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Review article

Chemistry, biology, surface properties and detection of H_2S

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Abstract: Hydrogen sulfide plays a fundamental role in the biochemistry of living cells, yet it is also a toxic molecule. Its sources can be either exogenous or endogenous. Many analytical methods are used to detect hydrogen sulfide from both sources and to control and monitor its concentration, given its dual nature. The interaction between H2S and biological complex systems, such as skin, underlies the therapeutic effects of balneotherapy during warm spring water treatments. The main objectives of our review are to provide an integrated analytical view at the chemistry, biology, surface chemistry, and surface tensiometry properties of H2S. Furthermore, we aim to offer a perspective on the integrated characterization of hydrogen sulfide, with particular attention to the skin/sulphur water inter-face.

Keywords: hydrogen sulfide; gas chromatography; Integrated Analytical Approach; surface chemistry; surface tensiometry; surface free energy; stratum corneum ; balneotherapy

1. Introduction

H_2S is considered toxic when inhaled in a ratio of 1 part gas to 200 parts air [1], while if its concentration falls in the range 20÷50 parts per million (ppm), it causes irritation of the eyes and of the respiratory system.

Adverse effects such as cognitive and motor impairment are compared after a short-term inhalation of H_2S in the range 100÷250 parts per million (ppm), while concentrations in the range 150÷200 parts per million (ppm) cause olfactory issues, anosmia, and inability to perceive the characteristic odour of H_2S.

Inhalation of H_2S at a concentration of 500 parts per million (ppm) for 30 min causes pulmonary oedema and pneumonia in the subject, and at the concentration of 800 ppm, it is fatal to humans [1], while concentrations >600 ppm can cause death within 30 min due to paralysis of the respiratory system.

In the human body, H_2S produces significant inhibitory effects on specific enzymes and processes related to oxidative phosphorylation, ultimately leading to suffocation of the cell [1].

For example, H_2S can inhibit the mitochondrial cytochrome c oxidase (COX), leading to chemical asphyxia. It can be oxidised to sulfates and thiosulfates, which are successively excreted in the urine. For this reason, the urinary thiosulfates could be considered as one of the biomarkers of H_2S poisoning [2].

On the other side, H_2S has cytoprotective properties against oxidative stress and inhibits the cytotoxicity caused by peroxynitrite (ONOOH/ONOO-) or hypochlorite (HOCl/-OCl) in SH-SY5Y cells comparable to that of glutathione (GSH) [3].

Furthermore, activated neutrophils can convert H_2S into sulfite, depending on NADPH oxidase activity,which is inhibited by ascorbic acid. On this basis, the direct H_2S scavenging could explain its protection mechanism as an antioxidant, since this compound is a nucleophile and can react with oxygen (O_2), hydrogen peroxide (H_2O_2) and HOCl/-OCl [3].

H_2S also delays the formation of accumulation products derived from hemin-induced lipid peroxidation. As a consequence, lipid hydroperoxide (LOOH) decreases in the oxidised lipids after H_2S treatment, which is correlated with a decrease in cytotoxicity of the same oxidised lipids [3].

H_2S interacts with COX by binding to the copper's oxidised state (CuB)/heme (a3) iron binuclear site, where oxygen (O_2) binds. H_2S-mediated COX reduction leads to its inhibition, which may represent a good characteristic, even if its respiratory inhibition is comparable with other inhibitors that causes death [3].

Also notably, H_2S and methemoglobin (metHb), an oxidized form of haemoglobin uncapable to bind oxygen due at the presence of different substances such as benzocaine, dapsone, nitrates, nitrites, toxic compounds that cause methemoglobinemia, orm a stable complex (H_2S-metHb) capable to protect against H_2S toxicity *in vivo* because it binds metHb instead of the cytochrome c (Cyt-C), rescuing the capability of cells to produce ATP [3].

Likewise, H_2S interacts with haemoglobin or myoglobin in presence of O_2 or H_2O_2 with production of sulfhemoglobin or sulfmyoglobin, respectively, by covalent heme modification. [3].

Although H_2S is described as an antioxidant that can scavenge HClO/ClO- and its ability is comparable to GSH and cysteine, the direct scavenging of oxidants by H_2S is unlikely, due to its lower concentration compared to other antioxidants [3].

H_2S can perform post-translational modifications of proteins through the generation of sulfane sulfur (S^0) that has the property of transsulfuration, which is a reaction where the transfer of S^0 occurs between sulfur structures through thiosulfoxide tautomer formation and interaction with a nucleophile [3].

This property allows S^0 to have a high potential for signal trasduction. The metabolism of sulfur-containing molecules, such as cysteine, GSH, H_2S, and S^0-containing molecules, is well studied, and fluorescent probes can detect S^0, which could be used to estimate sulfur flow upon H_2S addition [3].

Exogenous H_2S: the interaction with skin

The skin is [a tissue] composed of the epidermis and the dermis. The epidermis is the uppermost part, composed of five cell layers (Figure 1); the most abundant cells are keratinocytes. The dermis, located under the epidermis, consists of fibrous, collagenous, and elastic tissue that encompasses blood vessels, sensory receptors and nerves [4].

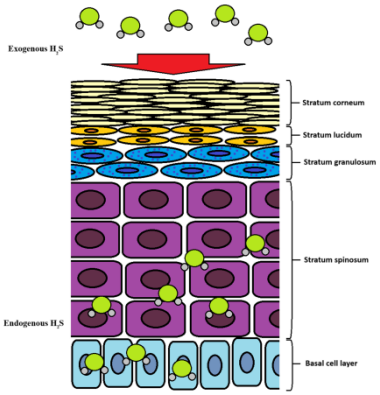

Figure 1: Epidermis and its five layers of cells, dermis, and deepest layer (hypodermis) (Adapted from [4]).

For example, H_2S could act on the proliferation and differentiation of the epidermis, which are important processes for wound healing. An increase of exogenous sodium hydrosulfide (NaHS) concentrations from 0 to 100 μM, with an increase of stimulation times (0-6 days) during the skin treatment, could increase cell proliferation in primary human epidermal melanocytes [4]. Also, exogenous H_2S promotes keratinocyte proliferation and differentiation in a dose-dependent manner [4].

H_2S might have a role in inflammation resolution, which is related to oxidative stress in those skin diseases causing skin hypoxia. In fact, it has been demonstrated that administration of NaHS to keratinocytes has a cytoprotective effect against hypoxia and inflammation by inhibiting the NF-κB/COX-2 signalling pathway induced by reactive oxygen species (ROS) [4].

The ROS damage the healing of wounds because they inhibit cytokine production and angiogenesis. In this context, the antioxidant and cytoprotective activity of H_2S could be effective in promoting wound healing. For example, the pretreatment of wounds with NaSH reduced ROS and induced the production of vascular endothelial growth factor (VEGF), thus accelerating wound healing [4]. As NaHS is a H_2S donor, it has a crucial role in multiple types of cancers, including human melanoma with a long-term anticancer effect [4].

For example, the treatment of human melanoma cell lines (A375 and SK-MEL-28) with 2 mM NaHS for 24 hours reduced cell proliferation and migration, blocking the cell cycle and inducing autophagy [4]. The treatment with H_2S donors such as NaHS could decrease the protein expression of p-PI3K, p-Akt and mTOR, suggesting that suppression of the PI3K/AKT/mTOR pathway is correlated with the inhibition of the development of human melanoma cells [4].

H_2S can directly cross skin cell membranes via passive diffusion because the stratum corneum (SC) of skin has an extracellular pH that ranges from 4.5 to 6.0 and a temperature of 34–35 °C, conditions where H_2S is present mainly in its gaseous form [5].

In this form, it diffuses passively through cell membranes following the concentration gradient, and for this reason, the gasotransmitter ability of H_2S is directly linked to its chemical form and the acidic nature of the tissue [5].

H_2S exerts its biological actions only after the absorption, without toxic effects, because it doesn't reach cumulative levels thanks to an optimal hydration state of the deepest ayers of the epidermis; this is a fundamental aspect for the conversion of sulfur into pentathionic acid ($H_2S_5O_6$), which is an antibacterial and antifungal agent [4].

Endogenous production of H_2S

As noted above, there are external and internal H_2S sources. In fact, the body produces H_2S in its anabolic metabolism through three main enzymes,–that is, cystathionine β-synthase (CBS), cytoplasmatic cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) [5].

Enzymatic H_2S synthesis occur from L-cysteine, homocysteine and mercaptopyruvate, respectively by CSE, CBS and 3MST activity [1, 2, 5] where cysteine aminotransferase (CAT) also plays a role acting on L-cysteine in tissues such as the liver, brain, endothelium, and skin (Figure 2).

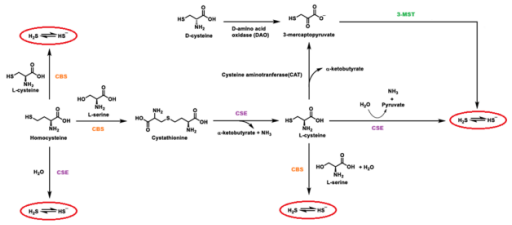

Figure 2: H_2S synthesis mediated by the enzymes CBS, CSE and 3-MST (adapted from [1].

CBS is located mainly in the central nervous system (CNS), such as cerebellum and hippocampus [2], but it is also present in the liver, kidney, ileum, uterus, placenta and pancreatic islets [1].

CSE is located in the cardiovascular and respiratory systems [2] and is expressed in the liver, kidney, ileum, uterus, brain, pancreatic islets, placenta, nd smooth muscle [1]. Both enzymes are located in the cytoplasm and use pyridoxal-5-phosphate (PLP) (vitamin B6) as a cofactor, and their concentrations change in different tissues. CBS and CSE convert homocysteine to cysteine, generating H_2S through the reverse sulfur conversion pathway [2]

Human CBS is a homotetrameric hydrolase that catalyses the cleavage of a CO bond in serine and belongs to the family of carbon-oxygen-lyases [1]. This enzyme can produce H_2S in three ways: hydrolysis of cysteine, formation of serine, and condensation of cysteine and homocysteine with the formation of cystathionine, or condensation of two cysteine molecules to form lanthionine [1].

On the other side, CSE is a homotetramer that produces H_2S from cysteine (70%) and homocysteine (30%), and its catalytic mechanism is very similar to that of CBS [1].

In addition, the transsulfuration pathway contributes to the production of H_2S from homocysteine, which represents a risk factor for cardiovascular and neurocognitive diseases, or L-cysteine [1].

3-MST is an enzyme that belongs to the family of sulfurtransferases and catalyses the transfer of a sulfur from a sulfur donor to a thiophilic acceptor by the formation of a protein cysteine persulfur intermediate. The sulfur atom donor is 3-mercaptopyruvate (3-MP), while thioredoxin is the favourite acceptor under physiological conditions [1].

The 3-MST uses zinc as a cofactor and is localised in mitochondria together with cysteine aminotransferase (CAT). CAT catalyzes the transamination of cysteine and α-ketoglutarate, producing 3-MP and glutamate. Then, 3-MST transfers one sulfur atom from 3-MP to the active site of a cysteine, producing pyruvate and a persulfide bound to the enzyme. The compound bound to 3-MST reacts finally with thioredoxin, producing H_2S [1].

The Selenium-Binding Protein 1 (SELENBP1) supports H_2S biosynthesis and adipogenesis. Although its cellular regulatory functions are not fully understood, this is another pathway by which H_2S is produced using D-cysteine as a substrate in conjunction with the enzymes D-amino acid oxidase and 3-MST [1].

After production, H_2S can be tored as sulfane sulfur or released immediately after synthesis in response to a physiological signal [1].

Sulfur can be deposited in cells in two forms: acid-labile and sulfane sulfur. Acid-labile sulfur is constituted mainly from atoms of iron-sulfur complexes and is released when the pH is below the critical value of 5,4 [1].

The fact that in physiological conditions, the mitochondrial pH is in the range 7-8 suggests that in these conditions, acid-labile sulfur may not be released. Sulfane sulfur is a form of storage localised into the cytoplasm, which encompasses compounds like polysulfides, thiosulfate, thiosulfonates and elemental sulfur [1].

Sulfane sulfur is released under reducing conditions, suggesting that the adjustment of its bioavailability is regulated by the redox state in the cell [1].

For example, the polysulfide is a sulfane sulfur that can release H_2S under cellular reducing conditions. Alkaline conditions favour the activity of reducing substances, thus the release of sulfane sulfur occurs when the intracellular pH becomes alkaline.

Also, free H_2S can be incorporated and stored into proteins as sulfane sulfur because its divalent sulfur form binds only with elemental sulfur, persulfides and polysulfides [1].

The erythrocytes– elemental sulfur to HS- in a non-enzymatic manner. Although the non-enzymatic manner is less important with respect to the enzymatic one, this form derives from the reduction of elemental sulfur to H_2S using reductants derived

from glucose oxidation, such as lactate, nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) [1].

The NADPH produced by the glycolysis is used for the reduction of glutathione disulfide (GSSG) to glutathione, and this process allows H_2S release from sulfane sulfur [1].

The H_2S can also be produced by the gut microbiota, mainly in the intestine, and has an important impact on the physiological processes of the host organism and microbial community (50% of H_2S production) [1].

The production of H_2S occurs through the metabolism of bacteria in the digestive tract [1] that utilise the sulfate as the final acceptor in the respiratory chain to produce H_2S. The quantity of H_2S produced depends on the diet and the type of microorganisms that inhabit the gut.

In fact, the 50% of H_2S found in colonocytes is produced by sulfate-reducing bacteria and the other 50% derives from CBS and CSE activity [1]. The microbiota's sulfate reduction takes place in assimilatory and dissimilatory pathways [1].

In the assimilatory pathway, sulfate is used to produce cysteine as the terminal product, and so the sulfate is necessary to give a substrate for the production of amino acids, while the dissimilatory pathway produces H_2S from sulfate; H_2S can also be generated from the degradation of cysteine [1].

In particular, sulfate-reducing bacteria such as *Desulfovibrio* and *Bilophila wadsworthia* are responsible for H_2S synthesis in the colon through fermentation of sulfur-rich proteins. Then, H_2S can modulate the intestinal epithelium and exert pro- or anti-inflammatory effects depending on dose. The biological impact of colonic H_2S is concentration and compartment-dependent [5].

In the luminal phase (~0.3–2 mM), H_2S acts as an electron sink that supports anaerobic energy metabolism, while at low micromolar diffusion into epithelial cells, it activates Nrf2-mediated antioxidative protective responses and tight-junction reinforcement [5]. Naturally, the production of H_2S by microbiota depends on the diet and on the type of bacteria, with influence on digestive health.

For example, high-protein diets or dysbiosis can cause an increase of H_2S exposure at the mucosal level (>500 μM), affecting the detoxification activity of mitochondrial Sulfide Quinone Oxidoreductase (SQR) in colonocytes, inhibiting butyrate oxidation and triggering DNA damage pathways, causing a cytotoxic pro-inflammatory profile [5].

The interaction between H_2S and Short-Chain Fatty Acids (SCFAs) represents a complex relationship that balances the beneficial metabolic effects of this gas with its potential concentration-dependent inflammatory properties. SCFAs include acetate, propionate and butyrate and are produced by bacteria through fermentation of dietary fibre [6].

Butyrate enhances epithelial oxygen consumption by upregulating the colonic SQR, thereby increasing the H_2S detoxification threshold. The sulfide-overload by suppressing β-oxidation of butyrate favours the expansion of sulfate-reducing bacteria (SRB) [5]. A diet with fibres combined with prebiotic supplementation elevates the butyrate, shifting the balance toward a tolerogenic environment due to the fact that butyrate has an important role in dendritic cell differentiation, maturation, and function toward a tolerogenic phenotype [7], helping the management of the immunological response during health and disease status [8].

For example, specific drugs and nutraceuticals (such as NaHS, GYY4137, diallyl trisulfide in garlic, and sulforaphane in broccoli) can achieve controlled release of H_2S, exerting beneficial effects in hypertension, neurodegeneration, chronic inflammation, and cancer [5].

In respect of excretion, in the respiratory tract, H_2S is expelled as gaseous molecules; in the urinary tract, it is eliminated as thiosulfate (or free sulfate in the urine); in the gastrointestinal tract, H_2S is excreted as free sulfide in the faeces [2].

Furthermore, the excretion of H_2S in mammalian tissues should be adjusted and kept at nanomolar levels to assure its signalling function and avoid inhibition of metal-containing proteins. Since the excretion of H_2S depends on the kind of system involved, its concentration varies between different tissues [1].

At the cellular level, more specifically in mitochondria, the elimination of H_2S takes place in the mitochondrial sulfide oxidation pathway. The process happens in the mitochondrial matrix and it is characterised by the action of four enzymes: SQR, rhodanese, persulfide dioxygenase (PDO) and sulfite oxidase (SOx) [9].

SQR catalyzes the transfer of two electrons from H_2S to the oxidised form of ubiquinone (Qox), which occurs alongside the transfer of the sulfur to GSH, forming glutathione persulfide (GSSH). GSSH is then converted into sulfite by Ethylmalonic Encephalophaty 1 protein (ETHE1) , which requires oxygen [9].

Therefore, the sulfite can be used by rhodanese or SOx to produce thiosulfate or sulfate, respectively, while rhodanese utilises GSSH as a cosubstrate [9].

On the other side, diseases including hypertension, atherosclerosis, gastrointestinal ulcers, cirrhosis of the liver, diabetes, inflammation, Alzheimer's disease, cancer, and others may develop when the concentration of H_2S decreases too much. As a consequence, pharmacological interventions with H_2S or its derivatives are strongly needed [1].

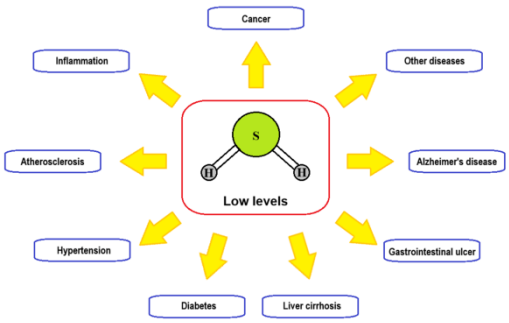

Figure 3: Examples of diseases related to low levels of H_2S (Adapted from [1])

1.1. Aims of the work

Considering the above reported roles of hexogenous and endogenous H_2S, the main aims of our work are: (a) to give an integrated view of this molecule by linking together its biochemistry, chemistry, surface chemistry, surface tensiometry; (b) to provide a wide overview on detection methods of H_2S present in the external environment and at living cells level; (c) to analyse new perspectives in the investigations of the phenomena that occurs at H_2S-H_2O/skin interface with particular attention at the hydrogen sulfide treatments in thermal medicine.

The approach chosen here to reach these objectives was based on the new concept of the Integrated Analytical Approach (IAA), introduced for the first time by Rossi et al. as "*an integrated approach to study the bulk chemical composition (e.g., by spectroscopic methods) bulk structure (e.g. by rheological measurements) and surface characteristics (e.g. by surface tensiometry) on the same material sample and at the same time*" and now applied in the fields of beauty and cosmetics [10], structure-surface correlations of ointments, pharmaceutical technology [11] and of pharmaceuticals [12].

The final target of our work is to give new perspectives in the study of warm spring waters containing H_2S in the behalf of balneotherapy, using new analytic approaches and focusing the attention on the water/skin interface phenomena.

2. Chemistry of H_2S

The above-reported activity of H_2S at exogenous and endogenous levels strongly relies on its physicochemical characteristics. The electronic configuration for S is of the

type $1s^2$, $2s^2$, $2p^6$, $3s^2$, $3p^6$, so in H_2S the sulfur atom completes its valence shell by borrowing an electron from each H atom, like in water [1].

However, the covalent radius for S (105 pm) is larger than that for O (66 pm) [1], hence, even if H_2S and H_2O have similar structures, the sulfur valence electrons occupy larger and more diffuse orbitals. Consequently, S is less polar and it is characterised by a higher degree of polarizability, also witnessed by the lower value of the Pauling electronegativity (2.5 compared to 3.5 for O).

In the same way, the smaller difference between the H (2.1) and S electronegativity makes the dipole moment in H_2S (0.97 D) also smaller than that of water (1.85 D), thus weakening intermolecular interactions [1] and preventing the formation of efficient hydrogen bridges (Figure 4).

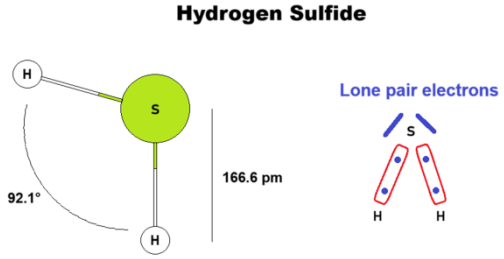

Figure 4: H_2S molecule and hydrogen bonds (Adapted from [1])

In aqueous solution, H_2S behaves as a weak (diprotic) acid and is in equilibrium with the HS^- and S^{2-} anions. At 25° C, reported pKa_1 and pKa_2 values vary from 6.97 to 7.06, and from 12.35 to 15.00, respectively; the pKa_1 of 7.02 appears to be the most reliable value [3]. Along with this basis, at **pH 7.4** in an aqueous solution 28% of the total hydrogen sulfide exists as H_2S, while 72% is in the form of HS^-; the high[er] pKa_2 value, on the other hand, indicates that the presence of the S^{2-} anion in solution is negligible [3].

Anyway, it must be noted that the pKa of H_2S depends on conditions such as temperature and solution composition. The three equilibria described in Equation 1 represent the dynamics of the H_2S solution.

$$H_2S_{(g)} \leftrightarrow H_2S_{(aq)} \leftrightarrow HS^- + H^+ \leftrightarrow S^{2-} + 2H^+ \quad (1)$$

Following Equation 1, these equilibria continuously shift towards the formation of $H_2S(g)$, leading to a decrease of $H_2S(aq)$ concentration, and to an increase of the solution pH [3], accordingly, the water solubility of the volatile H_2S equilibrating between the gas phase (g) and the aqueous phase (aq) at the interface l/v [3] depends on pressure, temperature and the composition of the solution. Following the above, H_2S is moderately soluble in water, with a solubility of 2.77 volumes in 1 volume of water at 20°C or 110 millimoles per atmosphere at standard room temperature [1].

In biology, the pH of H_2S solutions is of uttermost importance. pH values in the range 5.5-6.5 favour the presence of molecular H_2S, which can be absorbed by passive diffusion. When pH becomes more alkaline, the transcutaneous bioavailability of H_2S decreases, due to its dissociation into HS^- [5].

It is important to keep in mind that the concentration of H_2S at 20°C at physiological pH (7.35-7.45, mean 7.40), related to human blood plasma and extracellular fluids, (pKa_1=6.98) doubles at the human body temperature of 37°C– [3].

In fact, unlike in generic aqueous solutions, in biological conditions (37°C and pH 7.40), the pKa_1 is 6.76 (ΔpKa_1=-0.24), with an increase of H_2S equal to 80% and a decrease of HS^- equal to 20% , while S^{2-} remain negligible in extracellular fluid [3].

At the biological level, H_2S has a solubility of 80 mM at human body temperature of 37 °C, 100 mM at 25°C, and 122 mM at 20°C [3]. On the other hand, the $H_2S_{(g)}$ is a highly lipophilic gas molecule that can pass through the lipid bilayer structure of the

cell membrane and diffuse directly, because it is characterised by a lipid bilayer permeability of PM≥0.5±0.4 cm/s [3].

Half $H_2S_{(g)}$ can be lost from solution in 5′ in cell culture wells and 3′ in a bubbled tissue bath, which explains the variations of H_2S concentrations in tissues and plasma [3].

2.1. Chemical and biological detection of H_2S

From a chemical viewpoint, the determination of H_2S via gas chromatography (GC) is widely used, because of its high detectability and precision. In Figure 5, the typical GC-FPD (Flame Photometric Detector) peak of H_2S in the gas phase [13].

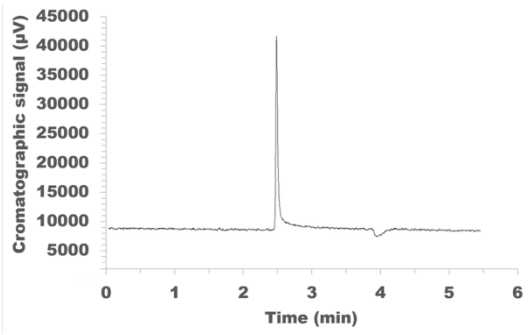

Figure 5: The GC-FPD chromatogram of H_2S, whose peak has a retention time of 2.5 min and great selectivity of flame photometric detector (Adapted from [13]).

Although the FDP has a great selectivity for H_2S, in general, the GC method is characterised by a complex protocol; it's not suitable for the detection of short-term variations due to the variability of environmental conditions and it is affected by the presence of biases, such as sorptive loss in association with its high reactivity [13].

2.1.1. Chemical sensors

On the other hand, chemical sensors for H_2S detection have been widely used in many applications. These instruments are based on easy-to-use sensors such as metal-oxide semiconductors (MOS) and other electrochemical, optical, and array sensors, with many advantages, such as high sensitivity, fast response, and low cost [13]. Metal-oxide sensors operate on the principle of conductivity, while electrochemical sensors operate on amperometry, potentiometry, cyclic voltammetry, and impedance measurements [13].

However, the detection principles for optical sensors are based on the observation of fluorescence-labelled systems or direct optical detection in the heterogeneous phase; for this reason, many H_2S gas sensors are developed on colourimetry, absorption and fluorescence principles.

2.1.2. Colorimetry methods

A novel colourimetric probe (C8-ISPBI) was recently developed to improve the detection of H_2S at the naked-eye level [14]. This new method is based on the isatin Schiff base. The C8-ISPBI probe showed fast response time (<15 s), low detection limit (1.2 μM), and high selectivity. Furthermore, this probe demonstrated high detection capability in different environments, such as solutions, films, and dyed textiles [14].

2.1.3. Fluorescence methods

With respect to the fluorescence probes for H_2S environmental detection, a portable sensor employing a diode emitting a light at 470 nm and a microfiber optic spectrometer was developed and is currently used [15].

This sensor is based on fluorescein mercury acetate (FMA), which impregnates a sensing layer onto the surface of a micropaper analytical device positioned in the optical detection system. FMA is employed for monitoring H_2S released from wastewater [15].

The determination of H_2S concentration is based on the quenching of fluorescence intensity after direct selective reaction between the gas and FMA (range 17–67 ppb of H_2S; detection limit of 3 ppb; RSD 6.5%; response time 60s) [15].

2.1.4. Biological detection methods

The methods for the detection of H_2S in biological systems are based on the development of specific chemical tools for the identification of H_2S signalling functions in various organs and systems [16].

H_2S can react with electrophilic species because in this molecule sulfur (for which are possible oxidation states from -2 to +6) is a reductant (oxidation state -2) that reacts with oxidants by giving up electrons, thus lowering their oxidation state [16]. Due to these properties, the quantity of H_2S generated can be determined analytically [16].

As an example, the methylene blue method is useful for H_2S determination, because the acidic condition allows the liberation of H_2S from zinc sulfide complex [17]. Once liberated, H_2S reacts with N,N-dimethyl-p-phenylene diamine (N,N-dpd) in the presence of $FeCl_3$ as oxidizing agent [17].

This reaction produces methylene blue that absorbs at 670 nm, with the absorbance value proportional to the sulfide concentration. However, this method requires the use of toxic and corrosive agents that limit the possibility of applying this approach to live cells [17].

Another way is to enhance the detection of H_2S produced in human cell culture using the agar trap method. In this method, the H_2S produced after the incubation of intact cell culture (e.g. human endothelial-like immortalized cells Ea.hy 926 type) can be trapped as zinc sulfide in the zinc-agar layer and successively released as zinc sulfide that can be analyzed in situ utilizing a modified methylene blue reaction [17].

Although this method is more convenient than conventional methylene blue method, it can be affected by interference due to colored substances that cause a decrease of its sensitivity [17].

Another simple method for the detection of H_2S in living cells is based on *in situ* formation of silver nanoparticles (AgNPs) on the surface of Ag_2S nanoparticles (NPs) (Ag_2S@Ag) [17]. Ag_2S nanoparticles– are formed by reacting Ag^+ ions with H_2S gas in a polyelectrolyte multilayer film. H_2S can be sensitively detected by monitoring the UV-vis absorbance of the newly formed AgNPs. This method allowed the measurement of the $H_2S_{(g)}$ generated from e.g. cell line HepG2 after treatment with Cys and pyridoxal phosphate (PLP) [17]. Although this method is very sensitive, it needs a long reaction time (2h) to reach the amplification of Ag [17].

The colorimetric system based on Ag-embedded Nafion/PVP membrane applied onto a polystyrene microplate cover is a simple and economic mean for the selective H_2S detection in live cells [17]. Following this colorimetric method, the detection of $H_2S_{(g)}$ is based on the reaction between Ag and sulfide that form brown-colored Ag_2S, while the use of optical probes based on gold (Au)/AgI dimeric NPs immobilized in agarose gels consent the detection of H_2S trough their reaction with production of Ag_2S that can be detected by UV–vis spectroscopy (in the plasmonic band of the AuNPs) [17].

More recently, many kind of H_2S-responsive fluorescent probes (based on different reactions such as azide-to-amine reduction, nitro-to-amine reduction, copper sulfide precipitation, and nucleophilic addition) have demonstrated their applicability in the detection of H_2S in tissues or cells thank to their nondestructive properties [17].

As example, the sulfidefluor-7 acetoxymethyl ester allows the visualization of the endogenous H_2S that is released in the endothelial cells of human umbilical vein [17],

while the dinitrophenyl ether operates as fluorescence quencher and H_2S-reaction trigger and its capacity in the detection H_2S in live cells was demonstrated using HeLa cells and NaHS as H_2S source [17].

Very interesting are the fluorescence nanoprobe based on quantum dots and consisting in p-amino thiophenol-capped AgNPs and thioglycolic-acid-stabilized quantum dots (QD/AgNP nanocomplexes). They have great sensitivity (detection limit 15 nM) and high ability to enter into cellular lysosome in live HeLa cells [17], while the ratiometric fluorescent probes, such as quinoline quaternary ammonium salt derivative-based ratiometric probe (QL-N3), shows two fluorescence emission peaks at 525 and 605 nm (excitation wavelengths of 385 and 521 nm). The ratio between the fluorescence intensities of two peaks correspond at the H_2S concentration [17].

These probes can detect the changes in exogenous and endogenous H_2S in live HeLa cells; however, a biocompatible two-photon fluorescent probe for H_2S, able to increase the selectivity, was recently developed [17]. This kind of biocompatible fluorescent probe optimizes the interactions between biothiols and the probe, reaching high sensitivity and fast response time.

Another method for in live detection of H_2S is represented by the Surface-Enhanced Raman Spectroscopy (SERS), an highly selective, ultrasensitive spectral analysis technique based on molecular fingerprinting, in which the high sensitivity is linked to single-molecule detection levels [17]. The SERS is more resistent to photobleaching and phototoxicity than fluorescence-based methods [17].

The SERS can increase the signal to many orders of magnitude in respect of the classic Raman spectroscopy and is commonly used for DNA, protein, metal ions, and pesticides analysis [17]. As an example, the SERS developed functionalizing AuNPs with 4-acetamidobenzenesulfonyl azide (AuNPs/4-AA) is used for the detection of endogenous H_2S in live cells, where the AuNPs/4-AA can respond to H_2S within 1 min, with a 0.1 μM level of sensitivity [17].

Paper-based sensors lead to a simple and economical development of point-of-care (POC) diagnostics. These sensors guarantee a passive liquid transport, are compatible with chemicals or biochemicals and consent a naked eye colorimetric detection [17].

So, innovative cheap colorimetric paper-based sensors for the detection of endogenous H_2S gas in live cancer cells were developed using a microplate constituted by 3D printed 96 wells device (4mm). In particular, this methodology measures the difference in H_2S levels between prostate cancer LNCaP and PC-3 cells; however, it cannot measure a too low amount of H_2S released from LNCaP cells within 24 h [17].

To overcome the limitations of colorimetric-based methdologies as well as to improve the sensitivity of the traditional techniques, dual- mode H_2S detection methods based on Colorimetric/SERS, Colorimetric/Fluorescence and Colorimetric/wettability approaches were also developed [17].

A sensitive Bioluminescense (BL) probe (called Luc-H_2S) for *in vivo* monitoring of H_2S levels was also recently introduced. The complex system Luc-H_2S is obtained by incorporating D-luciferin (D-Luc) into a part of H_2S-recognizing 2-thiophenecarboxylic acid, acting as a BL quencher [16]. The Luc-H_2S as such doesn't generate BL signal because of the quenching effect of the caged hydroxyl group, but the 2-thiophenecarboxylate bond of Luc-H_2S is cleaved after the addition of H_2S to release D-luciferin that when is recognized by luciferase (fLuc) emits a strong BL signal [16]. The Luc-H_2S has high selectivity and excellent sensitivity toward H_2S with a low detection limit (LOD = 0.337 μM) and can be applied for endogenous H_2S imaging in live cells [16].

As regards blood plasma, sensitive fluorescence methods of measuring the presence of H_2S in mice blood plasma at sub-nanomolar concentration levels were developed [18]. One such methods uses an excess of monobromobimane (MBB) involving the derivatization of the sulfide. The fluorescent product sulfide-dibimane (SDB) can be analyzed by RP-HPLC [18].

Hexogenous H_2S in the human blood plasma can be detected using fluorescence (FL) and a microfluidic device [19]. This approach is based on a plasma-compatible dansyl azide (DNS-Az) probe which has a very fast reaction time. When it comes in contact with H_2S, it produces the fluorescent compound dansyl amine (excitation wavelength at 360 nm, emission peak at 530 nm) [19]. The microfluidic device can detect H_2S in plasma in the range 33 – 330μM (smallest and highest concentrations, respectively) in a quick and accurate manner [19].

3. Surface chemistry of H_2S

The surface chemistry of hydrogen sulfide (H_2S) and water (H_2O) depends on adsorption, dissociation and reactivity of H_2S on various surfaces as well as on the presence of H_2O, that acts as a solvent or surface-modifying agent [20].

The knowledge of surface chemistry of the H_2S-H_2O system is fundamental to understand variations in physico-chemical properties at different conditions and their behaviours when the system ome in contact with solid, biocomposite and biological surfaces [20].

3.1. Adsorption

The interface properties of H_2S can be characterised using molecular dynamics (MD) simulations by the Drude Polarizable Force Field (DPFF) [20].

The DPFF study is based on the fact that H_2S has low hydrogen bonding and electrostatic interactions with water solvent ($\Delta G_{hydr}°$ of −0.5 kcal mol^{-1}), as a consequence, H_2S in water behave as a real hydrophobic solute [20].

The transition of H_2S across the water–vapour interface (or in general l/v interface) during its adsorption shows that H_2S has a measurable surface excess (Γ) (ΔG_{surf} of 1.3 kcal mol^{-1}), intended its concentration at the boundary, that corresponds to a decrease in the ST of liquid (20 dyn cm^{-1} under high densities of $H_2S_{(g)}$, while the dipole moment increases (0.98 to 1.25 D) [20].

In particular, at the l/v interface, the sulfur atom of the adsorbed H_2S is oriented toward the vapour (v) phase while the entire molecule is oriented perpendicular to the interface. This configuration characterises the adsorption of H_2S on the water surface [20].

Adsorption of H_2S on solid surfaces results from a combination of both physical and microchemical adsorption mechanism [21]. In the first steps of the adsorption process, the functional groups of the solid substrate attract H_2S gas molecules through electrostatic and van der Waals forces.

After that, , an electron transfer occurs between functional groups of the solid, such as carboxyl, carbonyl, hydroxyl, pyridine, sulfone, sulfoxide and H_2S molecules (microchemical interactions) [21]. The curves of the potential energy of functional groups and that of H_2S molecules are in accord with the Lennard-Jones theory [21].

The Lennard–Jones potential is a mathematical approximation of the potential energy applicable to the interaction of non-bonding particles and incorporates the attractive London dispersion forces and the repulsive forces due to atomic, or molecular, orbital interpenetration when the particles approach each other (Equation 2).

$$U(r) = 4\varepsilon[(\sigma/r)^{12} - (\sigma/r)^{6}] \quad (2)$$

Where ε indicates the strength of the attractive forces, σ is the distance at which the intermolecular potential is zero and r is the distance between the centers of the two particles [22]. These adsorption principles are at the base of interaction between H_2S and overall solid substrates.

3.1.1. Adsorption on solid surfaces

The solid sorbents for H_2S adsorption operate according to physisorption, which is related to the formation of weak bonds between H_2S and the substrate, and chemisorption, which is based on chemical bonds between the adsorbate and the solid substrate [23].

In general, the solid sorbents are represented by various composite materials such as metal foams, zeolites, laterites, kaolin, silica, carbon of miscellaneous sources and other solid phases activated with metal salts to enhance the H_2S sorption capability [23].

The adsorption mechanism of H_2S on solid surfaces plays a fundamental role in the capture and removal of this molecule by different solid substrates such as hematite-based sorbents (Fe_2O_3) [23], activated carbon (AC) [24], defective and perfect Cu(110) surface for molecular and dissociative adsorption of H_2S and H_2O [25], and defective and perfect CdS (110) surfaces [26].

Other examples of H_2S adsorption on solid surfaces are represented by cleaved hydroxylated (0001 with 4 axis C) α-quartz SiO_2, where the adsorbed H_2S breaks into H and HS groups (not an anionic dissociation) [27], Fe (110) solid surface, where H_2S can be adsorbed as a molecule or in its dissociated form (HS+H), and different γ-Fe(111) surfaces, where the H_2S molecules could dissociate and adsorbed because the S–H bond breaks at the vacancy catalitically site [28].

3.1.2. Adsorption on biocomposite substrates

The bacterial cellulose (BC) is an extracellular polymer synthesised by specific aerobic bacteria (*Komagataeibacter, Azotobacter, Pseudomonas, Salmonella, Sarcina, Agrobacterium*) and characterised by mechanical strength, excellent water retention and biodegradability [29].

In particular, a specific bio-composite membrane material was developed by inoculating an engineered strain of *K. oryzendophytica* FY-07 into the AC-fermentation medium to produce BC under various environmental oxygen conditions. *K. oryzendophytica* FY-07 was modified by introducing the genes encoding sulfide quinone oxidoreductasi (SQR) and Persulfide Dioxygenase (PDO) of *C. pinatubonensis* JMP134 to improve its sulfur oxidation activity toward the H_2S adsorbed on the biofilm [29].

These BC/AC biocomposite membranes exhibited high adsorption capacity; they can adsorb completely H_2S, at a rapid rate, in 24h. BC/AC surpasses both AC and BC alone in the volumetric adsorption [29]. This great adsorption ability is due to the water-holding capacity that leads to the formation of a water film on their surface by which the H_2S is absorbed and successively dissociated into HS^- with a reduction of the H_2S transfer rate from the water film to the gas phase [29].

Other porous biological-derived materials able of adsorbing H_2S are represented by chitosans/zeolite complexes (5A@Chi) having pores of 5 nm (Figure 6) [30].

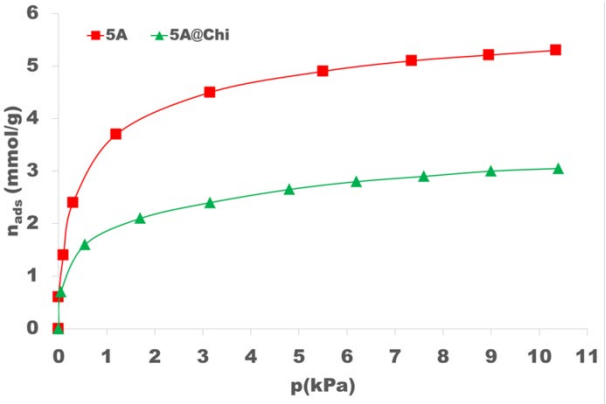

Figure 6: Adsorption isotherms of 5A zeolite and 5A@Chi (Adapted from [30]).

The higher adsorption of 5A is due to the presence of the Ca^{2+} cation in its structure that increases the H_2S adsorption capacity on zeolites, while the chitosan can delay the release of the gas in water, because it reaches a balance between the adsorbed and release kinetics [30].

3.1.3. Adsorption on biological surfaces: the skin surface

To understand the physical basis of the interactions between H_2S and the skin surface, it is fundamental to know the chemical composition of the stratum corneum (SC) because it affects the uptake of this molecule. The SC lipid composition is constituted by ceramides, fatty acids and cholesterol, together with low levels of glucosylceramides, cholesterol sulfate and esters; in SC, the ceramides and fatty acids are saturated with an average chain length between C20 and C22 [31].

There are 25 different ceramide classes that are present in the extracellular lipid matrix or protein-bound ceramides that surround the corneocytes. In particular, there are 1327 unbound and 254 protein-bound ceramides that vary according to the number of hydroxyl groups and carbon atoms, what makes the SC a multitude of lipid classes [31]. For example, the CER EO acylceramides class has a linoleic acid linked to a very long fatty acid chain in the omega position [31].

The SC lipids tend to form solid phases because of the high content of long and saturated acyl chains, although a small fraction of the lipids is present in a disordered fluid state (fluid lipid) with nanosecond dynamics, while the presence of cholesterol sulfate in the SC promotes the lipid mixing, preventing the segregation of crystalline cholesterol [31].

In particular, the amount of fluid lipids depends on temperature, hydration, and the presence of small penetration enhancers. With respect to hydration, the presence of water in SC causes an increase of fluid lipid fraction, osmolytes, polar Natural Moisturising Factors (NMFs) and NMF-like compounds such as urea, glycerol, lactate and other penetration enhancers [31].

With respect to the fluid lipids, it can be noted that if they are represented by isolated droplets embedded in the centre of the solid lamellae, they don't move continuously across the layered structure, and consequently, the permeability of SC is low [31].

On the contrary, if the fluid lipids move over a large area, their movement is continuous, and the permeability of SC increases because they can reach high amounts [31].

From an analytical viewpoint, the ^{13}C and ^{1}H analyses of model lipids by NMR has shown that fluid segments of CER EOs lipids are only present in the terminal part of their hydrocarbon chains, while the headgroup region corresponds to the discontinuous fluid droplets [31].

The NMR approach permits the study of the behaviour of SC in hydrated conditions relating to the mobility of CER headgroups, hydrocarbon chains and cholesterol, which influence the fluid lipids' movement across the lamellae. As a consequence, partially and fully hydrated SC can influence the transport of different solutes [31].

In the light of these evidencies, it is important to highlight that if the environmental relative humidity (RH) is under 100%, a gradient in water concentration forms within the SC, but when the skin is immersed in water, the SC start to swells absorbing about ten times more respect the dry weight while the permeability initially increases rapidly and then slows down to a steady state diffusion, during which the highly hydrated SC remains quite water impermeable [32].

During the contact between H_2S-H_2O and skin over the time, the interaction between H_2S and other skin components, such as cholesterol, involves mainly passive diffusion, lipophilic absorption and metabolic interaction that are more representative than surface adsorption models (colloidal sulphur) (Figure 7) [5].

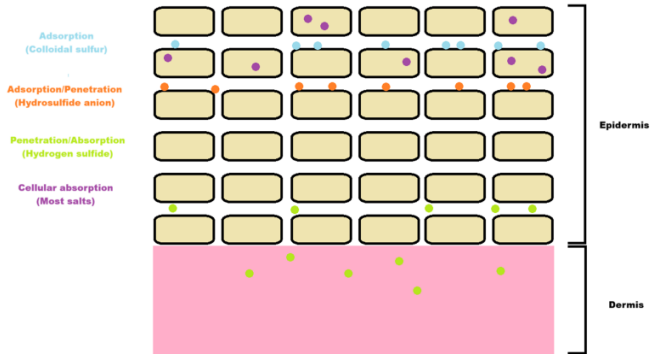

Figure 7: Adsorption/penetration/absorption of hydrogen sulfide (Adapted from [5])

Figure 6 shows the different phases that characterizes the permeation of H_2S across the skin as adsorption, penetration, absorption [5], demonstrating that the uptake of hydrogen sulfide at skin level occurs mainly by absorption process.

3.2. The H_2S dissociation

Generally, the dissociation of H_2S follows its adsorption when it comes in contact with solid surfaces,– an exothermic transformation that leads to surface sulfidation and leads to-the formation of adsorbed radical SH_{ads} (bisulfide), S_{ads} (sulfide) atom and hydrogen atom (H_{ads}) [33], according to a two-steps process– (Equations 3 and 4).

$$H_2S \rightarrow SH_{ads} + H_{ads} \quad (3)$$

$$SH_{ads} \rightarrow S_{ads} + H_{ads} \quad (4)$$

The first dissociation step occurs at E =1.6 kcal/mol with ΔE of -19.6 kcal/mol, while the second step occurs at E = 0.7 kcal/mol with a ΔE value of -18.2 kcal/mol [33]. Notably, the H_2S dissociation is a kinetically and thermodynamically favorable process.

The efficiency of H_2S dissociation improves when the solid catalyst is immersed in a liquid solvent (absorber) because the volumetric and surface concentration of H_2S increase with an improvement of the rate of chemical transformation due to an increase in the adsorbed H_2S coverage on the catalyst surface [33]. For example, the aqueous monoethanolamine (MEA) solution is a typical H_2S absorber that promotes an high efficiency dissociation (97.9%) at room temperature (Equation 5).

$$H_2S + R\text{-}NH2 \leftrightarrow HS^- + R\text{-}NH3+ \quad (5)$$

An alternative catalyzed dissociation process happens instead over a platinum surface such as Pt(111), in which the adsorbed H_2S dissociates following Equation 6.

$$H_2S \rightarrow 2H_2 + S_{2(g)} \quad (6)$$

The S_2 producted in this manner is well soluble in water (>5 g/L) and, if the reaction showed in Equation 6 occurs in water, white sulfur globules can be obtained when the water solution is saturated.

Another biologically relevant process is possible in presence of sulfur bacteria, in which H_2S can be oxidized to elemental sulfur-, following the Equation 7.

$$2\ H_2S + O_2 \rightarrow 2S + 2H_2O \quad (7)$$

The S_0 globules obtained following Equation 7 are components of S_8 crown-shaped molecule (ciclooctasulfur). The biological S_8 globules produced by colorless sulfur bacteria suggests that the biological H_2S assimilation can be also resulted in the formation of S_2 (Equation 8).

$$2H_2S \rightarrow 2H_2{}^* + \text{condensed-phase } S_2 \ (S_8) \quad (8)$$

Where the activated $H_2{}^*$ react with CO_2 to form HCOOH in bacteria during the chemiosyntesis process [33].

3.2.1. Dissociation on biological surfaces

The dissociation of H_2S on surfaces is a complex, multi-step process that has been investigated in metal-based catalysts and in chemical biology to understand H_2S toxicity and signalling.

For example, in biological systems, H_2S interacts with membrane surfaces and cytosolic proteins to produce reactive and unstable persulfides (R-SSH) that are fundamental intermediates in many biochemical interactions.

In the case of skin, undissociated H_2S, a lipophilic molecule, is directly absorbed when comes in contact with the SC surface via passive diffusion due to the presence of lipids (ceramides), because the skin pH is inside the range 5.5-6.5 (see paragraph 3.3.), as reported in the above 3.3 paragraph. As a consequence, the dissociation processes at the skin surface are negligible [5].

4. Surface tensiometry of H_2S in acqueous systems

It is well known since the 70s that the surface tension (ST: dyne/cm or mN/m) of anhydrous liquid H_2S in the 25÷40°C temperatures range varies from 11.3 to 8.7, in accord with the Guggenheim equation [34]; for example, addition of $H_2S_{(g)}$ bubbles in water at pressures over 300 psi and in this same temperature range causes a great decrease in ST (γ) of the H_2S-H_2O system [34].

The γ decrease of the system towards the lowest value corresponds to the increase of the adsorption process of $H_2S_{(g)}$ (monolayer) at the water surface (liquid/vapour interface) that reaches the monolayer adsorption at about ½ of the saturation pressure [34] (Figure 8). The effects of $H_2S_{(g)}$ adsorption on the γ of water can be measured by using the differential capillary rise method [34].

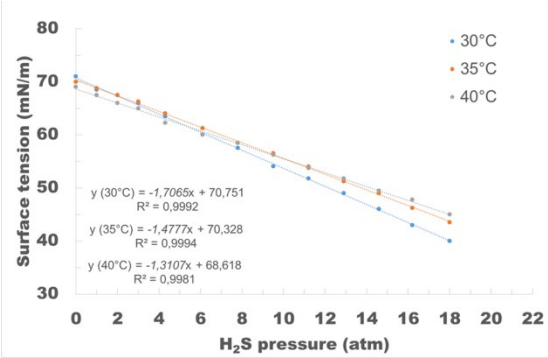

Figure 8: Surface tension (γ) of water saturated with H_2S against H_2S pressure (Adapted from [34]).

The H_2S-H_2O interface obtained after its saturation with H_2S can be characterised in a temperature range of 25÷130°C, using the pendant drop method (PD) [35]. After the PD characterisation of the interface, the γ of H_2O saturated with H_2S can be calculated according to Equation 9.

$$\gamma = g^*(\rho_1-\rho_2)d_e^2/H \quad (9)$$

where γ is the ST (mN/m), g is the gravity acceleration, ρ_1 is the density of H_2S phase (gas or liquid), ρ_2 is the density of aqueous phase, d_e is the maximum diameter of the drop [35].

The γ of H_2O saturated with H_2S decreases with the increase of pressure, however this dependence decreases with the increase of temperature (Figure 9), while the solubility of $H_2S_{(g)}$ in water and its adsorption process decreases in the range 25÷130°C [35].

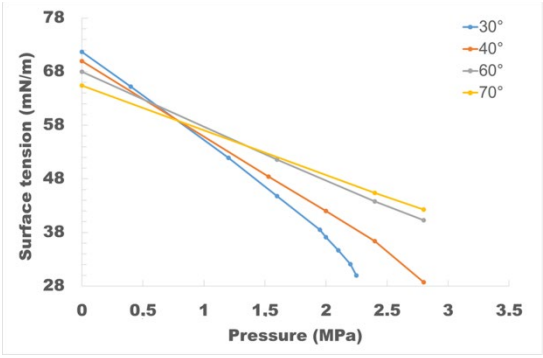

Figure 9: Surface tension variations in function of the pressure (Adapted from [35])

From a liquid/solid (l/s) interface's viewpoint, generally the variation of the concentration of H_2S in water causes alterations to viscosity, interfacial free energy (IFT; mN/m) and wettability contact angle (CA; deg) [36]. In fact, the IFT of the H_2S-H_2O system strongly decreases with the increase of H_2S concentration, while the CAs measured at the l/s interface depend strongly on the chemical properties of the substrates [36].

5. Surface tensiometry phenomena at water/skin interface

The CAs of several liquids on human skin (liquid/skin interface) can be measured before and after skin treatment with chemical substances to determine their effects on SC. The critical surface free energy (SFE; mJ/m^2) of the skin can be determined by extrapolating the cosines of the CAs of liquids such as acetone, water and diiodomethane as a function of their ST (mN/m) [37].

Wetting refers to the contact between a solid surface and a liquid; it depends on intermolecular interactions [38]. The degree of surface wetting is evaluated through the measurement of the CA θ. The smaller is θ, the better is the surface wetting. When $\theta = 0°$, the surface wets completely; the opposite is true for $\theta = 180°$ (dewetting). Partial wetting refers to θ ranging from 0° to 180° [38].

Young's equation correlates the surface tension between the liquid–vapour (γLV), the solid–vapour (γSV), the solid–liquid (γSL) interfaces with the free surface energy (γ) and contact angle (θ) (Equation 10).

$$\gamma LV \cos \theta = \gamma SV - \gamma SL - \pi e \quad (10)$$

where πe (external pressure) equals ~~to~~ 0 for low energy solids.

The surface hydrophobicity plays an important role in many biological processes, such as cellular adhesion, contact inhibition, elasticity, functionality of tissue membranes, functioning of intracellular structures, and adhesion of infectious microorganisms [38].

The skin hydrophobic balance, represented by surface hydrophobia/surface hydrophilia ratio (Ho/Hi), is quantified by a relationship between γc and the water γ (Equation 11),

$$Hi = \gamma c/\gamma H_2O \quad (11)$$

where Hi is the surface hydrophilia. This parameter is expressed by the ratio of its critical surface tension γc to the normalizrd water surface tension γH_2O.

Studies on the skin wettability clearly show the role of the hydrolipidic layer on the skin's hydrophobicity. The suppression or the alteration of this layer increases the skin's hydrophobicity. This property of the cutaneous lipids, which increases skin wettability, appears due to the free fatty acids, especially those composing the sebum. Skin wetting by lipids was found to increase with the amount of squalene and paraffin in sebum [38].

The *in vivo* quantification of physicochemical parameters can be performed also analysing the wettability of water onto nails surfaces representing a potential value in the field of research.

These studies are increasingly in the core attention of researchers because of the practical interest of investigations on the penetration of drugs and transdermal barrier function. The development and selection of films, including antifungal treatments (varnish), depend on the knowledge of these parameters [38].

More recently, Rossi and Bettero defined the skin as an „Integrated Chemical Cutaneous System" constituted by many surface tensiometry dispersion components (γ^d) and polar components (γ^p) due to the presence of different molecules derived from their contact with skin (equation 12) [39].

$$\gamma_{SKIN} = (\gamma^d_A + \gamma^d_B + \gamma^d_C + \gamma^d_D + ...) + (\gamma^p_A + \gamma^p_B + \gamma^p_C + \gamma^p_D + ...) \quad (12)$$

Where γ_{SKIN} is the Surface Free Energy (SFE mJ/m^2) of skin and γ^d_A, γ^d_B, γ^d_C, γ^d_D are the dispersion components (DC; mJ/m^2) of A, B, C, D chemical compounds present in the SC of the skin, and γ^p_A, γ^p_B, γ^p_C, γ^p_D are the correspondent polar components (PC; mJ/m^2) [39]; accordingly, it can be envisaged– the possibility that the presence of H_2S (γ_{H2S}) can modify the γ_{SKIN} during its permeation through the SC [39].

6. Role of H_2S in balneotherapy.

In nature, H_2S dissolves in various amounts in many thermal spring waters, which have different chemical compositions, depending on the geographical area of their source (e.g. near the sea, volcanic, geothermal or mountain areas, etc.) [40].

The main sources of H_2S in sulfidic environments are represented by: (a) deep-sea hydrothermal vents, where the sulfate seawater penetrates through the earth's crust and transforms into H_2S under reductive conditions; (b) cold seep sulfide springs, where methane-, hydrocarbon- and sulfate-enriched water is forced upwards through sediments by pressure gradients and the activity of sulfate-reducing bacteria, under anoxic conditions, produce H_2S; (c) freshwater sulfide spring waters, which are constituted by water of meteoric origin that accumulate H_2S during its ground flow (groundwater water reservoirs) by sulfate-reducing bacterial activity [40].

In thermal waters, the H_2S may be present under certain conditions as different reactive sulfur species. For example, the interaction between sulfur and oxygen radicals leads to the formation of subproducts, such as pentathionic acid, which may represent the source of the antibacterial and antifungal activity of thermal water on the skin that can be particularly important in the management of thermal waters for recreational use [41].

The activity of sulfur in the skin seems to be related mainly to its interaction with cysteine and its catabolites [41]. Accordingly, bathing in sulfur-rich spring water can treat many immune-mediated skin diseases, such as psoriasis and atopic dermatitis (AtD). Due to the sulfur and H_2S in thermal water, this therapy induces a decrease of pruritus and inflammation in patients with psoriasis, exerting anti-inflammatory, keratoplastic, and antipruriginous effects [41].

Carbajo et al. proposed the "*bioregulatory effect of balneotherapy*", a mechanism which diminishes systemic pro-inflammatory mediators but preserves an effective innate immune response, ensured by stimulation of neutrophil-mediated defences such as phagocytosis and microbicidal activity [5].

Furthermore, the use of sulfur water has been proposed to relieve not only AtD but also leg ulcers, acne, and hidradenitis suppurativa lesions. This is because sulfur can interact with ROS in the deeper epidermis.

Specifically, exogenous H_2S from balneotherapy promotes the proliferation and differentiation of human keratinocytes in a dose-dependent manner by regulating autophagy. In addition, sulfur waters are also used to treat acne, mainly due to their keratolytic effect. Due to the peculiar skin physiology, which lead to the optimisation of H_2S cellular absorption, treatment with sulfurous waters is really a key therapeutic strategy in balneotherapy. The beneficial properties of bathing in thermal spring water have been known since ancient times, and nowadays this kind of therapy remains a popular treatment for chronic skin and rheumatologic diseases.

In general, the waters used in balneotherapy have different physical and chemical compositions and are rich in H_2S, sulfur, sulfates, and other ions [5]. Therefore, bathing in sulfur-containing hot springs acts as an important alternative dermatological therapy; multiple recent *in vivo* and *in vitro* experiments have shed light on the biological and pharmacological roles of H_2S under a variety of physiological and pathological conditions.

The effects of balneotherapy can be partly explained by hormetic phenomena associated with non-specific factors such as heat, which activates the heat shock response and stimulates the synthesis of heat shock proteins (HSPs), while specific biochemical factors present in certain waters, such as H_2S, contribute to the modulation of oxidative stress and inflammatory pathways [5].

When skin and warm water spring come into contact with each other during balneotherapy, the cutaneous absorption of H_2S increases significantly at the water/skin interface because the continuous contact at the skin surface during immersion stimulates the opening of porous channels with an increase in the cutaneous blood flow and the gas solubility [5].

During the balneotherapy, H_2S acts in synergy with other components such as magnesium, sodium, sulfates, bicarbonate, carbon dioxide (CO_2), or trace elements, inducing vasodilation, improving the epidermal hydration and increasing the transdermal absorption of the gas [5]. In particular, CO_2 activates the endothelial nitric oxide synthase (eNOS), which improves H_2S effects by stimulating oxygenation and perfusion [5].

In this context, the skin hydration state should play a critical role in enhancing H_2S skin absorption-, allowing this gas ~~it~~ to penetrate deeper into the dermis, to reach internal organs, and also act as a gasotransmitter.

6. Perspectives of research

On the basis of what is reported in our review, the study of surface chemistry and surface tensiometry of warm spring waters (WSW) for balneotherapy purposes highlights the opportunity of undertaking a surface tensiometry investigation of waters containing H_2S (WSW-H_2S) using contact angle method (CA; deg) and different solid substrates having diverse surface free energy (SFE; mJ/m^2), dispersion component (DC; mJ/m^2), and polar component (PC; mJ/m^2). The surface tensiometry approach at the study of WSW-H_2S aims to characterize their surface tensiometry properties through the determination of surface tension (ST; mN/m), dispersion component (DC; mN/m), and polar component (PC; mN/m).

The results of these new surface tensiometry studies could be compared with those performed by Carlyle et al. in the range of 25-40°C and using the differential capillary rise method [34]. Furthermore, CAs and ST of WSW-H_2S using different solid substrates could be correlated at those obtained by Strathdee and Given [34] performed using the pendant drop method (PD) within the calculation of the interfacial free energy (IFE; mN/m) in the same range adopted by Carlyle et al. [34].

Along with this perpective, the CAs of different WSW-H_2S could be also determined by solid-like methdology (SLM) using a liquid film of Polyperfluoromethylisopropyl Ether (PFPEf) as universal "solid substrate" to avoid friction forces and surface roughness at water/PFPEf interface, which can affect– CAs measurements performed on conventional solids test [42-44].

It is important to highlight that the determination of the CAs/ST of each sample of WSW-H_2S should be performed simultaneously with other analytical techniques such as GC-FPD and employing chemical, colourimetric and fluorimetric methods [13-15] following the concept of Integrated Analytical Approach (IAA) *"on the same material sample and at the same time*" [10].

The second fundamental element is the skin. The skin corneum strate (SC) is the main "gate" through which H_2S can diffuse, reaching the system during balneother-

apy. Following the relationships between skin hydration (SH) [39] and H_2S skin permeation (SP) [32], the evaluation of the SH state, performed using the same surface tensiometry techniques employed for WSW-H_2S characterization, should represents an essential key for improving the uptake of H_2S and other natural subtances having potential therapeutical efficacies during balneotherapy.

Thus, in our opinion, the simultaneous, fast and non-invasive measurement of WSW-H_2S and SH with the same surface tensiometry approach represents the main perspective showed in our work. To reach this objective, the surface tensiometry control of SH (water CA<90°) performed using Tenskinmeter® [39] and the analysis of the skin pH (5.5-6.5) [5] of each patient before balneotherapy represents the essential prerequisite to ensure the optimal SP of H_2S.

In case of dry skin (water CA<90), the increase of SH should represent a fundamental condition to prepare the patient at the balneotherapy. This could be ensured following a regular body hydration with 2L of water per day and/or the application of skins natural moisturizers based on thermal elements respecting the skin pH range.

At the end, the effects of exogenous H_2S uptake after a cycle of balneotherapy should be monitored chemically and/or clinically to correlate them with surface tensiometry properties of WSW-H_2S and surface tensiometry properties of skin [19].

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