;255(1):1-7. doi: 10.1006/abio.1997.2293.

10. Bradshaw MP, Barril C, Clark AC, Prenzler PD, Scollary GR. Ascorbic acid: a review of its chemistry and reactivity in relation to a wine environment. Crit Rev Food Sci Nutr. 2011;51(6):479-98. doi: 10.1080/10408391003690559.

11. Retsky KL, Frei B. Vitamin C prevents metal ion-dependent initiation and propagation of lipid peroxidation in human low-density lipoprotein. Biochim Biophys Acta. 1995;1257(3):279-87. doi: 10.1016/0005-2760(95)00089-u.

12. Farriol M, Mourelle M, Schwartz S. Effect of vitamin C and vitamin E analog on aged fibroblasts. Rev Esp Fisiol. 1994;50(4):253-7.

13. Maellaro E, Bello BD, Comporti M. Protection by ascorbate against apoptosis of thymocytes: implications of ascorbate-induced nonlethal oxidative stress and poly(ADP-ribosyl)ation. Exp Cell Res. 1996;226(1):105-13. doi: 10.1006/excr.1996.0208.

14. Gemperline P. Practical Guide to Chemometrics. Boca Raton, FL: CRC Press; 2006.

15. Mojtahedi M, Parastar H, Jalali-Heravi M, Chamani J, Chilaka FC, Moosavi-Movahedi AA. Comparison between two different hemichromes of hemoglobins (HbA and HbS) induced by n-dodecyl trimethylammonium bromide: chemometric study. Colloids Surf B Biointerfaces. 2008;63(2):183-91. doi: 10.1016/j.colsurfb.2007.11.018.

16. Jaumot J, Gargallo R, de Juan A, Tauler R. A graphical user-friendly interface for MCR-ALS: a new tool for multivariate curve resolution in MATLAB. Chemometr Intell Lab Syst. 2005;76(1):101-10. doi: 10.1016/j.chemolab.2004.12.007.

17. Fotouhi L, Yousefinejad S, Salehi N, Saboury AA, Sheibani N, Moosavi-Movahedi AA. Application of merged spectroscopic data combined with chemometric analysis for resolution of hemoglobin intermediates during chemical unfolding. Spectrochim Acta A Mol Biomol Spectrosc. 2015;136(Pt C):1974-81. doi: 10.1016/j.saa.2014.10.120.

18. Riggs A. Preparation of blood hemoglobins of vertebrates. Methods Enzymol. 1981;76:5-29. doi: 10.1016/0076-6879(81)76111-1.

19. Gebicka L, Banasiak E. Interactions of anionic surfactants with methemoglobin. Colloids Surf B Biointerfaces. 2011;83(1):116-21. doi: 10.1016/j.colsurfb.2010.11.017.

20. Härdle W, Simar L. Applied Multivariate Statistical Analysis. Cham: Springer; 2007.

21. Sánchez FC, Toft J, van den Bogaert B, Massart DL. Orthogonal projection approach applied to peak purity assessment. Anal Chem. 1996;68(1):79-85. doi: 10.1021/ac950496g.

22. Shen H, Liang Y, Kvalheim OM, Manne R. Determination of chemical rank of two-way data from mixtures using subspace comparisons. Chemometr Intell Lab Syst. 2000;51(1):49-59. doi: 10.1016/s0169-7439(00)00054-x.

23. Maeder M. Evolving factor analysis for the resolution of overlapping chromatographic peaks. Anal Chem. 1987;59(3):527-30. doi: 10.1021/ac00130a035.

24. Windig W, Guilment J. Interactive self-modeling mixture analysis. Anal Chem. 1991;63(14):1425-32. doi: 10.1021/ac00014a016.

25. Grande BV, Manne R. Use of convexity for finding pure variables in two-way data from mixtures. Chemometr Intell Lab Syst. 2000;50(1):19-33. doi: 10.1016/s0169-7439(99)00041-6.

26. Vashishat R, Chabba S, Mahajan RK. Surface active ionic liquid induced conformational transition in aqueous medium of hemoglobin. RSC Adv. 2017;7(22):13041-52. doi: 10.1039/c7ra00075h.

27. Tajima H, Ikeda S, Matsuda M, Hanasaki N, Oh JW, Akiyama H. A light-emitting diode fabricated from horse-heart cytochrome c. Solid State Commun. 2003;126(10):579-81. doi: 10.1016/s0038-1098(03)00305-3.

28. Bharmoria P, Trivedi TJ, Pabbathi A, Samanta A, Kumar A. Ionic liquid-induced all-α to α+ β conformational transition in cytochrome c with improved peroxidase activity in aqueous medium. Phys Chem Chem Phys. 2015;17(15):10189-99. doi: 10.1039/c4cp06044j.

29. Peng W, Liu X, Zhang W, Li G. An electrochemical investigation of effect of ATP on hemoglobin. Biophys Chem. 2003;106(3):267-73. doi: 10.1016/s0301-4622(03)00212-6.

30. Cid A, Mejuto JC, Orellana PG, López-Fernández O, Rial-Otero R, Simal-Gandara J. Effects of ascorbic acid on the microstructure and properties of SDS micellar aggregates for potential food applications. Food Res Int. 2013;50(1):143-8. doi: 10.1016/j.foodres.2012.10.009.