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RESEARCH ARTICLE

The Effect of Water of Crystallization on Isomerization of Liposome using Sodium Sulfide Nonahydrate

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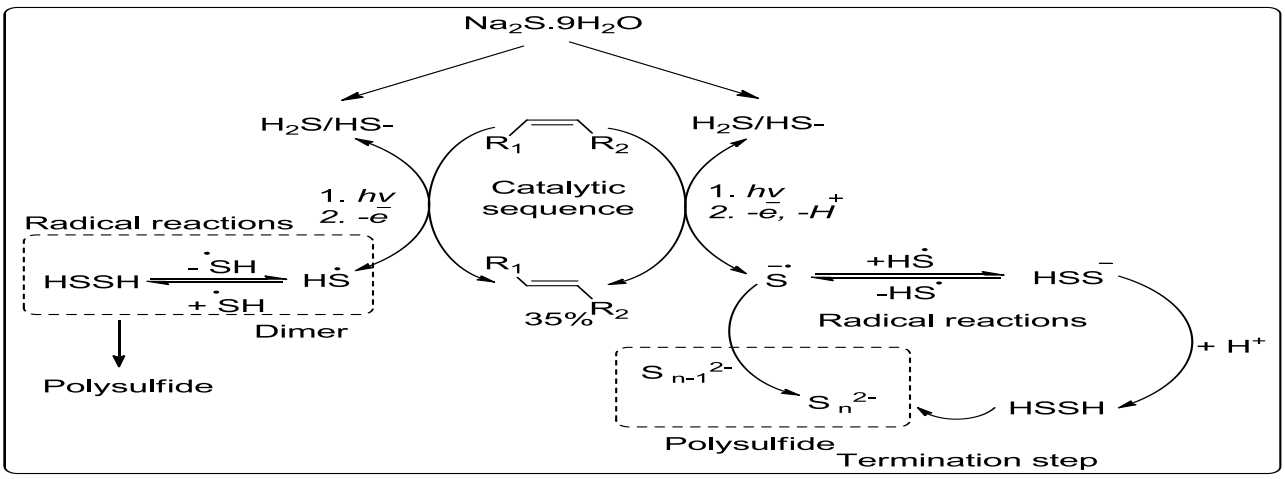
Abstract

The endogenous sulfide radicals ($HS^{\cdot}$/ $S^{\cdot-}$) play an important role in the biological system. They have cyto-protective antioxidant and pro-oxidants adverse effect. Sulfide radicals can produce isomerization of fatty acid physiologically and pathologically. In terms of biomimetic model using liposome, these radicals can produce *trans* fatty acid. The production of thiyl radical via photolysis was reported using sodium sulfide nonahydrate ($Na_2S.9H_2O$) *'H_2S donor'* in a phosphate buffer (PH=5.0). The efficacy of $Na_2S.9H_2O$ to produce thiyl radical ($HS^{\cdot}$/ $S^{\cdot-}$) was evaluated by GC using POPC vesicle in phosphate buffer (PH=5). It has been noticed that the maximum isomerization rate was up to 35% of trans oleic acid after (30 minutes). The declining in the percent of isomerization on what was previously reported is attributing to the low diffused concentration of the H_2S, that is due to the crystallization nature of sodium sulfide nonahydrate as well as the low solubility profile of hydrated sodium sulfide. Furthermore, it also refers to the high affinity of thiyl radicals to make weak isomerizing species such as sulfide dimer (HSSH) and polysulfide (S_n^{2-}) in a heat releasing process. Polysulfides themselves have the ability to scavenge the thiyl radical ($HS^{\cdot}$/ $S^{\cdot-}$) and then inhibit the isomerization process (Scheme 1).

Keywords: *Liposomes, Sodium sulfide nonahydrate, Water of crystallization, Trans oleic acid, Trans fatty acid (TFA), Omega-9, POPC vesicle, Polysulfide, Thiyl radical.*



Scheme 1: Reaction of liposomal (POPC) vesicle with thiyl radical and formation the polysulfide

Introduction

Water is a fascinating molecule that can interact within different organic compounds. It can systemically organize itself inside or around the lattice structure of other molecules and hence forming the hydrated form. The water of hydration plays a critical role in the solubility profile and the chemical stability of the organic compounds [1]. Sodium sulfide (Na_2S) can be converted to a hydrated form, to increase its chemical stability, via incorporating nine water molecules to its original structure and becomes ($Na_2S.9H_2O$) [1]. Sodium sulfide nonahydrate is crystalline compound with polyhedral existence [2, 3].Every sodium ion is surrounding by four H_2O molecules [2].

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The octahedral form of [NaOH] would comprise two chains in which, one sodium ion is surrounded by four water molecule such as $[Na(H_2O)_4]$ wherease the another ion is surrounded by five water molecules as $[Na(H_2O)_5]$ [2]. These chains are connected via hydrogen bonds with sulfide ion [2, 4]. We used sodium sulfide nonahydrate as 'H_2S donor' and liposome as a models of biological membranes and as a source of cis-fatty acid in a biomimetic model [5, 6].Sodium sulfide nonahydrate ($Na_2S.9H_2O$) can release H_2S gas [4]. The ubiquitous reactivity of H_2S makes it as a reactive intermediate in the synthetic organic chemistry and biological process.

Thiol plays a crucial role as antioxidant. It protects the tissue from oxidative damage and preserves the redox status of the cells [7]. H_2S is present in the brain with range (10- 160) µm. It is manufactured from the amino acid *L*-cysteine and homocysteine by cystathionine-*β*-synthetase inside cerebellum and hippocampus. The low endogenous level of H_2S in human brain is associated with Alzheimer disease [8]. In context of repair reaction, H_2S can effectively stop the cellular radical cascade via donating its hydrogen and converting to ($HS^{\cdot}$/ $S^{\cdot-}$) [5]. The radicals that derived from thiol often termed as a thiyl radical [9].

The S-H hemolytic bond dissociation energy (BDE) is about 91 kcal/ mol in hydrogen sulfide [9, 10]. Thiol can present in biomolecules as disulfide (RSSR), thioester (RSCOR) and thioether functionalities (RSR) [11].Polysulfides are the compounds that have at least 3 sulfurs bounded to each other in sequence with the end sulfur bounded to a hydrocarbon of 3 carbons [12].

Furthermore, thiyl radical can also be produced as a diffusible gas. Lykakis et al. (2007) approves the isomerization of fatty acid, namely 1-palmitoyl- 2-oleoylphosphotidylcholine (POPC), to *cis/ trans* isomers after using a freshly prepared solution of sodium sulfide (Na_2S) with radiolysis (Dose = ~ 10.5 Gy min^{-1}) or photolysis (5.5W, low pressure Hg lamp) [11, 13].

It is also emphasized that fatty acid isomerization has been decreased with increasing PH of the reaction mixture and/or the concentration of H_2S and Na_2S, as well [11, 13].

It is also emphasized that the double bonds located closest to the membrane polar region in phospholipid compartment of liposome vesicle are the most involved in the reaction with the diffusible thiyl radicals [11]. In terms of trans fatty acid (TFA), the presence of trans lipids in the cells is considered as a signaling marker in the human. They are associated with coronary heart diseases, higher serum lipoprotein, cholesterol and triglyceride levels. Human exposes to trans lipids exogenously and endogenously.

The exogenous exposure is resulting from destroying cis- lipid during industrial deodorization/ purification process as well as during the heating process of food [6, 14]. However, prokaryotes such as *Vibro cholera* and *Psedomoneus aerogenosa* produce the trans lipid to resist unpleasant environmental condition via decreasing their membrane fluidity and permeability.

Regarding the endogenous source of TFA in human, they can be produced due to the radical stress during physiological and pathological condition using, most importantly, thiyl centered radical ($HS^{\cdot}$/ $S^{\cdot-}$) [5, 6]. This paper is conducted to report the production of thiyl radical using sodium sulfide nonahydrate in a phosphate buffer (PH=5) and the efficiency of producing thiyl radical was tested and evaluated by isomerizing cis-oleic acid of liposme (POPC-vesicle).

Experimental Section

All materials such as POPC, $Na_2S.9H_2O$ (98%) were bought from Sigma Aldrich and used without further purification. All solvents such as methanol, n-hexane, HCl and chloroform were bought from Sigma Aldrich. The phosphate buffer (Na_2HPO_4) was prepared by dissolve a phosphate buffer tablet in mili Q water (200 mL) and the PH was adjusted to PH= 5. Thiyl radical was produced by direct photolysis using UV- light (5.5 W, λ = 250- 260 nm). All experiments were accomplished under nitrogen gas. The liposomes were prepared by use Avanti extruder. All samples were examined by GC (Agilent Gas Chromatography) for 30 minutes/ sample.

Production of Liposomes

In a test tube 2 mL, POPC (50 mg, 0.07 mmol) was dissolved in chloroform (1 mL). POPC solution was evaporated slowly using

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N2 gas and the colorless film on the wall of tube was obtained. Then, the tube was put under vacuum for (20) minutes.

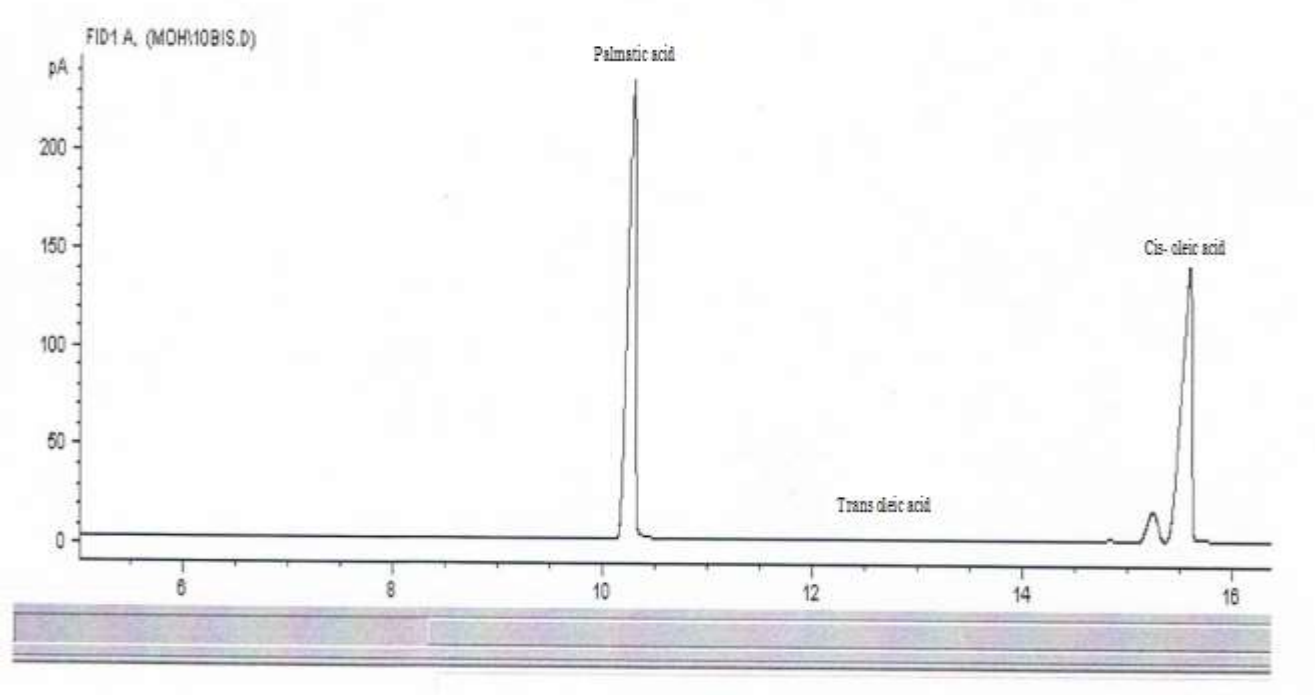
Milli Q water (1 mL) was added to the tube and stirred for (5 minutes) to produce a white suspension of POPC. The suspension was transferred to an Avanti Extruder and injected for 19 times through (100 nm) film. Then, the liposome was collected in small vials and kept in the fridge.

Isomerization of Liposomes using a UV Lamp

Liposomal POPC (184.2 µL) was added to phosphate buffer (6 mL, PH= 5). The reaction mixture was left flushing under the nitrogen atmosphere for (20) minutes. Then, $Na_2S.9H2O$ (0.2 Mm, 10 µL) was added to reaction mixture and the reaction was left flushing under nitrogen atmosphere for (5) minutes. After that, the UV light (5.5 W) was applied and five samples were collected after (10) minutes, (20) minutes, and (30) minutes respectively and so on. Then, the samples were worked up individually using $CHCl_3$:MeOH (2:1) and water. The organic layer was washed with water (100 µL x 3); and the aqueous layer was re-extracted by using $CHCl_3$:MeOH (2:1) (100 µL x 2). Then, the organic layer was dried using anhydrous Na_2SO_4, filtered and evaporated and putted under vacuum for (20) minute to give a colorless layer of isomerized liposomes.

Transesterification of Isomerized Liposomes

The isomerized layer of liposome was dissolved in MeOH/KOH (0.5 M, 200 µL). The reaction mixture was stirred by use a sonicator for (10) minutes at 800 RPM. Then, *n*- hexane (250 µL) was added and the mixture was stirred for extra (2) minutes. Milli Q water (100 µL) was add to quench reaction. The organic layer was washed with the water (100 µL x 3) and the aqueous layer was re-extracted by n-hexane (300 µL x 3). Then, the organic layer was dried by using Na_2SO_4 anhydrous, filtered and then evaporate to get a colorless layer of methyl ester. Then, each sample was inject to the GC for 30 minutes by use n-hexane as a solvent.



#	Time	Area	Height	Width	Area%	Symmetry
1	15.242	140.2	17.4	0.1341	**12.932**	1.532
2	15.583	944.2	140.5	0.112	**87.068**	3.852

Figure 1: Isomerization of cis- oleic acid by $Na_2S.9H_2O$ after (10) minutes of photolysis

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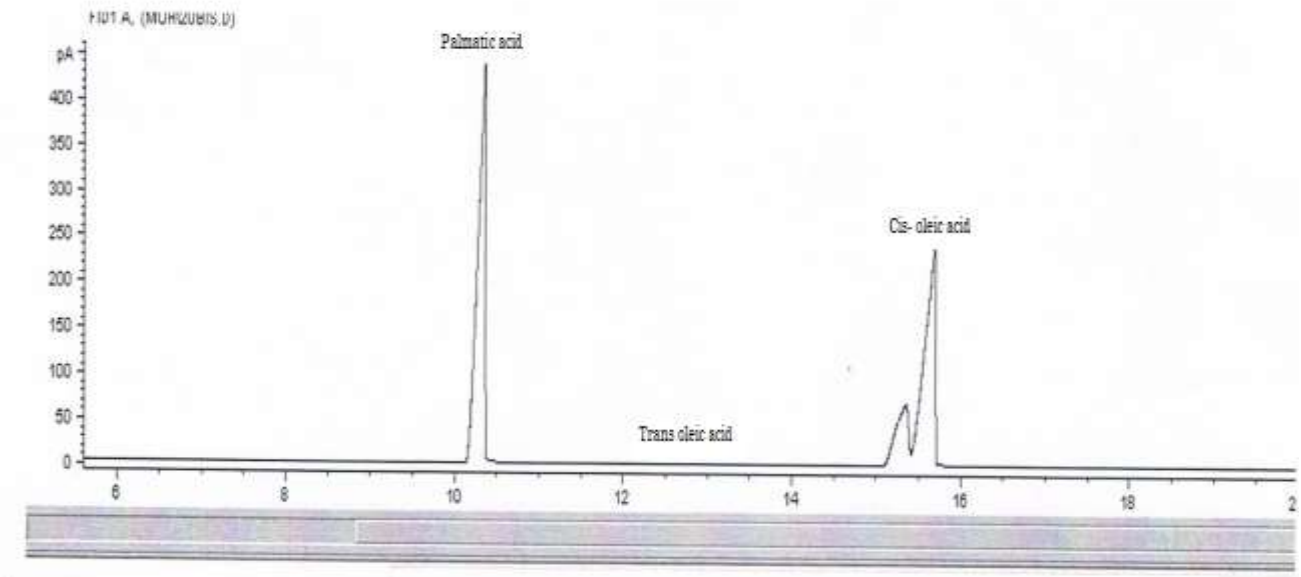
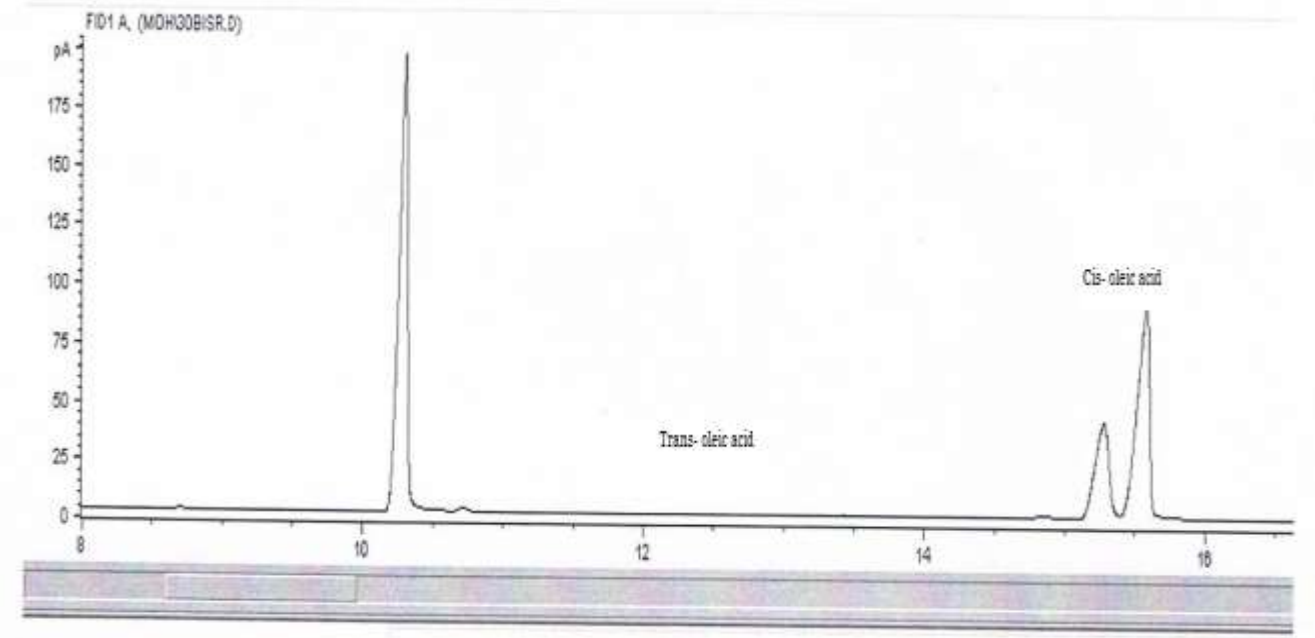


#	Time	Area	Height	Width	Area%	Symmetry
1	15.363	716.4	69.4	0.1721	**25.520**	0
2	15.694	2090.8	240.2	0.1451	**74.480**	8.385

#	Time	Area	Height	Width	Area %	Symmetry
1	**15.363**	**716.4**	**69.4**	**0.1721**	**25.52**	**0**
2	**15.694**	**2090.8**	**240.2**	**0.1451**	**74.48**	**8.385**

Figure 2: Isomerization of cis- oleic acid by $Na_2S.9H_2O$ after (20) minutes of photolysis



#	Time	Area	Height	Width	Area%	Symmetry
1	15.272	284.7	41.5	0.1144	**34.590**	1.58
2	15.578	538.5	92.2	0.0974	**65.410**	4.071

#	Time	Area	Height	Width	Area %	Symmetry
1	**15.272**	**284.7**	**41.5**	**0.1144**	**34.59**	**1.58**
2	**15.578**	**538.5**	**92.2**	**0.4097**	**65.41**	**4.071**

Figure 3: Isomerization of cis- oleic acid by $Na_2S.9H_2O$ after 30 minutes of photolysis:

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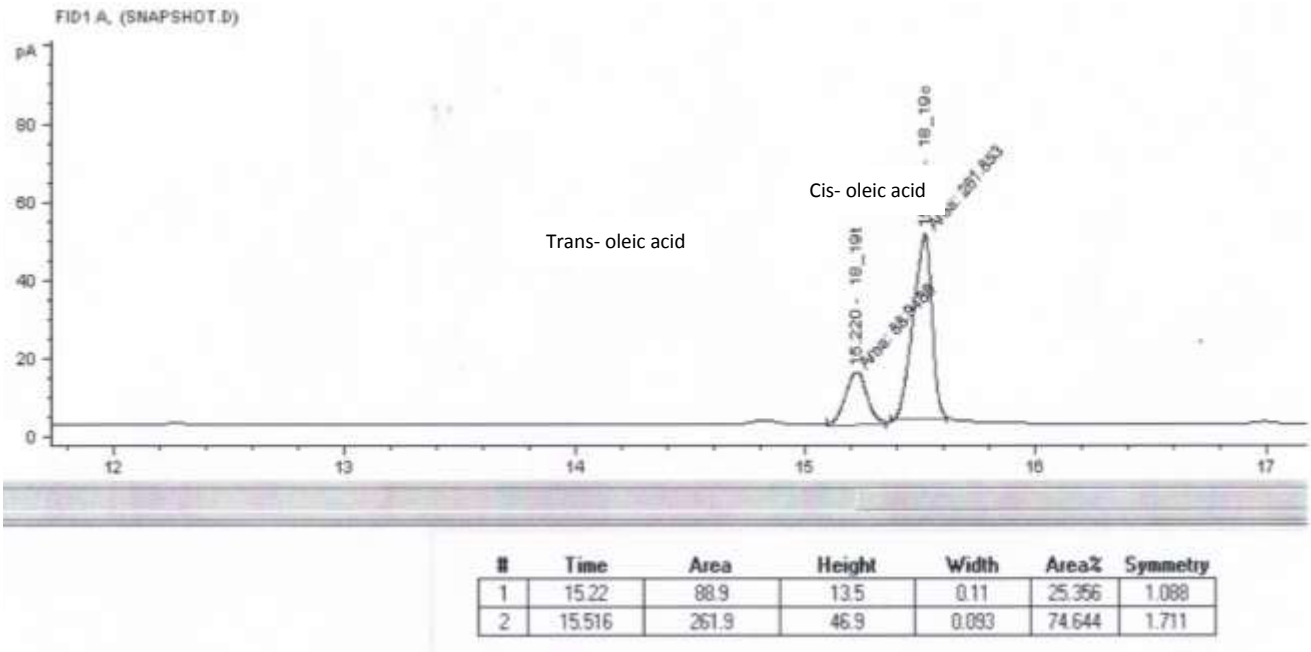
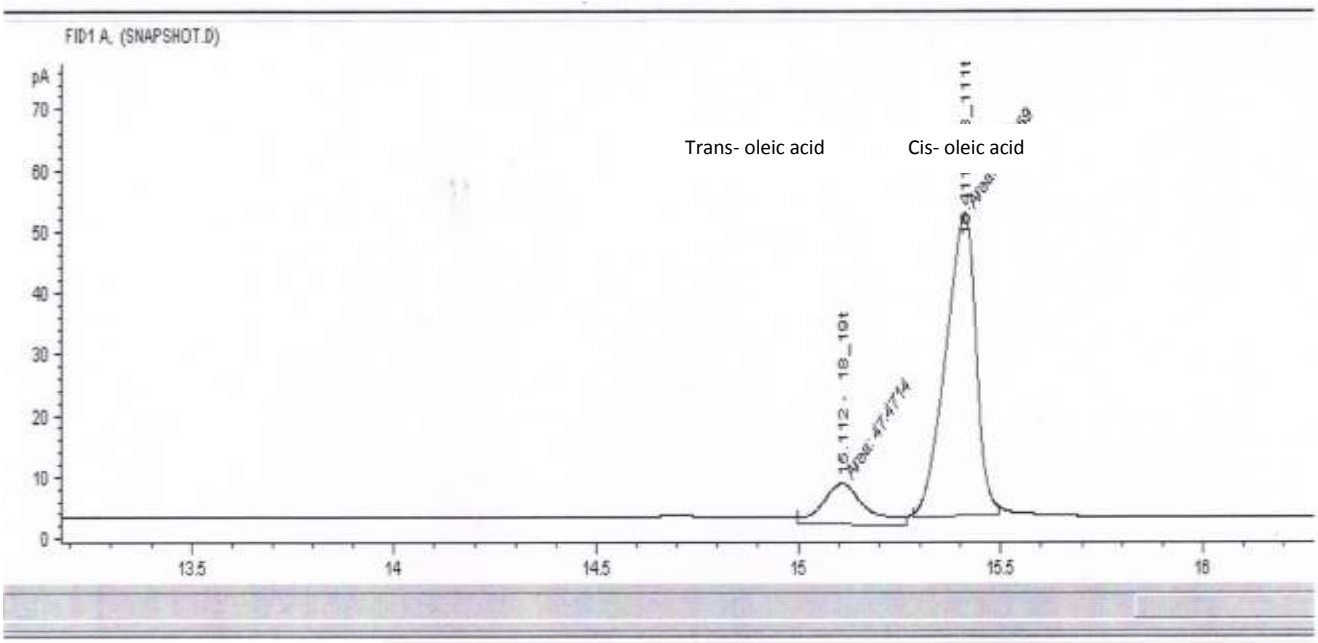


#	Time	Area	Height	Width	Area%	Symmetry
1	15.22	88.9	13.5	0.11	25.356	1.088
2	15.516	261.9	46.9	0.093	74.644	1.711

#	Time	Area	Height	Width	Area %	Symmetry
1	**15.22**	**88.9**	**13.5**	**0.11**	**25.356**	**1.088**
2	**15.516**	**261.9**	**46.9**	**0.093**	**74.644**	**1.711**

Figure 4: Isomerization of cis- oleic acid by $Na_2S.9H_2O$ after 40 minutes of photolysis



#	Time	Area	Height	Width	Area%	Symmetry
1	15.112	47.5	6.5	0.1213	15.548	0.911
2	15.411	257.9	49.3	0.0872	84.452	1.66

#	Time	Area	Height	Width	Area %	Symmetry
1	**15.112**	**47.5**	**6.5**	**0.1213**	**15.548**	**0.911**
2	**15.411**	**257.9**	**49.3**	**0.0872**	**84.452**	**1.66**

Figure 5: Isomerization of cis- oleic acid by $Na_2S.9H_2O$ after (50) minutes of photolysis

Result and Discussion

It has been approved that the addition of thiyl radical is involving in the isomerization of cis- oleic acid in biomimetic model of liposome.

Thiyl radical is reversibly added to the double bond to form an intermediate (propagation step). Then thiyl radical is ejected by β- scission to form trans oleic acid due to steric effect (Scheme 2).

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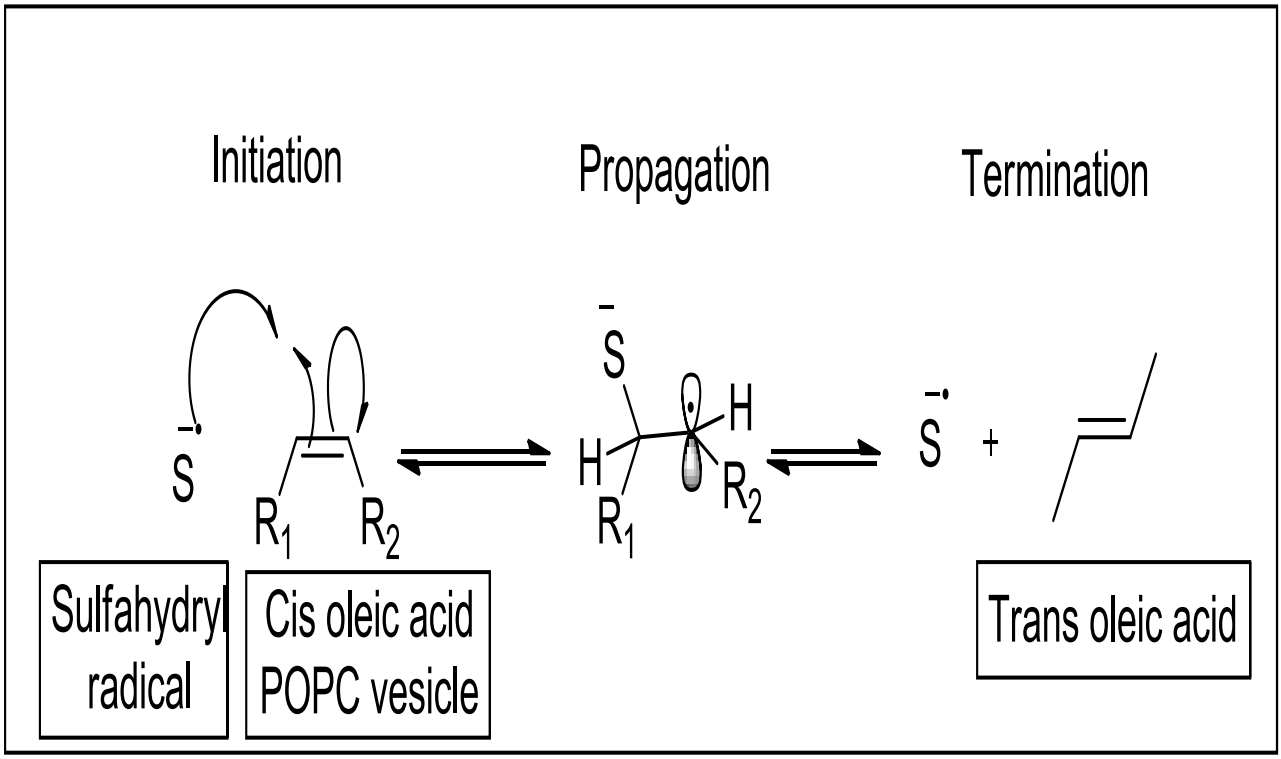
The trans isomer is considered as thermodynamic product. The reaction is depending on time, PH value and the concentration of the diffusible thiyl radical [5, 14].The high percent of isomerization was reported with anhydrous sodium sulfide[Na_2S] was more than 70 % at PH= 5 in phosphate buffer after 30 minutes of reaction [13]. However, the isomerization of cis oleic acid has not been reported using sodium sulfide nonahydrate [$Na_2S.9H_2O$].



Scheme 2: Reaction of sulfhydryl radical with Cis- oleic acid (POPC vesicle)

To study the effect of water of crystallization on the diffused concentration of thiyl radical and subsequently the percent of isomerization of oleic acid, both PH value and concentration were constant in our reaction condition. It has been reported that the reaction was accompanied with heat and the highest percent of isomerisation was up to 35% after (30) minutes of reaction (Figure 1). This could prove the ability of sodium sulfide nonahydrate to produce the thiyl radicals as well as its ability to interact with the biomimetic vesicle of liposome. However, the percent of isomerization was less in a half than what was previously reported using Na_2S [13].

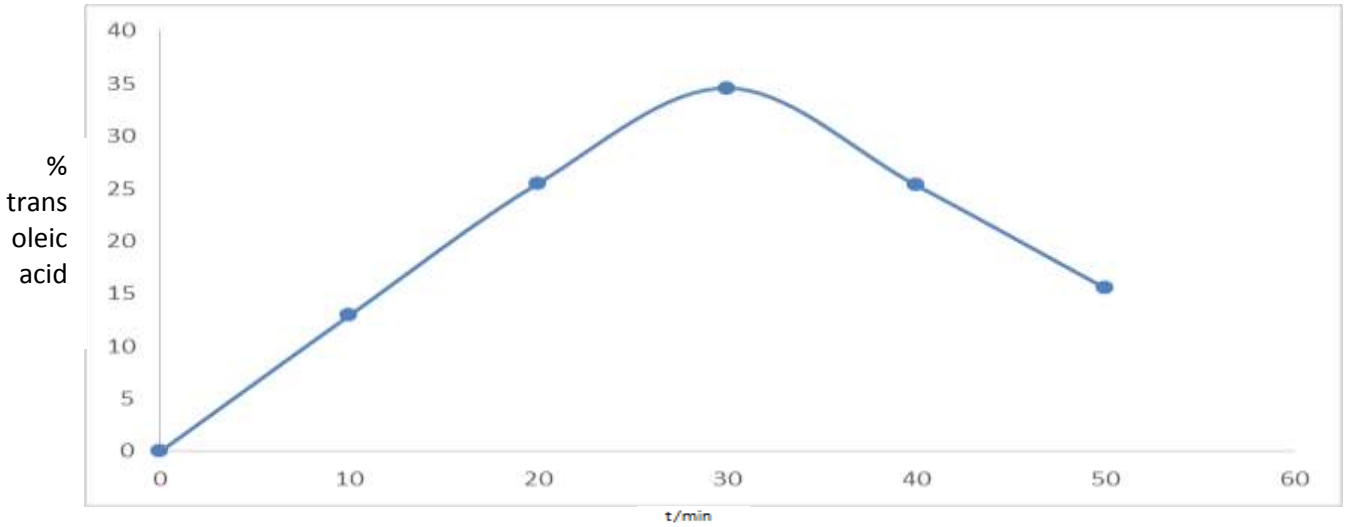


Figure 1: Formation of the trans oleic acid upon the photolysis of POPC vesicles (2 mm, PH=5) in the presence of Na2S.9H2O in a phosphate buffer

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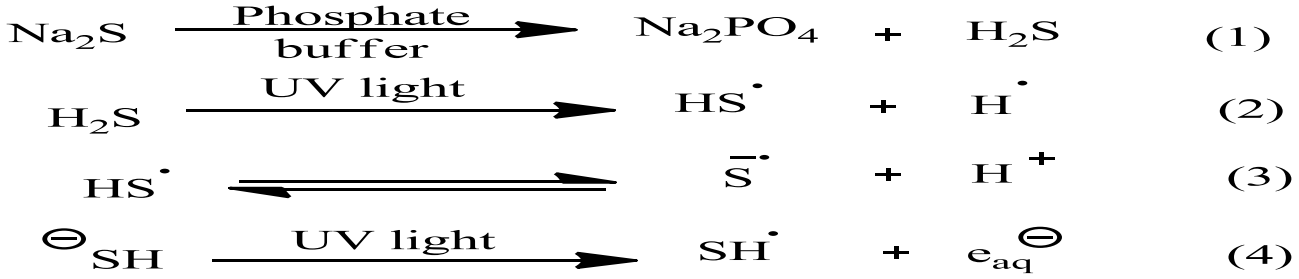
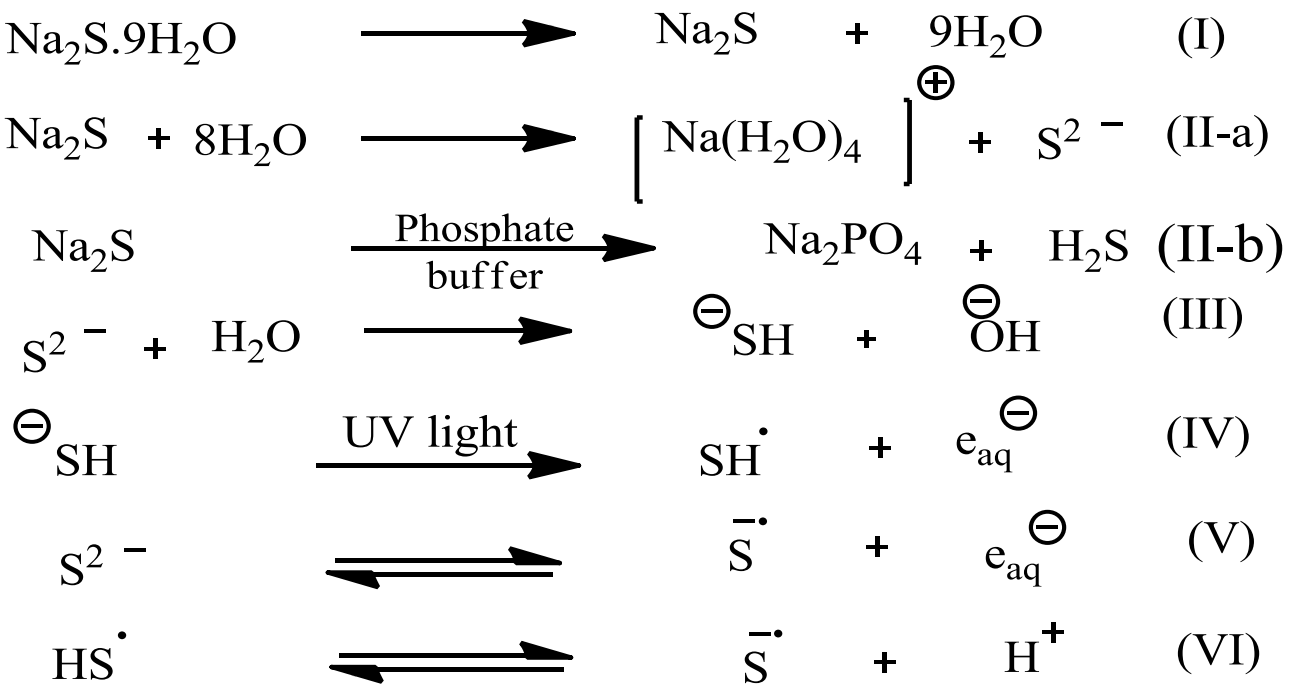
There are many reasons that can explain the dropping in the percent of isomerization. It has been noticed that sodium sulfide nonahydrate ($Na_2S.9H_2O$) production of thiyl radical was slower than the anhydrous form (Na_2S). It can deduce from the mode of dissolution of Na_2S that the thiyl radicals ($HS^{\cdot}$/ $S^{-\cdot}$) were produced faster than those produced from $Na_2S.9H_2O$. In Na_2S, thiyl radical ($HS^{\cdot}$/ $S^{-\cdot}$) were produced in eq. (2) and eq. (3); respectively (Scheme 3).



Scheme 3: The mode of dissolution of sodium sulfide (Na2S) in phosphate buffer

While for sodium sulfide nonahydrate ($Na_2S.9H_2O$), the thiyl radicals ($HS^{\cdot}$/ $S^{-\cdot}$) were produced in later photolytic steps (Scheme 4). In other words, the thiyl radicals of Na_2S were produced faster than that for ($Na_2S.9H_2O$) in a two dissociation steps during the photolysis. The thiyl radicals ($HS^{\cdot}$/ $S^{-\cdot}$) were produced in eq. (IV) and eq. (V) for sodium sulfide nonahydrate ($Na_2S.9H_2O$), (Scheme 4) [15].

We can deduce from the (scheme 4) that $Na_2S.9H_2O$ needs to dissociate from its crystalline structure and then the released Na_2S interacts with phosphate buffer, which can prolong the reaction time and delay release of thiyl radicals. The delay in thiyl radicals production can significantly effect on the production of thiyl radicals and subsequently the percent of isomerization of POPC vesicle.



Scheme 4: The mode of dissolution and photolysis of sodium sulfide nonahydrate (Na2S.9H2O) in phosphate buffer

What is more, the sharp dropping in the percent of isomerisation can attribute to the affinity of sodium sulfide nonahydrate to form a dimer, such as disulfhydryl dimer or higher order ($HSSH$ /S_n^{2-}). It has been reported that that the percent of isomerization was dropped sharply after leaving the reaction for (40- 50) minutes. The reaction was accompanied by releasing heat. This could be explained by polysulfide formation which are considered as a radical inhibitor. The polysulfides can scavenge the thiyl radicals to produce (S_n^{2-}, n= 3-8) (Scheme 5) and hence the isomerization was dropped and then stopped [13, 15].

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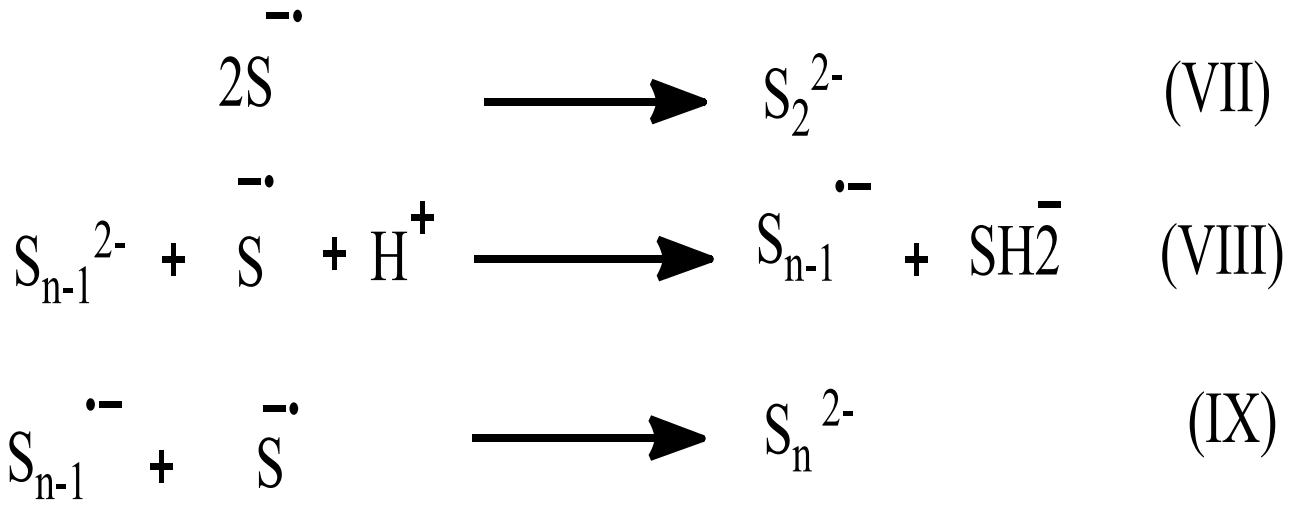

Scheme 5: Formation of polysulfide during the photolysis of Na2S.9H2O

Furthermore, the low percent of isomerzation can be refered to the low solubility profile of the hydrated form of sodium sulfide and the crystalline nature of sodium sulfide nonahydrate. In many cases the hydrated form of organic compound has a low water solubility and low dissolution rate [1]. In terms of the electrooptic coefficient, these water molecules of sodium nonahydrate are electrooptically quasi inactive molecules [3]. The hydrated form ($Na_2S.9H_2O$) is generally described as a thermodynamic product while the anhydrous form (Na_2S) is called as a kinetic product.

The kinetic product has a higher water solubility and dissolution rate than the thermodynamic product. On another hand, this can be supported by intrinsic dissolution rate of carbamazepine dihydrate which is equivalent to two third of dissolution profile for anhydrous form [1]. Moreover, the less solubility profile of the hydrated sodium sulfide could be attributed to the high hydration number which makes an equilibrium with these electrolyte at the room temperature [16]. It shows that sodium sulfide nonahydrate is surrounded by a hydrated shell of the water molecules [4].

At high temperature reaches to 48 ℃, the hydrated form of sodium sulfide is decomposed via losing their water molecules and leaving the sodium ions is directly connected to the sulfide ion; whereas it forms octahedral structure of six water molecules surrounded sodium atoms which is connecting to sulfide ion by hydrogen bonds at 110 ℃ [4]. This could be called a metal-ion coordinating water that characterized by a high dehydration temperature[1].

These shell, low dissolution rate, the crystalline nature of $Na_2S.9H_2O$ and their stability to a high temperature would negatively effect on the concentration of diffused H_2S as well as the ability of photolytic process to break S-H bond. The low concentration of thiyl radical would ultimately produce the low isomerization percentage of trans oleic acid. In conclusion, the experiment can consider as a good answer on how the condition of radical stress can work in the biological compartment. $Na_2S.9H_2O$ was worked as a source to a gaseous H_2S, which works as a precursor for diffusible thiyl radicals.

Liposome vesicle was used as a protocell to study the efficiency of ($HS^{\bullet}$/ $S^{\bullet-}$) to produce trans lipid. In a context of lipodomic, it has noticed that the low released concentration of thiyl radical from H_2S can produce a lipid modification which have a role in determining biomarkers for a health condition or specific disease. As well as, this research opens the door to study the effect of water of crystallization of different organic compounds not only in the biomimetic context, but also to other organic reactions.

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References

1. Jørgensen AC, Strachan CJ, Pöllänen KH, Koradia V, Tian F, Rantanen J (2009) An insight into water of crystallization during processing using vibrational spectroscopy. Journal of pharmaceutical sciences, 98(11):3903-32.
2. Bedlivy D, Preisinger A (1965) Die Struktur von Na2S· 9H2O und Na2Se· 9H2O. Zeitschrift für Kristallographie-Crystalline Materials, 121(1-6):114-30.
3. Bohatý L, Haussühl S (1984) Elastic, electrostrictive, and electro-optic properties of tetragonal sodium sulfide nonahydrate, Na2S· 9H2O. Zeitschrift für Kristallographie-Crystalline Materials, 167(1-4):299-302.
4. Scheel H (1974) Crystallization of sulfides from alkali polysulfide fluxes. Journal of Crystal Growth, 24(25):669-73.
5. Chatgilialoglu C, Ferreri C, Melchiorre M, Sansone A, Torreggiani A (2013) Lipid geometrical isomerism: from chemistry to biology and diagnostics. Chemical reviews, 114(1):255-84.
6. Ferreri C, Chatgilialoglu C (2015) Membrane lipidomics for personalized health: John Wiley & Sons.
7. Pogocki D, Schöneich C (2001) Thiyl radicals abstract hydrogen atoms from carbohydrates: reactivity and selectivity. Free Radical Biology and Medicine, 31(1):98-107.
8. Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, et al (2004) The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'? Journal of neurochemistry, 90(3):765-8.
9. Subramanian H, Moorthy R, Sibi MP (2014) Thiyl Radicals: From Simple Radical Additions to Asymmetric Catalysis. Angewandte Chemie International Edition, 53(50):13660-2.
10. Denes F, Pichowicz M, Povie G, Renaud P (2014) Thiyl radicals in organic synthesis. Chemical reviews, 114(5):2587-693.
11. Ferreri C, Kratzsch S, Landi L, Brede O (2005) Thiyl radicals in biosystems: effects on lipid structures and metabolisms. Cellular and Molecular Life Sciences CMLS, 62(7-8):834-47.
12. Forester DR, Malik BB (2001) H2S scavengers for polysulfide products and methods for scavenging H2S from polysulfide products. Google Patents.
13. Lykakis IN, Ferreri C, Chatgilialoglu C (2007) The sulfhydryl radical (HS./S.−): A contender for the isomerization of double Bonds in membrane lipids. Angewandte Chemie International Edition, 46(11):1914-6.
14. Ferreri C, Costantino C, Perrotta L, Landi L, Mulazzani QG, Chatgilialoglu C (2001) Cis-Trans Isomerization of polyunsaturated fatty acid Residues in phospholipids catalyzed by thiyl radicals. Journal of the American Chemical Society, 123(19):4459-68.
15. Steudel R (1996) Mechanism for the formation of elemental sulfur from aqueous sulfide in chemical and microbiological desulfurization processes. Industrial & engineering chemistry research, 35(4):1417-23.
16. Licht S (1988) Aqueous solubilities, solubility products and standard oxidation-reduction potentials of the metal sulfides. Journal of the Electrochemical Society, 135(12):2971-5.

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