Development of Chemical- and Bio-sensor for Environmental Monitoring

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Abstract We introduced the parts of the research results on the organic semiconductor as a stress sensor., as a part of "Membrane Stress Biotechnology". Organic semiconductor doped with thiol self-assemblied membrane/cupper particle could detect the ammonium gas with high sensitivity and could differentiate the species of gases (ammonia, chloroform, ethanol, *i*-buthane, methane) by combining the heat condition.

Keyword Membrane Stress Biotechnology, sensor, conducting polymers, thiol self-assemblied membrane/cupper particle

Introduction

Organic semiconductor has been applied to the gas sensor units or electronic units (eg. Organic EL units) and to the biosensor. In our laboratory, the conducting polymers including polythiophene and polypyrrole, and artificial cell membrane such as liposome, have been selected as sensor elements to study the sensor properties of their sensor against the odor, stressor gass[1,2] and biopolymers[3-5] and the improvement methodology[6] of their responsibility. Furthermore, the sensor property for solution systems including cell fermentation liquids under stress conditions has been investigated[7]. Here, we report the recent research results obtained in the flow of the new field "Membrane Stress Biotechnology" that our group has developed.

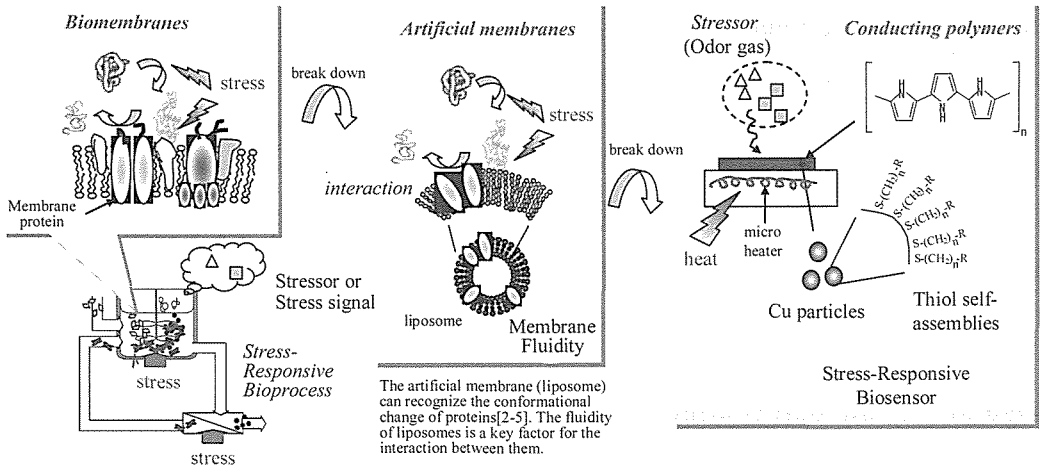


Fig.1 Break-down of cell membrane to organic semiconductor sensor structure

Table 1 Chemical structure of various conducting polymers

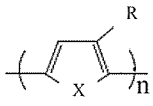
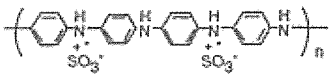
polymer	Chemical structure
polythiophene	X=S R=H
polydodecyl thiophene	X=S $R=C_{12}H_{25}$ 
polypyrrole	X=N R=H
polyaniline	

Table 2 Various thiol self-assemblied membranes

Sensor	Thiol adsorbed with Cu
S1	no Cu
S2	Cu only
S3	$HS-(CH_2)_2-OH$
S4	$HS-(CH_2)_9CH_3$
S5	
S6	calix
S7	PPO-calix
S8	NIPAM-calix

Membrane Stress Biotechnology[8-10)]

First of all, we note the outline of the membrane stress biotechnology. Biomembrane is the frontline for the cell and tissues against the outer world and always exposed to the stresses. Herewith, a stress means the variation of environmental condition by thermal (temperature change), reactive oxygen species(ROS), pH change, ionic strength, and addition of the chemical reagents. The kernel of Membrane Stress Biotechnology is to contribute the solution on potential function of cell membrane as well as the application to material design/ function exhibition/ process design by a methodology pointing to a break-down of a function of cell membrane (**Fig.1**). Among these, this is a artificial cell membrane, liposome, that plays an important role. Liposome is a closed-vesicle made of lipid bilayer membrane of surfactants including phospholipid and detergents and its diameter is ranged from 30 nm to several hundreds μm. The function of liposome under stress conditions is interesting from the viewpoints of bio-inspired functional materials since its function depends on its physical properties and components, on the intermolecular interaction, and on the curvature of liposome membrane. We have presented this as "Membrane Stress Biotechnology " to organize the aforesaid perspectives.

As one of the elemental technology to achieve the above purposes, the stress sensing technology is needed. Since the stress sensing is wide field, we focus on the sensing of stressor gas by the conducting polymer film.

Design of organic semiconductor with fluctuation/hydrophobicity

Firstly, thiophene, pyrrole, and aniline were used as sensor materials (**Table 1**). We prepared the sensor films with an electropolymerization method. In the case of

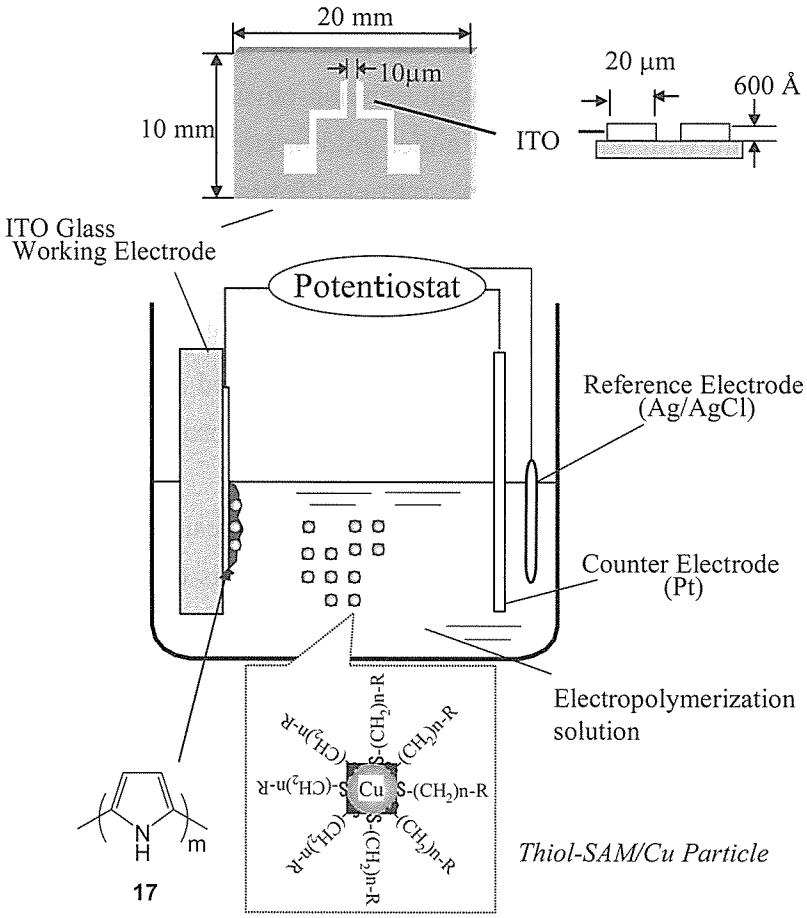


Fig. 2. Preparation of a PPy film doped with thiol-SAM/Cu particles.

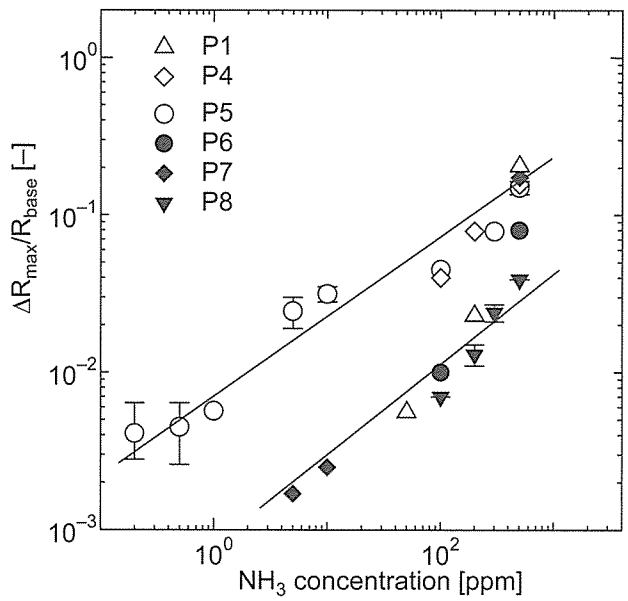


Fig. 3. The dependence of $[NH_3]$ versus change in normalized resistance for various sensor films.

ammonium gas that is a typical gas in the experiment, it is difficult to improve the responsibility of the sensor film. Then, we tried to use the thiol derivatives as a hydrophobic material. Sulfur molecule with rich non-covalent electron pair can form the coordination bonding with 3d-orbital of either gold or cupper. This permits us to obtain the thiol self-assembled membrane/cupper particle (**Table 2**) and these were doped with the conducting polymers to obtain the hybrid sensor films. The remarkable change in resistance was observed at the exposure of sensor films to ammonium gas. We considered the doping of thiolated-Cu is effective for this purpose. Thiol and its derivatives with bulkiness or flexibility (S1-S8) were doped into the sensor membrane to modify the resistance (**Fig.3**). This method could improve the previous detection limitation of ammonia by nearly 1000 times [3].

The sensor film with as fine structure as possible is desirable from the viewpoints of its application to the chemical/bio-sensor. However, it is quite difficult to fabricate the sensor film structure in the case of electropolymerization in bulk phase as shown in **Fig.2**[1]. Then, ITO electrode prepared with a photolithographic technique was designed (electrode gap: 10μm). We tried to use the micromanipulation technique for this preparation method. Firstly, we injected the electropolymerization solution (thiophene or pyrrole) onto the gap of ITO electrode with a micropipette to form the droplet with about 50μm squared. Next, a fine gold electrode (ϕ ~ 10μm) was contacted with the droplet not to directly contact with ITO electrode (**Fig. 4**). Furthermore, the necessary

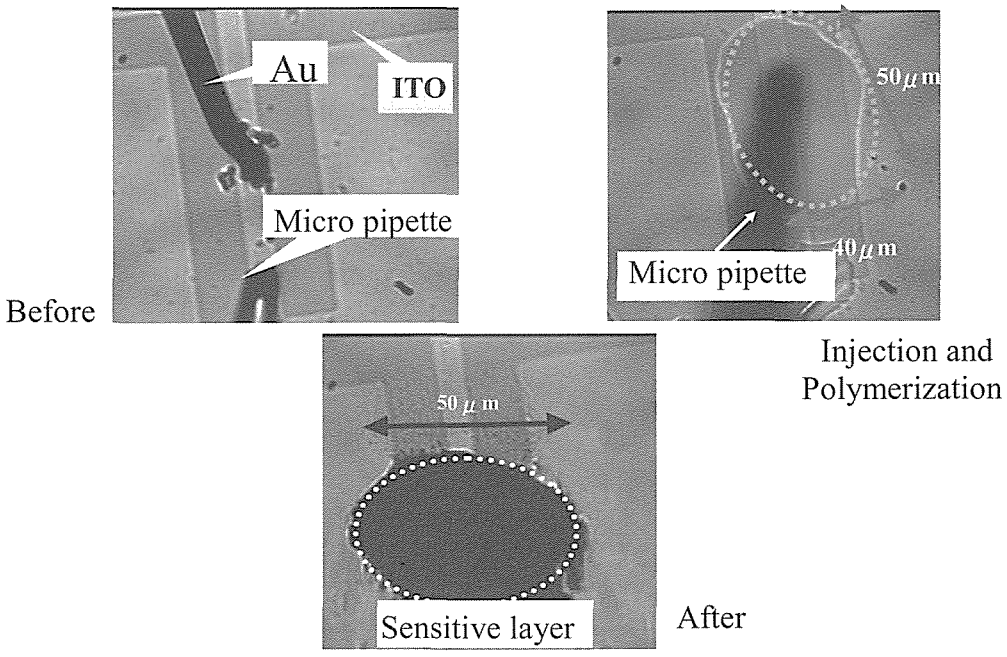


Fig.4 Microelectropolymerization with a micromanipulation. Polymerization time is about 1min. Solvent: acetnitrile/propylene carbonate, working electrode: ITO, counter electrode: Au, reference electrode: Ag/AgCl, surpporting electrolyte: Bu_4NClO_4

potential was added to make a sensor film, resulting that the production of electropolymerized film (pTh, pDpTh) with 50μm squared was possible [2]. This method permits us to produce the micro fabricated sensor film on the micro domain with several tens μm squared.

Pattern recognition of gases based on stress conditions (Stress sensing) [1]

The resistance of the pTh and pDpTh sensitive layers was varied considerably depending on temperature and the type of gases (**Fig.5a,b**). This characteristic of the responsive behavior differentiated each gas. Therefore, we tried to discriminate the responses for the gases using pattern recognition with a principal component analysis.

In the present case, a six-dimensional space that was made from two types of membrane and three different operating temperatures was reduced to a two-dimensional plane. The second principal component (PC2) is plotted against the first principal component (PC1) (**Fig.5c**). The presented data show that the sample gases were mainly classified into three groups, which were ammonia, chloroform and others. It is, however, necessary to discriminate alcohol from other hydrocarbons such as methane and isobutane.

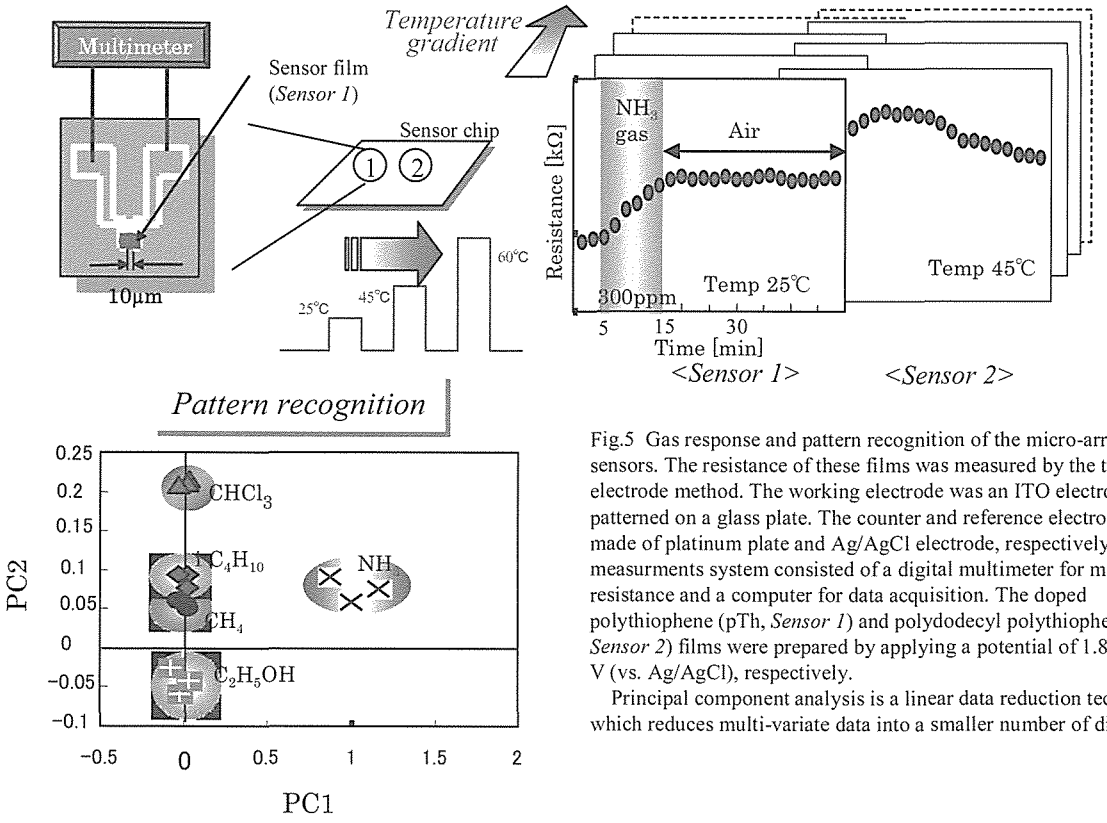


Fig.5 Gas response and pattern recognition of the micro-array gas sensors. The resistance of these films was measured by the two-electrode method. The working electrode was an ITO electrode patterned on a glass plate. The counter and reference electrodes were made of platinum plate and Ag/AgCl electrode, respectively. The measurments system consisted of a digital multimeter for measuring the resistance and a computer for data acquisition. The doped polythiophene (pTh, *Sensor 1*) and polydodecyl polythiophene (pDpTh, *Sensor 2*) films were prepared by applying a potential of 1.8 V and 2.0 V (vs. Ag/AgCl), respectively.

Principal component analysis is a linear data reduction technique, which reduces multi-variate data into a smaller number of dimensions.

Perspectives

We introduced the parts of the research results on the organic semiconductor as a stress sensor. Based on the stress responsive dynamics of cell membrane, we are developing the novel stress sensor, stress-responsive material production[7], the drug delivery system[8] and the bio-inspired functional material, as a part of "Membrane Stress Biotechnology".

References

1) Sakurai, Y. et al., *Sensors and Actuators B*, **83**, 270 (2002)
2) Jung, H.-S. et al., *Electroanalysis*, **15(18)**, 1453 (2003)
3) Kuboi,R. et al., *Sensors & Materials*, **16(5)**, 241 (2004)
4) Jung, H.-S. et al.: *Solv. Extr. Res. Dev. Japn.*, **10**, 123 (2003)
5) Morita, S. et al.: *J. Biosci. Bioeng.*, **95**, 252 (2003), Morita, S. et al., *J. Biosci. Bioeng.*, **90**, 157 (2003)
6) Shimanouchi, T. et al., *Sensors and Materials*, **16(5)**, 255-265 (2004)
7) Ngo, K.X. et al., *J. Biosci. Bioeng.*, 100(5), 491 (2005)
8) Kuboi, R., *KEMICAKENJINIYARINGU*, **47**, 742 (2002), Shimanouchi, T. et al., ibid, **47**, 748 (2002), Kuboi, R. et al., ibid, **49**, 255 (2004)
9) Kuboi, R. et al., *SEIBUTSUKOGAKU*, **82(5)**, 201(2004), Shimanouchi, T. et al., ibid, **82(5)**, 208(2004), Shimanouchi, T. et al., *BUNRIGIJUTSU*, **35(6)**, 391 (2005)
10) Kuboi, R. and Shimanouchi, T., Immobilized liposome chromatography and the sensing of the abnormality of protein conformation (9. 2) 『LIPOSOME OYOU NO SHINTENKAI』 NTS, p483