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SELF ASSEMBLED MONOLAYER OF ALKANETHIOL AND ITS APPLICATION IN BIOSENSORS

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Introduction

Surfaces, interfaces and interfacial phenomena are of great interest to people of diverse fields like chemistry, physics, biology and material science. Adsorption of different organic molecules on certain surfaces to form two-dimensional systems of molecular dimensions are of great importance to the present scientific community from the point of view of its potential application in the field of catalysts, sensors, electro-optics, molecular electronics and other nanosized materials.

The Self-Assembled monolayer is the spontaneous adsorption process of organic molecules on some substrates to yield structurally well defined monolayers. One type of organized monolayers can be formed on noble metal surface like gold, silver and copper by the self assembly of alkanethiol molecules. Such organized SAMs of alkanethiol molecules exhibit several interesting properties and have several potential applications like corrosion inhibitions, lubrications, wetting, sensors, molecular electronics etc.

The phenomenon of self-assembly has been known for the last 50 years, but not until recently people took interest to this versatile field. Self-

Assembled Monolayers are ultrathin organic films of few nanometers thickness, which are formed spontaneously by immersion of appropriate substrate into dilute solution containing organic molecules. There are several methods of self-assembly that yield organic monolayers. These include organosilicon on hydroxylated surfaces alkanethiols on gold silver and copper , dialkyl sulphide on gold alcohols and amines on platinum and carboxylic acids on aluminium oxides and silver.

From the energetic point of view self-assembly can be divided into three steps. The first step is the chemisorption of the head group onto the substrate. The bonding can be covalent in the case of alkyltrichlorosilanes on hydroxylated surfaces, covalent but slightly polar for alkanethiol monolayers on gold, ionic bond for carboxylic acids on AgO/Ag. The energies associated with such chemisorption are of the order of tens of kcal/mol (in case of thiolate on gold it is 40-50 kcal/mol). The second step is the inter chain van der Waals interactions among the alkyl group, where the energy associated with it is only few kcal/mol. The third and the final step is the reorientation of the terminal groups. In the case of simple alkanethiol, methyl is the terminal group. These surface groups are thermally disordered at room temperature, which are evident from the FTIR studies on monolayers .

Process of alkanethiol self assembly

The process of formation of SAMs of alkanethiol on gold is very simple. A clean, fresh gold substrate is immersed into a dilute solution of alkanethiol molecules in an organic solvent. The time of immersion varies from few minutes to several hours. The concentration of the thiol molecules and the immersion time of the substrate in the adsorption solvents play very important role in the self-assembly process. Typically, the concentration of the thiol is at millimolar levels, although concentration from micromolar to neat liquid thiols have been reported. Ethanol has been the most common solvent for the formation of alkanethiol monolayers on gold, although several other solvents like THF, acetonitrile, hexane have been used by some people. Formation of monolayer is extremely fast and electrochemical impedance studies based on interfacial capacitance measurements have clearly shown that the monolayer typically forms in a few seconds. It is also possible to form monolayer by gas-phase deposition. An early work of self-assembly in the gas phase was reported by some workers. In addition to simple alkanethiol SAMs, monolayers with different terminal functional groups, aromatic thiols, thiol with attached polymers, lipids, proteins, peptides and redox centers have been studied.

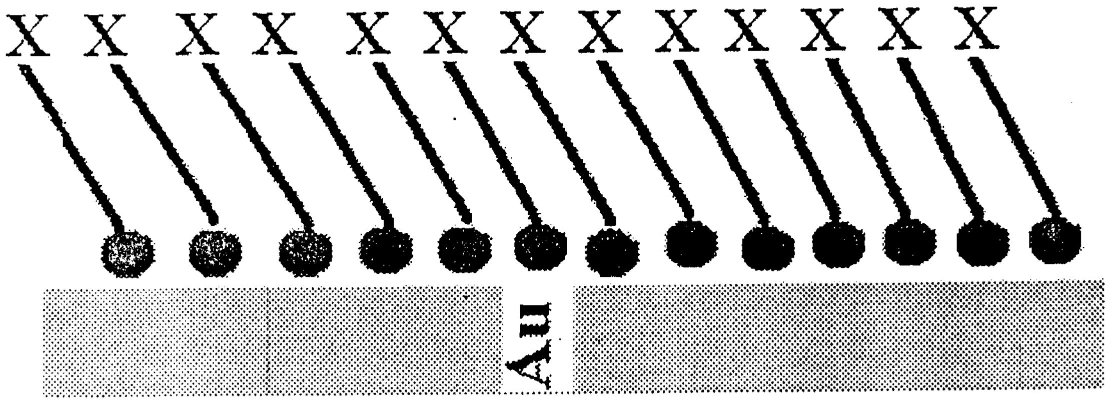

Figure 1 : Alkanethiol SAM on gold surface

X = TERMINAL FUCTION such as – CH_3 , - COOH , -OH , -SH etc.,

The versatility of the organic thiol self assembly arises essentially due to the fact that it is possible to functionalise the terminal group to have well defined properties. For example if one attaches carboxyl or hydroxyl functional group as a terminal group, one can get a surface which is hydrophilic as opposed to the normally hydrophobic methyl group. This also provides exciting possibilities of attaching other covalent molecules to the SAM to exploit the potential advantage of a solid surface for the development of sensors.

Nature of bonding :

The nature of bonding between the alkanethiol molecules and Au substrate is still a matter of considerable research. Both alkanethiols and disulphides adsorbed on gold to form the same gold thiolate (RS^-) species.The reaction of dialkyldisulphides with gold is an oxidative addition,

$$RS{-}SR + Au_n^0 \Rightarrow 2R{-}S^-{-}Au^+ + Au_n^0$$

In the alkanethiol case, the reaction may be considered as an oxidative addition of the S-H bond to the gold surface, followed by a reductive elimination of the hydrogen. When a clean gold surface is used, the proton probably ends as H_2 molecule.

$$R{-}S{-}H + Au_n^0 \Rightarrow R{-}S^-{-}Au^+ + Au_n^0 + \tfrac{1}{2} H_2$$

The combination of hydrogen atoms at the gold surface to give H_2 molecule may be an important exothermic step in the overall chemisorption energies. During the chemisorption, formation of thiolate have been verified by XPS, FTIR spectroscopy, FT-mass spectrometry, electrochemistry and Raman spectroscopy . The bonding of the thiolate group to the gold surface is very strong (bond strength is approximately 40 kcal/mol).

Since the discovery of SAMs of alkanethiol, a lot of efforts have been taken to obtain the most blocking and highly oriented monolayers on gold by varying different parameters of self-assembly process like substrate morphology, substrate cleanliness, substrate pre-treatment, alkanethiol concentration, adsorption time, adsorption solvent, alkanethiol purity etc.

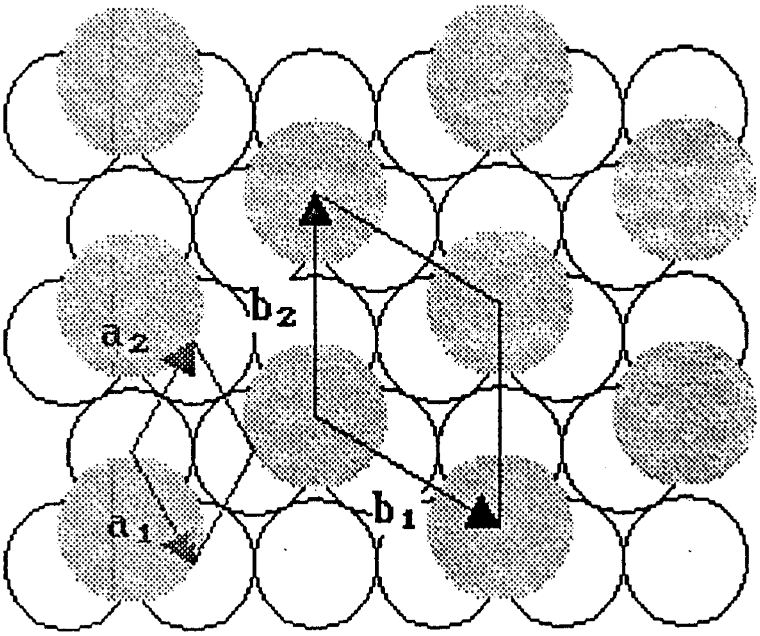

Figure 2 ($\sqrt{3}$ x$\sqrt{3}$) R 30° overlayer structure of alkanethiol on Au (111) surface

The process of self-assembly of alkanethiol on the surface of gold is known to occur in three steps. The first step is the chemisorption of the head group onto the substrate. In a molecular self-assembly process, the bonding can be covalent as in the case of alkyltrichlorosilanes on hydroxylated surfaces or covalent but slightly polar as in alkanethiol monolayers on gold. The energies associated with such chemisorption process are of the order of tens of kJ mol^{-1} (in the case of thiolate on gold it is about 200 kJ mol^{-1}). The next step is the inter chain van der Waals interactions among the alkyl groups, where the energy associated with it is a few kJ mol^{-1}. The third and the final step is the reorientation of the terminal groups.

Self-assembled monolayers of alkanethiols form a ($\sqrt{3}$ x$\sqrt{3}$) R 30° overlayer structure on Au (111) surfaces as observed from the electron diffraction and Scanning Tunneling Microscopic (STM) studies . This means that the vectors a_1 and a_2 which define the adsorbate unit cell is longer than b_1 & b_2 of the Au (111) substrate unit cell by a factor of $\sqrt{3}$ and the angle between b_1 & a_1 vectors (also b_2 & a_2 vectors) is 30 ^{0}. The spacing between the adjacent sulphur atoms in this structure (4.99 Å) is about 3 times larger than the van der Waals diameter of sulphur atom (1.85 Å). This distance is also greater than the distance of closest approach of the alkyl chains (4.24 Å). Therefore, the chains tilt by an angle 30^0 with respect to surface normal to maximize their van der Waals interactions . Depending on the chain length and the terminal group, various superlattice structures are superimposed on the ($\sqrt{3}$×$\sqrt{3}$)R30^0 overlayer structure. The most common superlattice is the c(4×2) reconstruction.

Bio-analytical sensors with SAM:

Self-assembled monolayers (SAMs) provide many interesting ways of development of solid-phase bioanalytical techniques. The well ordered monolayers formed by alkanethiols on metal surfaces act as a receptors to immobilise proteins close to an electrode surface in a definite way. This provides a degree of control over the molecular architecture not commonly achievable with immobilisation within polymers membranes. As a consequence of this ability, SAMs have been used for fundamental investigations of the interactions of proteins with surfaces and in the development of biosensors . These monolayers are found to be suitable for biocompatibility and biosensing. The use of SAMs is associated with various advantages in in the development of biosensors such as a large reduction in double layer charging current thus increasing the sensitivity due to this. SAM modified electrodes are finding application in the development of amperometric enzymatic biosensors for the determination of different analytes.

The development of chemical modification strategies for controlling the specific and non-specific adsorption of biopolymers (e.g., synthetic polypeptides, proteins and DNA molecules) onto metal surfaces is one of the important research areas of growing interest in recent times. . The formation of biologically active biopolymer monolayers at metal surfaces is an essential step in the fabrication of a number of important bioanalytical devices such as biochemical sensors, bioelectronic switches and gates, chemical separation and purification surfaces, and enzymatically controlled electrochemical interfaces.

There are two basic approaches to immobilising enzymes onto electrodes using the characteristic of alkanethiols to self-assembled on gold surfaces. The enzyme can be either bonded to a existing SAM or the enzyme can be modified to contain thiol functional group which then can bond to the gold surface. Electrostatic binding also provides a simple and gentle method for immobilising proteins onto surfaces. The electrostatic bond does provide the potential for control over the orientation of the immobilised protein molecules depending on the charge distribution of the protein. Therefore this method has been used to investigate direct electron transfer between enzymes or redox proteins more than for the development of sensors directly.

For example a glucose sensor can be assembled on a a carboxylate-terminated alkanethiol SAM on a gold electrode. Poly(allylamine), modified with ferrocene to form a cationic polymer (PAA-Fc), was deposited electrostatically onto the SAM followed by anionic glucose oxidase. By repeating the process of depositing poly(allylamine) followed by glucose oxidase (GOD), a multilayer enzyme electrode can be built up in a highly controlled manner. As another example of the process, a gold disc electrode can be modified with alkanethiol SAM and glucose oxidase and a redox mediator such as tetrathia fulvalene (TTF).

Alkanethiols can act as hydrophobic monolayer substrates for the formation of phospholipid containing bilayers. Phospholipid vesicles were allowed to fuse to alkanethiol monolayers, resulting in stle, solvent -free lipid bilayers on gold electrodes. The capacitance of these bilayers has been determined by impedance measurements, and the effect of the pore-forming peptide, melittin, on the bilayer has been determined by the measurement of Faradaic current. These supported self-assembling phospholipid/alkanethiol bilayers demonstrate properties consistent with fluid membranes, and provide a useful way to study the electrical characteristics of membranes in the absence of solvent. It has been suggested that cytochrome c can be irreversibly bound to a SAM of thiols terminated by carboxylic acids.

Covalent Attachment

Covalent attachment has the greatest potential for the development of commercial enzyme sensors. This is because of the stability of the resultant bond. A frequently used method for covalently attaching proteins to SAMs is by using carbodiimide coupling. In this method amines are coupled to carboxylic acids.

For example this method can be used to covalently attach glucose oxidase to a SAM of 3-mercaptopropionic acid where the bond occurs through the free lysine chains(coupling moiety) on the enzyme and the SAM. The enzyme electrode, mediated with p-benzoquinone in this case, has a large dynamic range, responding to changes in glucose concentration over 50 mM.

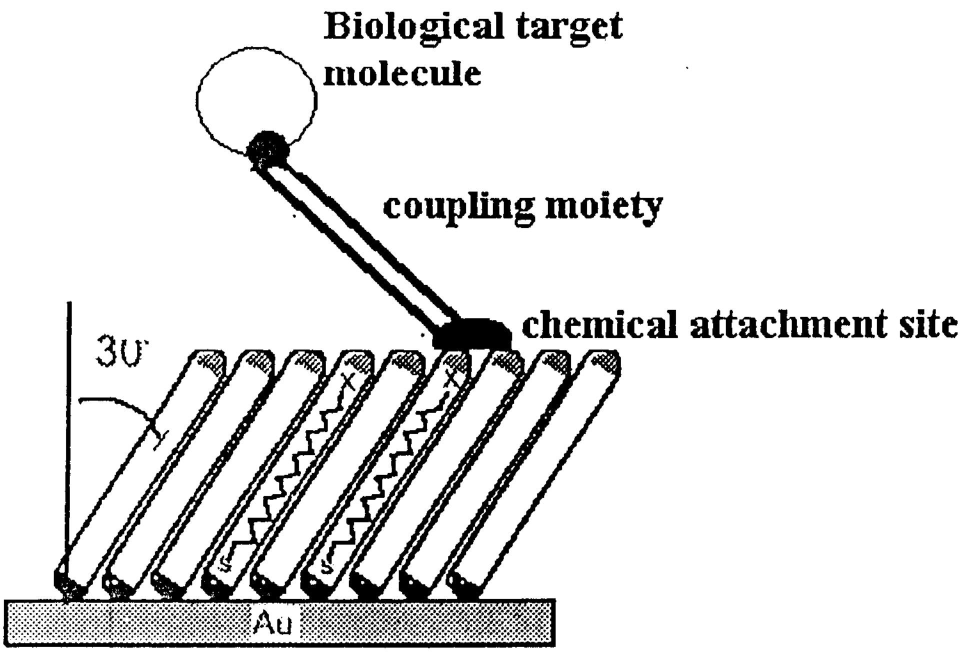

Figure 3 : Example of an enzyme electrode formed by coupling the thiol adsorbed on gold surface and the enzyme .

The formation of SAMs with homogeneous distribution from mixtures of alkanethiols is important for the construction of sensors as large recognition elements can be spaced apart from each other if necessary. Also important for electrochemical sensors is the stability of the film at the potential of the underlying electrode. SAMs are stable at potentials between approximately –0.8V to +1.0 V Vs SCE, which is compatible with most enzyme electrode applications. Outside this range most of the thiols are oxidatively or reductively desorbed.

The use of alkanethiols as immobilisation matrices for enzyme biosensors have shown that they have the potential to construct highly ordered two-dimensional arrays of enzymes and hence reproducible devices. The covalent immobilisation of enzymes with alkanethiols has the very attractive feature of providing generic immobilisation technologies.

Conclusions :

Some of the important issues associated with this method of constructing enzyme layers are for improving the sensitivity and selectivity, but the construction of enzyme biosensors from immobilised enzymes in precisely-controlled monolayers is still open for lot of research and creative applications. The realisation of the potential of self-assembled monolayers to manufacture reproducible immobilisation matrices for enzyme should see many more biosensors make the transition from the research laboratory to the market place.

The alkanethiol and other functionised thiols SAMs on noble metal surfaces have come a long way from being a research tool for understanding many fundamental phenomenon in electron transfer studies. These Self-assembled monolayers provide exciting possibilities in the area of sensors development due to their flexibility ,versatility and simplicity . A new class of nanometer scale low power solid state devices are now being fabricated for the detection of various hazardous vapours in the atmosphere using nanoparticles of gold encapsulated with the monolayers of alkanethiols. These are immobilized on interdigitated microelectrodes. The adsorbed vapour molecules affect the electronic properties of the interdigitated microelectrode assembly. These so called metal-insulator-matal ensembles (MIME) are being investigated for their selectivity and sensitivity in sensor applications.

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