# Comparison of electrochemical behaviour of poly anions with that of biological species

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**Abstract :** Biological electron transfer processes are usually facile, since the agents are capable of adopting appropriate redox potential values, modulated and tuned by the immediate environment. To mimic this in the laboratory one needs a chemical agent whose redox potential is completely tunable with minimum alteration of the nature and environment of the species. The purpose of this presentation is to highlight how the electrochemistry of the polyanions can be exploited in this direction. Though the possibilities are many, this presentation considers only a selected simple reactions and devices to highlight the point that the electrochemistry of poly anions can be made use of to understand some of the biological electrochemical transformations.

## Introduction

Electron transfer is an important key step in large number of biological and chemical processes. The oxidation-reduction reactions that take place in biological systems are characterized by stepwise electron transfer through a number of redox active biomolecules or proteins. This is achieved by altering and adjusting the redox potential of the species by appropriate combination of species at the required place to the requisite level i.e., the reduction potentials are tunable according to the requirement.

In this regard, heteropoly anions can mimic the redox behaviour of biological systems owing to their tunable nature of redox properties and stepwise electron transfer by the cluster. Heteropoly anions possess energy levels that are provided by the cluster contributed by several species. This makes their electrochemical behaviour different and distinct from the simple metal oxides that possess discrete energy levels.

The outline of this presentation include (1) brief introduction to heteropoly anion, (2) example of redox reaction in biological systems and comparison of the same with electrochemical behaviour of heteropoly anions (heteropoly anions mimic the biological systems essentially in two distinct ways i.e., exhibit stepwise multiple electron transfer and tunable redox potential), (3) examples where heteropoly anions are employed as electrocatalysts for biologically relevant reactions (*in vivo*, enzymes carry out these reactions), (4) example of a bioelectronic device where the redox property of biomolecule is exploited and demonstration of the same application with heteropoly anion.

## 1. Heteropoly anions

Polyanions are characterized by the principal building block, $MO_x$, usually $MO_6$ octahedron that are linked together by oxygen atoms. There are two classes of polyanions, the isopoly anions that contain only the $d^0$ metal cations and oxide anions (general formula: $M_xO_y^{n-}$) and the heteropoly anions, that contain, one or more d or p block 'heteroatom' cations $X^{n+}$, in addition to the metal

cations and oxide anions (general formula: $X_xM_mOy^{n-}$). Heteropoly anions are the focus of this presentation as they are more versatile and more easily tunable with respect to their composition and in turn their redox potential.

A heteropoly complex contains a high atomic proportion of one kind of atom in positive oxidation state ("addenda atoms") and much smaller proportions(s) of other kind of atom(s) in positive oxidation state ('heteroatoms")[1]. Over 60 elements including nonmetals and transition metals, can function as heteroatoms[2].

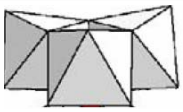
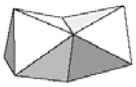
The addenda atom should satisfy the following requirements:

1. change its coordination with oxygen from 4 to 6.
2. have high positive charges and radius range should be 0.53 to 0.70Å.

1.1. Classification of Heteropoly anions

Heteropoly anions can be regarded as packed arrays of octahedral $MO_6$ units around the central $XO_4$ or $XO_6$ moiety. Based on the type of sharing between individual $MO_6$ units, they are classified as shown in Table.1. The most stable union between two octahedra is edge or corner sharing in which the $M^{n+}$ ions are far enough from each other and their mutual repulsion is modest. Keggin and Dawson type heteropoly anions are formed by $M_3O_{13}$ clusters around the central $XO_4$ tetrahedron.

Table. 1. Classification of heteropoly anion based on the type of cluster

| Type of cluster | Central group | Type of HPA | Molecular formula |
|---|---|---|---|
|  **$M_3O_{13}$** | $XO_4$ | Keggin | $X^{+n}M_{12}O_{40}^{(8-n)-}$ |
| | $XO_4$ | Dawson | $X_2^{+n}M_{18}O_{62}^{(16-2n)-}$ |
| | $XO_6$ | Waugh | $X^{n+}M_9O_{32}^{(10-n)-}$ |
|  **$M_2O_{10}$** | $XO_6$ | Anderson | $X^{+n}M_6O_{24}^{(12-n)-}$ |
|  **$M_2O_9$** | $XO_{12}$ | Silverton | $X^{+n}M_{12}O_{42}^{(12-n)-}$ |

The formation of a heteropoly anion from the basic $MO_6$ and $XO_4$ units can be understood as shown in the Fig.1. Three $MO_6$ units form a $M_3O_{13}$ unit by edge sharing and four $M_3O_{13}$ moieties share the edges through the oxygen atoms with the central heteroatom to form a Keggin type heteropoly anion.

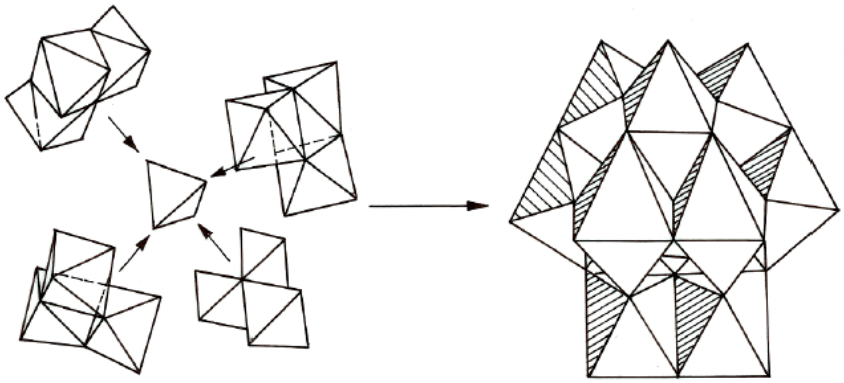


Fig.1. Formation of a Keggin type heteropoly anion

1.2. Properties of Heteropoly anions

Variations in the composition of heteropoly anions can be achieved with ease. Many heteropoly compounds have high solubility in polar solvents and fairly high thermal stability in the solid state[3]. The addenda metals are present in their highest oxidation state in $d^0$ or $d^1$ electronic configuration and there is little ligand-to-metal electron donation in the structure. Hence they can undergo photo and electrochemical reduction with retention of the geometry[4]. The electrons are accepted by the addenda ions and if they are identical, the electrons are delocalized on the structural framework by rapid electron hopping or through bridging oxygen atoms[2]. They can undergo rapid multiple reversible reduction, which is often accompanied by colouration. These reduced species are called heteropoly blues[2]. The redox processes of heteropoly anions can be studied by several electrochemical methods. By a smooth change of their composition, their redox potentials can be chosen to span a wide range. The similarities in the electrochemical behaviour of heteropoly anions and the biological systems are considered in this paper.

## 2. Electrochemical behaviour of heteropoly anions and biological species

2.1. An example of redox process in biological system (oxidative phosphorylation or cellular respiration).

*Stepwise electron transferin biological systems*

Fig.2 shows the overall organization of oxidative phosphorylation that is taking place in mitochondria. Oxidative phosphorylation is the production of ATP (Adenosine tri phosphate) using energy derived from the transfer of electrons in an electron transport system. In mitochondria, the food molecules get metabolized and they give high-energy electrons, which are first carried by activated carrier molecules NADH (Nicotinamide adenine dinucleotide) and $FADH_2$ (Flavin adenine dinucleotide). The electrons are quickly passed along the electron transport chain by several electron carriers and finally to molecular oxygen to form water ($(1/2)O_2 + 2H^+ + 2e^- \rightarrow H_2O$; E = +0.82V)[5]. The

energy released during the passage of the electron along the electron transport chain is harnessed to pump protons and thus synthesize ATP. Mitochondrial electron transport chain is grouped into five complexes each containing multiple individual proteins. The five respiratory complexes are (1) the NADH dehydrogenase complex, (2) the ubiquinone system, (3) the cytochrome reductase complex, (4) the cytochrome c and (5) the cytochrome oxidase complex. Throughout the chain, the redox potential of each of the electron carriers has been tuned to facilitate efficient electron transfer by binding the carrier atom or molecule to a particular protein, which adjusts its normal affinity for electrons to fit its position in the respiratory chain
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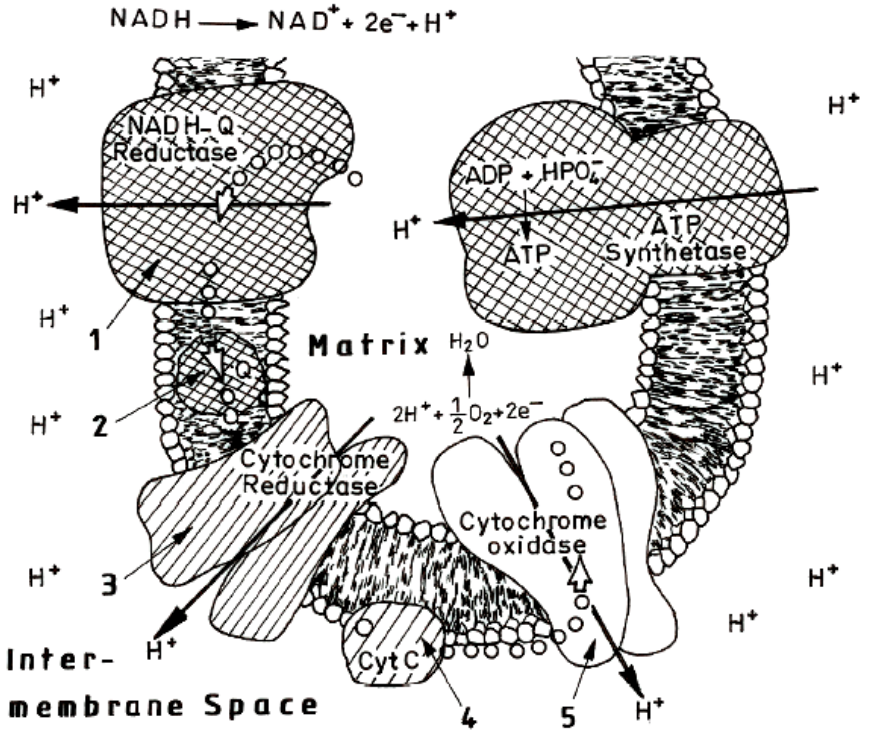


Fig.2. Oxidative phosphorylation in mitochondria

The transfer of electrons along the chain is energetically favourable. In the respiratory chain, the electron carriers are arranged in order of increasing redox potential thus making possible the gradual release of the energy stored in NADH electrons.

Fig. 3 shows the direction of flow of electrons in the chain and the redox potentials involved in the process[5]. Electrons will tend to flow from a relatively electronegative redox pair, such as $NADH/NAD^+$ (E = -0.32 V), to more electropositive electron acceptors, such as reduced cytochrome c / oxidized cytochrome c (E = +0.23 V). Similarly, they will also tend to flow from the cytochrome c redox pair to the water-oxygen pair (E = +0.82 V). The tendency for electrons to flow from electronegative towards electropositive systems is the result of the loss of free energy, since electrons always tend to move in such a direction that the free energy of the reacting system decreases.

The greater the difference in the standard potentials between two redox pairs, the greater the free energy loss as electrons pass from the electronegative to the electropositive couple. Therefore, when electrons flow down the complete electron transport chains from NADH (E = -0.32 V) to oxygen (E = +0.82 V), via several electron carrying molecules of the electron transport chain, they lose a large amount of free energy because the difference between the standard potentials of the redox pairs $NADH/NAD^+$ and $H_2O/1/2O_2$ is relatively great.

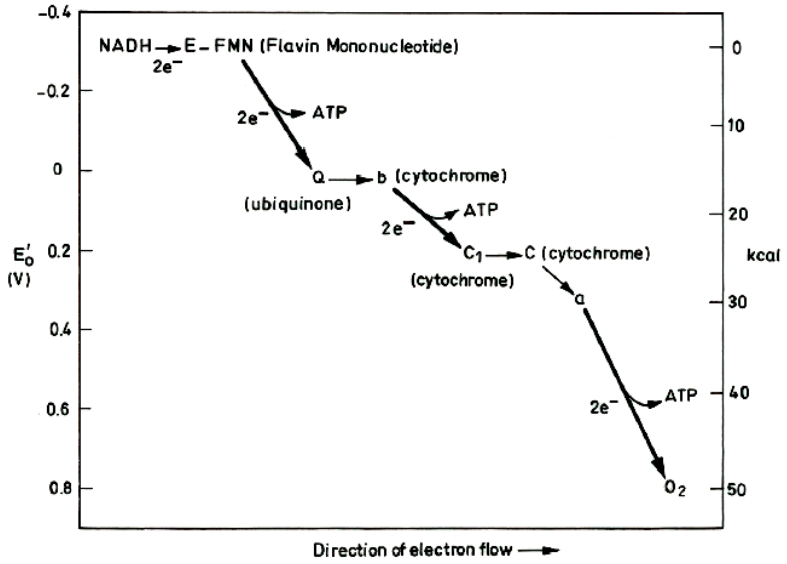


Fig. 3. Stepwise electron transfer and energy relationships in respiratory chain (reproduced from ref. 5)

This example demonstrates how the stepwise redox process facilitates a reaction that requires a high potential and also one can observe how the biological systems are capable of tuning the redox proteins and biomolecules with increasing redox potential so as to make the process facile.

*Stepwise electron transfer in heteropoly anions*

The redox behaviour of heteropoly anions was studied and it is observed that heteropoly anions mimic the properties of biological systems in two distinct ways i.e., similar to biological redox systems, they also exhibit stepwise multiple electron transfer and tunable redox potential. As representative examples, cyclic voltammograms of a Keggin and Dawson type heteropoly anions are shown in Fig.4a and b respectively[6-7]. Fig.4 shows multiple electron transfer (1:1:2 electron ratios) and the peak separation values (55 mV, 55 mV and 30 mV) of the three-redox couples show that the processes are reversible. In general, all heteropoly anions show unique electrochemical behaviour where they undergo stepwise, reversible multiple electron transfer processes

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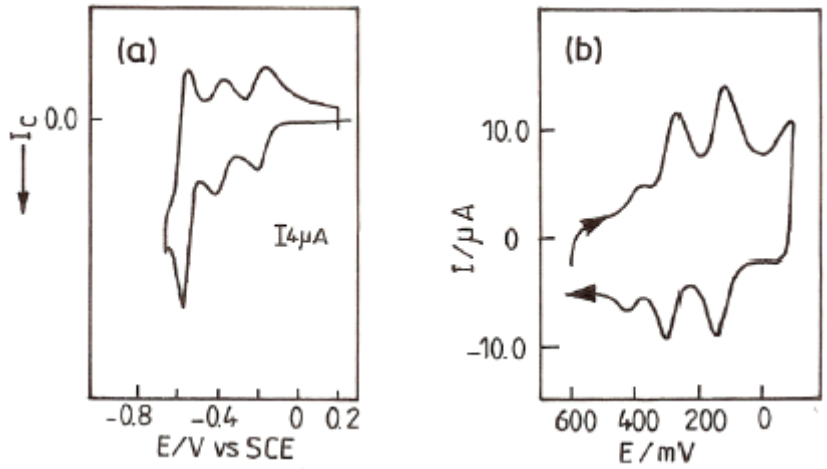


Fig.4. Cyclic voltammograms of (a) Keggin type heteropoly anion $SiW_{12}O_{40}^{4-}$ in 1 M $HClO_4$ as supporting electrolyte at a glassy carbon electrode. Scan rate:100 mV/s. (b) Dawson type heteropoly anion $P_2Mo_{18}O_{62}^{6-}$ in 0.5 M $NaHSO_4$ at ITO electrode. Scan rate:50 mV/s (reproduced from ref.6 and 7).

2.2. Tunable redox potentials

A potential scale diagram is drawn from the cyclic voltammetric data obtained for several heteropoly anions with varying heteroatom and addenda atoms (left side of fig. 5)[8-9]. For comparison, the potential scale for iron system in different cytochromes is shown in fig.5 (right side of the scale)[10]. One can observe a wide range of redox potential in both biological systems and heteropoly anions from the figure. In case of biological systems, this is attributed to the immediate environment in which the active metal center i.e., iron is present. In each cytochrome, different protein mantles surround the active metal center i.e., iron. The protein molecules encapsulating the active sites induce the distortion of symmetry of the metal complex[10]. This distortion causes characteristic splitting of d orbitals of coordinated transition metal centers. The extent of distortion varies according to the protein mantle that encapsulates the metal and hence it leads to altered redox potentials of metal (iron) complex in various protein mantles.

In heteropoly anions, this tunability in the redox potentials can be achieved by varying the compositions or by varying the conditions like pH, concentration of medium and solvents.

*(i) Effect of heteroatom*

The half wave potentials for first one-electron reduction of $XW_{12}O_{40}^{n-}$ with different heteroatoms are shown in Table. 2[11]. The half wave potentials for these one-electron reductions show a linear dependence upon the ionic charge with a slope of -0.16 V/ unit charge. In this series of isostructural ions of similar size, the main cause of the variation of the reduction potentials will lie in the electron affinity variations. Keggin anion behave in solution as sphere with a radius of about 5.6 Å and the electron affinity of a sphere of charge -ne in a medium of dielectric constant ε is the same as its electrostatic potential, ne/εr. The difference in electron affinity for two such spheres differing by unit charge will then be e/εr. Hence with

variation in the composition, change in the reduction potentials can be achieved .

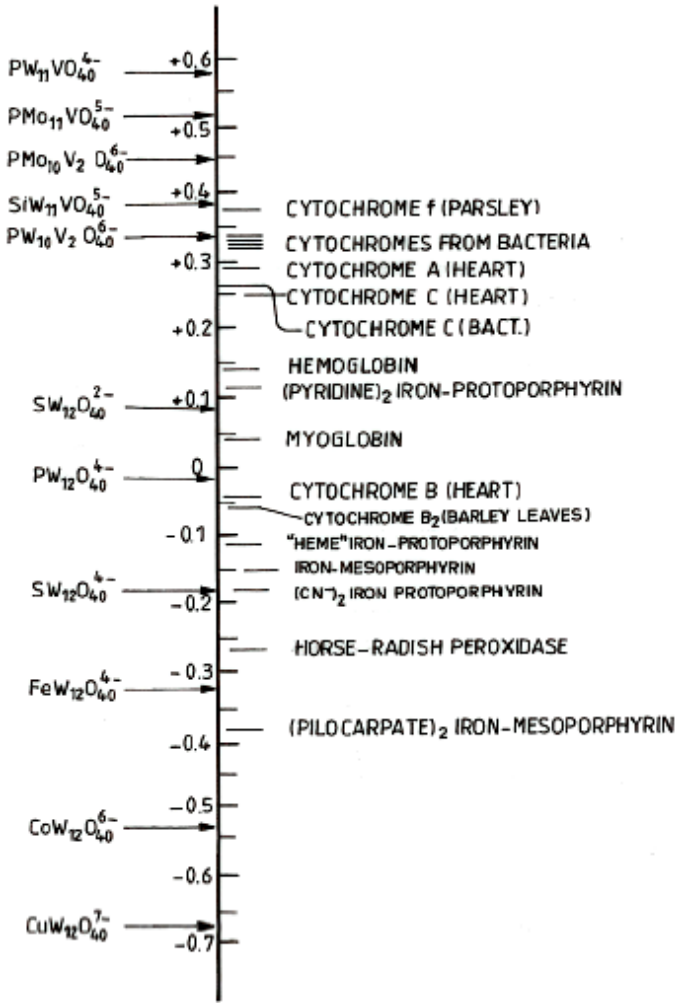


Fig.5. Redox potentials (in Volts) of $Fe^{2+}/Fe^{3+}$ in different biological systems (right side of the scale) and heteropoly anions ( left side of the scale) with varying composition (values obtained from ref. 8-10).

*Manipulation of redox potential in heteropoly anions*

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same as its electrostatic potential, ne/εr. The difference in electron affinity for two such spheres differing by unit charge will then be e/εr. Hence with variation in the composition, change in the reduction potentials can be achieved.

Table. 2. Half- wave potentials for first one-electron reduction of $XW_{12}O_{40}^{n-}$

| **Anion** | **$E_{1/2}$ , V** |
|---|---|
| $[PW_{12}O_{40}]^{3-}$ | -0.023 |
| $[SiW_{12}O_{40}]^{4-}$ | -0.187 |
| $[FeW_{12}O_{40}]^{5-}$ | -0.349 |
| $[CoW_{12}O_{40}]^{6-}$ | -0.510 |
| $[H_2W_{12}O_{40}]^{6-}$ | -0.581 |

*(ii) Effect of pH*

Heteropoly anions undergo reversible reduction and during the process the negative charge density at the heteropoly anion increases. This leads to an increase in the basicity of the species and as a result the reduction can be accompanied by protonation depending on the pKa of the produced oxometalates. In Fig.6, the effect of pH on the reduction potential of $PCr(III)W_{11}O_{40}^{4-}$ is shown[12]. With decreasing pH, the shift in the reduction potentials to positive potentials can be observed with a slope of ~59 mV/pH

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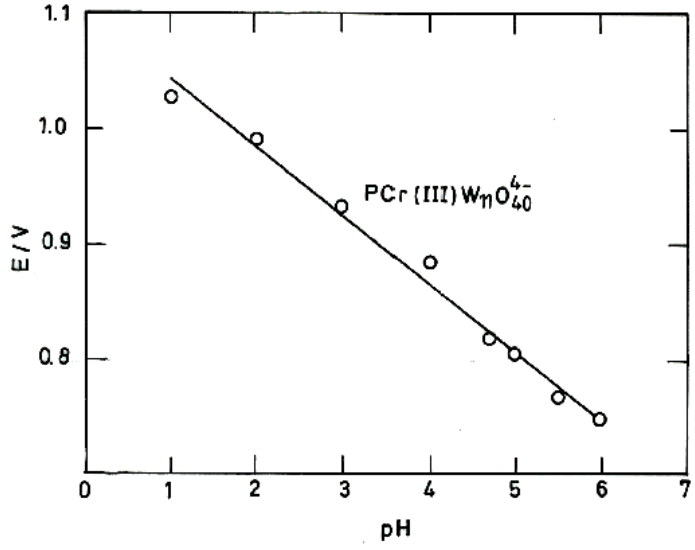


Fig.6. Effect of pH on reduction potential of $PCr(III)W_{11}O_{40}^{4-}$ ( reproduced from ref.12)

*(iii) Effect of solvent*

The solvent effects on the reduction potentials of α-$SiW_{12}O_{40}K_4$ ($SiW_{12}$) and α-$P_2W_{18}O_{62}K_6$ ($P_2W_{18}$) are shown in Fig.7. The plots of reduction potentials of the heteropoly anions vs. the dielectric constant or the dipole moment of the solvents showed no correlations. On the other hand, a good correlation of the reduction peak potential of $SiW_{12}$ and $P_2W_{18}$ with the acceptor numbers of the solvents was obtained[13]. Lewis-type interactions are expected between solvents and the oxygen atoms of heteropoly anions. Anions are considered as electron pair donors (Lewis bases) interacting with the solvents which are considered as electron-pair acceptors (Lewis acids). The reduced form is more strongly affected by the interaction with the solvent than the oxidized form. Larger the acceptor number, more is the ability to withdraw negative charge from the ionic species. This results in a shift in redox potentials towards positive values with increasing acceptor number.

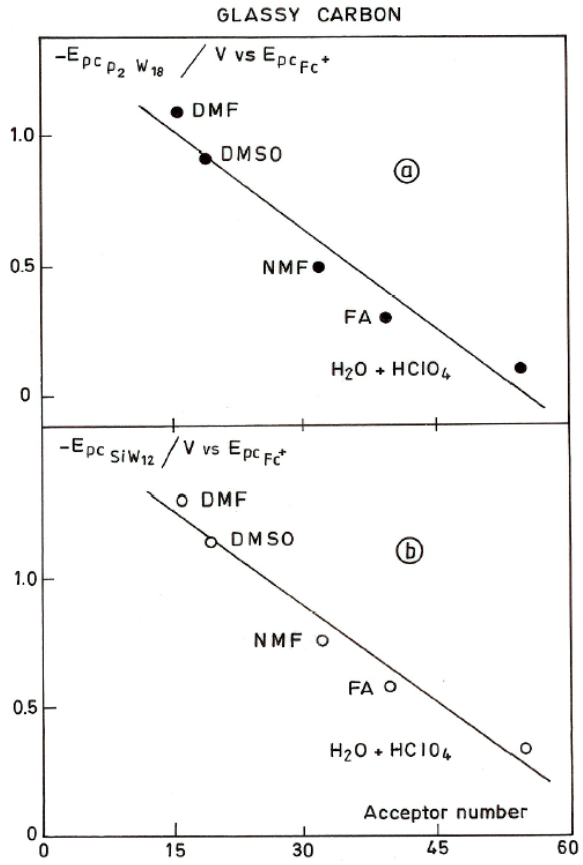


Fig.7. Relationship between the reduction peak potential, Epc of (a ) $P_2W_{18}$ and (b) $SiW_{12}$ vs. EpcFc+, of ferricinium, and the acceptor number of the solvents (reproduced from ref.13).

*(iv) Mixed addenda atoms – substitution with transition metals*

Lacunary heteropoly anions are derived by removing one MO unit from heteropoly anions. Many transition elements can fit into this octahedral binding site, giving rise to transition metal substituted heteropoly anions. Such species are called mixed addenda heteropoly anions. In these species, both the parent metal atoms and the substituted transition metals are redox active and hence the redox couples of both these metals can be observed. This can be observed from the Table.3. for $PTW_{11}0_{40}^{n-}$ where T is the transition metal. In addition to the peak at –0.035 V for the $W^{6+}/W^{5+}$ couple and +0.340 V for $Mo^{6+}/Mo^{5+}$, the mixed addenda species show peak in the positive potentials for the substituted transition metal[14-15].

Table.3a. Redox potentials of mixed addenda atoms in $PTW_{11}O_{40}^{n-}$ and 3b. Redox potentials for vanadium substituted $PTMo_{11}O_{40}^{n-}$

| $PTW_{11}O_{40}^{n-}$ | E (V)Vs. Ag/AgCl |
|---|---|
| $PW_{12}O_{40}^{3-}$ | -0.035 |
| $PFe(III)W_{11}O_{40}^{6-}$ | +0.039 |
| $PV(V)W_{11}O_{40}^{4-}$ | +0.394 |
| $PMn(III)W_{11}O_{40}^{4-}$ | +0.924 |
| $PCr(III)W_{11}O_{40}^{4-}$ | +1.064 |

| $PTMo_{11}O_{40}^{n-}$ | E (V) Vs. Ag/AgCl |
|---|---|
| $PMo_{12}O_{40}^{3-}$ | 0.340 |
| $PVMo_{11}O_{40}^{4-}$ | 0.510 |
| $PV_2Mo_{10}O_{40}^{5-}$ | 0.480 |
| $PV_3Mo_9O_{40}^{6-}$ | 0.476 |

From these electrochemical studies, it can be observed that heteropoly anions can exhibit reversible multi electron redox transformations under neutral to acidic conditions. The second important observation that can be made is their redox properties can be varied over a wide range by changing the chemical composition and reaction conditions. In these two ways, one can see them mimicking the redox behaviour of biological systems.

Biological molecules that are redox active find applications as catalysts for biological redox reactions. Also, recently their redox properties are exploited in bioelectronic devices. Since, heteropoly anions mimic the redox behaviour of biological systems, they might be capable of carrying out similar catalytic reactions and device applications in place of biomolecules. In the following section, some examples are illustrated wherein heteropoly anions can be used in place of biomolecules.

## 3. Heteropoly anions as electrocatalysts for biologically relevant reactions

### 3.1. Heteropoly anions in hydrogen peroxide reduction

In the respiratory chain (Fig.2), oxygen is the end acceptor molecule, which undergoes reduction to give water, and if this process is incomplete, it produces reactive species such as superoxides and peroxides. Oxygen reduction is carried out by cytochrome c oxidase and although cytochrome c oxidase and other proteins that reduce oxygen are remarkably successful in not releasing intermediates, small amounts of superoxide anion and hydrogen peroxide are unavoidably formed[16]. Superoxides, peroxides and species that can be generated from them are collectively referred to as reactive oxygen species(ROS). *In vivo*, Superoxide Dismutase (SOD) and catalase are the enzymes that catalyses the reduction of superoxide anions to hydrogen peroxide and hydrogen peroxide into water and oxygen respectively. Heme moiety containing Fe(III) metal in catalase is the active center for hydrogen peroxide detoxification. Oxidative damage which is caused by the ROS is believed to cause, at least in part, a growing number of diseases such as bronchitis, Parkinson disease, alcohol liver disease etc.,[16]. Hydrogen peroxide is determined *in vitro* from the patients' blood and exhaled air by several methods like titrimetry, chemiluminescence and spectrometry. Apart from these methods, biosensors containing enzymes of similar active moiety as that of catalase has also been reported for determination of hydrogen peroxide *in vitro*[17-19]. Since, these biosensors are capable of sensing hydrogen peroxide *in vitro*, they have also been further extended from clinical control to environment and industrial control of hydrogen peroxide. However, the drawback associated with these biosensors involving enzymes or biomolecules, is their stability in acidic conditions[20].

Heteropoly anions can be a better alternative in these biosensors to sense hydrogen peroxide *in vitro* since they can withstand acidic conditions as well as they can exhibit similar redox behaviour like biological systems. There are several reports where heteropoly anions are employed as electrocatalysts for hydrogen peroxide reduction and determination[21-22]. The advantages of using heteropoly anions as electrocatalysts for this purpose are (i) they can minimize the activation energy and hence allow an electrode reaction to occur at high density close to the equilibrium potential or even considerably below it[23] and (ii) they are generally nontoxic to normal cells[24]. There are several reports available where heteropoly anions are used *in vivo* for medicinal purposes[24].

12-molybdophosphoric acid ($PMo_{12}$) has been employed as an electrocatalyst for this purpose. $PMo_{12}$ is immobilized on the Pt electrode (working electrode) by

sol-gel method, which is formed using tetraethoxysilane (TEOS), polyethylene glycol benzylethyl ether and $PMo_{12}$[25]. The electrochemical behaviour of the modified electrode is characterized using cyclic voltammetry and found that they show three two-electron redox couples and the electrode is stable for several runs of experiment. Using this modified electrode, hydrogen peroxide is determined by amperometric measurements (Fig.8.a)[25]. It can be seen from the figure, that rapid and sensitive responses to changes in the concentration of $H_2O_2$ were achieved and this can be attributed to the thin active film and short penetration depth of $H_2O_2$. Using the steady state current obtained from this measurement, a calibration graph (Fig.8.b) is achieved. The electrode response was found to be linear for $H_2O_2$ within the concentration range of 20 to 30 mM. The detection limit was 7x $10^{-6}$ M.

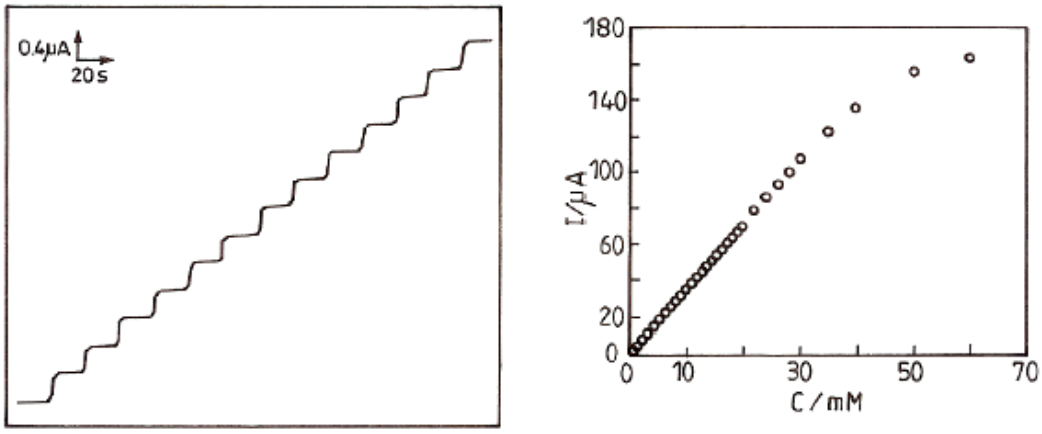


Fig.8.a. Current –time response curve for successive additions of 1x$10^{-4}$ M $H_2O_2$. Operating potential 0.0 V vs. SCE (b) Calibration graphs for $H_2O_2$ at a $PMo_{12}$ gel film electrode (reproduced from ref.25).

3.2. Electrochemical oxidation of NADH using heteropoly anion

Nicotinamide adenine dinucleotide NAD(H) is a cofactor that is involved in a large number of biochemical processes. It acts as an electron and hydrogen donor in most of the biochemical reactions catalyzed by redox enzymes (dehydrogenases or oxidoreductases). In its reduced and enzymatically active form (1,4-NADH), the molecule transfers two electrons and a proton to a substrate in the presence of a suitable enzyme to form NAD+. NADH/NAD+ pair can be regenerated using enzyme modified electrodes[26]. Biosensors employing enzymes on electrodes for regenerating NADH/NAD+ pair is a promising method but this approach has difficulties related to immobilization of an enzyme and electron mediator at the electrode surface, loss of the enzyme activity and electron-mediator leakage. In order to avoid these disadvantages, chemical species can also be employed to regenerate NADH by electrochemical means.

In the following example, a heteropoly anion functioning as an electrocatalyst

for this process is illustrated. In this example, a Dawson-type mixed heteropoly anion, $\alpha_2$- $P_2W_{17}VO_{62}^{8-}$ has been used for oxidation of NADH[27]. Fig.9.(i) shows the cyclic voltammogram associated with the first redox system of heteropoly anion, featuring a one-electron diffusion-controlled process. Fig.9.(ii) shows the cyclic voltammogram of NADH in absence of heteropoly anion. Fig.9.(iii) – (vi) are the cyclic voltammograms of $\alpha_2$-$P_2W_{17}VO_{62}^{8-}$ ($10^{-3}$ M) + $\gamma$ M NADH where $\gamma = c^o_{NADH}/ c^o_{HPA.}$ This ratio $\gamma$ was varied between 0.5 and 50. Fig.9.(iii)- (vi) represents NADH oxidation in the presence of $\alpha_2$-$P_2W_{17}VO_{62}^{8-}$ and it can be observed that on addition of increased amounts of NADH, the anodic current of the redox pair $\alpha_2$- $P_2W_{17}VO_{62}^{7-}$ /$\alpha_2$- $P_2W_{17}VO_{62}^{8-}$ increased substantially. The shift in the potential for NADH oxidation in presence of heteropoly anion and the increasing anodic current shows how NADH is electrocatalysed by heteropoly anion.

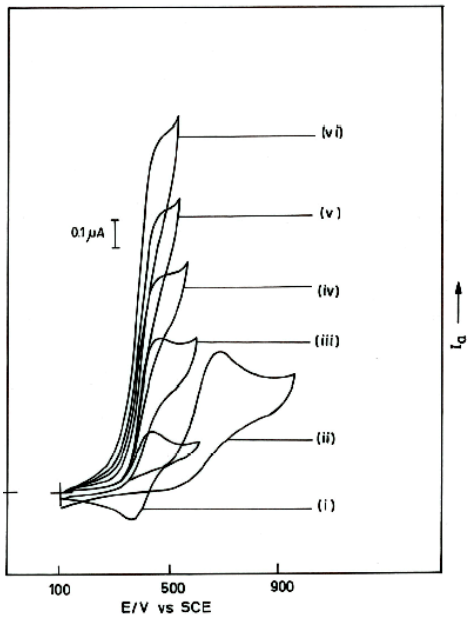


Fig.9. Cyclic voltammograms obtained for $P_2W_{17}VO_{62}^{8-}$/NADH. (i) $10^{-3}$ M $\alpha_2P_2W_{17}VO_{62}^{8-}$ in pH 7 phosphate buffer. (ii) $10^{-3}$ M NADH alone and (iii)- (vi) $10^{-3}$ M $\alpha_2P_2W_{17}VO_{62}^{8-}$ + $10^{-3}$ x $\gamma$ M NADH where $\gamma = C^0$ NADH/ $C^0$ HPA (reproduced from ref.27).

## 4. Device applications of biological species and heteropoly anions

4.1. Electrochromic Devices

(i) *Biomolecules as active component*

Some bioelectronic device applications wherein the redox properties of biomolecules are employed are considered in this section. Biomolecules have tremendous capabilities in molecular recognition and catalysis that can be integrated with solid-state materials such as electrodes to produce bioelectronic devices. These bioelectronic devices can be used in information processing. In these devices, the information is processed using electron transfer by signal

transducing mechanism. The biomolecular materials are characterized by a) an electroactive host matrix, b) the existence of ohmic contacts between the host matrix and the redox-active biomolecules for electron transfer, c) the presence of locally restricted areas for physical separation of molecules in different oxidation states and d) the feasibility of electrode contacts with the matrix at the bulk level for connection to the external electronic circuit.

Biomolecules that can show different colours in their oxidized and reduced states can be an active component in bioelectronic devices where the signal is transduced by electron transfer. Such species with sharp optical contrast at two different potentials are called as electrochromic materials. An electrochromic material is one whose light-absorbing properties are altered upon reduction under the influence of an externally applied field.

In this example, the Sn-doped silica sol-gels are electroactive matrices that are used to encapsulate biomolecules. The behaviour of film containing flavin mono-nucleotide (FMN) investigated. When a reduction potential –0.5 V is applied, it undergoes reduction with a colour change from yellow to red (Fig.10.a)[28]. The colour change takes place in ca. 20 min. On reversing the potential to +0.6 V, the original form is regenerated. This is supported with the absorption spectrum where the original form (oxidized) shows an absorbance at 400 nm and the reduced species shows a new peak at 490 nm (Fig.10.b). The regenerated oxidized form when the potential is reversed to +0.6 V, shows the peak as that of the original form. The change in colour of the film with respect to the applied potential shows that these materials are electrochromic in nature, where the active response is generated by the redox-active molecules. These materials are used as signal transducers for converting an electric signal to an optical output.

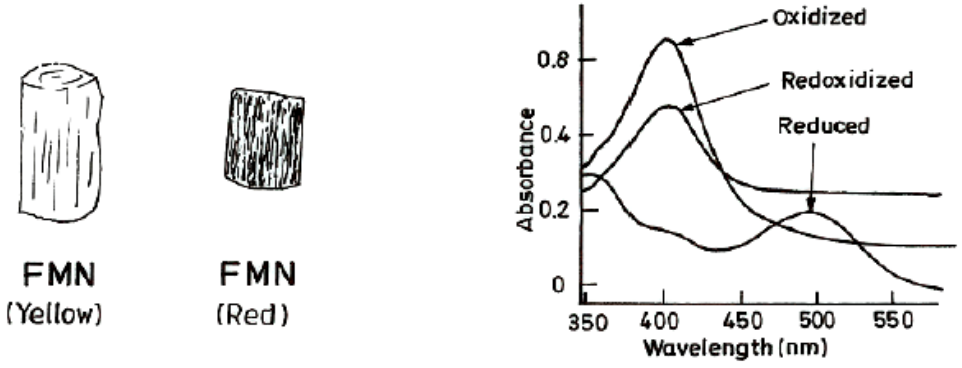


Figure. 10. (a). Oxidized and reduced forms of FMN and (b) optical absorption spectrum of FMN (reproduced from ref.28).

(ii) *Heteropoly anions as active component*

Similarly, redox behaviour of heteropoly anions has been exploited in electrochromic devices. Since they can show reversible electron transfer, optical contrast at two potentials with suitable response time, they can be used as electrochromic material.

When a potential more negative than the vacant orbitals of heteropoly anions are applied, reduction occurs and it changes from $d^0$ to $d^1$ configuration. The $d^1$ electron facilitates the absorption of visible light via intervalence charge transfer among metal centers and d-d transitions.

In the example below, a thin film of heteropoly anion formed by layer-by-layer assembly method is found to show electrochromism. The device is fabricated by layer by layer assembly of $Na_{12}[P_2W_{15}O_{56}]\cdot 18H_2O$ ($P_2W_{15}$), polyallyl amine hydrochloride(PAH), polystyrenesulphonate (PSS)[29]. A visually noticeable optical contrast (transparent to blue) during potential scanning manifests the clear electrochromism of the film. At potential –800 mV, it shows a band at 585 nm. The absorbance increases with increasing number of multilayer films. From the absorbance study, the colouring time and bleaching time is found to be 5.5 s and 6.0 s. The electrochemical reversibility of the multilayer film was evaluated by performing repetitive double-potential steps (Fig.11). This film shows strong colour contrast at maximum absorbance and the low voltage (-800 mV), which is more suitable to be applied in ECDs. The multilayer has good stability and reversibility as the CVs, the response times for colouration and bleaching, and the absorbance do not change noticeably even after 300 cycles (Fig.11 dotted line). The UV-vis spectra of the film have hardly changed after various tests of this work, suggesting that the electrochromic film is robust and environmentally stable.

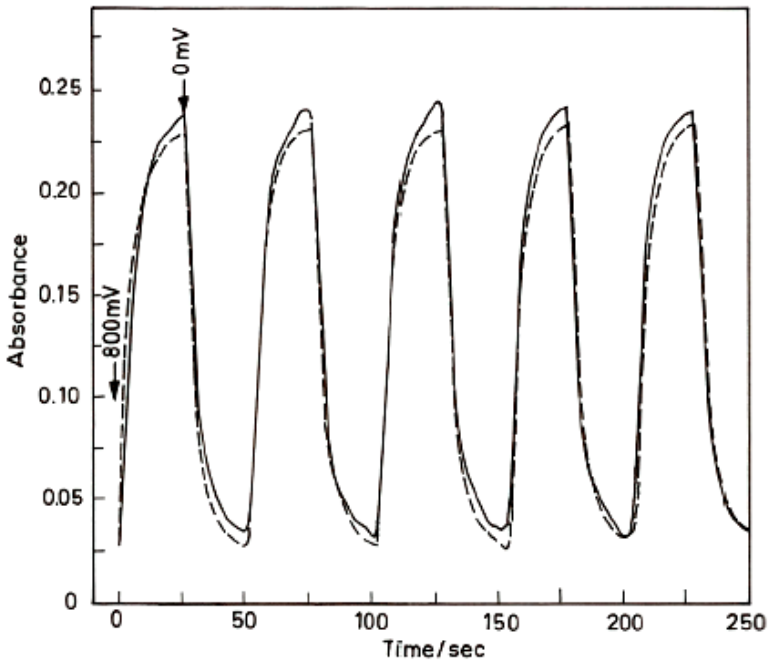


Figure.11. Absorbance of PSS/PAH/$(P_2W_{15}$/PAH$)_{20}$ at $A_{max}$ = 585 nm (from ref.29).

4.2. Surface patterning

In bioelectronics, in the example discussed it has been shown, how the redox properties of biomolecules are exploited in fabricating electrochromic devices. These biomolecules can be molecularly manipulated on the surface of the device by fabricating desired patterns. Such patterns can be achieved directly on the device by self-assembly of the biomolecules on the device followed by etching. But, if the same patterns can be achieved through a mediator such as heteropoly anion, the following advantages can be attained. The biomolecules can be anchored on a stable heteropoly anion moiety, and at the same time they can function as signal transducer since they are redox-active molecules.

Several strategies such as lithography, replication against moulds, self-assembly etc., have been explored to fabricate patterned nanostructures[30]. The concept of patterning originates from biological processes such as the folding of polypeptide chains into functional proteins and chains of RNA into functional t-RNAs, the formation of the DNA double helix, and the formation of cell membranes from phospholipids[30].

In the following example, it is demonstrated how a heteropoly anion can function as molecular ink to pattern copper on the surface of ITO (Fig.12), which is an optically transparent electrode. The concept of microcontact printing and self-assembly have been used to direct electrocrystallization of copper on the substrate.

ITO electrode surface is chemically modified by dipping the electrode in 1mM aqueous solution of $Na_6P_2Mo_{18}O_{62}$ for 10 min. In cyclic voltammetry, this modified electrode showed a typical two electron waves indicating the presence of $P_2Mo_{18}O_{62}^{6-}$ ion and monolayer coverage is found from the current integral calculation[31].

This substrate in the presence of $Cu(NO_3)_2$ solution, shows the onset potential for $Cu^{2+}$ reduction at +300 mV in voltammetry. Same experiment in absence of heteropoly anion shows an onset potential at +200 mV. This indicates that the presence of heteropoly anion catalyzes the electrocrystallization. When a part of ITO is modified with $P_2Mo_{18}O_{62}^{6-}$ and when this electrode is applied with the potential of +300 mV in a $Cu(NO_3)_2$ solution, well defined spots of copper appears on the location corresponding to the area where the electrode had been exposed to $P_2Mo_{18}O_{62}^{6-}$. This observation indicated that the presence of heteropoly anion catalyzes the electrocrystallization of copper and therefore leads to its preferential deposition on the monolayer modified sites.

Based on all these observations, copper was patterned by microcontact printing as shown in the Fig. 12[31]. As seen in the scheme, using a rubber stamp, the pattern of heteropoly anion is imprinted on the surface of ITO. This substrate which has the pattern of heteropoly anion was immersed into a $Cu(NO_3)$ solution. When an adequate potential is applied to the substrate, the substrate started to exhibit a visible metal deposit at the patterned heteropoly anion spots as shown in the Fig.13. This concept can be extended to the patterning of biomolecules for bioelectronic devices if they can interact specifically with the heteropoly anion.

## Conclusion

It appears that complex biological electrochemical reactions can be mimicked in the laboratory by heteropoly anions by appropriate modulation of the electron energy levels of the cluster species brought forth by substitution or by alteration of the environment. This may provide a pathway to study a variety of chemical transformations of biological relevance. It is also possible that these systems lead to some devices which can mimic a few of the natural process. The level of this comparison and the extent to which these systems can run parallel to natural processes are issues that awaits imagination and execution.

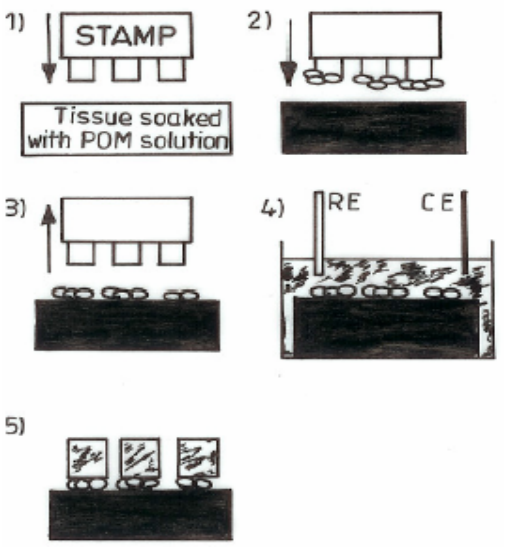


Fig. 12. Various steps (1-5) involved in electrodeposition of copper on ITO. Steps 1-3: Working electrode is patterned with heteropoly anion using rubber stamp. Steps 4-5: Electrodeposition of copper on the patterns of heteropoly anion by applying suitable reduction potential (from ref.31).

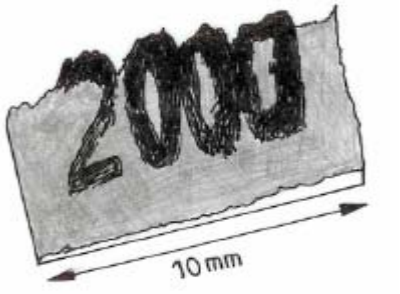


Fig. 13. Copper pattern on ITO surface modified with a “2000” heteropoly anion layer (reproduced from ref.31).

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