

Appendix

Formulas and extinction coefficients used for the determination of NAD(P)H oxidation and quinone reduction (hydroquinone accumulation) in NADPH/quinone mixtures. Suitable wavelengths pairs (λ_1, λ_2) were selected from absorbance (A) spectra of the quinones (100 μM) dissolved in 20 mM HEPES, pH 7.5, with 0.1% DMSO (0.1% ethanol + 2% Tween 20 in the case of vitamins k_1 and k_2) before and after exhaustive reduction with solid NaBH_4 . NAD(P)H was oxidized with catalytic amounts of methoxyphenazine methosulfate. Disregarding possible impurities of the commercially obtained quinones, differential (ox \rightarrow red) extinction coefficients ($\epsilon_{\lambda_1}, \epsilon_{\lambda_2}$) were calculated and used for the estimation of concentration changes ($\Delta c_a, \Delta c_b$, measured in a 1-cm cuvette) in a mixture of components a, b according to the general formulas:

$$\Delta c_a = \frac{\epsilon_{\lambda_2}^b}{x} \Delta A_1 - \frac{\epsilon_{\lambda_1}^b}{x} \Delta A_2, \text{ and } \Delta c_b = \frac{\epsilon_{\lambda_1}^a}{x} \Delta A_2 - \frac{\epsilon_{\lambda_2}^a}{x} \Delta A_1,$$

whereby ΔA_1 = measured absorbance change at λ_1 , ΔA_2 = measured absorbance change at λ_2 and $x = \epsilon_{\lambda_2}^b \cdot \epsilon_{\lambda_1}^a - \epsilon_{\lambda_1}^b \cdot \epsilon_{\lambda_2}^a$. $\Delta c_b, \epsilon_{\lambda_1}^b, \epsilon_{\lambda_2}^b$ refer to $\text{NAD(P)H} \rightarrow \text{NAD(P)}^+$.

These formulas can be derived from the principle that the concentrations of the two pigments a, b in a mixture of these pigments can be calculated from the absorbance spectrum at two wavelengths (λ_1, λ_2) demonstrating large differences in ϵ_λ . From $A_1 = \epsilon_{\lambda_1}^a \cdot c_a + \epsilon_{\lambda_1}^b \cdot c_b$, and $A_2 = \epsilon_{\lambda_2}^b \cdot c_b + \epsilon_{\lambda_2}^a \cdot c_a$, the equations defining c_a and c_b can be derived by mutual substitution and rearrangement. This procedure has been exemplified in detail for the estimation of chlorophylls *a* and *b* in plant extracts (Strain HH, Svec WA, 1966).

Component a (ox \rightarrow red)	λ_1 [nm]	λ_2 [nm]	$\epsilon_{\lambda_1}^a$ [mM ⁻¹ cm ⁻¹]	$\epsilon_{\lambda_2}^a$ [mM ⁻¹ cm ⁻¹]	$\epsilon_{\lambda_1}^b$ [mM ⁻¹ cm ⁻¹]	$\epsilon_{\lambda_2}^b$ [mM ⁻¹ cm ⁻¹]
NQ	290	340	2.23	-0.35	-0.92	-6.21
2-Methyl-NQ	290	340	2.18	-0.085	-0.92	-6.21
5-Hydroxy-NQ	366	425	-0.98	-3.15	-3.38	0.00
5-Hydroxy,2-methyl-NQ	420	366	-3.79	-1.41	0.00	-3.38
2-Hydroxy-NQ	450	366	-2.92	1.52	0.00	-3.38
2-Methoxy-NQ	282.3	340	-14.1	0.86	0.00	-6.21
2,3-Dimethoxy-NQ	282.3	340	-8.16	-0.16	0.00	-6.21

Vitamin k ₁	282.3	366	-1.24	-0.32	0.00	-3.38
Vitamin k ₂	282.3	366	-1.24	-0.32	0.00	-3.38
BQ	290	340	1.41	-0.008	-0.92	-6.21
2-Methyl-BQ	282.3	340	2.61	-0.38	0.00	-6.21
2,5-Dimethyl-BQ	282.3	340	3.11	-0.075	0.00	-6.21
2,6-Dimethyl-BQ	282.3	340	2.54	-0.26	0.00	-6.21
Ubiquinone 0	290	340	0.70	-0.25	-0.92	-6.21
Tetramethyl-BQ	290	340	1.22	-0.17	-0.92	-6.21
2,6-Dimethoxy-BQ	282.3	340	-11.2	-0.31	0.00	-6.21
2,5-Dihydroxy-BQ	290	366	3.14	-0.31	-0.92	-3.38
Tetrahydroxy-BQ	483	340	-16.9	-0.22	0.00	-6.21

(NQ=1,4-naphthoquinone, BQ=1,4-benzoquinone)