Methods Absorbance, absorptance and friends

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Introduction

Most photobiologists sooner or later have to measure light absorption by objects such as plant leaves, optical filters or solutes in a liquid medium. The physical quantities we measure may vary: absorbance, optical density, absorptance, transmittance and reflectance. For each of these quantities there is also variation in how they are defined and in the symbols used to represent them. The main authority for chemical notation is the International Union of Pure and Applied Chemistry (IUPAC) and as photochemistry is closely related to photobiology, IUPAC definitions are suitable and broadly used in plant physiology (Braslavsky 2007). I will use the definitions and symbols recommended by IUPAC (Braslavsky 2007; Cohen et al. 2007) and the Système international d'unités (SI units). Johnsen (2012) discusses the proliferation of units and describes a subset of them, based on the uses in his field of research, and several of the definitions he gives are not consistent with those currently recommended by IUPAC. Even if in the field of plant photobiology the IUPAC definitions are usually followed, as I will do here, researchers should be very attentive both as readers and writers about the existence of alternative definitions and the use of the same symbols for different physical quantities. In addition, some of the consistently used and named quantities can be difficult to distinguish from each other for non-experts. My aim here is to provide guidance for the use of these quantities in research on plants.

Reflectance

Reflectance is the fraction of the incident radiation that is reflected,

 $\rho = P_{\text{refl}}/P_0$,





Figure 10.1: An integrating sphere in two different configurations, as used to measure total transmittance and total reflectance, respectively. Source: Wikimedia Commons. Creator: cmglee. Revised by: P. J. Aphalo. License: CC BY-SA 3.0.

where P_{refl} is the reflected radiation and P_0 the incident radiation¹. Simple enough, but in most cases ρ depends on the angle of incidence of the illumination, so for ρ to be interpretable this angle must be known. How we collect the reflected light also matters, giving rise to two different quantities, specular reflectance ρ_{specular} and total reflectance ρ_{total} . For measuring ρ_{total} we use in most cases collimated light for illumination at only a small angle of incidence (θ_1) and collect all reflected light with an integrating sphere with its port seated against the illuminated side of the object (Figure 10.1). For ρ_{total} we use as white reference ($\rho_{\text{total}} \approx 1$) a surface that scatters the light. To measure $ho_{
m specular}$ we use collimated light for illumination and measure reflected light over a narrow angle and on a plane normal to the light beam used illumination, using a probe usually based on a coaxial arrangement of optical fibres. In this second case, we can easily take readings at different angles to describe how ρ_{specular} varies. For objects that scatter light, $\rho_{\text{specular}} < \rho_{\text{total}}$. Reflectance (ρ) is defined as a "summary" over a broad range of wavelengths, a range that depends on the light source and sensor used. To measure a reflectance spectrum we combine a light source with a wide and "featureless" emission spectrum with the use of a spectrometer as sensor. The quantity we obtain is spectral reflectance, given by $\rho(\lambda) = P_{refl}(\lambda)/P_0(\lambda)$, where λ stands for wavelength.

For a plane interface, such as that between air and a polished glass plate, the reflectance at different angles can be calculated from the refractive indexes (Figure 10.2). It depends on the relative refractive index between two media, such as air and glass. I assumed an interface with a relative refractive index of 1.5, which is close to that between crown glass or acrylic and air. If light is moving from air into the glass or acrylic, $\rho \leq 0.1$ for small incidence

¹We use *P* as symbol, instead of the usual *E* or *Q* as the discussion is valid for radiation expressed on both an energy- or photon basis, and for both irradiance and exposure, as long as units are used consistently for the different terms in each equation.





Figure 10.2: Reflectance of a single plane interface as a function of the angle of incidence (θ_1). Computations are for an interface between air and crown glass, i.e., for relative refractive index n = 1.5. See the Appendix for code used.

angles ($\theta_1 < 30^\circ$) and then increases rapidly reaching $\rho \approx 0.5$ at $\theta_1 = 75^\circ$ and $\lim_{\theta_1 \to 90^\circ} \rho = 1$ when the light beam is close to parallel to the interface surface (Figure 10.2). In most cases we are dealing with two interfaces, one on each face of the glass or acrylic pane, resulting in a further decrease in transmittance. The dependency on the angle of incidence is, obviously, important when using wavelength-selective filters but also crucial for the design of glass-houses at medium and high latitudes.

The same formulae apply to metals, but in the case of metals the refractive index is given by a complex number with a Real component n and an imaginary component k. Reflection of diffuse, i.e., Lambertian, light at plane interfaces and reflection of collimated light by scattering media are beyond the aims of this paper.

Transmittance

Total transmittance is the fraction of the incident radiation that is transmitted through an object,

$$\tau = P_{\rm tr}/P_0,$$

where $P_{\rm tr}$ is the transmitted radiation and P_0 the incident radiation. In practice we usually measure τ with normal illumination and collect all the transmitted light, which in the case of objects that scatter the transmitted light



requires an integrating sphere for measurement (Figure 10.1). Transmittance can be also expressed as internal transmittance, $\tau_{\text{internal}} = P_{\text{tr}}/(P_0 - P_{\text{refl}})$, i.e., using as reference the light actually "entering" the object, rather than the incident one. For some objects which do not scatter light, such as glass filters with a polished surface, ρ varies little with λ and a constant conversion factor can be used to inter convert τ_{internal} and τ_{total} values. For objects like plant leaves, the conversion requires that $\rho(\lambda)$ is known. As above if measured across the spectrum, we obtain the spectral equivalents, $\tau(\lambda)$ and $\tau_{\text{internal}}(\lambda)$. Light extinction corresponds to $1 - \tau$ and its use is frequent in atmospheric sciences or when considering light in plant canopies.

Absorptance

Absorptance is the fraction of the incident radiation that is absorbed by an object,

$$\alpha = P_{\rm abs}/P_0,$$

where P_{abs} is the absorbed radiation and P_0 the incident radiation. As above if measured across the spectrum, we obtain the spectral equivalents, $\alpha(\lambda)$.

As there is no other fate possible for incident radiation, $\rho + \tau + \alpha = 1$, and consequently, in theory, each of ρ , τ and α can take values in the range zero to one. If we exclude reflectance we get $\tau_{\text{internal}} + \alpha = 1$. On the other hand $\rho_{\text{specular}} + \tau + \alpha \leq 1$, as $\rho_{\text{specular}} \leq \rho_{\text{total}}$ (This is so because ρ_{specular} does not include the scattered component of reflectance.)

The easiest way of demonstrating the importance of the difference between internal and total transmittance is using an example. In Figure 10.3.A $\rho(\lambda)$, $\tau(\lambda)$ and $\alpha(\lambda)$ are plotted as a stack, showing that their sum is always equal to 1. In Figure 10.3.B we plot only $\tau(\lambda)$, or total spectral transmittance, which is identical to the lower layer of the stack in Figure 10.3.A. In Figure 10.3.C we plot $\tau_{internal}(\lambda)$, where we see that $\tau_{internal}(\lambda) + \alpha(\lambda) = 1$.

Absorbance

In this case we have two definitions in use, mostly in different fields of research: (decadic) absorbance, A_{10} or A, and napierian absorbance, A_e . The definition of (decadic) absorbance is

$$A_{10} = log_{10}(1/\tau_{\text{internal}}),$$

or its equivalent

$$A_{10} = -log_{10}(1 - \alpha).$$

In the case of napierian absorbance, we need only substitute log_{10} by log_e ,

$$A_{\rm e} = log_{\rm e}(1/\tau_{\rm internal}),$$



Figure 10.3: Optical properties of the adaxial side of an Arabidospis (Ler) leaf. A. Total spectral transmittance, spectral absorptance and spectral reflectance from the same leaf; B. Total spectral transmittance; C. Internal spectral transmittance. One observation from (Wang et al. 2020). See Appendix for code used.

or its equivalent

$$A_{\rm e} = -log_{\rm e}(1-\alpha).$$

From these equations it follows that $A_{10} = A_e \cdot log_e(10)$ and $A_e = A_{10} \cdot log_{10}(e)$.

While absorbance is defined as

 $A_{10} = log_{10}(1/\tau_{\text{internal}}),$

optical density is denifed as

$$OD = log_{10}(1/\tau_{total}),$$

i.e., optical density is the equivalent of absorbance but based on *total* transmittance instead of *internal* transmittance.

As for ρ , τ and α above, if *A* (or OD) is measured across the spectrum, we obtain the spectral equivalents, $A_{10}(\lambda)$ and $A_e(\lambda)$. With the definitions above becoming dependent on wavelength (λ), for example spectral (decadic) absorbance is defined as

$$A_{10}(\lambda) = log_{10}(1/\tau_{\text{internal}}(\lambda)),$$

or its equivalent

$$A_{10}(\lambda) = -log_{10}(1 - \alpha(\lambda)).$$

Absorption of light by homogeneous semi-transparent media is a cumulative process along the light pass, resulting in exponential decay, as described by Lambert-Beer's law,

$$I_l = I_0 e^{-a \cdot l}.$$

This curvilinear relationship is the reason why absorptance is not proportional to solute concentration or path length while absorbance is. The attenuation of radiation passing through homogeneous media is an exponential process with respect to both the length of the light path (*l*) and with increasing values of the absorption coefficient, *a* (*K* also used), where *a* is expressed in m⁻¹. In other words, while, *a* is an intensive property of a material, A_{10} is an extensive property of an object.

When we are interested in the concentration of a solute, we define the molar extinction coefficient $\epsilon = a/c$, where *c* is the molar concentration, resulting in an alternative formulation of the Lambert-Beer's law,

$$I_l = I_0 \,\mathrm{e}^{-c \cdot l \cdot \epsilon},$$

The coefficient ϵ is expressed² in m² mol⁻¹, assuming concentration *c* is expressed in mol m⁻³.

The data in Figure 10.4 simulate the effect of thin layers of flavonoid solutions at two different concentrations. We can see that attenuation per unit of path length is strongest immediately below the illuminated surface. We can

²We show here SI units only, although some other units are still in use.

UV4Plants Bulletin, 2020, no. 1



Figure 10.4: Light attenuation in a homogeneous semi-transparent medium. Relative irradiance (I_l) is plotted as a function of the length (l) of the light path. Plotted values were computed using Lambert-Beer's law assuming solutions of quercitrin at concentrations of 10 and 25 molm⁻³ and extinction coefficient $\epsilon = 16 \times 10^4 \,\mathrm{m^2 \,mol^{-1}}$ (ϵ for $\lambda = 350 \,\mathrm{nm}$ from Latouche et al. 2012, Figure 1). See Appendix for code used.

also see that the effect of solute concentration on the transmitted irradiance is most noticeable deeper into the layer. The depth into the layer at which attenuation is 50% depends on the concentration (Figure 10.4). It should be remembered that the Lambert-Beer's law does not apply to scattering media like plant tissues and colloidal suspensions.

Units and symbols

All of ρ , τ , α , A and OD are unitless quantities, describing ratios between values expressed in the same units. While A and OD are always expressed as some small positive number, ρ , τ , and α can be expressed either as fractions of one (/1) or as percentages (%).

The symbols *R*, *T* and *A* are also commonly used in place of ρ , τ , and α . However, although IUPAC accepts this use of *R* and *T*, it reserves *A* for absorbance. Not being these quantities fundamental or directly derived from such quantities, no symbols are defined for them in the SI standard.



Box 10.1: Estimating epidermal UV-screening with the Dualex Because absorbance, A, is proportional to the concentration of a lightabsorbing solute, $A_{10} \propto$ [solute], it is used widely in spectrophotometry. Similarly, the Dualex instruments (Force-A, Orsay) measure a quantity that approximates the absorbance of the epidermis of leaves on a band centred at $\lambda = 375$ nm (Goulas et al. 2004). This index quantity is assumed to be useful as a proxy of the concentration flavonoids in the epidermis. However, when we are interested in the degree of protection, transmittance, τ , is more informative than absorbance. This instrument measures the attenuation of radiation reaching the chlorophyll in the leaf mesophyll by comparing the excitation of chlorophyll fluorescence by radiation of different wavelengths. The conversion of $A(\lambda = 375 \text{ nm})$ into τ (λ = 375 nm) is straightforward. As τ = 10^{-A₁₀}, it follows that a value of $A_{\text{epidermis}} = 2$ from the Dualex can be interpreted as meaning that $\approx 1\%$ of the UVA at $\lambda \approx 375$ nm impinging on the epidermis reaches the mesophyll and $\approx 99\%$ is attenuated. Because of the way the Dualex works, comparing two wavelengths, only the difference in epidermal reflectance between $\lambda \approx 375$ nm and $\lambda \approx 655$ nm is measured and consequently the A estimate from the Dualex is not a true absorbance neither a true optical density, OD, estimate but instead something in-between. This must be taken into consideration when discussing protection for leaves that are highly reflective in the visible, because true UVA protection will be significantly better than that estimated by Dualex instruments.

Practical considerations and applications

Depending on the aims of a study, or the problem at hand, ρ , τ , α , A or OD may be the most informative quantity. Depending on the object measured and equipment used, τ_{internal} or τ_{total} may be easier to obtain. In many cases by default or as only option an instrument may provide values for a quantity that is not the one most appropriate for our study. In such cases, the relationships and equations described above may allow us to convert the measured values (see, Box 10.1).

If we measure a solution in a cuvette with a spectrophotometer and we use as reference the same or an identical cuvette with solvent as reference, we can assume that we have discounted the effect of reflections. Instead if we measure a filter, such as a piece of polyester film, and the reference is no film, our measurement includes the effect of reflections at the film surface. If we express the readings as transmittance, in the first case we have measured τ_{internal} while in the second cases τ_{total} . If we use logarithms then we obtain absorbance *A* and optical density OD, respectively.





Figure 10.5: Effect of celullose diacetate film thickness on total transmittance. See Appendix for code used.

Internal transmittance, τ_{internal} , makes it easy to compute the effect of changes in transmission with changes in the length of the light pass, such as when using different spectrophotometer cuvettes, or the effect of ionic filter glass of different thickness. This is easy to understand from first principles: ρ in non-scattering media is defined by the surface, so ρ is not affected by the thickness of the material. That in the formula below we use the ratio between the thicknesses of the filters as an exponent, stems from the exponential extinction relationship described by the Lambers-Beer law.

$$au_{ ext{internal}, ext{d}2} = au_{ ext{internal}, ext{d}1}^{(d1/d2)},$$

where d1 is the thickness corresponding to the known τ_{d1} and d2 is the thickness for which we want to compute the corresponding τ_{d2} . Figure 10.5 shows measurements of transmittance for cellulose diacetate. Increasing the thickness four times alters the shape of the curve and shifts the wavelength for 50% transmittance by 8.6 nm towards longer wavelengths and decreases the UV-A transmittance by 20%.

If we compare a standard spectrophotometer cuvette with 10 mm light path to a cuvette with a path of 50 mm, a solution that yields A = 0.2 in the first cuvette will yield $A = 0.2 \cdot 5 = 1.0$ in the second cuvette. When we need to measure very low concentrations using a longer light path is very useful and using a short light path helps when concentrations are high. Cuvettes with light-paths lengths betteen 1 mm and 100 mm are easily available, and can greatly increase the range of concentrations that can be measured with a given instrument, as long as they physically fit into the spectrophotometer.



Box 10.2: Measuring reflectance of semi-transparent objects When we measure reflectance from objects that transmit some of the incident radiation, we need to ensure that no light inpings on the back of the object. For example, the opposing integrating spheres in the SpectroClip-TR (Ocean Insight, Dunedin, FL, formerly Ocean Optics), create an important problem. Part of the transmitted photons will bounce on the lower integrating sphere impinging onto the lower surface of the object and may be transmitted back through the object into the upper sphere. This means that some photons will contribute to both the transmittance and reflectance measurements, which can result in erroneous measurements that seem to indicate that $\rho + \tau + \alpha \ge 1$. The problem is more apparent when measuring samples with high values of τ . For example, in spectral measurements of leaves in the far-red region ($\lambda \gtrsim 700$ nm) α is very small and τ nearly 50%, a situation where unless a black object is put behind the leaf during the measurement of ρ , the estimate of ρ will be biased towards values larger than the true ones. It is also possible to apply a correction when processing the data. To obtain the data in Figure 10.3 a black object was put behind the leaf during the measurement of ρ , and the minimum calculated $\alpha(\lambda)$ was very close to zero. The best light absorber that is easily available and thin, is the black flocking sold for covering the inside of optical instruments and cameras (Arax, Kiev; https://araxfoto.com/) or special black paint for this same purpose such as Kameralack Spray (Tetenal, Norderstedt; https://www.tetenal.com/) sprayed on a suitable base material. Not being aware of the limitations introduced by the Spectro Clip's design can lead to substantially wrong data being reported. The same problem will be introduced by the presence of any reflecting material behind the leaf being measured, e.g. a white sheet of paper behind the sample even when using a reflectance probe with a narrow angle of acceptance. Obviously, when measuring at wavelengths that we cannot see, we cannot choose an object that looks black, e.g., black anodised aluminium has hight reflectance in the near infrared.



In the first part of this section we have considered only non-scattering materials. This is the simplest case because if we measure in a normal spectrophotometer a non-scattering material like an homogeneous solution or a piece of glass or acrylic with well polished surfaces estimates of τ will be reliable as the light beam direction will not be disturbed. In contrast, if we measure a suspension of particles in a solvent or a thick film of polythene or similar plastic, we will grossly underestimate τ . The reason is simple, the transmitted light that is no longer collimated will not reach the sensor and will not be measured. In this case, to obtain a reliable measurement, we need to use an integrating sphere to collect the photons leaving the measured object in all possible directions. The obvious way to recognize that scattering is biasing the measurements is to look at the measured transmittance at wavelengths were the material is known to have very high transmittance such as the visible region for polythene. If the measured transmittance is less than 0.9, then the measurement has been biased by the scattering and the reading obtained wrong. Of course, unless scattering is minimal, we can also see its effect when looking through the materials. Depending on how the integrating spheres are attached to the sample, additional complications may arise (see, Box 10.2).

In the previous examples in this section we have considered objects that attenuate irradiance mainly through absorption of light that travels through them. There are filters that attenuate light through selective reflection. With such filters thickness of the base material only minimally affects transmittance, i.e., $\alpha \ll \rho$. Interference filters are produced by deposition of very think layers on the surface of the substrate and $\rho(\lambda)$ is controlled by their thickness. The opposite effect is also possible, and is used to produce anti-reflection (AR) coatings in glass and plastic filters and windows. AR multicoating (MC) can achieve $\rho < 0.5\%$ over the whole visible region. If we "stack" filters of either type, as long as air gaps remain between them, they can be thought as "functioning independently" of each other.

To estimate $\tau(\lambda)$ for such a stack of filters separated by air gaps, we need to convolute the spectra—i.e., we need to multiply them wavelength by wavelength. The stacking order is in theory and frequently also in reality irrelevant—i.e., it is transitive as for multiplication in algebra:

$$\tau_{1+2}(\lambda) = \tau_1(\lambda) \cdot \tau_2(\lambda).$$

In the case of absorbances we have to add them instead because of the log transformation:

$$A_{1+2}(\lambda) = A_1(\lambda) + A_2(\lambda).$$

Normally transmittance is measured for a light beam impinging on the surface of a filter at 90°. However, the angle of incidence can affect in various ways light attenuation. We will first consider τ_{internal} and how the length of the light path through the filter depends on the angle of incidence of the



Figure 10.6: Effect of the angle of incidence on the internal transmittance of polyester film 0.125 mm-thick. See Appendix for code used.

light beam (Figure 10.6). If we discount the effect of refraction at the airfilter interfaces, and assume the direction of the beam remains the same inside the filter, we can use simple trigonometry to compute the approximate path length. As an example we will consider the spectral transmittance of polyester 0.125 mm-thick, and that the sun will shine on it at $\theta_1 = 0^\circ$ at noon but later in the afternoon at an angle that doubles the path length of the light through the filter.

Similar considerations apply to the path of solar radiation through the atmosphere and its dependence on the solar zenith angle. In this case the path length is described using (relative) air mass (AM) traversed in the light path. For example, spectral irradiance for AM1.5 is frequently used to characterize solar cells. To derive AM values from solar zenith angle empirical equations are used in most cases instead of geometrical rules.

Above I mentioned that reflectance, ρ , depends on the angle of incidence, and it increases with increasing values of θ_1 . So these two effects add up. The angle of incidence of the solar beam on the filters used tends to be infrequently explicitly considered when designing outdoors UV filtration experiments. Although the path length may not have a huge effect for good quality glass or acrylic, reflection will decrease PAR even for clear materials.

In the case of reflective or interference filters which wavelengths are transmitted and which reflected, depends on the angle of incidence, so spectral transmittance in specifications is given at a specific angle of incidence. Usually $\theta_1 = 0$ is used, but some filters, in particular many of those reflecting



infrared radiation, or "hot mirrors", are designed to be installed at other angles, such as 45° so that the thermal radiation is not reflected back towards the light source but instead to the side. Interference filters are available only in small sizes and expensive, and consequently are used for imaging, sensors and some rather small light sources.

The optics of leaves and flowers

The same analysis as above could be, in principle, applied to an object with a heterogeneous internal structure, like a plant leaf with its multiple internal air-water interfaces, but one would have to consider the multiple internal interfaces and their positions. The presence of these interfaces at different angles, plus small particles, cause strong scattering, and thus in this case ρ depends on both surface and internal properties of the leaf. We can still measure ρ , τ and α but predicting based on optical theory the effects of changes in leaf thickness or pigment concentration becomes daunting.

One way of demonstrating the role of air-water interfaces within leaf tissues is to infiltrate a leaf with water using a vacuum chamber. The effect is most spectacular in a variegated leaf such as those from some clones of English ivy: after infiltration with water the green areas become translucent green and the white areas almost transparent. The internal structure of leaves is extremely efficient at trapping light, to the point that it has been copied in a recent design for high efficiency solar cells (Yun et al. 2019). There is also evidence that shade leaves are better light traps per unit dry mass than sun leaves. Modelling of the optical properties of leaves using a ray-tracing approach can be computationally expensive (see the book by Jacquemoud et al. 2019, for an up-to-date account). The optics of leaves, flowers and fruits are described in detail in the book *Nature's palette : the science of plant color* (Lee 2007).

Concluding remarks

Obviously in photobiology, but also in other fields of biology, light- and UVradiation-based measurements are very frequent. Being aware of key principles of how radiation interacts with objects can be very useful in research. Knowing the different physical quantities in use and how to interconvert them, opens the door to the comparison of results from different studies even across disciplines allowing the more effective review and integration of knowledge. I hope those readers who have reached this far will find the time spent worthwhile.



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Appendix

R code for this article, which uses data and functions published as part of the *R* for photobiology suite (Aphalo 2015).



Code for loading R packages.

```
library(grid)
library(magick)
library(tibble)
library(photobiologyFilters)
library(ggspectra)
library(patchwork)
library(wrapr)
```

Code for drawing Figure 10.2.

Code for drawing Figure 10.3.

set_annotations_default("boundaries")
(autoplot(Ler_leaf.spct) /
 autoplot(Ler_leaf_trns.spct) /
 autoplot(Ler_leaf_trns_i.spct))

Code for drawing Figure 10.4.

```
k <- 16e4
# concentration 1 mM
beerlamb_InmM.tb <- data.frame(z = 0:100 / 100 * 1e-3)
beerlamb_InmM.tb$I_z <- exp(-1 * 10e-3 * beerlamb_InmM.tb$z * k)
beerlamb_InmM.tb$Concentration <- "10"
# concentration 5 mM
beerlamb_5mM.tb <- data.frame(z = 0:100 / 100 * 1e-3)
beerlamb_5mM.tb$I_z <- exp(-1 * 25e-3 * beerlamb_5mM.tb$z * k)
beerlamb_5mM.tb$Concentration <- "25"
# both
beerlamb.tb <- rbind(beerlamb_InmM.tb, beerlamb_5mM.tb)
ggplot(beerlamb.tb, aes(z * 1e3, I_z, linetype = Concentration)) +
geom_line() +
expand_limits(y = 0) +
labs(x = "Path length (mm)", y = "Relative irradiance (/1)") +
theme_bw()</pre>
```

Code for drawing Figure 10.5.

Code for drawing Figure 10.6.

```
polyester.spct %.>%
    convertTfrType(., "internal") -> short_path.spct
# compute transmittance assuming radiation path-length doubles
polyester.spct %.>%
    convertTfrType(., "internal") %.>%
    convertThickness(., thickness = 0.250e-3) -> long_path.spct
list(midday = short_path.spct, afternoon = long_path.spct) %.>%
    filter_mspct(.) %.>%
    autoplot(., range = c(280, 450))
```

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60