About units and quantities – 1.

In the last editorial (Photosynthetica 50/1, 2012) I wrote about some quantities and units. My intention is to keep the rules of physics and physical chemistry and, if possible, also to use the recommended SI units in our journal. In our Notice to Contributors we ask the authors: "Units, dimensions, terms, symbols, abbreviations *etc*., recommended by the Systéme International d´Unités (SI – the International System of Units) should be used."

Enzyme activity

Different units appear in the literature and also our authors use a wide range of them. I sometimes ask the authors to change the units or specify their definition. The internationally suggested unit (SI) of enzyme activity is **katal**. The authors from some unknown reasons refuse to use this unit. I am sorry to say, I can recall only one paper in Photosynthetica using katal as a unit of the enzyme activity. Dybkaer (2001) names two basic reasons for refusing the SI units – the inertia induced by habit and the lack of recognition by the General Conference on Weights and Measures (GCWM). I would add another reason, a lack of information in the books on biochemistry. Even the large Encyclopedia of Biological Chemistry (Lennarz and Lane 2004) or Lehninger Principles of Biochemistry (Nelson and Cox 2000) do not contain any information about katals. Students probably have no chance to find out about katals.

The former international unit of the **enzyme activity** designated as **U** represents such amount of the sample in which the active enzyme converts 1 μmol of substrate in 1 min, *i.e.*: $\mathbf{1\ U = 1\ \mu mol\ min^{-1}}$

This unit has been introduced on the recommendation of the International Union of Biochemistry (IUB) in 1961. The conditions at which the measurements are done have to be specified: usually at the standard pressure, a temperature of 25°C and the pH value and substrate concentration that yield the maximal substrate conversion rate. As can be shown theoretically, there should be excess of the substrate so that the maximal value of the enzyme activity is reached. Under the excess of the substrate, the rate of substrate conversion is proportional to the amount of the active enzyme. In other case, also the substrate concentration is of importance. Under saturating conditions, the zero-order kinetics is achieved giving a constant rate of conversion (Dybkaer 2001).

However, in 1966 the IUB suggested to use another basic unit – **katal**. One reason was that the minute used in the definition of U is not an SI unit. The other reason claimed was that kat is a more suitable unit for the newly discovered and studied enzymes. Katal was then recommended as the SI unit by the GCWM in 1978 and officially adopted in 1999 (Resolution 12, *see* Dybkaer 2002).

The enzyme activity of **1 katal (kat)** means such amount of the sample which converts 1 mol of a substrate in 1 s (under defined conditions), *i.e.*: $\mathbf{1\ kat = 1\ mol\ s^{-1}}$.

The so called **specific enzyme activity** should be expressed as kat per 1 kg of total protein (sometimes also related to kg of dry mass, unit leaf area, unit volume, *etc.*). That is, the correct unit for the presentation of the specific enzyme activity is: $\mathbf{kat\ kg^{-1}(protein)}$.

Because 1 kat is quite a large unit, often nanokatals (nkat) per mg or μg of protein are used to present the specific enzyme activity. **Most of our authors usually mean by the term enzyme activity this specific enzyme activity**. However, the correct term specific enzyme activity is recommended although practically not used.

Interestingly, the conversion of U to kat is very simple. A simple calculation shows that

$$\mathbf{1\ U = 1.666 \times 10^{-8}\ kat = 16.67\ nkat \quad or\ 1\ kat = 6 \times 10^{7}\ U}.$$

I have to mention that in many enzyme activity measurements (*e.g.* the Rubisco activity) other purchased enzymes are used and their amount is presented in U. This means that the authors are aware of the unit U. However, this fact does not prevent them to use the same symbol U for their own defined units.

That is why, if the authors define their own unit U, they come into contradiction with the internationally introduced former unit U and may cause confusion. The reader has to realize that the used unit U is not that one agreed by the community to present the above-mentioned activity of 1 μmol of substrate changed by the sample in 1 min. Own units are not recommended since a comparison with the other papers is more complicated then. Use of the internationally recognized units helps compare the results of different laboratories.

Even if the authors do not use katals or the former U units, their paper may have been accepted. Then, the basic rule must be obeyed; the units should contain the substrate (or product) amount (*e.g.* μmol) converted in a time unit (second or minute) and related to a defined quantity (protein, dry mass, fresh mass, leaf area).

Especially the time unit is of basic importance, because the enzyme activity is defined as a rate of substrate conversion. Here, I have to admit that I do not understand the units of SOD (superoxide dismutase) activity presented by some authors. These units generally do not contain the time units. A usual form of the definition is "One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction rate of NBT as monitored at 560 nm" (no information about the light intensity and NBT concentration is given). I have asked several

groups of authors presenting the SOD activity to use the corresponding time units but I did not succeed. So there is some discrepancy either in the concept of the enzyme activity or in the internationally used approach for the SOD activity estimation somehow ignoring the time parameter. I would appreciate an explanation by those who understand better this problem, *e.g.* in a form of a *Letter to editor*. In my opinion, in this case the quantity defines the content but not the activity of the enzyme. The term "content" is also used in the frequently cited paper of Giannopolitis and Ries (1977).

Some authors do not use the amount of substrate (*e.g.* μmol) in the units and use the change in the absorbance (dimensionless) at certain wavelength instead. Sure, the change in the absorbance (in a 1-cm cuvette) should be proportional to the change in the substrate (product) concentration. Then, knowing the molar absorption coefficient of the substrate at the set wavelength and the amount of the extract, the amount of the substrate changed by the sample can be estimated. This approach should lead to a more exact presentation of the units. If, for instance, the amount of the extract is not presented, the results are not comparable to those published by other papers. Even in this case, there is a value in the comparison of this relative enzyme activity of the exposed sample and a control one. Only the possibility to relate the results to those of other authors is lost.

SPAD units

There is a new phenomenon, the use of the SPAD units for presentation of the chlorophyll concentration in a leaf (on the area basis). For readers not having the SPAD chlorophyll meter (*e.g. SPAD-502DL, Konica Minolta Sensing Inc*., Osaka, Japan) at their disposal, this unit may not be understandable and accessible. In a more exact approach, the factory units of SPAD should be calibrated by a separate measurement in which the real amount of chlorophylls (*a* and *b*) per unit leaf area is measured both by the chlorophyll meter and by the extraction to a solvent followed by an exact spectrophotometric estimation (*e.g.* in μg cm^{-2}). The measured SPAD units range from about 10 (for yellowish leaves) to about 60 (for very green leaves). These values very roughly correspond to the values expressed in μg cm^{-2} (Nauš *et al*. 2010). In the calibration procedure, the leaves of the given species having different chlorophyll amounts but conserving approximately the same leaf structure should be used. The reason is that the SPAD units are species-specific and may reflect also the cultivation conditions. We have done such a calibration for tobacco leaves showing the effect of chloroplast movement (Nauš *et al*. 2010). Most of our authors omit the calibration procedure using the SPAD units as the only information about the chlorophyll content (based on the leaf area). Then, the results may be obtained very fast and in a statistically sufficient amount, but the possibility of direct comparison with other papers is again more problematic. I have also to mention that the dependence of the SPAD data on the area chlorophyll content is generally not linear and should be carefully checked for the interval used. I think that the calibration procedure does not take so much time (about 1 day) and may substantially improve the quality of the paper. Moreover, some reviewers reasonably argue that SPAD units are not the SI units.

Dry matter (DM) *vs*. fresh matter (FM)

In some cases the authors are criticized due to use of fresh matter (FM) as a reference quantity instead of dry matter (DM). It is clear that detection of the fresh matter by a simple weighing of the detached fresh leaf (or a segment) is the simpliest and fastest way how to get a reference quantity. However, in some cases this reference quantity may be misleading and may present an obstacle of acceptance of a manuscript for publication. This regards mainly the studies of water, salinity, or osmotic stresses. The basic phenomenon of the drought or osmotic effect is a loss of leaf water. In this case, the fresh mass reflects the effect of water loss. A relation of the other measured quantity (*e.g*. the enzyme activity) to the fresh- and dry matter may lead to opposite conclusions. In my opinion, use of the fresh mass as a reference quantity in case of water-loss studies is a mistake and a relation to dry mass should be preferred.

J. NAUŠ (*Olomouc*)

References

Dybkaer, R.: Unit "katal" for catalytic activity (IUPAC technical Report). – Pure Appl. Chem. **72**: 927-931, 2001.
Dybkaer, R.: The tortuous road to the adoption of katal for the expression of catalytic activity by the General Conference on Weights and Measures. – Clin. Chem. **48**: 586-590, 2002.
Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases. I. Occurence in higher plants. – Plant Physiol. **59**: 309-314, 1977.
Lennarz, W.J., Lane, M.D. (eds): Encyclopedia of Biological Chemistry. Elsevier, 2004.
Nauš, J., Prokopová, J., Řebíček, J., Špundová M.: SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. – Photosynth. Res. **105**: 265-271, 2010.
Nelson, D.L., Cox, M.M. (eds.): Lehninger Principles of Biochemistry (3rd edition), Worth Publishers, New York, 2000.