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RELAXATION PHENOMENA IN CAROTENOID FILMS AT THE OIL/WATER INTERFACE

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ABSTRACT. The kinetic analysis of the relaxation phenomena in carotenoid films adsorbed at the oil/water interface is presented for several carotenoid concentrations in the oil phase. A new kinetic approach is applied and it addresses the diffusion adsorption associated with a reversible interfacial reaction of reorientation of carotenoid molecules between two conformations The new model contains two characteristic times, i.e., *diffusion relaxation* and *kinetic relaxation times* and it describes with high accuracy the experimental data of dynamic interfacial tensions for the adsorption of two carotenoids: ethyl ester of β – apo – 8' – carotenoic acid and β-carotene, both all-*trans* isomers, from n-hexane phase to the n-hexane/water interface. The calculations give the two characteristic times for the relaxation phenomena in carotenoid adsorbed films at the oil/water interface. The data are in substantial agreement with the molecular structure of these carotenoids.

Keywords*:* relaxation kinetics; dynamic interfacial tensions; ethyl ester ofββ– apo - 8' – carotenoic acid; β -carotene; hexane/ water interface; diffusion relaxation time; kinetic relaxation time

INTRODUCTION

Carotenoids are important biological pigments that play vital role in cell membrane stability, cellular differentiation, growth control, photooxidative protection, vision process, photosynthesis and nutrition [1-4]. It clearly appears that the varied biological activities of carotenoids in living organisms are determined by the biophysical and chemical properties of these molecules [1-7]. Among them, the adsorption properties of carotenoids seem to be defined by their molecular structure and their molecular geometry [1, 5]. In addition, specific conformations of carotenoid derivatives and their correct orientation [5] at various biological interfaces [6, 7] will determine their proper function within cellular and subcellular structures [1].

Generally, a surfactant solution is characterized by the adsorption of surfactant molecules in monolayers at the fluid interface [8-26]. The adsorption kinetics can be controlled by surfactant molecular diffusion from the bulk to the subsurface [11-14, 19-22] and/or by an energy barrier, which controls the transfer of the surfactant molecules from the subsurface to the interface [9, 19].

In the present study, we focus on the relaxation kinetics in carotenoids films adsorbed from oil solutions at the oil/water interface, by using interfacial tension measurements as an experimental tool. For our systems containing carotenoids, the dynamic interfacial tensions versus time show long time effects which cannot be understood by diffusion from the bulk to the subsurface and the transfer from the subsurface to the interface.

These time effects can be described by a surface reaction of reorientation of surfactant molecules. This type of behavior is also found with some sulfosuccinates [20] at the air/water interface and with proteins [26] at the oil/water interface.

The kinetic analysis of the interfacial tension relaxation becomes more complex when the surfactant molecules participate in an interfacial reaction of molecular reorientation [8, 26- 29] or when they form aggregations or clusters in the bulk phase or in the interfacial adsorbed layer [30, 31]. Although relevant for many important applications in various fields, like, pharmaceutical formulations, food processing, and emulsification processes, the relaxation kinetics in carotenoid films at fluid interfaces has received so far only a marginal attention [29].

The major goal of this study is to describe the long time effects recorded for the dynamic interfacial tensions of carotenoid systems at the oil/water interface. To reach this goal, a new kinetic theoretical model is applied to allow for a diffusion process to be associated with a surface reaction of reorientation.

KINETIC MODEL OF RELAXATION PHENOMENA

The kinetic model applied in this study, consists of a bulk diffusion process, given by the diffusion penetration theory [12-14, 20, 32], which is associated with a first order reversible interfacial reaction of surface reorientation.

The surfactant molecules are adsorbed at the liquid/liquid interface as conformation 1, soluble in oil phase, and at the interface they undergo a transformation to the conformation 2. The conformation 1 is exchangeable with the bulk oil phase, but the conformation 2 is not, being stable only at the interface. Both conformations are assumed practically insoluble in the aqueous phase.

To model the relaxation phenomena in adsorbed films at the liquid/liquid interface, we start from the mass balance at the interface for conformation 1 written as:

$$\frac{d\Gamma_1}{dt} = D\,\frac{c_0 - c_s}{\sqrt{\pi D t}} - k\left(\Gamma_1 - \Gamma_1^e\right) \tag{1}$$

or written in an equivalent form:

and for conformation 2, given by:

$$\frac{d\Gamma_1}{dt} = \left(\frac{D}{\pi t}\right)^{\frac{1}{2}}\left(c_0 - c_s\right) - k\left(\Gamma_1 - \Gamma_1^e\right) \tag{2}$$

$$\frac{d\Gamma_2}{dt} = k\left(\Gamma_2^e - \Gamma_2\right) \tag{3}$$

where, c_0 and c_s are the surfactant bulk and subsurface concentrations, respectively, at the time t: π is 3.1415; D value is the diffusion coefficient of the surfactant molecules in the bulk solution; Γ_1 and Γ_2 are the adsorption of conformation 1 and of conformation 2, respectively, at the time t; Γ_1^e and Γ_2^e represent the adsorption of conformation 1 and of conformation 2, respectively, at the equilibrium; k is the rate constant for the surface reaction of molecular transformation or reorientation. The subsurface layer is situated only a few angstroms away from the interface and still belonging to the oil bulk solution.

Eq. (3) is readily integrated, taking in to account that at t =0, Γ_2 =0, whence:

$$\Gamma_2 = \Gamma_2^e - \Gamma_2^e e^{-kt} \tag{4}$$

The subsurface concentration (noted c_s or simply c) in equilibrium with the composition at the liquid/liquid interface depends on $\Gamma_1$and Γ_2, as follows:

$$dc = \left(\frac{\partial c}{\partial \Gamma_1}\right)_{\Gamma_2} d\Gamma_1 + \left(\frac{\partial c}{\partial \Gamma_2}\right)_{\Gamma_1} d\Gamma_2 \tag{5}$$

or

$$c_0 - c_s = \left(\frac{\partial c}{\partial \Gamma_1}\right)_{\Gamma_2} \left(\Gamma_1^e - \Gamma_1\right) + \left(\frac{\partial c}{\partial \Gamma_2}\right)_{\Gamma_1} \left(\Gamma_2^e - \Gamma_2\right) \tag{6}$$

The Eq. (6) can be further written in another equivalent form:

$$c_0 - c_s = \left(\frac{\partial c}{\partial \Gamma_1}\right)_{\Gamma_2} \left[\left(\Gamma_1^e - \Gamma_1\right) + \frac{\left(\partial c/\partial \Gamma_2\right)_{\Gamma_1}}{\left(\partial c/\partial \Gamma_1\right)_{\Gamma_2}} \left(\Gamma_2^e - \Gamma_2\right)\right] \tag{7}$$

We note by ν:

$$\nu = -\frac{\left(\partial c/\partial \ln \Gamma_2\right)_{\Gamma_1}}{\left(\partial c/\partial \ln \Gamma_1\right)_{\Gamma_2}} = -\frac{\left(\partial c/\partial \Gamma_2\right)_{\Gamma_1} \Gamma_2^e}{\left(\partial c/\partial \Gamma_1\right)_{\Gamma_2} \Gamma_1^e} \tag{8}$$

and it obtains

$$\frac{\left(\partial c/\partial \Gamma_2\right)_{\Gamma_1}}{\left(\partial c/\partial \Gamma_1\right)_{\Gamma_2}} = -\nu \frac{\Gamma_1^e}{\Gamma_2^e} \tag{9}$$

With Eq. (9), Eq.(7) becomes:

$$c_0 - c_s = \left(\frac{\partial c}{\partial \Gamma_1}\right)_{\Gamma_2} \left[\left(\Gamma_1^e - \Gamma_1\right) - \nu \frac{\Gamma_1^e}{\Gamma_2^e} \left(\Gamma_2^e - \Gamma_2\right)\right] \tag{10}$$

Further, with Eqs.(4) and (10), Eq. (2) becomes:

$$\frac{d\,\Gamma_1}{d\,t} = \left(\frac{D}{\pi\, t}\right)^{\frac{1}{2}} \left(\frac{\partial\, c}{\partial\, \Gamma_1}\right)_{\Gamma_2} \left[\left(\Gamma_1^e - \Gamma_1\right) - \nu\, \Gamma_1^e\, e^{-kt}\right] - k\left(\Gamma_1 - \Gamma_1^e\right) \tag{11}$$

We introduce the *diffusion relaxation time* [32-34], noted τ_D or τ, defined as:

$$\frac{1}{(\tau)^{1/2}} = D^{1/2}\left(\frac{\partial c}{\partial \Gamma_1}\right)_{\Gamma_2} \tag{12}$$

and in this way Eq.(11) is written as:

$$\frac{d\left(\Gamma_1^e - \Gamma_1\right)}{d\,t} + \left[\left(\frac{1}{\pi\,\tau\, t}\right)^{1/2} + k\right]\left(\Gamma_1^e - \Gamma_1\right) = \frac{\nu\, \Gamma_1^e\, e^{-kt}}{(\pi\,\tau\, t)^{1/2}} \tag{13}$$

The Eq. (13) is a linear differential equation and its integral is:

$$\Gamma_1^e - \Gamma_1 = e^{-\left(\frac{4t}{\pi\tau}\right)^{1/2}}\, e^{-kt}\left[K + \nu\, \Gamma_1^e\, e^{\left(\frac{4t}{\pi\tau}\right)^{1/2}}\right] = K\, e^{-\left(\frac{4t}{\pi\tau}\right)^{1/2}}\, e^{-k\,t} + \nu\, \Gamma_1^e\, e^{-k\,t} \tag{14}$$

From the initial conditions, t=0 and Γ_1=0, the integration constant K is obtained:

$$K = \Gamma_1^e (1 - \nu) \tag{15}$$

Finally:

$$\Gamma_1^e - \Gamma_1 = \Gamma_1^e\ (1 - \nu)\ e^{-\left(\frac{4\,t}{\pi\tau}\right)^{\frac{1}{2}}}\ e^{-k\,t} + \nu\, \Gamma_1^e\, e^{-\,k\,t} \tag{16}$$

Further, since the surface tension depends on Γ_1 and Γ_2, it results:

$$\sigma - \sigma_e = \left(\frac{\partial\sigma}{\partial\Gamma_1}\right)_{\Gamma_2}\left(\Gamma_1 - \Gamma_1^e\right) + \left(\frac{\partial\sigma}{\partial\Gamma_2}\right)_{\Gamma_1}\left(\Gamma_2 - \Gamma_2^e\right) \tag{17}$$

By using Eqs.(4) and (16), the Eq. (17) gives:

$$\sigma - \sigma_e = \left\{-\left(\frac{\partial\sigma}{\partial \ln\Gamma_1}\right)_{\Gamma_2}\left[(1 - \nu)\, e^{-\left(\frac{4t}{\pi\tau}\right)^{1/2}} + \nu\right] - \left(\frac{\partial\sigma}{\partial \ln\Gamma_2}\right)_{\Gamma_1}\right\} e^{-k\,t} \tag{18}$$

By noting:

$$-\left(\frac{\partial\sigma}{\partial\ln\Gamma_1}\right)_{\Gamma_2} = \varepsilon_{01}$$

$$-\left(\frac{\partial\sigma}{\partial\ln\Gamma_2}\right)_{\Gamma_1} = \varepsilon_{02} \tag{19}$$

the Eq. (18) becomes:

$$\sigma-\sigma_e = \left[\varepsilon_{01}(1-\nu)e^{-\left(\frac{4t}{\pi\tau}\right)^{1/2}} + (\nu\varepsilon_{01}+\varepsilon_{02})\right]e^{-kt} \tag{20}$$

By making the notations:

$$\begin{aligned}&\varepsilon_{01}(1-\nu)=\Delta\sigma_{01}\\&\nu\varepsilon_{01}+\varepsilon_{02}=\Delta\sigma_{02}\\&(\sigma-\sigma_e=\Delta\sigma)\end{aligned} \tag{21}$$

the Eq. (20) becomes:

$$\sigma = \sigma_e + \left[\Delta\sigma_{01}\,e^{-\left(\frac{4t}{\pi\tau}\right)^{1/2}} + \Delta\sigma_{02}\right]e^{-kt} \tag{22}$$

where, for t=0, it results:

$$\Delta\sigma_{01}+\Delta\sigma_{02}=\Delta\sigma_0=\sigma_0-\sigma_e \tag{23}$$

σ_0 being the surface tension of the pure interface without surfactant. It should be remarked that we have linearized the mathematical problem, i.e., $\left(\frac{\partial c}{\partial\ln\Gamma_1}\right)_{\Gamma_2}$; $\left(\frac{\partial c}{\partial\ln\Gamma_2}\right)_{\Gamma_1}$; $\left(\frac{\partial\sigma}{\partial\Gamma_1}\right)_{\Gamma_2}$ and $\left(\frac{\partial\sigma}{\partial\Gamma_2}\right)_{\Gamma_1}$ are considered as constants. In practice, however, Eq. (22) describes the experiments quite well, as it will be detailed in this investigation. It is also noteworthy here that the dynamic interfacial tension, σ(t), appears to be determined mainly by the two characteristic times, *namely*, τ the diffusion relaxation timeand τ_k the reaction relaxation (k^{-1}) time, k being the rate constant of the interfacial reaction.

In Eq. (22), the term $\exp - \left(\frac{4t}{\pi\tau}\right)^{1\!/\!2}$ accounts for diffusion relaxation with τ (noted also as τ_D) the diffusion relaxation time and e^{-kt} accounts for the surface reaction of reorientation at the oil/water interfacewith τ_k the reaction relaxation (k^{-1}) time.

The Eq. (22) describes the dynamic surface tension with time. For k = 0 (no surface reaction) and $\Delta\sigma_{02} = 0$, $\Delta\sigma_{01} = \Delta\sigma_0$, and Eq. (22) becomes:

$$\sigma - \sigma_e = \Delta\sigma = \Delta\sigma_0 \; e^{-\left(\frac{4t}{\pi\tau}\right)^{1\!/\!2}} \tag{24}$$

The Eq. (24) is the equation for a pure diffusion controlled process.

Further Eq. (22) can be used to derive simple analytical expressions describing the relaxation behavior in the short and long time limits of the relaxation diffusion time. For some situations it may happen that diffusion equilibration is so fast $\tau_D \; \langle\langle \; \tau_k$, that we only observe the transformation step and the Eq. (22) becomes:

$$\sigma = \sigma_e + \Delta\sigma_{02} \; e^{-kt} \tag{25}$$

The Eq. (25) shows an exponential decrease of $\Delta\sigma$ with time. The extrapolation of Eq. (25) at t=0 do not yield σ_0 value, but the following relation results:

$$\sigma\left(t \to 0\right) = \sigma_e + \Delta\sigma_{02} \tag{26}$$

If diffusion is slow $\tau_D \; \rangle\rangle \; \tau_k$, Eq. (22) gives:

$$\Delta\sigma = \Delta\sigma_0 \; \exp\left(-\frac{t}{\tau_k}\right) \tag{27}$$

and the diffusion is the rate controlling step and, for $\tau_D \to \infty$, Eq. (22) extrapolates to an equivalent form of Eq. (27) given by:

$$\Delta\sigma \;\; (t) = \left(\Delta\sigma_{01} + \Delta\sigma_{02}\right) e^{-kt} = \Delta\sigma_0 \; e^{-kt} \tag{28}$$

and the$\Delta\Delta\sigma$ shows an exponential decrease with time, which for $t \to 0$ extrapolates to the σ_0 value. The decay is again exponential but the interfacial tension for $t \to 0$ extrapolates to σ_0 = 50 mN m^{-1}. Between these two limiting cases, the adsorption relaxation is affected by both diffusion and surface reorientation processes at liquid/liquid interfaces.

To describe the relaxation phenomena in adsorbed films at the oil/water interface we apply our new kinetic approach to the dynamic interfacial tension data for two carotenoids at the hexane/water interface and the characteristics time τ_D and τ_k are determined.

EXPERIMENTAL

The compounds used, *namely* ethyl ester of β - apo – 8’-carotenoic acid and β - carotene, were synthetic commercial products of analytical grade. They were purchased from Hoffmann La Roche. Both carotenoids are all-*trans* isomers and they are insoluble in the water phase. Therefore, their transfer through the interface towards aqueous phase can be neglected. The n-hexane was purchased from Merck. All chemicals were used without additional purification. Two distilled water was used as aqueous phase.

The equilibrium (σ_e) and the dynamic interfacial tensions, σ(t), were measured by Du Nouy ring method and Wilhelmy plate method as described elsewhere [1, 20, 34-36]. The agreement between the two methods is excellent and the deviations do not exceed the error of the individual method. The accuracy of interfacial tension measurements was ± 0.1 mN/m, in agreement with literature data on different systems [20, 34-36]. All measurements were carried out at constant temperature of $25 \pm 0.1\,^{o}$C.

RESULTS AND DISCUSSION

Dynamic interfacial tensions

The dynamic interfacial tension, σ(t), as a function of time for several concentrations of the ethyl ester of β - apo - 8’ - carotenoic acid in n-hexane solutions at the hexane/water interface are presented in Figs. 1 and 2.

The results of measurements for different β - carotene concentrations in hexane phase at the hexane/water interface are presented in Figs. 3 and 4. As can be seen, in Figs. 1-4, both carotenoids possess a high surface activity as indicated by the equilibrium interfacial tensions(σ_e), values considered at the time of 120 minutes.

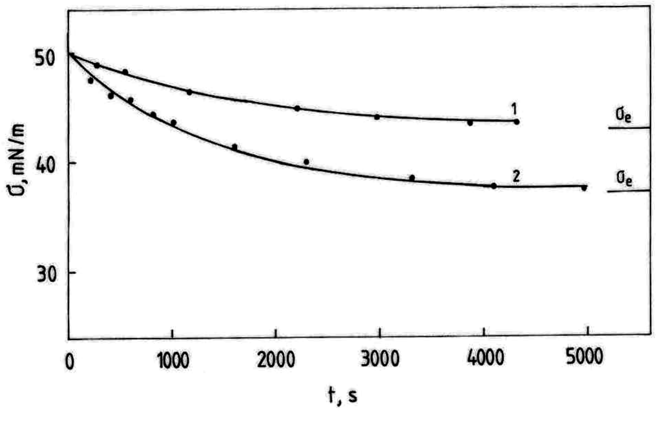

Fig. 1. *Dynamic interfacial tensions,* σ *(t), as a function of time, for hexane solutions of ethyl ester of β - apo-8’- carotenoic acid at the interface with water, for two ester concentrations. Curve (1) for* $c_0 = 3 10^{-8}$ *mole* cm^{-3} *and (2)* $c_0 = 7.9\ 10^{-8}$ *mole* cm^{-3}*. Full line calculated by Eq. (22) using constant parameters given in Table 1. Horizontal line indicates* σ_e *value for each ester concentration.*

Figs. 1-4 show that the relaxation process illustrated by the dynamic interfacial tension measurements is long time dependent for the two selected carotenoids. To investigate the relaxation process in adsorbed films at the hexane/water interface for the two carotenoids we use our new kinetic model developed in this paper.

Kinetic analysis of dynamic interfacial tensions

The dynamic interfacial tensions, shown in Figs. 1-4, are analyzed applying our new kinetic approach, which considers diffusion relaxation associated with surface reorientation relaxation in carotenoid films at the oil/water interface, by using Eq.(22). The total jump in interfacial tension is$\Delta\sigma_0$defined as $\Delta\sigma_0 = \sigma_0 - \sigma_e$ (σ_0 is the interfacial tension of the pure hexane/water interface of 50 mN m^{-1}), and $\Delta\sigma_0 = \Delta\sigma_{01} + \Delta\sigma_{02}$, where $\Delta\sigma_{01}$ and $\Delta\sigma_{02}$ are the amplitudes of the interfacial tension.

Ifτ is *very small*, the interfacial tensions with time seem to extrapolate for $t \rightarrow 0$ to $\sigma \rightarrow \sigma_e + \Delta\sigma_{02}$, given by Eq. (26). Here the diffusion equilibrium is established, *i.e.,* the equilibrium between the initial surface configuration and the bulk concentration is established, but the surface reaction did not start.

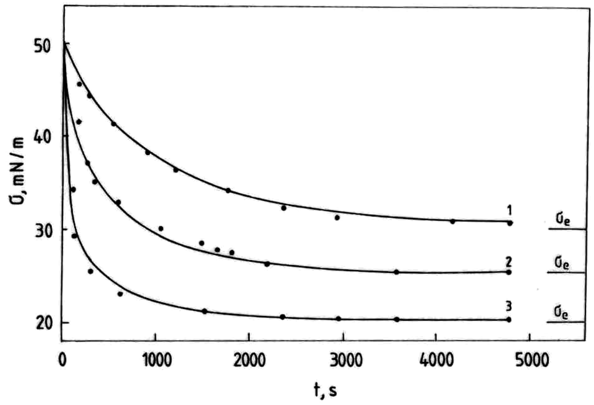

Fig. 2. *Dynamic interfacial tensions (σ versus t) at the hexane/water interface, for several concentrations of ethyl ester of β - apo-8'-carotenoic acid in hexane solution. Curve (1) for c_0 =1.76 10^{-7} mole cm^{-3}; (2): c_0 =2.97 10^{-7} mole cm^{-3} and (3): c_0 =5.07 10^{-7} mole cm^{-3}. The full line calculated with Eq. (22) using constant parameters given in Table 1. Symbols as in Fig. 1.*

The decay of the interfacial tension with time is exponential and it is given by Eq. (25). If τ is *very large*, diffusion is the rate controlling step and, for $\tau \rightarrow \infty$, Eq. (22) extrapolates to Eq. (28).

The decay is again exponential but the interfacial tension for $t \rightarrow 0$ extrapolates to σ_0 = 50 mN m^{-1}. We have analyzed the dynamic interfacial tensions for both carotenoids and the data are well fitted by the Eq. (22) and the standard deviation of experimental data from the calculated ones is within the experimental errors. The parameters of Eq. (22) that fit the experimental data for ethyl ester of β-apo-8'-carotenoic acidare given in Table 1 and for β-carotene in Table 2.

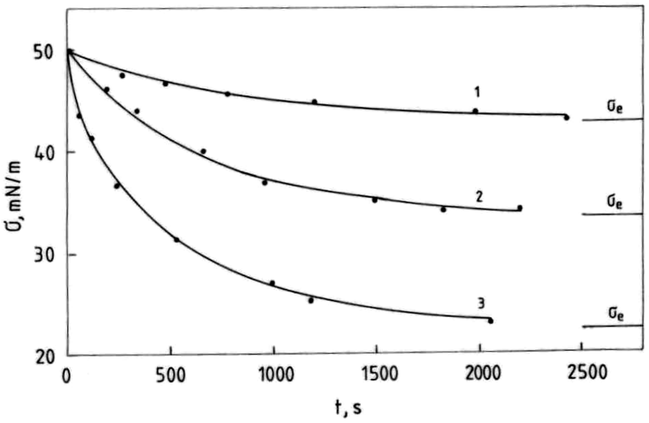

Fig. 3. *Dynamic interfacial tensions for hexane solutions of β- carotene at the interface with water, for different β- carotene concentrations. Curve (1): c_0=6.7 10^{-8} mole cm^{-3}; (2): 1.34 10^{-7} mole cm^{-3}; (3): 3.43 10^{-7} mole cm^{-3}. Full lines calculated with Eq. (22) using constant parameters given in Table 2.*

Horizontal line indicates σ_e value for each β- carotene concentration.

From Tables 1 and 2, it is seen that for $\tau \to \infty$, $\Delta\sigma_{01}$ =0. In these cases and for t $\to 0$, Eq. (28) extrapolates to Eq. (26) and again $\sigma \to \sigma_e + \Delta\sigma_{02}$. Therefore, it is evident that the (σ_e + $\Delta\sigma_{02}$) values represent the interfacial tensions when the diffusion equilibrium is established, but the interfacial reaction did not start. The reaction rate constant k is nearly concentration independent for each of the two carotenoids. For the adsorption behavior of ethyl ester of β-apo-8'-carotenoic acid, the mean value of k is $\langle k \rangle$ = 8.2 $10^{-4}s^{-1}$ (Table 1) and corresponds to a kinetic relaxation timeof $\tau_k = k^{-1} \sim$ 1.2 10^3s (approx. 20 minutes). For β-carotene, the reaction rate constant k is again nearly independent on concentration and the mean value is $\langle k \rangle$=1.3$10^{-3}s^{-1}$ (see Table 2) and $\tau_k \sim$760 s (approx. 13 minutes). The τ_k for the ethyl ester of β-apo-8'-carotenoic acid is higher than its value for β-carotene. This result is in substantial agreement with the molecular structure of these carotenoids and reflects the flexibility of the terminal group of the ethyl ester with possibilities of both the interfacial conformational changes and the polar group hydration against the terminal hydrocarbon β-ionone rigid ring from β-carotene molecule.

Both carotenoid compounds possess the same type of conjugated polyene hydrocarbon moiety, carrying the lateral methyl groups [37]. The β-carotene molecule has a symmetric structure with a long hydrocarbon chain with a terminal β-ionone ring at each end. The ethyl ester of β-apo-8'-carotenoic acid is an asymmetric molecule with a shorter hydrocarbon chain with only one terminal β-ionone ring and at the other extremity with an ethyl ester group.

The *diffusion relaxation time* for β-carotene (Table 2) is higher than its value for ethyl ester of β-apo-8'-carotenoic acid (Table 1) for a comparable carotenoid bulk concentration. This is plausible because the β-carotene molecule is longer than the molecule of ethyl ester of β-apo-8'-carotenoic acid.

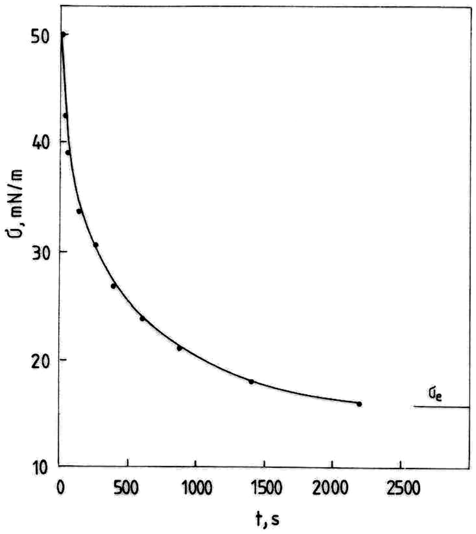

Fig. 4. *Dynamic interfacial tensions for hexane solutions of β - carotene at the interface with water, for the β - carotene bulk concentration c_0=5.99 10^{-7} mole cm^{-3}. Full lines calculated with Eq. (22) using constant parameters given in Table 2.*
Horizontal line indicates σ_e value.

Table 1

The parameters for the relaxation kinetics of dynamic interfacial tensions caused by the diffusion adsorption with surface reorientation of the ethyl ester of β-apo- 8'-carotenoic acid from hexane solutions to the hexane/water interface at 25 °C, calculated by Eq. (22).

c_0 mole cm^{-3}	σ_e mN m^{-1}	$\Delta\sigma_{01}$ mN m^{-1}	$\Delta\sigma_{02}$ mN m^{-1}	τ_D s	k s^{-1}	$\sigma_e+\Delta\sigma_{02}$ mN m^{-1}
5.07 10^{-7}	20.0	26	4.0	120	8.9 10^{-4}	24
2.97 10^{-7}	25.3	9	15.7	60	1.1 10^{-3}	41
1.76 10^{-7}	30.0	2	18.0	30	8.0 10^{-4}	48
7.90 10^{-8}	37.0	0	13.0	∞	7.2 10^{-4}	50
3.00 10^{-8}	43.0	0	7.0	∞	6.0 10^{-4}	50

Similar long time effects are also observed for other surfactants, soluble in the oil phase only, *such as*, lauric acid and palmitic acid at the oil/water interface [19]. Due to a few fatty acid concentrations reported it is not possible to evaluate the relaxation times by using our kinetic model. Sometimes, long time effects are also observed for adsorbed surfactants at the air/water interface [20, 21].

Table 2.

The parameters for the relaxation kinetics of the dynamic interfacial tensions generated by the diffusion adsorption of β-carotene from hexane solutions to the hexane/water interface associated with surface reorientation of β-carotene molecules at 25 ^oC, calculated by Eq. (22).

c_0 mole cm^{-3}	σ_e mN m^{-1}	$\Delta\sigma_{01}$ mN m^{-1}	$\Delta\sigma_{02}$ mN m^{-1}	τ_D s	K s^{-1}	$\sigma_e+\Delta\sigma_{02}$ mN m^{-1}
$5.99\ 10^{-7}$	15.6	27.0	7.7	450	$1.10\ 10^{-3}$	23.3
$3.43\ 10^{-7}$	22.5	8.6	18.9	160	$1.50\ 10^{-3}$	41.4
$1.34\ 10^{-7}$	33.8	0	16.2	∞	$1.55\ 10^{-3}$	50.0
$6.70\ 10^{-8}$	42.5	0	7.5	∞	$1.10\ 10^{-3}$	50.0

In the foreseen future we intend to deepen the description of the relaxation phenomena in adsorbed films at fluid interfaces and to extend this investigation on a large variety of biosurfactants.

Molecular mechanism of the surface relaxation

Among other factors, the hydrophobic effect [17, 38] seems to have an important role in the relaxation process of the dynamic interfacial tensions due to the adsorption of carotenoid molecules from oil phase at the oil/water interface and due to the molecular reorientation of carotenoids at the interface. It is important to mention that according to the hydrophobic effect, the hydrocarbon chains anchored in aqueous solutions are surrounded with structured water. We further suggest that the interfacial water is also structured around the polar groups (*e.g.*, ethyl ester group in the ethyl ester of β-apo-8'-carotenoic acid) and around the hydrocarbon β-ionone ring (in β-carotene), these moieties being oriented towards the water phase at the hexane/water interface or somehow the molecular chains are penetrating into the aqueous phase [5, 39].

Consequently, the two states of adsorption related to the conformer 1 and conformer 2 give different molecular interfacial areas, which can be evidenced thermodynamically (unpublished results). The molecular area at the initial states of the diffusion equilibrium is almost identical for both carotenoids and corresponds to the value of the cross section area of the hydrocarbon moiety existing in both carotenoid molecules. The cross section area determined by X-ray analysis [37] is around 15 Å^2/molecule and it almost equals the collapse molecular area for β-carotene spread at the air/water interface [39]. This indicates that the carotenoid molecules are vertically orientated in the adsorbed monolayer at the hexane/water interface at the initial states of the diffusion equilibrium.

We also suggest, as an alternative, that the conformation 1 corresponds to the non – hydrated molecules oriented vertically at the hexane/water interface and the conformation 2 corresponds to the carotenoid molecules vertically oriented at the interface having the structured water around their polar groups and around their hydrocarbon extremity anchored in the water phase. It is evident that in the conformation 2, the carotenoid molecule with its anchoring terminal group in the aqueous phase and with structured water around it, is irreversibly adsorbed at the interface being insoluble in hexane phase or in water phase.

At the final state of the equilibrium of adsorption with surface reorientation probably the predominant molecular species are the hydrated forms of carotenoid molecules vertically oriented at the oil/water interface. It is evident that the mean molecular area of conformation 2 is higher than its value for the conformation 1. Thus, it seems that the carotenoid molecules are initially adsorbed from n-hexane in a very compact film at the interface with water, and then, they relax at the interface into an equilibrium interfacial film having their molecular terminal groups surrounded with structured interfacial water.

This new kinetic model sheds light on the relaxation phenomena of carotenoid films at the oil/water interface and it leads to the *diffusion relaxation times* and the *reaction relaxation times* in substantial agreement with their molecular structure. The understanding of the interfacial behavior may contribute to the design of new carotenoid derivatives with high surface activity that will determine an increased stability of multi-phases systems with a remarkable impact on the designing of new carotenoid delivery systems for therapeutic purposes.

CONCLUSIONS

In this paper, we present a new kinetic approach applied to the dynamic interfacial tensions, which are generated by the adsorption of two carotenoids (*e.g.*, ethyl ester of β-apo-8’-carotenoic acid and β-carotene, all *trans*-isomers) at the oil/water interface for several carotenoid concentrations in the n-hexane solutions. For these systems it is found that the relaxation of the dynamic interfacial tensions is basically governed by two processes, *namely*, the diffusion process and the interfacial reaction of surfactant re-orientation and hydration.

In the theoretical description of this new kinetic model are included two characteristic relaxation times, *namely,* the relaxation diffusion time and the relaxation reaction time. The relaxation phenomena of the dynamic interfacial tensions are very well described by the new Eq. (22) within the limits of the experimental errors. The two specific relaxation times, *i.e.,* diffusion and surface reaction relaxation times are determined and they are in substantial agreement with the molecular structure of these biosurfactants.

Thus, it seems that the carotenoid molecules are initially adsorbed from hexane phase in a very compact film at the interface with water. Then, they seem to relax at interfaceintoan equilibrium interfacial film having their terminalgroups oriented towards water phase and finally surrounded with structured water molecules. This is also related to the hydrophobic effect or hydrophobic bonds. At the equilibrium, it is plausible that the interaction of carotenoid molecules with the interfacial water molecules leads to an expended interfacial lattice in adsorbed films at the hexane/water interface.

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