

Biohazardous Impact of Hydrogen Sulfide Gas Exposure on Tear Film Constituents: Structural Characterization by Ultraviolet Spectroscopy

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Abstract

Background/aim Humans can be exposed to hydrogen sulfide gas due to either endogenous or exogenous sources. The health effects associated with exposure to hydrogen sulfide is dependent on its concentration and the duration of exposure. It includes -among many other effects- eye irritation, tearing and inflammation. This study aims to clarify the ocular bio-hazardous effects induced in tear film due to H₂S exposure by using Ultraviolet spectroscopy.

Materials and methods: Sixty Chinchilla rabbits were randomly grouped into six subgroups. Group one served as control. Animals were exposed to hydrogen sulfide gas with concentrations of 75, 90, 115, 250 and 500 ppm. After exposure, tears were collected from the lower lid using glass micro capillaries and its structural characteristics were investigated immediately with ultraviolet spectroscopy. The curve enhancement procedure using Fourier deconvolution was also applied to resolve the obtained absorption peaks.

Results: The control pattern indicates the presence of three absorption peaks at 203±5 nm, 273±3 nm and 323±4 nm, which represent the native lipids, trine conjugation and retinol respectively. After exposure to the specified concentrations of H₂S the general observation is that the number of detected peaks varied with increasing the H₂S dose.

Conclusion: Tear's lipid is the primary target for hydrogen sulfide; this was associated with changes in the retinol content.

Keywords: Hydrogen sulfide, Tear film, Eye, Ultraviolet spectroscopy, Fourier transformation.

Introduction

Hydrogen sulfide (H₂S) is a flammable, colorless gas that smells like rotten eggs is a toxic gas responsible for the second highest number of occupational gas-related deaths, after carbon monoxide. People usually can smell hydrogen sulfide at low concentrations in air, ranging from 0.0005 to 0.3 parts hydrogen sulfide per million parts of air but at high concentrations, a person might lose their ability to smell (Guidotti, 2010).

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Hydrogen sulfide occurs in nature and due to human-made activities. It is emitted from volcanoes, sulfur springs, undersea vents, swamps and stagnant bodies in water (Pope et al., 2017). It is also emitted due to crude petroleum and natural gas production. Hydrogen sulfide is also associated with municipal sewers and sewage treatment plants, swine containment and manure-handling operations, pulp and paper operations (Farahat and Kishk, 2010). Endogenously, bacteria found in mouth and gastrointestinal tract produce hydrogen sulfide during the digestion of food containing vegetable or animal proteins (Whiteman et al., 2010).

The metabolism of H₂S can be divided into three discrete pathways: oxidation to sulfate, methylation, and reaction with metallic- or disulfide-containing proteins (Jones, 2010).

The health effects resulting from H₂S exposure varies from irritation eye and respiratory system to death depending on the level and duration of exposure (Guidotti, 1996).

Eye irritation is one of the earliest occurring. In sub-acute poisoning, the eyes are affected by palpebral edema, bulbar conjunctivitis and mucopurulent secretion with, perhaps, a reduction in visual. There is some variation in the concentration at which it is termed as starting, ranging from 25 ppb to 100 ppm, possibly depending on individual exposure and the duration of exposure. Such exposures occur mainly in industrial situations and are associated with extensive release of the gas (Bates et al., 2017).

Tear film is a complex mixture of proteins, lipids and small bioactive molecules. Their concentrations and relative distribution represent not only the metabolic state of the ocular surface but also the systemic and local homeostasis of the outer eye and the human body. The tear film lubricates the ocular surface and also nourishes the avascular tissues of the cornea. Since tear film is also an optical refractive medium, its stability is pivotal in achieving appropriate vision (Rentka et al., 2017). This study aims to clarify the ocular bio-hazardous effects induced in tear film due to H₂S exposure.

Materials and Methods

Chemicals

Hydrogen sulfide (H₂S) saturated solution was obtained from the ministry of environment, Cairo, Egypt. The concentration (exposure dose) of the saturated H₂S solution was obtained as 75, 90, 115, 250 and 500 ppm. The concentration was monitored and kept constant in the exposure tank using gas alert micro 5 gas detector (Brandt Instruments, Inc. LA, USA).

Experimental animals

Chinchilla rabbits weighing 2 - 2.5 kg were used in the present study. The total number of animals involved in the study was 60 rabbits randomly assigned into six groups, ten animals each. The animals comprised both sexes and were selected from the animal house facility of Research Institute of Ophthalmology, Giza, Egypt. Rabbits were maintained in a standard 12 hr. light-dark cycle with free access to water and balanced diet at a temperature of 22 ± 2°C and 50% humidity. The research protocol was approved by the local ethical committee that applies the ARVO (the association for Research in vision and ophthalmology) statements for using animal in vision and ophthalmic research.

Hydrogen sulfide exposure

Animals previously divided into six subgroups (n=10) were exposed to specific H₂S concentration in a specially designed chamber. The lid of the glass chamber has two ventilation holes to allow excess H₂S vapor and oxygen to flow. The concentration of the H₂S gas inside the chamber was monitored by the alert micro 5 gasdetector (Brandt Instruments, Inc. LA, USA). Animals were introduced into the chamber one by one and kept exposed for one hour.

Tearcollection

After exposing the animals to the desired concentrations of H₂S, tearing was induced by smelling glacial acetic acid. Tears were collected from the lower lid using glass micro capillaries. The collected tears were then stored at -20° C for further analysis by UV spectrophotometer.

Recording of tears by UV spectra

Five µl of freshly collected tears was diluted with to 1 ml with distilled water. The spectra were recorded in the UV range 190 – 400 nm using U.V/VIS spectrometer (evaluation 600, THERMO FISHE, USA).

Statisticaevaluation

Results were displayed as the mean±SD. To obtain a comparison between groups, investigation of fluctuation was done by using the commercially available software program (SPSS-11 for windows, SPSS Inc., Chicago, Illinois, USA), where the significance level was set at P<0.05. All the spectral were averaged using OriginPro9 program (Origin Lab Corporation, Northampton, Massachusetts, USA).

Results

Figure1 showsthe overlaid typical ultraviolet spectra of rabbit tears that were collected from all groups involved in the study. The contour of absorption curves indicates the presence of mean absorption peak at 203 nm. The detailed analysis of the control pattern indicates the presence of three absorption peaks at 203±5 nm, 273±3 nm and 326± 4 nm.

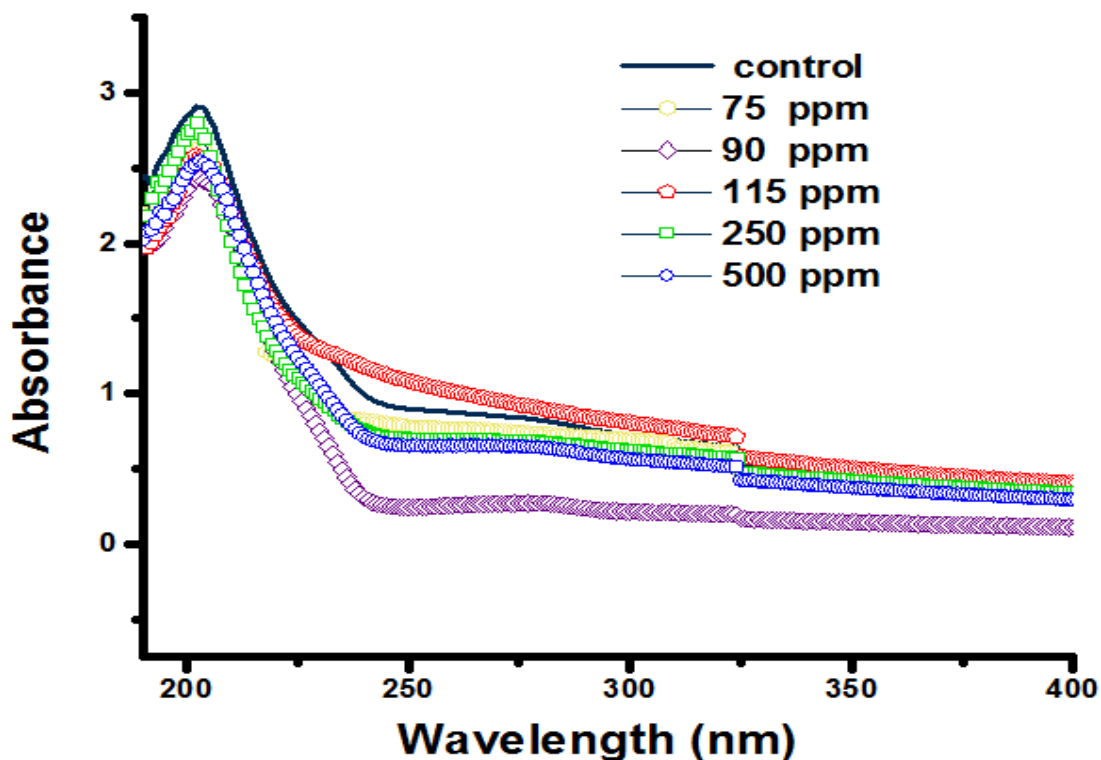


Fig. (1): UV absorption spectra of control tears and tears from exposed animals to specific concentration of H₂S.

To resolve the overlap between peaks and between different groups; curve enhancement procedure was applied. The resulting deconvoluted curves are given in figure 2.

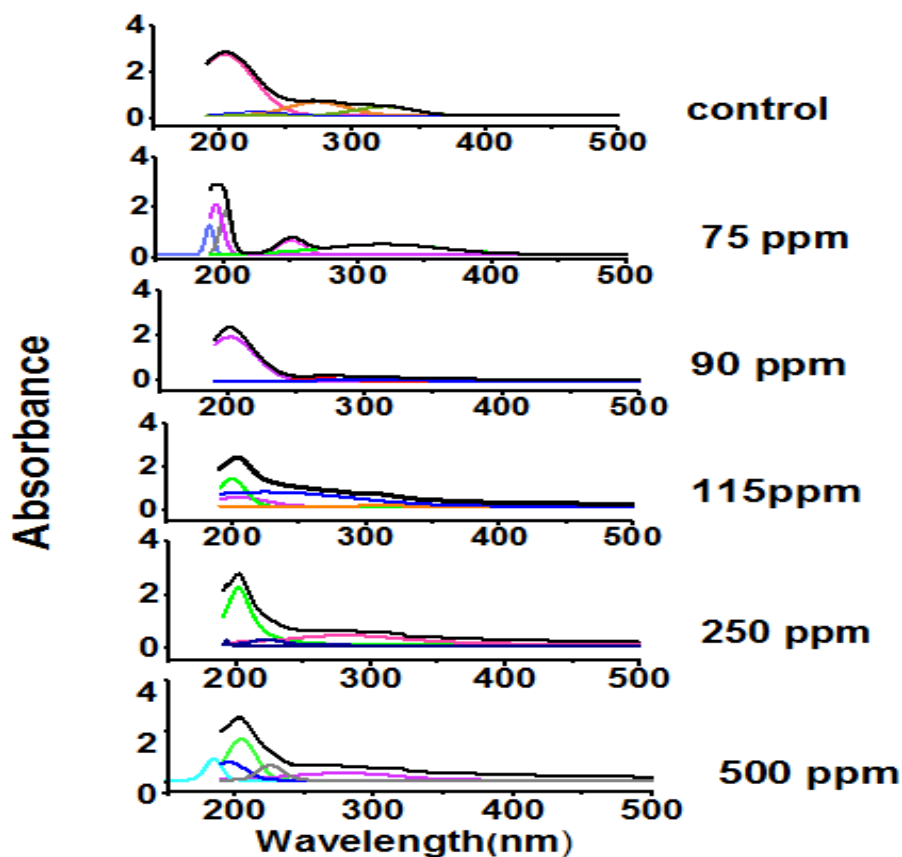


Fig. (2): Deconvoluted-fitted UV spectra of control tears and tears from exposed animalsto specific concentration of H₂S.

The control pattern indicates the presence of three absorption peaks at 203±5nm with area 164± 10that represent the native lipids (Depciuch et al., 2017) and (Zigman et al., 1984). 273±3nm with an area 33± 2 which reflects thetrine conjugation (Schmid, 2001) and (Mahmoudand Aly, 2003).and 326± 4nm with an area 21 ±5 which represents the retinol BaruaandFurr, 1998) and (Tee andKhor, 1995). Upon deconvolution an additional peak can be noticed at 227± 5nmwith an area 5±1that represents diene conjugate(Mahmoudand Aly, 2003).as given in table (1).

Table (1): Tear film UV spectroscopy- Deconvolution analysis after exposure to H₂S.

Groups and doses	Native lipids			Diene Conjugate	Trine Conjugate		Retinol
	Gr. I Control			203±5 164±10	227±5 5±1		273±3 33±2
Gr. II 75ppm	189±9 9±3	194±5 25±8	201±3 18±6 [†]		250±4 13±6		319±1 44±3 [†]
Gr. III 90ppm			201±4 61±8 [†]			271±4 3.17±2 [†]	314±2 [†] 4±2 [†]
Gr. IV 115ppm		194±6 188±8	203±2 146±5	220±3 98±6 [†]			314±2 [†] 33±2 [†]
Gr. V 250ppm		193±7 0.7±0.1	201±2 77±5 [†]	222±4 14±2 [†]		280±4 22±1 [†]	
Gr. VI 500ppm	185±4 1.2±0.6	196±3 104±5	203±6 29±4 [†]	226±2 17 ±3 [†]		275±2 19±3 [†]	

[†]Statistically significant relative to control at P<0.05.

After exposure to the specified concentrations of H₂S the general observation is that the number of detected peaks varied with increasing the H₂S dose. The newly detected peaks in the wavelength range 185 – 196 nm can be assigned to the native lipids family. These peaks appeared in the tear solution when rabbits were exposed to 75, 115, 250 and 500 ppm of H₂S gas.

The peak that represents the diene conjugate (220 – 227 nm) was not detected after exposing rabbits to 75 and 90 ppm, and then was detected again at H₂S doses of 115, 250, and 500 ppm with a significant increase in its area as compared to control. In this context, the interesting finding is that the increase in the peak area was reduced as the exposure dose increased.

Regarding the trine conjugate peak (273 – 280 nm), it was detected after exposing rabbits to 90, 250 and 500 ppm of H₂S. These detected peaks were associated with significant reduction in its peak area as compared to control. For the H₂S dose of 75 ppm, a newly detected peak at 250 nm was found.

The last peak in the UV absorption pattern was detected at 316 – 326 nm. This peak is assigned to retinol moiety as previously mentioned (Barua and Furr, 1998). At the beginning of exposure to H₂S (75 ppm), the area of this peak was significantly increased as compared to the control. Then its area was dramatically reduced due to exposure to 90 ppm. After exposing the rabbits to 115 ppm, the retinol peak area was significantly increased relative to the control one. As the exposure to H₂S increased to 250 and 500 ppm, the retinol peak cannot be detected in the UV absorption pattern. In addition to these changes in the area, the wavelength (λ_{max}) of the retinol peak was also affected by H₂S exposure where a significant decrease (blue shift) was noticed. Finally, the peak associated with H₂S dose of 90 ppm was similar to the control pattern except its area was significantly decreased compared to that of control.

Discussion

Ultraviolet spectroscopy is widely used to detect the structural changes of molecules. The results clearly show that the native lipid of tear film is affected by exposure to all doses of H₂S. The main peak of the control tears reflects the presence of one-structural related molecules and/or family. This lipid family was splitted into three structurally different molecules after exposure to the lowest H₂S dose (75 ppm) and the highest dose (500 ppm). On the other hand, two structurally different molecules were associated with the following doses of H₂S: 90, 115 and 250 ppm. In UV- spectroscopy, the area under the peak is used to determine the concentration. Accordingly, after exposure to all H₂S doses the content of the main peak at 203 nm was significantly decreased as compared to the normal content. This decrease in the area was concomitant with the detection of structurally-different lipid molecules.

When the native lipids are subjected to oxidation, the degree of unsaturation is increased and two additional lipid peroxides can be detected in the wavelength 230 nm (secondary lipid peroxides; diene conjugate) and 270 nm (tertiary lipid peroxides; trine conjugate). Our results indicate that due to exposure to H₂S the degree of unsaturation (diene conjugate content) was increased concomitant with reduced content of trine conjugate (Tertiary lipid peroxides). Also, H₂S dose of 75 ppm induces different effect than the other H₂S doses. It was associated with characteristic absorption at 250 nm that can be assigned to structurally-different unsaturated lipid. Retinoid have been defined as a class of compounds consisting of four isoprenoid units (H₂C=C (CH₃)-CH=CH₂) joined in a head-to-tail manner. The retinoid molecule can be divided into three parts: a trimethylcyclohexene ring (retinol), a conjugated tetraene side chain (retinaldehyde) and a polar carbon-oxygen functional group (retinoic acid).

The absorption of retinol at 326 nm is mainly due to *All-trans* retinol. Exposing the rabbits to H₂S doses induce marked changes in the structure of retinol (vitamin A₁). The blue shift in the absorption of the retinol peak after exposure to 75 ppm (319 nm) is indicative that this absorption is mainly due to 11-*Cis* retinol (Barua and Furr, 1998). And its content was higher than the normal as given by the area in table 1. As the exposure increased to 90 and 115 ppm, the absorption peak was also characterized by blue shift (314 nm), this absorption shift is due to α -retinol as previously given by (Barua and Furr, 1998). The content of α -retinol dramatically decreased after exposure to 90 and contradictory increased after exposure to 115 ppm.

Conclusion, UV results indicate that exposed eyes to H₂S in concentrations ranging from 75 ppm to 500 ppm resulted in structural alterations in the tear film and was associated with increased lipid peroxides and dramatic changes in the structure as well as the content of the retinol. The native lipid of the tear solution is the primary target of H₂S.

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الملخص باللغة العربية

تأثير التعرض لانبعاث غاز كبريتيد الهيدروجين الحيوي على المواد المكونة للدموع: التوصيف البنيوي بواسطة التحليل الطيفي للأشعة فوق البنفسجي

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الهدف من هذه الدراسة : يمكن تعرض البشر إلى غاز كبريتيد الهيدروجين إما بسبب المصادر الداخلية أو الخارجية. وتعتمد الآثار الصحية المرتبطة بالتعرض لكبريتيد الهيدروجين على تركيزه ومدة التعرض له. ويشمل ذلك - العديد من الآثار الأخرى - تهيج العين ، وزيادة نسبة الدموع والالتهابات. تهدف هذه الدراسة إلى توضيح التأثيرات الحيوية الخطيرة الناتجة في فيلم الدموع بسبب التعرض لغاز كبريتيد الهيدروجين باستخدام التحليل الطيفي فوق البنفسجي.

المواد والطرق المستخدمة: تم تجميع ستين من أرانب شينشिला عشوائياً في ست مجموعات فرعية كانت المجموعة الأولى تعمل تحت السيطرة. تعرضت الحيوانات لغاز كبريتيد الهيدروجين بتركيز 75 ، 90 ، 115 ، 250 و 500 جزء في المليون. بعد التعرض ، تم جمع الدموع من الغطاء السفلي باستخدام الشعيرات الدقيقة الزجاجية وتم فحص خصائصها الهيكلية على الفور باستخدام التحليل الطيفي للأشعة فوق البنفسجية. كما تم تطبيق إجراء تحسين المنحنى باستخدام تقنية فورييه من أجل حل قمم الامتصاص التي تم الحصول عليها.

النتائج يشير نموذج التحكم إلى وجود ثلاثة قمم امتصاصية عند 203 ± 5 نانومتر ، 273 ± 3 نانومتر 326 ± 4 نانومتر والتي تمثل الدهون المحلية ، اقتران التريني والريتينول على التوالي. بعد التعرض لتركيزات محددة من غاز كبريتيد الهيدروجين و الملاحظة العامة هي أن عدد القمم المكتشفة يتفاوت مع زيادة جرعة الغاز.

الخلاصة : تشير نتائج الأشعة فوق البنفسجية إلى أن العيون المعرضة إلى غاز كبريتيد الهيدروجين من 75 جزءاً في المليون إلى 500 جزء في المليون نتج عنها تغيرات هيكلية في فيلم الدموع وارتبط ذلك بزيادة البيروكسيدات الدهنية وتغيرات دراميتيكيه في الشكل الهيكلية وكذلك محتوى الريتينول.