The fundamental problems regarding the relative hydrogen (rH) score in biochemistry

Note I. Theoretical bases of rH determination

Z. Gârban

Department of Biochemistry and Molecular Biology, Faculty of Food Products Technology, University of Agricultural Sciences and Veterinary Medicine of Banat Timișoara

Abstract
In living organism there are numerous oxido-reductive systems (i.e. redox systems) essential for the development of reactions specific for the diverse biochemical pathways, integrated into the biotransformation processes, that is biodegradation and biosynthesis. Such processes are specific for the metabolisation of nutrients in biochemistry (i.e. catabolism / anabolism) and for the biotransformation of xenobiotics in xenobiochemistry (i.e. xeno-biodegradation / xenobiosynthesis). In redox systems determinations can be made on the oxidation reduction potential (ORP) evaluated by the electrode redox potential (E), on pH and on the relative hydrogen (rH) score. The value of rH constitutes a typical expression for such systems. Data referring at rH present some fundamental and applicative aspects. Fundamental data refer at calculation modalities, evaluation criteria, physico-chemical acceptance of notions etc. Applicative problems extend over numerous domaines of biochemistry and molecular biology, ecology, nutrition, pharmacology etc. The present note refers to fundamental problems regarding the rH.

Keywords: redox reactions, rH score in biochemistry

1. Introduction
Oxidation reduction systems, generically named „redox systems” stay at the origin of numerous reactions met in chemistry as well as in biochemistry. Currently there are distinguished reversible redox systems – in most cases and irreversible redox systems – in the usually called combustion reactions (e.g. combustion reactions in organic chemistry, biodegradation reactions in biochemistry).

Determinations of oxidation reduction potential (ORP), can be assimilated with electrode potential (E). In redox systems the value of pH can also be determined. The ORP and pH determinations are important in the calculation of the rH score (Stumm, 1967; Dal Beek and Sprung, 1994; Pinto, 2008).

1. General characterization of redox systems
Oxido-reductive reactions, usually named redox reactions, characterise the chemical transformation in which a reactant is oxidized by gaining electrons and oxygen, loosing hydrogen and another one is reduced by reversed mechanisms than those above presented.

In order to evaluate the specificity of reaction in a redox system, is determined the oxidation reduction potential (ORP). For this purpose the redox potential (E) can be determined using the Nernst relation. Currently the pH is meantime determined.

Also, in the evaluation of redox systems in aqueous environment , the pH (hydrogen potential) is usually determined in order to explain the acido-basic balance (on a scale ranging 0-14, where neutrality is at pH=7).

Corresponding author: e-mail address: zgarban@yahoo.com
Especially in redox systems is also possible the measurement of the relative hydrogen (rH) score. This can be carried out even by calculations based on the values of ORP and pH. The domaine of rH explains the oxido-reduction balance (on a specific scale between 0-42, where neutrality is at rH=28).

1.1. Physico-chemical characterisation

Considering, for example, that in a solution there is a reversible redox system formed of ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$) ions, the modifications given by the electron exchange can be represented as follows:

$\text{Fe}^{2+} - e^- \rightarrow \text{Fe}^{3+}$

Experimentally was established that a standard electrode also named inert electrode (of platinated-platinum) introduced in the solution of a redox reversibl system could register, reported to a reference electrode, the difference of potential that depends on the concentration of the reduced form and of the oxidised one. This difference of potential represents the oxidation reduction potential (redox potential).

In the case of the redox system Fe$^{2+}$ / Fe$^{3+}$ it is found that an increase of the Fe$^{2+}$ concentration would determine a decrease of the redox potential, and an increase of the Fe$^{3+}$ concentration would determine an increase of the redox potential.

The redox potential (E) of the redox system (systems) is calculated starting from the value of the standard redox potential ($E_0$) of the system, the value of some physico-chemical constants and the ratio of chemical activities $a_{\text{ox}}/a_{\text{red}}$ or chemical concentrations $[\text{ox}] / [\text{red}]$. Nernst relation is used for calculations:

$$E = E_0 + \frac{RT}{nF} \ln \left( \frac{a_{\text{ox}}}{a_{\text{red}}} \right)$$

where:
- $R$ – gas constant (8,315 Joule/kmol K)
- $T$ – absolute temperature (e.g. at 20° exist 293°K)
- $F$ – Faraday constant (96,500 C/eq gr)
- $a_{\text{ox}}, a_{\text{red}}$ - chemical activities of the oxidised and reduced form
- $\nu_{\text{ox}}, \nu_{\text{red}}$ – stoichiometric coefficients of the oxidised and reduced form
- $n$ – number of electrons that take part in reaction.

It is mentioned that the normal redox potential, usually named standard redox potential ($E_0$) is a constant of the redox system and characterises the situation of equilibrium between the oxidised and reduced form : $[\text{ox}] = [\text{red}]$.

In order to express the redox potential (E) using the Nernst equation, one can also use the values of chemical concentrations of the oxidised $[\text{ox}]$ and reduced $[\text{red}]$ form. It results:

$$E = E_0 + \frac{0.058}{n} \log \left( \frac{[\text{ox}]}{[\text{red}]} \right)$$

Further on the redox potential at 20°C (i.e. 293°K) can be calculated, replacing the R, F constants and using decimal logarithms ($\log = \log 2,303$). The value of redox potential will be:

$$E = E_0 + \frac{0.058}{n} \log \left( \frac{[\text{ox}]}{[\text{red}]} \right)$$

One can see that at equal concentrations $[\text{ox}] = [\text{red}]$, the measured potential E is even the normal redox potential ($E_0$).

1.2. Redox systems in biochemistry

If in an aqueous environment that contains Fe$^{2+}$ and Fe$^{3+}$ ions an inert electrode is introduced, (e.g. platinated – platinum electrode) the reduction of hydrogen ions will take place, forming gasous molecular hydrogen. Thus, two reversible redox systems will exist:

$$2\text{Fe}^{2+} - 2e^- \leftrightarrow \text{Fe}^{3+}$$  (system I)
$$2\text{H}^+ + 2e^- \leftrightarrow \text{H}_2$$  (system II)
Such redox systems are also found in biochemistry (e.g. metabolism of carbohydrates, lipids, protids etc.) and in molecular biology (e.g. interactions of free radicals with DNA). These are experimentally constituted (in vitro) or exist in tissues (in vivo).

In the above situations the redox systems reach the equilibrium, the corresponding redox potential (E) will be for the first system:

\[
E_1 = E_{(H_2O)}^0 + 0.058 \log \frac{\text{Fe}^{3+}}{\text{Fe}^{2+}} 
\]

and for the second system:

\[
E_{II} = E_{(H_2)}^0 + 0.029 \log \frac{[H^+]^2}{[H_2]} 
\]

As it was shown above, the redox potential is more positive as the concentration of the oxidised form is greater as compared to that of the reduced form and vice versa (Clark, 1960).

In biological systems and in biochemistry in general, are redox systems with different potentials situated in the vicinity of zero potential. Table 1 shows the normal potentials of the main redox systems in biochemistry. It is also given the value of pH at which determinations were made.

The reactions presented in table 1 are met in the material metabolism (e.g. of carbohydrates, lipids, protids, bioelectrolites etc) and in the energetic metabolism (Gârban, 2004).

Thus, for a redox system the value of its standard redox potential is determined \(E_0\).

This value is conditioned by the electron exchange that takes place between the oxidised and the reduced form of the compounds present in the system. The electron exchange takes place at the level of the inert electrode.

<table>
<thead>
<tr>
<th>System</th>
<th>(E_0) (volt)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{H}_2\text{O}_2 \rightleftharpoons \text{H}_2\text{O}+1/2 \text{O}_2)</td>
<td>0,30</td>
<td>7,0</td>
</tr>
<tr>
<td>Cytocrome a (\text{Fe}^{2+}) \rightleftharpoons Cytocrome a (\text{Fe}^{3+})</td>
<td>0,29</td>
<td>7,0</td>
</tr>
<tr>
<td>Cytocrome c (\text{Fe}^{2+}) \rightleftharpoons Cytocrome c (\text{Fe}^{3+})</td>
<td>0,25</td>
<td>7,0</td>
</tr>
<tr>
<td>Hemoglobine \rightleftharpoons Methemoglobine</td>
<td>0,17</td>
<td>7,0</td>
</tr>
<tr>
<td>Ascorbic acid \rightleftharpoons Dehydroascorbic acid</td>
<td>0,08</td>
<td>6,4</td>
</tr>
<tr>
<td>Cytocrome b (\text{Fe}^{2+}) \rightleftharpoons Cytocrome b (\text{Fe}^{3+})</td>
<td>0,04</td>
<td>7,4</td>
</tr>
<tr>
<td>Succinic acid \rightleftharpoons Fumaric acid</td>
<td>0,03</td>
<td>7,4</td>
</tr>
<tr>
<td>Malic acid \rightleftharpoons Oxalilacetic acid</td>
<td>-0,17</td>
<td>7,0</td>
</tr>
<tr>
<td>Lactic acid \rightleftharpoons Pyruvic acid</td>
<td>-0,19</td>
<td>7,0</td>
</tr>
<tr>
<td>Ethilic alcohol \rightleftharpoons Acetic aldehyde</td>
<td>-0,20</td>
<td>7,0</td>
</tr>
<tr>
<td>Reduced gluthatione redus \rightleftharpoons Oxidised gluthatione</td>
<td>-0,34</td>
<td>7,0</td>
</tr>
</tbody>
</table>

2. Evaluation of the relative hydrogen (rH) score

After determining the redox potential using the Nernst relation, a correlation was made with other physico-chemical parameters of a given redox system (the reaction environment).

In this manner the so called relative hydrogen (rH) score can be determined which gives information on the „redox capacity” of the system.

In chemical and biochemical systems it was observed that the degree of oxidation and reduction are dependent on the values of temperature and pH.
2.1. General principles

In order to evaluate the specificity of the redox environment of a solution, between the electrode potential and the pH of solution, a specific factor was decelated named rH score, that can define the state of a system that reached the equilibrium (Clark, 1923, Clark, 1960). Thus, rH characterizes the redox capacity of a redox system.

The use of the relation given by Nernst – integrating Clark’s conception – to establish the relative hydrogen (rH) score is discussed by Pinto (2008).

The factor can be easily understood starting from relation (5). If \(E^0(H_2)\) potential is zero (conventionally), the relation becomes:

\[
E = 0.029 \log \frac{[H^+]^2}{[H_2]} \tag{6}
\]

and:

\[
E = 0.058 \log[H^+] - 0.029 \log[H_2] \tag{7}
\]

It is known that the value of pH is expressed by the relation:

\[
pH = – \log[H^+]\]

The concentration of \([H_2]\) equals the pressure of gasous hydrogen in solution, \(P_{H2}\) (i.e. partial pressure of hydrogen).

By analogy with the relation for pH it can be considered that the expression of hydrogen concentration \([H_2]\), therefore its partial pressure, can represent the value of rH. This can be expressed as follows:

\[
rH = - \log[H_2] = - \log P_{H2} \tag{8}
\]

In such conditions relation (7) becomes:

\[
E = 0.029 \ rH – 0.058 \ pH \tag{9}
\]

resulting:

\[
rH = \frac{E + 0.058 \ pH}{0.029} \tag{10}
\]

If \(pH = 0\) the relation (9) becomes:

\[
rH = \frac{E}{0.029} \tag{11}
\]

As a definition it can be affirmed that: rH is the logarithm with changed sign of the partial pressure of gasous hydrogen expressed in atmospheres at an inert electrode introduced in a solution containing a redox system.

2.2. Applicative orientations

Data refering at the relative hydrogen (rH) score show that its value can be determined when the redox potential (E) and the pH values are known.

From relation (10) is exemplified the way of determining the rH value in a \(Fe^{2+} / Fe^{3+}\) redox system. Thus if \(E = 0.63V, \) at pH = 1 is obtained:

\[
rH = \frac{0.63 + 0.058}{0.029} = 23.45 \tag{12}
\]

and for the same solution at pH = 2 results:

\[
rH = \frac{0.63 + 0.116}{0.029} = 25.72 \tag{13}
\]

In a redox system in aqueous environment the reaction of water ionisation is:

\[
H_2O \rightleftharpoons H^+ + OH^-\]

By reducing the \(H^+\) ions and oxidation of \(OH^-\) ions a balance is reached between gasous hydrogen (\(H_2\)) and gasous oxygen (\(O_2\)). At the origin lays the reaction:

\[
2H_2O \rightleftharpoons 2H_2 + O_2\]

For gasous hydrogen and gasous oxygen partial pressures can be evaluated.

If the relative hydrogen (rH) score is given by the logarithm with changed sign of the partial pressure of gasous hydrogen, it can be accepted – by analogy - that there also exists a rO factor – given by the logarithm with changed sign of the partial pressure of gasous oxygen (expressed in atmospheres).

The rO value can be calculated in function of the concentration of oxygen [\(O_2\)] or the
rO = \(-\log [O_2]\) = \(-\log P_{O_2}\) \hspace{1cm} (14)

The value \(rH\) has an extreme limit electrochemically deduced at 18°C with the relation:

\[ rH = \frac{rO}{2} = 42.4 \] \hspace{1cm} (15)

That is analogue with the relation:

\[ pH + pOH = 14 \] \hspace{1cm} (16)

In order to evaluate the role of partial pressure of hydrogen \((P_{H_2})\) and of oxygen \((P_{O_2})\) there will be presented the limit situations in which:

1. partial pressure of hydrogen is of 1 atmosphere, so that \(P_{H_2} = 1\), \(rH = -\log 1 = 0\), and \(rO = 84.8\) – when a certain compound with redox properties will be totally in the reduced form.
2. partial pressure of oxygen is of 1 atmosphere, so that \(P_{O_2} = 1\), \(rO = -\log 1 = 0\), and \(rH = 424\) – when a certain compound with redox properties will be totally in the oxidised form.

If \(rH = rO\) out of relation (15) is obtained:

\[ rH + \frac{rH}{2} = 42.4 \] \hspace{1cm} (17)

resulting:

\[ rH + \left(1 + \frac{1}{2}\right) = 42.4 \text{ and } rH \frac{3}{2} = 42.4 \]

resulting the final value:

\[ rH = 28.3 \] \hspace{1cm} (18)

having in view that \(rH = -\log P_{H_2}\) we can write:

\[ \log P_{H_2} = 28.23, \text{ respetively } P_{H_2} = 10^{-28.3} \] \hspace{1cm} (19)

representing the pressure of gasous hydrogen at 18°C of the redox system where \(rH = rO\).

In the study of the reaction of environment (acidity/alkalinity) at \(pH = pOH\) is considered the system neutral.

By analogy one can similarly evaluate the relative score of hydrogen of the environment \(rH = rO\) - considering the „neutrality” of the redox capacity.

### 3. Determination of the relative hydrogen score - general applications

For determining the relative hydrogen \((rH)\) score of the environment is important to know the redox potential \((E_0)\) of the system. Determinations can be made using the potentiometric method, determining the electrode potential of the platinated-platinum electrode – representing the inert electrode related with a calomel electrode – representing the reference electrode.

In this way determinations of the relative hydrogen \((rH)\) score of the environment can be made, using certain substances. These substances permit the determination of the standard redox potential \((E_0)\). In order to determine the relative hydrogen \((rH)\) score of a certain system (environment) „redox indicators” can be used, for example. Table 2 presents data refering at diverse redox indicators.

The classical used method for the first determinations was the spectrophotometric one that used indicators for redox systems. The spectrophotometric method uses organic substances whose colour is changed at structural modifications when passing from the oxidised to the reduced form or viceversa.

In the studies of redox systems, empirical mathematical relations were discovered between ORP, \(pH\) and \(rH\).

For example, after Holmes-Farley (2008) the value of \(rH\) was calculated starting from the empirical relation:

\[ rH = \frac{ORP}{29} + 2pH + 6,67 \]

With the analytical determined values \((pH)\) and calculated values \((rH)\) were established the variations of the environment in aquarium in water of sea and ocean (Skopintsev et al., 1966; Delbeek and Sprung, 1994; Heinson et al., 2007).
The experiments lasted one week and allowed the representation of the ratio rH / pH vs. time. This diagramme, after Holmes-Farley (2008) is given in fig. 1.

The problem of the relative hydrogen (rH) score is of special interest for biochemistry because in numerous biochemical pathways specific for biodegradation / biosynthesis processes are redox reactions.

Limited strictly at biologic systems, the ORP value can be expressed by the redox potential E – see relation (3). In this situation the ratio [ox] / [red] is in fact, represented by the ratio [H⁺] / [H₂].

### Table 3. Substances used as redox indicators

<table>
<thead>
<tr>
<th>Indicators for redox systems</th>
<th>E₀</th>
<th>rH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral red</td>
<td>-0.340</td>
<td>2.3</td>
</tr>
<tr>
<td>Ianus green</td>
<td>-0.258</td>
<td>5.2</td>
</tr>
<tr>
<td>Cresyl violet</td>
<td>-0.167</td>
<td>8.3</td>
</tr>
<tr>
<td>Nil blue</td>
<td>-0.142</td>
<td>9.2</td>
</tr>
<tr>
<td>Disulphonated Indigo</td>
<td>-0.125</td>
<td>9.9</td>
</tr>
<tr>
<td>Trisulphonated Indigo</td>
<td>-0.081</td>
<td>11.3</td>
</tr>
<tr>
<td>Tetrasulphonated Indigo</td>
<td>-0.046</td>
<td>12.5</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>-0.011</td>
<td>14.4</td>
</tr>
<tr>
<td>Cresyl blue</td>
<td>+0.032</td>
<td>15.1</td>
</tr>
<tr>
<td>Toluidin (chlorhydrated) blue</td>
<td>+0.115</td>
<td>17.9</td>
</tr>
<tr>
<td>1 naphtol 2 indophenol sulphonic acid</td>
<td>+0.135</td>
<td>18.5</td>
</tr>
<tr>
<td>Orto cresol-indo 2',6-diclorphenol</td>
<td>+ 0.181</td>
<td>20.1</td>
</tr>
<tr>
<td>Orto-cresol-indophenol</td>
<td>+ 0.195</td>
<td>20.5</td>
</tr>
<tr>
<td>Meta-cresol-indophenol</td>
<td>+ 0.210</td>
<td>21.0</td>
</tr>
<tr>
<td>Orto-chlorphenol-indophenol</td>
<td>+ 0.233</td>
<td>21.8</td>
</tr>
<tr>
<td>Meta-bromphenol-indophenol</td>
<td>+ 0.248</td>
<td>22.3</td>
</tr>
<tr>
<td>Phenol-m-sulphonated-indo-2,6 dibromphenol</td>
<td>+ 0.273</td>
<td>23.1</td>
</tr>
<tr>
<td>Potassium ferrycianide</td>
<td>+ 0.430</td>
<td>28.4</td>
</tr>
</tbody>
</table>

**Figure. 1. Diagramatic representation of the relation pH / rH vs. time**
Relation (3) leads to the value for E in biologic systems given by the relation:

\[ E = E_0 + \frac{0.058}{n} \log \frac{[H^+]}{[H_2]} \]

The data on rH can indicate an increase of the potential in the studied fluids. For example rH calculations offer important information on the oxidative stress induced by the modification of the quantum of electrons in diverse reactions at cellular level. Characteristic are also the modifications in nutritional deficiencies, in the stimulation of the immune system exposed to radiations and toxic substances. In all these situations one can observe an increase of rH over the optimal values. We remind that the domain ranges between 0-42 with an average of 28.

A value lower than 28 indicates the presence of a greater number of donor electrons than acceptors. Similarly, a value over 28 shows that the fluid is an oxidant existing a lower number of donors than acceptors (Stephanson and Flanagan, 2004).

Even if apparently theoretic, above presented mentions find current applications in biochemistry, molecular biology, nutrition, pharmacology and, evidently, in the biotechnologies where redox reactions appear.

**Conclusive remarks**

1. For a certain environment (an aqueous solution): pH characterises the reaction of environment (i.e. acidity/alcalinity) and rH characterises the redox (i.e. reduction/oxidation) capacity of that environment.

2. A comparison between pH and rH values reveals following aspects:
   - in the case of pH – the higher the concentration of hydrogen ions, i.e. \([H^+]\), the lowest the pH, thus the environment is more acidic and reverse; the scale ranges between 0-14.
   - At pH=0 the acidity is maximum, at pH=14 the alcalinity is maximum, at pH=7 is a neutral state.
   - in the case of rH – the highest the hydrogen pressure in a redox system the lowest the rH, thus the environment is more reducing and reverse; the scale ranges between 0-42. At rH=0 the environment is strongly reducing; at rH=42 the environment is strongly oxidant. At rH=28 is a neutral state.

3. In the case of redox systems – having in view the criterium of „neutrality”- it can be considered: a) at \( rH < 28,3 \) – are reducing systems, that can release electrons to other systems with lower rH; b) at \( rH > 28,3 \) – are oxidant systems that can accept electrons from systems with higher rH.

**References**


Holmes-Farley R. – ORP and the Reef Aquarium, Reefkeeping – an online magazine for the marine aquarist, 2008, 7,(2), 1-20

