

SCREEN-PRINTED SENSOR FOR CHLORIDE QUANTIFICATION IN SWEAT FOR EARLY DETERMINATION OF CYSTIC FIBROSIS

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Abstract One-use screen-printed sensor capable to generate sweat and measure the chloride concentration is presented. Sweat is induced by iontophoresis, pilocarpine is forced to get into the skin and stimulate the sweat glands. Chloride concentration is measured by potentiometry. The performance of the devices has been tested by means of reproducibility studies. Finally, the application of these sensors in several volunteers has been carried out. Errors less than 10% have been obtained in real samples.

1 INTRODUCTION

Cystic fibrosis (CF) is an inherited chronic disease that affects the lungs and digestive system (Davis, 1993). Life expectancy of people with cystic fibrosis is between 30 and 40 years (Doering et al., 2007). Early diagnosis of CF is important, newborn screening can lead to fewer hospitalizations; minimized the symptoms, nutritional benefits (Rosenstein, 1998) and potentially better lung function throughout early childhood (Wang et al., 2002).

There is a close correlation between increased concentration of chloride and sodium in sweat and the presence of the disease (Rockville, 1974). Chloride concentration in sweat less than 40 mmol dm⁻³ is defined as normal but over 60 mmol dm⁻³ is indicative of CF. People showing values between 40 – 60 mmol dm⁻³ are considered as population in risk of CF. The sweat test offers a rapid diagnosis and permits an early CF determination (Warwick et al., 1990, Warwick et al., 1986).

The Gibson-Cooke sweat test (Gibson and Cooke 1959) is accepted as the most discriminatory test for diagnosis of CF. This method is based on iontophoretic sweat test. Pilocarpine is a reagent with the capacity to stimulate sweat glands (Katzung

2004). Sweat is collected either upon a gauze square or filter paper, and then the chloride present on the sample is analyzed on a laboratory. This test involves multiple steps for collection and analysis of sweat sample, and requires prescribed procedures for each step and high level of quality control.

In this work, the development and test of four electrodes configuration sensor, fabricated by thick film technology, with the capacity to generate sweat and measure chloride ion is presented. Two electrodes were used for sweat generation. Pilocarpine is immobilised over the cathode electrode using a hydrogel matrix, and applying a small current (iontophoresis), this reagent is forced to get into the skin in order to induce sweat (Davis, Wilson et al. 2005; Ortuno, Rodenas et al. 2007).

The other electrodes, working as ISE format, measure the chloride concentration in sweat by potentiometry. Both electrodes were made of Ag/AgCl ink. One acts as working electrode. The other one was covered with KCl-containing membrane in order to realise the miniaturized reference electrode. The organic matrix consists of KCl-containing poly(2-hydroxyethyl methacrylate) (pHEMA) membrane (Simonis, Dawgul et al. 2005).

The performance of the reference electrode as well as these sensors was checked by their reproducibility and the response in different synthetic solutions of chloride. Finally, these devices were tested in several volunteers. The chloride concentrations obtained were compared with the results achieved by a common method used by the hospitals.

2 EXPERIMENTAL

2.1 Reagents, Equipment and Software

Analytical grade chemicals were used. All the solutions were prepared from ultra pure deionised water (DI) (18 MΩ cm).

Polyvinyl alcohol (PVA) powder (Mowiol 28-99, Flucka, Steinheim, Germany), pilocarpine (Advanced instruments Inc., Norwood, USA) and sodium nitrate (Advanced instruments Inc., Norwood, USA) solutions were used to develop the hydrogel matrix for iontophoresis process.

To fabricate pHEMA solution the adequate amount of 2-hydroxyethyl methacrylate (Aldrich, Steinheim, Germany), ethilenglicol (Flucka, Steinheim, Germany) Tripropylene glycol diacrylate (TPGDA) (Aldrich, Steinheim, Germany) and Benzylidimethyl-ketal (irgacure 651) (Ciba, Basel, Switzerland) were mixed.

Potassium chloride (KCl) (Flucka, Steinheim, Germany) solutions were used on the fabrication, storage and test of the fabricated sensors.

Homemade equipment was developed in order to integrate current application and chloride measurement. Sweat chloride analyzer (Advanced instruments Inc., Norwood, USA) was used to contrast the measurements achieve with the homemade electrodes.

2.2 Electrode Preparation

2.2.1 Screen-printed Electrode Fabrication

A DEK 248 screen-printing system (DEK, UK), screen polyester mesh and polyurethane squeegees were used to fabricate the electrodes. Sequential layer deposition has been performed on a polyester substrate (0.15mm thickness). First, a layer of silver ink (Electrodag 418 SS) was deposited to define the conductive paths. Over these paths, a layer of Ag/AgCl ink (Electrodag 6037SS) was deposited to

form the electrodes. A drying cycle (80°/30 min + 120°/5 min) was subsequently applied (Gonzalo-Ruiz et al., 2007). Finally, a piece of polyester substrate was used to prevent the conducting paths from the solution.

These designs are made up of two parts, sweat generator made up of the two external electrodes (28.2 mm²) and potentiometric sensor composes of both internal electrodes (7.0 mm²) (Fig.1).

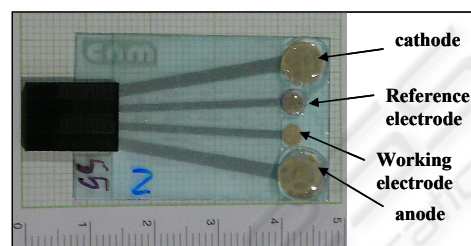


Figure 1: Picture of screen-printed sensor.

2.2.2 Electrode Modification Procedure

In order to fabricate the sweat generator, a hydrogel formulation containing polyvinyl alcohol (PVA) and pilocarpine was developed to entrap this drug over the cathode surface.

Aqueous solution of 17% by weight of PVA was prepared by adding a calculated amount of dry PVA powder into a mixing vessel and slowly dissolving it in water. The temperature of the solution was raised to 98 – 100 °C during 15 minutes with continuous stirring of the mixture. It was then transferred to pattern and frozen at -10 °C during 24h. Each pattern had a diameter of 6.2 mm, bit bigger than the electrode, and a thickness of 2 mm. The cured hydrogel samples were immersed, overnight, in a solution of 0.5% by weight of pilocarpine. These pieces were stuck on the cathode surface.

Hydrogel sample saturated with sodium nitrate solution (1% by weight), fabricated in the same way described above, was adhered onto the anode surface.

Sensing part is composed by two electrodes fabricated with Ag/AgCl ink. The surface of the electrode which acts as working one were not modified because of the high selectivity of this material to chloride ion activity (Ives and Janz, 1961).

In the case of reference electrode, it is necessary keep constant the chloride activity over the electrode. In order to do this, the surface was

modified with KCl containing matrix based on photocurable hydrogel. 80% of HEMA, 1,4% of ethilenglicol, 14,6% of TPGDA and 4% of irgacure solution (0.11gr l⁻¹ in EtOH) were mixed. 25% of 1 mol dm⁻³ of KCl solution was added to the mixture. Using an O ring seal, a drop of HEMA-containing solution was deposited on top of AgCl layer, which will act as reference electrode, and it was irradiated with UV light for 4.30 min to polymerise HEMA to pHEMA.

The electrode was stored over night and a glassy pHEMA layer was obtained. Before measuring, the sensor was immersed in 3 mol dm⁻³ of KCl solution.

3 RESULTS AND DISCUSSION

3.1 Potentiometric Sensor Test

First, the performance of the potentiometric sensor in synthetic samples was tested by its reproducibility. The potentiometric response of six different electrodes was checked. Calibration curves in the concentration range 0.01–0.1 mol dm⁻³ of KCl were carried out (Fig. 2). The slopes of these calibrations were used to evaluate the sensor reproducibility. The residual standard deviation (RSD) was 8.02 % (n=6 α= 0.05).

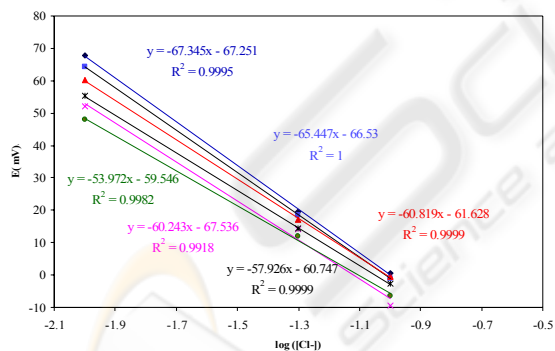


Figure 2: Calibration curves recorded to estimate the sensor reproducibility.

3.2 Application in Real Samples

These devices were used to chloride determination in sweat.

First, the sensor was stuck over the skin (Fig. 3), and then a current between 1 and 1.2 mA was applied during 10 min between the cathode and the anode to force the pilocarpine to get into the skin. Current over 1.2 mV may cause burns. After 10 min waiting, the skin started sweating. The sensing part

recorded potential values which can be related to chloride concentration by a calibration curve.

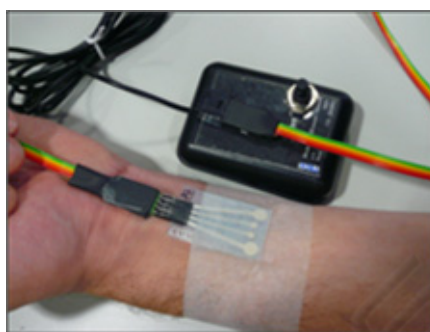


Figure 3: Picture of a sensor during measurement.

Chloride concentration was measured in six volunteers using 1-use screen-printed sensors (SPS). The results were compared with the values achieved by a common method (CM) used by the hospitals.

Table 1 shows the results obtained, as it can be seen, good agreement with the common method was obtained.

Table 1: Chloride concentrations obtained by two different methods in 6 volunteers.

Volunteer	[Cl ⁻] (SPS) (mmol dm ⁻³)	[Cl ⁻] (CM) (mmol dm ⁻³)	Error (%)
1	55.5	58	4.3
2	52.7	50	-5.4
3	60.1	56	-7.4
4	60.2	58	-3.9
5	56.8	58	1.9
6	74.5	70	-6.4

4 CONCLUSIONS

We have demonstrated that it is possible to develop a device capable to induce sweat and measure chloride concentration. The potentiometric sensor reaches acceptable values of reproducibility (8.02%) These sensors were applied in 6 volunteers with satisfactory results, using a rapid and low cost methodology for cystic fibrosis detection.

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