What's obscure with previtamin D Action Spectrum – a challenge for AI

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Abstract. The article discusses issues related to the significant difference between the Action Spectra of Vitamin D synthesis *in vivo* and *in vitro*. Experimental measurements of previtamin D photosynthesis *in vitro* under the quasy-monochromatic UV radiation at different wavelengths are presented, which indicate that nonlinear kinetics of previtamin D accumulation together with the wavelength effect on its side photoconversions, will lead to ambiguity in the Previtamin D Action Spectrum depending on the accepted erythema dose at which it was measured.

1 Introduction

As is known, solar ultraviolet (UV) radiation can cause both beneficial and adverse effects on health depending on the dose. The maximum admissible dose of UV radiation is associated with its erythemal biological activity and to date, the Erythema Action Spectrum adopted by CIE [1], Standard Erythema Dose (1 SED = 100 J/m2) [2] and the Global UV index [3], constitute the metrological basis for the use of AI for health protection.

The situation is less clear regarding the determination of the minimum required UV dose of sunlight required for natural synthesis of health-promoting vitamin D in human skin to balance the risks of vitamin D deficiency and skin cancer [4].

An important step towards this goal should be the construction of adequate Action Spectrum for calculating the vitamin D-synthesizing capacity of any UV radiation source and adequate assessment the so-called anti-rickets biologic dose of UV radiation in relation to the standard erythemal dose (SED) for various UV sources.

These are the issues that are discussed in this article.

2. Critical analysis of the Previtamin D Action Spectrum *in vivo*

The construction of the wavelength dependence for a given bioeffect, so called Action Spectrum, is one of the fundamental methods for analysis of an organism's responses to UV radiation [5]. By definition, the action spectrum (AS) of a specific biological effect of UV radiation is characterized by the spectral dependence of its magnitude, measured with monochromatic irradiation of different wavelengths with the same dose [5].

Hence, in theory, to determine the biologically effective irradiance E_{eff} (Wm^{-2}), the solar spectrum measured by a spectroradiometer is to be weighted (integrated over the wavelengths) according to the action spectrum of a specific biological effect [6]:

$$E_{eff} = \int E_{\lambda}(\lambda) * S_{\lambda}(\lambda) d\lambda \qquad (1)$$

Here $E_\lambda(\lambda)$ - solar spectral irradiance [$Wm^{-2}nm^{-1}$], $S_\lambda(\lambda)$ - action spectrum [relative units], λ - wavelength [nm].

The CIE erythema action spectrum [1] is widely used for estimation of the erythemal-active irradiance of sunlight and artificial UV sources.

An adequate assessment of the ability of sunlight to synthesize vitamin D also requires the Action Spectrum for vitamin D synthesis, especially in view of its widespread in the biosphere. It is known that vitamin D_2 is synthesized under sunlight from its precursor ergosterol (mainly in plants), and vitamin D_3 - from 7-dehydrocholesterol (7-DHC) in animals and humans. It is important that their synthesis occurs in the same way by two-stage monomolecular isomerization (Figure1).

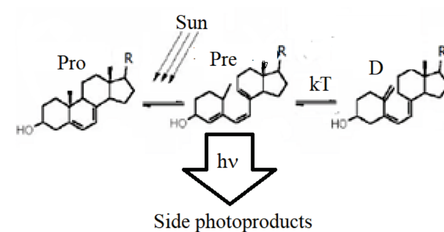

Fig.1 Simplified scheme of Vitamin D synthesis. Pro – Provitamin D, Pre – Previtamin D, D – Vitamin D. R = C_8H_{17} for vitamin D_3 series; R = C_9H_{17} for vitamin D_2 series.

At the first stage, absorption of UVB (280–315 nm) photons by provitamin D results in its conversion into previtamin D, which is then converted into vitamin D by heat. Thus, it is the amount of previtamin D accumulated during an UV exposure is a measure of the biologically effective anti-rickets dose of UV radiation.

In 2006, the CIE published an Action Spectrum for production of previtamin D_3 in human skin [7], based on the spectrum published by MacLaughlin et al. [8] with long-wavelength extrapolation up to 330 nm (Figure 2).

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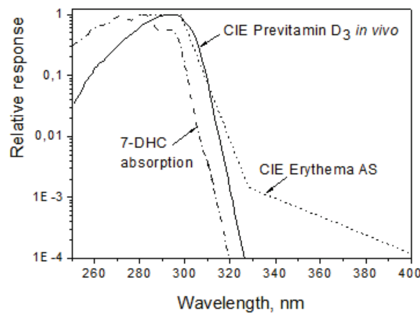

Fig. 2. The CIE Previtamin D Action Spectrum in human skin in comparison with absorption spectrum of provitamin D (extrapolated from 310 nm) and CIE erythema AS [1].

Several features in the graphs presented in Figure 2 are worthy of notice:

1. Difference in the wavelengths of maximum in the previtamin D AS (295 nm) and in the provitamin D absorption spectrum (282 nm),
2. Opposite relation of the two 'Vitamin D' spectra to the CIE erythema action spectrum around 310 nm,
3. Significant distinction between CIE erythema AS and both 'Vitamin D' curves in the UVA spectral range,
4. Starting from 305 nm and further towards longer wavelengths, the difference between the action spectrum of previtamin D and the absorption spectrum of provitamin D exceeds more than one order of magnitude (!), which contradicts the First Law of Photochemistry: '*Light must be absorbed for photochemistry to occur'*.

Moreover, the spectrum of previtamin D synthesis in human skin [8] does not correspond to the generally accepted methodology of measuring AS, since instead of measuring the spectral dependence of the amount of previtamin D synthesized under monochromatic irradiation of different wavelengths with the same dose, on the contrary, the dose required for the accumulation of no more than 5 percent of previtamin D was measured under irradiation at different wavelengths.

A simple mathematical analysis shows that with such a measurement, the AS should correspond to the absorption spectrum of the initial provitamin D (as was obtained for the AS *in vitro* [9]).

This discrepancy has been analyzed in detail [10], and the reason could be an incorrect concentration analysis that does not take into account the irreversible photoreactions of provitamin D, which play an important role during irradiation at the long-wavelength absorption edge of provitamin D [11].

In view of criticism [12,13], this AS of previtamin D synthesis in human skin has not yet been standardized by the CIE, but until a clear alternative becomes available, it is recommended [14] to use this existing CIE Action Spectrum to ensure spectral information on UV sources to enable comparison of results from different studies.

Another possible solution to this problem is discussed in this article, namely, measuring the amount of synthesized previtamin D *in vitro* in relation to the received erythema dose. The rationale for this approach is the linear correlation between *in vitro* previtamin D_3 synthesis and the increase in serum 25-hydroxyvitamin D_3 in humans after UV exposure [15].

3. Experimental technique

Experimental studies of the kinetics of previtamin D photosynthesis *in vitro* for radiometric characterization of the 'D-dosimeter' [16] were carried out within the framework of the BIODOS project at the Belgian Institute for Space Aeronomy (IASB) using a unique laboratory setup specially designed to characterize biological dosimeters of UV radiation. The laboratory facility has been described in detail [9], but only a small part of these measurements has been published until today [9,17].

3.1. UV source

Quasi-monochromatic UV radiation from the xenon arc lamp with a number of the narrow-band interference filters ($\Delta\lambda \sim 2.5$ nm) (Figure 3) was used to link physical and erythemal UV doses (Table 1) with concentrations of accumulated previtamin D.

Table 1. Characteristics of the UV irradiance with the narrow-band filters

Irradiation wavelength (nm)	Spectral irradiance (mW/m^2)	Erythema effective fluence rate (mW/m^2)	Irradiation time for 1 SED (min)
260	10.579	10.181	156
270	103.804	103.352	15
280	112.799	112.223	14
290	175.995	175.679	9
300	243.854	162.446	9.8
310	591.995	41.731	38

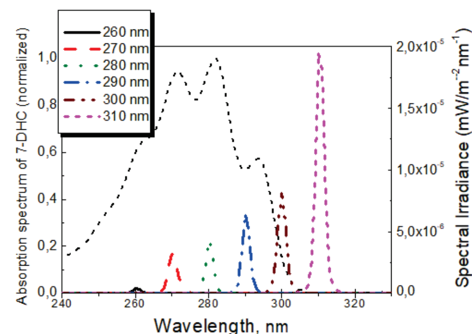

Fig. 3. Spectral characteristics of the narrow band filters in comparison with normalized 7-DHC absorption spectrum.

3.2. The measurements procedure

The ethanol solutions of 7-DHC (Sigma) ($C \sim 20$ µg/ml, $V = 2$ ml) were irradiated in standard quartz cuvettes (Helma) of 0.5 cm thickness located normally to the light beam. During the time when the cuvette with 7-DHC solution was exposed, the incident spectral irradiance

was measured by the spectroradiometer SPEX 1672M. The absorption spectra of 7-DHC were recorded within 230 – 330 nm before and after several exposures using upgraded Beckman-34 spectrophotometer for further computer processing with specially developed original software for concentration analysis [18].

3.3. Spectrophotometric concentration analysis

As is known, when provitamin D is irradiated with UV light, a multicomponent mixture of photoisomers is formed due to the side photoconversions of previtamin D and the overlap of their absorption spectra (Figure 4).

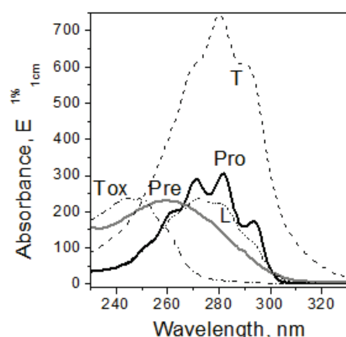

Fig. 4. UV absorption spectra of provitamin D and its photoisomers: T – Tachysterol, L – Lumisterol, Tox – Toxisterols. (The **Tox** spectrum was recorded after photolysis of 7-DHC in ethanol with excimer XeCl laser at 308 nm [19]) .

Concentration analysis of this multicomponent mixture is usually carried out using HPLC and the total concentration of the four main photoisomers is taken as 100%, i.e. irreversible photoconversions of previtamin D are not taken into account [8]. However, this neglect causes serious errors upon UV irradiation at the long-wavelength edge of provitamin D absorption band [20].

Therefore, in the described experiments, UV absorption spectroscopy was applied to monitor the course of the photoreaction, and the concentration composition of the photo-isomeric mixture was determined using an original spectrophotometric analysis [18, 21] developed with due consideration of its irreversible photodegradation.

Additionally, to verify the photoreaction model based on the system of rate equations [16, 20], the photoreaction kinetics was calculated at different wavelengths using the spectroradiometric data at the model input for comparison of the calculated and experimentally obtained kinetics.

4. Results and discussion

Due to the low intensity of UV radiation at 260 nm, only minor changes in the initial absorption spectrum of the 7-DHC solution occurred after almost three hours of exposure (Figure 5).

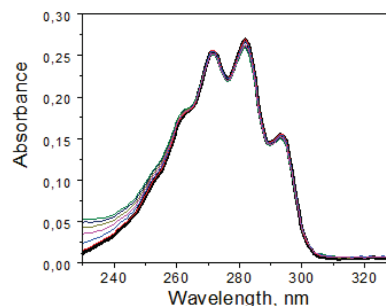

Fig. 5. Transformation of the 7-DHC initial spectrum upon UV irradiation at 260 nm recorded after 50, 80, 110, 140 and 170 min exposures.

The result of concentration analysis of previtamin D accumulation is presented in Figure 6.

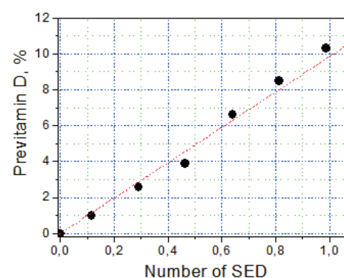

Fig. 6. Accumulation of previtamin D depending on the SED number: symbols – experiment, line – linear fit.

Note that Figures 4) and 5), indicating the sensitivity of the spectrophotometric analysis and the scatter of results, confirm previously published data on the dose of 0.5 SED required for the accumulation 5% of previtamin D [9].

Since the purpose of these experiments was not only calibration of the 'D-biodosimeter' [16], but also testing the adequacy of the photoreaction model, the exposure time was chosen to observe the full kinetics of previtamin D accumulation up to its maximum concentration, which could be indicated by the behavior of the optical density of the sample (its rise until the start of decline [22]), as is demonstrated in Figure 7.

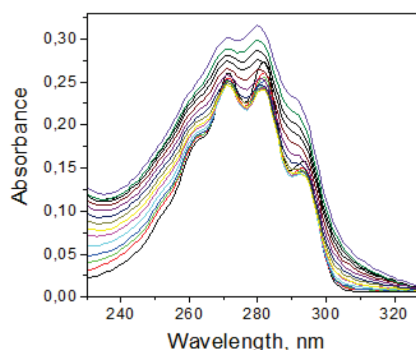

Fig. 7. Transformation of the 7-DHC initial spectrum upon UV irradiation at 280 nm with total exposure time 210 min; the exposures for individual spectra are visible from Figure 8.

The concentration kinetics obtained by computer processing of these spectra together with the results of model calculations are shown in Figure 8.

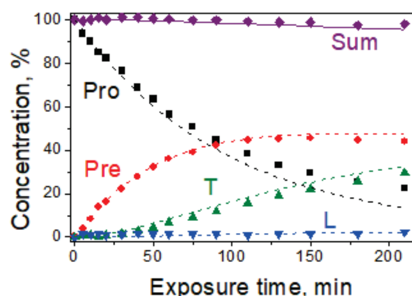

Fig. 8. Concentration kinetics: designations Pro, Pre, T and L as in Figs. 1) and 4); Sum – their summary concentration. Symbols – experiment, line – calculation.

It is important to note that in the longer wavelength region, where the efficiency of *cis-trans* isomerization is reduced, spectral transformation does not provide the possibility for fixation of Previtamin D maximum accumulation [22] (Figure 9).

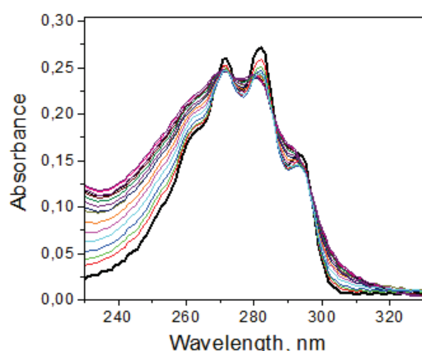

Fig. 9. Transformation of the 7-DHC initial spectrum upon UV irradiation at 300 nm with total exposure time 420 min.

This is demonstrated by Figure 10, where with a total irradiation time of 420 minutes, the maximum accumulation of previtamin D was not reached.

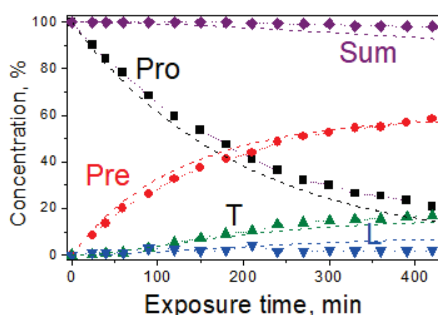

Fig. 10. Concentration kinetics: the notations are similar to Figure 8. Symbols – experiment, line – calculated kinetics.

Regarding the adequacy of the numerical modeling of the photoisomerization kinetics, from Figures 8 and 10 it can be concluded that there is rather good agreement (within 3%) with the experimentally measured kinetics at the initial stage of irradiation, but the match is slightly worse at the last stage.

The final results of the measurements of previtamin D accumulation in relation to the number of standard erythemal doses received during irradiation at all six wavelengths are shown in Figure 11.

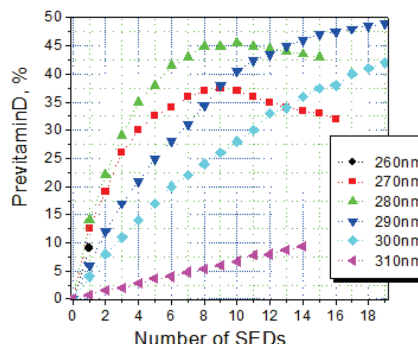

Fig.11. Previtamin D accumulation depending on the number of SED obtained

Analyzing the dependencies presented in Figure 11, one can come to an important conclusion about the absence of a monotonic increase in the concentration of accumulated previtamin D with an increase in the erythemal dose at all measured wavelengths. And when exposed to sunlight, the monotonous dependence is limited to a dose of no more than 8 SED, given that photons with a wavelength of less than 280 nm do not penetrate the earth's surface.

Based on the results shown in Figure 11, a dependence of previtamin D accumulation on the obtained SEDs number was constructed for different wavelengths (Figure 12) with the exception of $\lambda = 260$ nm, due to the lack of data for a dose exceeding 1 SED.

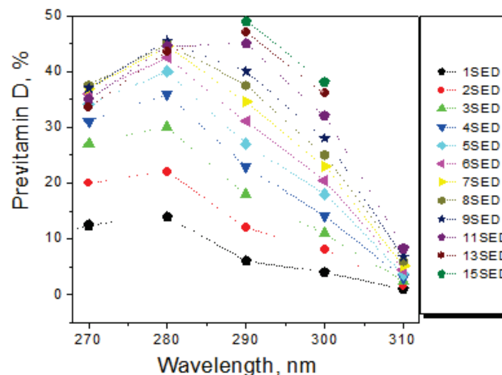

Fig.12. The wavelength dependence of previtamin D accumulated after irradiation with different SEDs.

The graphs in Figure 12 that are the basis for constructing the action spectrum of previtamin D synthesis, clearly demonstrate its ambiguity in relation to the maximum wavelength, i.e. its maximum is maintained at a wavelength of 280 nm only up to 10 SED, and with a larger number of SEDs it is gradually shifted to the longer wavelengths.

It is worth noting here that this result confirms the previously made conclusion, based on calculations of the photoreaction kinetics for different wavelengths [13,16], that the term ‘spectral effectiveness’ for the previtamin D photosynthesis can be considered in two aspects: either for the wavelength with the maximum rate of previtamin D formation, or for the wavelength with its maximum achievable concentration. A detailed examination of the ratio of the absorption spectra of provitamin D and previtamin D leads to the same conclusion [22].

5. Conclusions

The main result of these measurements is the revealed spectral dependence of previtamin D accumulation *in vitro* on the accepted UV dose at which it was measured that could suggest the ambiguity of its action spectrum.

To obtain an adequate action spectrum of previtamin D synthesis *in vivo*, further careful measurements using monochromatic UV irradiation are needed together with urgent development of non-invasive spectral-optical methods for measuring previtamin D formation in human skin.

In the meantime, in calculations of the vitamin D synthesis effective UV irradiance E_{eff} (W m^{-2}) it seems more appropriate to use the absorption spectrum of provitamin D (in relative units) as spectral weighting function $S_\lambda(\lambda)$ in formula (1) in accordance with the 1st Law of Photochemistry.

The simple procedure for determining the biologic dose of a UV source by integrating its effective irradiance E_{eff} over time [6], is unacceptable for an adequate assessment of the amount of synthesized previtamin D, that is, the anti-rickets UV dose should either be calculated using the photoreaction mathematical model, or measured with D-biodosimeter based on an *in vitro* model of vitamin D synthesis [23].

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