Advances in H₂ sensors for bioanalytical applications

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Tribute to Prof. Marco Mascini

Abstract

H₂ sensors have been receiving more attention, especially with the development of H₂ medicine and biodegradable alloys that produce H₂. The H₂ sensors discussed in this review are primarily the electrochemical, chemochromic metal oxides, and color indicator sensors. This review focuses on the bioanalytical application of these existing H₂ sensors. The performance parameters of these sensors are reviewed to aid in identifying and selecting appropriate sensors for practical applications.

Key words: H₂ sensors, H₂ detection, biological applications, electrochemical sensors, metal oxides, color indicator

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1. Introduction

Molecular hydrogen (H_2) has been receiving more and more attention due to its various novel medical and therapeutic applications with antioxidant and anti-inflammatory effects [1-5]. Inhaling H_2 gas has been shown to be effective with rat models of acute inflammatory and oxidative diseases, including brain and myocardial infarction and post-cardiac arrest syndrome [5]. Moreover, inhalation of H_2 gas strongly suppresses ischemic and reperfusion brain injury [1,2]. Additionally, consumption of water saturated with H_2 (H_2 water) prevents stress-induced impairments in learning tasks during chronic physical restraint [3] by buffering the effects of oxidative stress or superoxide formation [6]. H_2 has advantages as a potential antioxidant without adverse effects: it is mild enough to neither disturb metabolic redox reactions nor to affect reactive oxygen species (ROS), which function in cell signaling [7-10]. It has low affinity for physical ROS, and directly eliminates hydroxyl radical. In addition, it has favorable distribution characteristics because of its physical ability to penetrate biomembranes and diffuse through barriers into cellular components. Due to these important advantages, H_2 has been used for oxidative stress-mediated disease, such as brain stem infarction, diabetes mellitus, neurodegenerative diseases or rheumatoid arthritis. One of the vital parameter for molecular H_2 application is its concentration, making the detection of H_2 concentration *in vivo* and *in vitro* desirable. So far, the measurement of H_2 concentration in the blood for animals is mostly done by gas chromatography. The instruments are large, expensive and require trained personnel. Therefore, portable and less expensive detection methods are needed for *in vivo* and *in vitro* studies.

Biodegradable metals such as Mg and its alloys provide many advantages over the stainless steel and titanium permanent implants used today such as for bone repair [11]. Magnesium

biodegradation rate is one of the crucial parameters to evaluate for their biomedical applications.

Magnesium and its alloys degrade in aqueous environments, generating magnesium (Mg^{2+}) and hydroxyl (OH^-) ions, as well as H_2 gas. Therefore, measuring H_2 evolution rate is equivalent to measuring the biodegradation or corrosion rate of a magnesium alloy. So far, the measurement of H_2 evolution is mostly performed *in vitro* by measuring the generated H_2 volume. However, this measurement is cumbersome, and suffers from H_2 gas leaking. Therefore, a simple and effective H_2 sensing method *in vivo* is desirable.

So far, the research and review articles on H_2 sensors have focused on detecting H_2 leaks associated with transportation pipes and fuel cells. Although the application of H_2 sensors in bioanalysis is increasing, this growing area has yet to be reviewed. This review focuses on H_2 sensors as related to bioanalytical applications.

2. Electrochemical sensors

Electrochemical H_2 sensors are devices for detecting H_2 concentration that are dependent on electrochemical reactions at the sensing electrode. The signal from an electrochemical cell changes in proportion to the H_2 concentration at the electrode surface [12]. Electrochemical H_2 sensors offer the advantages of room temperature operation and low power consumption. A market survey found that electrochemical H_2 sensors are commercially more available than any other types [13]. The fundamentals of the major categories of electrochemical sensors (amperometric, potentiometric, and conductometric) have been reviewed in detail by Korotcenkov et al. [14]. Briefly, in the most commonly adopted two-electrode amperometric H_2 sensor system, H_2 diffuses through a gas permeable layer to the sensing electrode (Pt and Pd) at which the oxidation of H_2 takes place. The potential between the working and reference electrodes is maintained constant. The electric current generated through electron transfer with

H₂ is proportional to the concentration of H₂ according to Faraday's law [14]. Different from the amperometric sensors, H₂ determination with a potentiometric sensor measures the potential difference between the sensing and reference electrodes at zero current where the redox reaction is at equilibrium. The H₂ concentration is obtained based on the Nernst equation [13-14]. Unlike the amperometric and potentiometric H₂ sensors, the conductometric H₂ sensor detects solution resistance, therefore, it is not H₂ selective. However, the much higher conductivity of proton compared with ions generated from other gases enables it to achieve similar selectivity to H₂ as the other two electrochemical sensors [14].

A Clark-type H₂ microsensor was first described in 1991 [15]. It operated with a Pt anode in acidic electrolyte environment and responded to H₂ concentration. However, it was extremely unstable and could lose sensitivity to H₂ within hours. Later on, newer versions of the Clark-type H₂ sensor with higher stability were commercialized [16].

In vivo H₂ sensing was first applied to a study of correlating H₂ level with colonization of *H. pylori* in the stomach [17]. During the procedure, mice were kept fully anesthetized and the tissue was surgically exposed. H₂ concentration was determined by inserting the electrochemical H₂ microsensor (H₂-50, Unisense) into the mucus lining area of the mouse stomach through an incision. The amperometric H₂ microsensors had high sensitivity towards H₂ with a detection limit of 0.1 μM according to the manufacturer. The procedure was repeated for up to eight sites per mouse stomach. The H₂ level was calibrated by standard curves using a calibration chamber as described by the manufacturer [17]. This H₂ measurement strategy was adapted in a study subsequent to that, where the same type of H₂ microsensor was used for detecting H₂ in mouse liver [18]. This sensor was also used to determine bone blood flow by monitoring an H₂/O₂ mixture during its washout in the blood flow. It requires breathing an H₂/O₂ mixture until the H₂

levels of the gas equilibrate in the tissue. The gas mixture flow is then stopped, resulting in a washout rate of tissue H₂ that is proportional to the blood flow according to equation [19]:

$$C = C_0 e^{-Kt}$$

Where C is the H₂ concentration at time t , C_0 is the original H₂ concentration, $K = F/\lambda$, F is the flow rate (mL/min), λ is the partition coefficient of H₂ in tissue, and t is the time.

In the promising field of developing biodegradable metals for medical applications, H₂ sensing has become a unique application for evaluating biomedical materials because H₂ is a product of metal biodegradation. We and others are developing nontoxic, light weight, rapidly biodegradable metallic biomaterials to replace permanently implanted metals as internal fixtures to aid the healing of fractured bones and tissue [20]. Mg and its various alloys are the major materials of interest in this research, which have been fabricated and tested *in vitro* and *in vivo*.

The first application of H₂ measurement with the H₂-50 electrochemical sensor was to investigate the formation of gas cavities caused by Mg alloy (Mg-4 wt.% Y-0.5 wt.% Gd-2 wt.% Nd-0.5 wt.% Dy) implanted subcutaneously in mice for *in vivo* testing [21]. The formation of these cavities was widely reported and the composition and concentration of each component of the gas was the subject of much debate in the field [22]. It was often assumed that gas cavities that formed during Mg material corrosion *in vivo* contained primarily H₂ until a recent study carried out by our group, using the H₂-50 microsensor proved that H₂ exchanges rapidly with the surrounding tissue in mice [21]. A 1 cm long incision in the mouse skin was made to give access to the gas cavity adjacent to an implanted Mg sample. Measurements of H₂ showed that the levels were actually very low, less than 1.2 % v/v in either 2-day or 10-day monitoring. The high sensitivity and fast response enabled measurements both in the cavities and on the surface of the gas cavities using a micropositioner to hold the sensor (Fig. 1a). The results were confirmed by

collecting gas in the cavity for analysis by mass spectrometry [21]. In a subsequent study, Mg materials were subcutaneously implanted in mice and instead of making an incision for the H₂ measurement, detection was made non-invasively by pressing the sensor tip on the surface of the mouse skin. Because of the rapid exchange of H₂ through the skin, the H₂ concentration can indicate the rate of corrosion of an implanted Mg sample. Typical measurements made on the skin on top of the gas cavity and far away from the gas cavity are shown in Fig. 1b. As seen, the H₂ microsensor response is very fast - less than 1 min. Some variability in H₂ was found depending on exactly where the tip was placed on the cavity. When the sensor tip was moved off of the cavity, H₂ was still detected from the skin immediately adjacent to the cavity, but at considerably lower concentrations. The H₂ microsensor was used to map out the H₂ permeating through the skin. A good correlation between the H₂ levels with the corrosion rate was established based on this study [23-27].

Fig. 1. H₂ concentration measurements *in vivo*. (a) Experimental apparatus with an anesthetized mouse positioned on a swiveling table. A H₂ microsensor is mounted in a micromanipulator and positioned in an incision in the skin of the hairless mouse. (Reprinted from Acta Biomater., Vol 9, J. Kuhlmann, I. Bartsch, E. Willbold, S. Schuchardt, O. Holz, N. Hort, D. Hoche, W.R.

Heineman and F. Witte, Fast escape of hydrogen from gas cavities around corroding magnesium implants, p. 8714-8721 (ref 21). Copyright (2013), with permission from Elsevier.). (b) Noninvasive H₂ measurement through the skin above a gas cavity and far from the cavity on a mouse with a sample of Mg single crystal implanted subcutaneously on its back. (c) Response of the thin film visual H₂ sensor from Element One before and (d) after 60 min exposure to H₂ permeating through the mouse skin from implanted biodegrading Mg.

Although the H₂-50 microsensor is a very sensitive and relatively stable H₂ sensor, its fragility is a concern, especially when inserting into tissue is required for *in vivo* experiments. Even when non-invasive experiments are done, such as the measurement of H₂ escaping from mouse skin above a Mg implant, gentle movement of the mouse can damage the sensor tip. Recently a more robust needle type amperometric H₂ sensor (H₂-N needle sensor and H₂-NP needle sensor for piercing, Unisense) was introduced for biomedical applications. In the H₂-N sensor, instead of having a glass tip, a steel needle is mounted on the sensor for protection purposes. The first study using the H₂-N sensor was carried out by Hayashida et al. by directly placing the sensor into tissues to investigate H₂-dependent cardioprotection against ischemia-reperfusion injuries in rats in 2008 [28]. In a study conducted by Kajiya et al., intestinally produced H₂ was determined to suppress inflammation in the liver and prevent the development of dextran sodium sulfate-induced colitis [29-30]. The H₂-N sensor was placed into organs - small intestine, larger intestine, caecum, liver, spleen and brain - after sacrifice and blood H₂ levels were monitored by cardiac puncture using the H₂-N sensor [29]. Kamimura et al. used the H₂-N sensor to monitor the dynamic movement of H₂ in the liver after oral administration of H₂-water to assess the biomedical treatment for obesity, and levels of H₂ in the liver were monitored by directly

inserting a needle-type H₂ sensor into the liver for an hour [31]. The H₂ concentration profile gave a peak 5 min after administration of H₂-water in both fed and fasted livers (Fig. 2). A significant difference was found between the fed and fasted livers; the maximum H₂ level in the fed liver was two-fold that of the fasted liver. Moreover, the fed liver maintained a considerable H₂ level for an hour, while that in the fasted liver returned to the basal level after 25 min. A correlation between glycogen concentration and accumulated H₂ was found using a H₂-N microsensor. The study also showed that a fatty liver from rats drinking H₂ rich water contained less oxidative stress marker proteins and looked healthier compared to fat rats that did not receive H₂ enriched water.

Fig. 2. H₂ concentration measured in rat liver of a fed and an overnight-fasted rat. (Reprinted from Obesity, Vol 19, N. Kamimura, K. Nishimaki, I. Ohsawa, S. Ohta, Molecular hydrogen improves obesity and diabetes by inducing hepatic FGF21 and stimulating energy metabolism in db/db mice, p. 1396-1403 (ref 31). Copyright (2011), with permission from John Wiley & Sons, Inc.).

Terasaki et al. have used the H₂ microsensor to study the radioprotective effects of H₂ for human (*in vitro*) and mice (*in vivo*) lung fibrosis [32]. The protective effect of H₂ during lung damage induced by irradiation was also investigated with H₂-N by directly inserting the sensor

into mouse lung. They pointed out that H₂, with no known toxic side effects, effectively protects cells and lungs of mice from oxidative stress and damage caused by irradiation because of the rapid diffusion of H₂ across cell membranes and its reaction with cytotoxic ROS. In another study, an examination of the role of H₂ inhalation for O₂ deficit induced heart injuries also used the same sensor and approach [33]. Oharazawa and coworkers have used the H₂ microsensor to monitor the H₂ concentration in eye drops to study H₂'s protection of the retina [34]. The H₂ measurement was made by immersion of the H₂ sensor into the solution. Itoh et al. [have used the H₂-N microsensor](#) in a similar way to abolish an immediate-type allergic reaction in mice [35]. Furthermore, if H₂ monitoring has to take place under muscle, where H₂ detection is difficult [through skin, the tip protected](#) needle sensor (H₂-NP) can be used to pierce underneath the muscle without the danger of damaging the sensor tip.

In this relatively new field of biomedical sensing of H₂ using electrochemical sensors, considerable research is ongoing to further improve the sensitivity, selectivity, response time, reliability, life-time, and cost. A wireless sensor network was reported in 2003 for atmospheric H₂ sensing [36]. The sensor network consists of Ti nanotubes and Pt electrodes. [H₂](#) concentrations from 0 ppm to 500 ppm were correlated with the electrical resistance from 245 Ω to 10.23 kΩ. However, no wireless H₂ sensor has been developed for *in vivo* H₂ sensing so far. As more biological functions/applications of H₂ are discovered, we [believe new](#) electrochemical H₂ sensors, including wireless H₂ sensors will be developed to better serve biomedical applications.

3. Chemochromic semiconductor sensors

Although the direct optical detection of H₂ is not easy due to its lack of light absorption in the ultraviolet-visible or infrared region, various chemochromic devices that undergo color changes

upon exposure to H₂ have been developed [12, 37-40]. For example, WO₃ films change their color from greenish yellow to blue when exposed to H₂. WO₃, MoO₃, Nb₂O₅, V₂O₅ and noble metals such as Pd, Pt, Ir, and Rh are extensively used for this purpose. The sensing mechanisms involve reduction of the metal oxides by H₂ in the presence of a catalyst. Take Pd/WO₃ as an example where H₂ gas is dissociated at room temperature on the surface of Pd catalyst into H atoms (eq 1) that diffuse into the underlying transition metal oxide film to reduce WO₃ causing it to change color to [bronze \(eq 2\)](#). The reaction mechanism can be written as follows:

Hoagland et al. have developed a H₂ gas indicator with multi-layered structure that is commercially available from Element One. The sensor comprises layers of H₂ sensitive material such as the oxides of vanadium, yttrium, tungsten, and molybdenum, a catalyst such as Pd, Pt, Ni, and Rh, and a protective molecular diffusion barrier such as a polymer film [41]. A typical thin film indicator consists of a transparent polymer substrate, a 500 nm thick layer of tungsten oxide, a 3 nm layer of palladium catalyst, and a 100 nm thick protective coating of poly-tetrafluoroethylene (PTFE). Exposure to H₂ causes the color to change from light off-white to dark blue. They also developed a reversible H₂ sensor that returns to the original off-white color after exposure for 16 h in air due to oxidation by atmospheric oxygen. The reversible H₂ sensor is reusable, which reduces cost. The color change of the irreversible H₂ sensor can last for several weeks. The reversible/irreversible systems are based on different metal oxides/catalysts. These sensors can detect H₂ as low as 300 ppm, which has made them useful as simple visual sensors for detection of H₂ leaks in tank storage, pipe lines, fuel cells, and so on in industry. They have been developed in different configurations such as H₂ tapes, paints, decals, thin coatings,

indicator tags and warning signs for various applications. The detection method does not require electronic circuitry and is simple, visible, low cost, flexible, and easily implemented.

We have used the visual H₂ sensor developed by Element One to monitor the Mg alloy corrosion process during *in vivo* testing with the subcutaneous mouse model that was described in the previous section. The visual H₂ sensor was tested by positioning it on the skin directly above a Mg sample implanted under the skin of an anesthetized mouse. The color of the visual sensor changes from gray to dark blue as it is exposed to H₂ generated from the Mg alloy biodegrading process (Fig. 1c-d). The color response of the sensor outlines the region of H₂ permeation from the biodegrading Mg through the skin. This method provides a rapid, visual, noninvasive, easily performed measurement of H₂. It takes advantage of the degradation mechanism of the main metal component, Mg, which generates H₂ gas that permeates through the skin adjacent to the implant at a low level. This low cost, simple method of monitoring the dissolution of biodegradable implants would facilitate the development of biodegradable materials, especially in animal studies where *in vivo* biodegradation is tested and is potentially useful for monitoring biodegradation of implants in patients.

Seibert et al. have developed a screen procedure based on a chemichromic WO₃ film to identify H₂-producing mutants with increased O₂ pressure [42]. An agar plate containing different algal colonies was covered with filter paper onto which a grid was placed (Fig. 3a). The chemochromic film was placed on filter paper which covers the different algal colonies (Fig. 3b), then illuminated with light induced H₂ production by the individual algal colonies. The color of the WO₃ chemochromic film changed to purple (seen as black in Fig. 3c) in the presence of H₂ generated by the algal colonies. After exposure to air for 5 min, the chemochromic film returned to its original color (Fig. 3d), which indicates reversibility of the sensor film. The study

demonstrated that the chemochromic film is sensitive enough to detect nanomoles of H_2 and has the potential to rapidly screen large numbers of colonies for H_2 production capacity. The technique has been used to screen and isolate large numbers of H_2 producing mutants of *C. reinhardtii* colonies.

Fig. 3. Chemochromic film screen for H_2 -producing algal colonies. (a) Plate of individual algal colonies on agar. Grid placed on filter paper covering the algal colonies to allow easy identification of individual colonies. (b) Chemochromic film placed on top of the filter paper/grid and illuminated from the bottom of the plate to induce H_2 evolution by the algal colonies. (c) Chemochromic film and grid removed from the plate containing the algal colonies for better observation of the purple spots on the film, which correspond to areas sensitized by H_2 evolved by the colonies. Note differences in intensity of the spots, which correspond to the amount of H_2 produced by the colony. (d) Sensitized film after exposure to air for 5 min. The spots disappeared, demonstrating that they were exposed to H_2 . (Reprinted from *Biohydrogen*, Development of selection and screening procedures for rapid identification of H_2 -producing algal mutants with increased O_2 tolerance, Chapter 30, 1998, p. 227-234, M. Seibert, T. Flynn, D.K. Benson, C. Tracy, M.L. Ghirardi, (ref.42). With permission from Springer).

4. Color indicator sensors

Recently, a simple method employing a color indicator and H₂-oxidation catalyst has been developed and used for the detection of biological H₂ production [43-44]. The evaluated color indicators and catalysts are summarized in Table 1. This method involves hydrogenation of the color agent in the presence of the catalyst; the colors of the color agent before and after the hydrogenation are different. Generally, unsaturated double bonds between chromophore and auxochromes of a color agent are involved in the color change. For example, the color of methylene blue is blue in an aqueous solution; hydrogenation reduces the methylene blue to colorless leucomethylene blue as follows:

Table 1. Color indicators and catalysts

Color Indicator	Color change	Catalyst
Methylene blue	green→yellow ^a	sulfonated Wilkinson's catalyst ^a ;
Methylene blue	Blue → colorless ^b	colloidal Pt ^b ;
neutral red	red→brown ^a	sulfonated iridium cyclooctadiene
methyl orange	orange→brown ^a	triphenylphosphine ^c ;
crystal violet	violet→yellow ^a	sulfonated rhodium cyclooctadiene
Victoria blue	Pale violet→pale brown ^a	triphenylphosphine ^c ;
Evan's Blue	blue→ yellow ^a	sulfonated ruthenium triphenylphosphine ^c ;
Bromphenol blue	Pale violet→brown ^a	Azotobacter vinlandii ^c ;
Thymol blue	yellow→brown ^a	Ralstonia eutropha ^c
Orange II,	orange→pale brown ^a	
methyl purple,	-	
WST-1,3,5,9,11,	-	
XTT	-	

Note: ^c is the reported catalysts used for the color indicator hydrogenation, however, the color indicators are not specified.

Katsuda et al. examined a series of color indicators in the presence of water soluble sulfonated Wilkinson's catalyst for screening H_2 producing microorganisms [44]. The typical color changes of indicators in 0.8 mM sulfonated Wilkinson's catalyst aqueous solution before and after H_2 exposure are shown in Fig. 4. This method has been used for the detection of H_2 gas generated by H_2 -producing microorganisms *Rhodobacter capsulatus* ST-410, which is simple, visual and effective. The decoloring process has been characterized by UV-visible spectroscopy. The absorption band of methyl orange at 520 nm disappeared after exposure to H_2 generated by microorganisms *Rhodobacter capsulatus* ST-410 within 45 h. Although the procedure is somewhat time consuming, visual detection is much easier compared with gas chromatography.

Fig. 4. Color changes of indicators in the presence of sulfonated Wilkinson's catalytic hydrogenation in aqueous solution (0.8 mM) at room temperature. Left and right vials show the color of the solution after and before the H₂ exposure. Color indicators: (a) methyl orange; (b) methyl red sodium; (c) neutral red; (d) Evan's blue; (e) crystal violet; (f) methylene blue. (Reprinted from J. Biosci. Bioeng., Vol 102, T. Katsuda, H. Oshima, M. Azuma, J. Kato, New detection method for hydrogen gas for screening hydrogen-producing microorganisms using water-soluble Wilkinson's catalyst derivative, p. 220-226 (ref 44). Copyright (2006), with permission from Elsevier.).

Schrader and coworkers have developed a high-throughput screening assay for assessing biological H₂ production [43]. This assay comprises a 96-well micro plate with water soluble hydrogenation catalyst and the color indicator on the top (catalysts and color indicators are listed in Table 1), H₂ producing cyanobacteria in another 96-well microplate at the bottom, and a membrane in between. The H₂ generated by cyanobacteria diffused through the membrane into the upper plate, and changing the color of the indicator (WST-3) which is quantified with a microplate reader. This assay is reproducible and semiquantitative, sensitive down to the 20 nmol H₂, and largely unaffected by oxygen, carbon dioxide and volatile fatty acids in the biological systems. This setup avoids catalyst microbial inhibition, and the color change is more

stable due to the physical separation of the indicator solution from the cells so it is not affected by cellular interactions or nonvolatile metabolites. This assay provides a reliable, versatile method for screening H₂ production by a large number of microbial or algal cultures. It can be adapted to various physical configurations and diverse applications, such as screening mutant strains or environmental samples. It can also be potentially used for the rapid evaluation of the effects of various factors such as pH on production of H₂ in biological environments.

Recently, Seo and coworkers used colloidal platinum as the catalyst to hydrogenate methylene blue in an aqueous solution to determine H₂ concentration in water [45]. The H₂ concentration was also determined by oxidimetry and electrochemistry and the results were correlated. This detection method performs as effectively as the electrochemical method. The combination of the color indicator and the water soluble catalyst as the H₂ biological sensor is promising. The detection method is simple, effective and visible, and requires no expensive equipment such as for gas chromatography and electrochemistry. The hydrogenation of the color indicator does not involve other side reactions.

5. Comparison of electrochemical, chemochromic metal oxide and color indicator sensors

Electrochemical sensors have the highest sensitivity among these commercial H₂ sensors. The sensors can be calibrated by different H₂ concentrations in water solution, making the measurements easily quantified. Electrochemical sensors have been used for biological studies in a subcutaneous implant mouse model to monitor the biodegradation process of magnesium alloys and to determine H₂ concentrations in medical applications of H₂. The drawbacks of this sensor are fragility, cost, and decreasing sensitivity with time,.

Semiconductor metal oxide sensors are low cost, small, easier to fabricate, with high sensitivity for use in H₂ safety applications; however, the operating conditions are harsh. Most of

them require high temperature to obtain stable resistance or conductivity. These harsh operation conditions require more power consumption and pose potential hazards, which limits their application for bioanalysis such as *in vivo* measurements. With the addition of noble metals in the metal oxides, the operation temperature is significantly lowered to room temperature or near room temperature. This property makes them more useful for bioanalytical applications in the future. The H₂ chemochromic sensors have fast response and higher sensitivity. So far, the chemochromic sensors are based on two kinds of H₂ materials: Pt-doped WO₃ thin films or other formats and palladium-alloy thin films. The H₂ chemochromic sensors based on pure palladium are safe, and efficient; however, they usually suffer from the Pd film's fatal fracture due to the α - β phase transition. In addition, pure Pd films easily peel off with exposure to H₂ due to poor adhesive to the optic fiber. These behaviors contribute to more irreversible response, which leads to hysteresis effects during the H₂ adsorption and desorption process. Therefore, different films such as PTFE have been applied to protect the Pd films, and improve sensor lifetime.

The color indicator H₂ sensors are comprised of a catalyst such as sulfonated Wilkinson's catalyst and a color indicator. The response time for this type sensor is short and the sensors have good sensitivity. They have been used for detection of biological H₂ production. The drawback of this sensor is the use of heavy metal catalysts, which can cause microbial inhibition. The color indicator sensors can be used in aqueous solution, which is more compatible with the biological environment. Overall, none of these sensors demonstrate optimum performance under all conditions. Therefore, the selection of sensor type depends on the operating requirements of the application such as the response time, measuring range, detection limit, convenience, ruggedness, etc.

6. Conclusion

The increasing activity in H₂ sensing for bioanalytical applications is being driven by the growing interest in studying the potential biological and health benefits provided of H₂ that have been reported. The development of biodegradable implants made of Mg whose corrosion can be easily monitored by transdermal H₂ detection also provides impetus to the development of appropriate H₂ sensors. The H₂ detection properties of different categories of H₂ sensors based on electrochemistry, chemochromic metal oxides and color indicators have been reviewed. Electrochemical sensors that are very sensitive and very selective are commercially available. However some probes are fragile and loss of sensitivity in the long term is an issue. Metal oxide based sensors that are used for many industrial applications operate at very high temperatures, which is problematic for many biological applications. Doping the metal oxides with noble metal lowers the operating condition to room temperature, which provides promise for biological applications. However, selectivity is still a problem due to interference from other reducing gases such as hydrogen sulfide. H₂ detection by optical and color indicator sensors is simple, effective and provides a visible signal that can be observed by the naked eye. A limitation of this type of sensor is interference by heavy metals in cell cultures. Therefore, understanding the limitations of the different H₂ sensors is needed for good sensor selection to best serve a particular application. H₂ sensors will play increasingly important roles in biological applications such as studying the effects of H₂ on biological systems, monitoring biodegradable alloy corrosion *in vivo* and *in vitro* by a H₂ sensor to provide feedback for the design of new alloys and improvement in the current alloys, and monitoring H₂ concentration changes with time in blood to provide real-time measurement information for the study of H₂ benefits for medicine.

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