Use of Microcalorimetry in Monitoring Stability Studies. Example: Vitamin A Esters

Frank E. Runge[†] and Robert Heger*[,‡]

Fine Chemicals Division and Polymer Division, BASF Aktiengesellschaft, D-67056 Ludwigshafen, Germany

Vitamin A is very sensitive to chemical degradation caused by oxygen, light, heat, and other stress factors. If light and oxygen are excluded, the dominant degradation reaction for vitamin A derivatives is heat-induced formation of kitols, that is, dimers or higher oligomers. In this study vitamin A esters were used as model systems to evaluate microcalorimetry as a tool for monitoring the stability of heat sensitive substances. To obtain more knowledge about the model reaction, analytical investigations (supercritical fluid chromatography) were also performed. Because analytical and microcalorimetry data were consistent, a quantitative description of the kinetics and thermodynamics of the kitol formation reaction could be obtained. Aside from the academic motivation, this is important for practical purposes such as shelf life stability of vitamin A in feed, food, and pharmaceutical products. The vitamin A stability of a given sample can easily be predicted from the initial heat flow in a simple microcalorimetry experiment. Compared to conventional stability tests, this offers savings of money and time.

Keywords: *Vitamin A stability; kitols; microcalorimetry; supercritical fluid chromatography*

INTRODUCTION

Vitamins, particularly vitamin A (retinol) and the corresponding retinyl esters, are very sensitive to chemical degradation caused by oxygen, light, heat, moisture, and other stress factors (Ottaway, 1993). Therefore, pure vitamin A has to be handled in dark containers under an inert atmosphere. For practical purposes, special formulations have been developed to protect the vitamin A and derivatives from degradation in common food, feed, and pharmaceutical products (Schumacher and Grafen, 1982; Fukamachi et al., 1989; Chaundy et al., 1992).

Retinol and retinyl ester molecules are chemically reacting with one another, forming so-called kitols and kitol esters, that is, dimers of vitamin A derivatives (Giannotti et al., 1966; Mousseron-Canet et al., 1968; Burger and Garbers, 1973; Das, 1974; Pfoertner et al., 1988). Natural kitol has first been discovered in whale liver oil. Synthetically, these dimers can be formed in a thermally induced Diels−Alder reaction (Giannotti et al., 1966; Mousseron-Canet et al., 1968; Burger and Garbers, 1973) and in photochemically induced reactions (Mousseron-Canet et al., 1968; Das, 1974; Pfoertner et al., 1988). Under oxygen-free conditions, the kitol formation or vitamin A degradation will be much faster if the samples are illuminated. Therefore, in most papers on kitols and kitol esters, the material was either prepared from natural sources or synthesized by illumination of retinol derivatives. However, even under perfectly inert conditions the stability of vitamin A in the dark is not infinite, which is due to thermally induced kitol formation.

In the handling and processing of vitamin A, temperature stress is quite often involved. Therefore, it is important to learn about the vitamin A stability as a function of temperature under inert conditions. Also, the shelf life stability of retinyl esters in closed containers is expected to be determined by the Diels−Alder type of dimerization. Some investigations on the vitamin A stability in organic solutions (Paquette and Kanaan, 1985; Ammo et al., 1968) and food systems (Shah et al., 1976; Yoshioka et al., 1990; Laohasongkram and Thamjariyapan, 1995) have been published, but quantitative data for thermally induced kitol formation are not available so far.

The motivation for this study is to obtain physicochemical data on the temperature-induced formation of kitol esters from retinyl esters under inert conditions, that is, oxygen, light, and humidity are excluded. In stability experiments, accompanied by analytical investigations by means of supercritical fluid chromatography (SFC), a kinetic analysis was performed. Second, thermodynamic quantities were concluded from microcalorimetry studies. Finally, on the basis of this quantitative knowledge of the kitol formation, a method is explained for the prediction of the vitamin A stability in a given sample with a quick and simple microcalorimetry experiment. Thus, expensive and time-consuming shelf life tests may be replaced by this method.

MATERIALS AND METHODS

Retinyl Esters. The following specially prepared laboratory samples of different retinyl esters were used in this study: vitamin A acetate (VAA), vitamin A propionate (VAPr), and vitamin A palmitate (VAPal). The chemical formulas and some data for these vitamins are given in Figure 1 and Table 1. The all-E retinyl ester content in these samples was ≥90%.

Analytical Methods. The straightforward way to determine kinetic parameters is to study the chemical reaction under isothermal conditions, that is, to perform stability

* Author to whom correspondence should be addressed [telephone +49 (621) 60-45503; fax +49 (621) 60-72660; e-mail Robert.Heger@.basf-ag.de].

[†] Fine Chemicals Division.

[‡] Polymer Division.

10.1021/jf981163y CCC: $19.00
Published on Web 12/14/1999

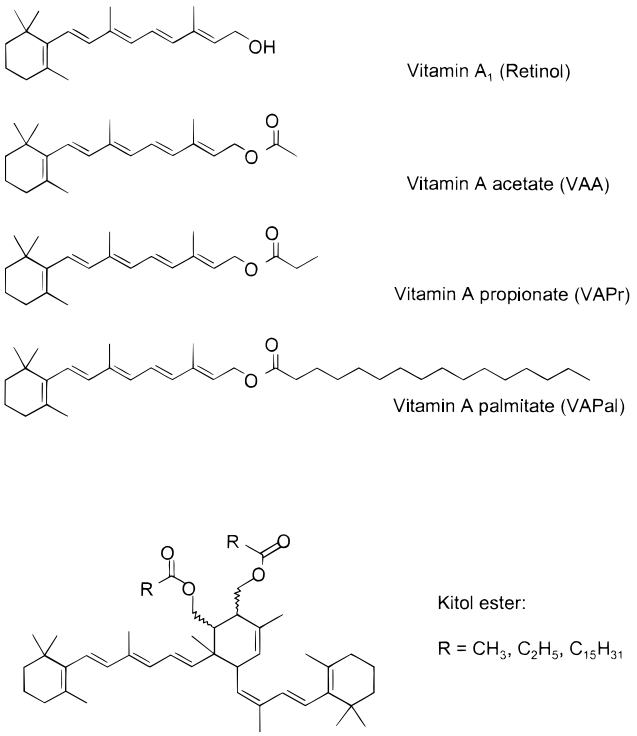

Figure 1. Chemical structures of the vitamin A derivatives investigated in this study.

Table 1. Characterization of the Used Vitamin A Esters (Retinyl Esters)

abbreviation	chemical name	molar mass (g/mol)	mp (°C)
VAA	vitamin A acetate	328.5	60
VAPr	vitamin A propionate	342.5	<20
VAPal	vitamin A palmitate	529.4	26

experiments of pure retinyl esters and their solutions in inert oils at constant temperature. The extent of reaction can be followed by the decrease of retinyl esters and the formation of kitol esters. From the rate of conversion a kinetic analysis can be performed. Also, the degree of conversion was compared to the thermal heat flow as determined by microcalorimetry.

Several papers have been published for the HPLC analysis of retinyl components (Albert et al., 1995; Schlotterbeck et al., 1997). In this study, SFC was used as the analytical method (Analytical Department, BASF Aktiengesellschaft, Ludwigshafen, Germany). For the SFC analysis CO_2 was used as mobile phase and biphenyl 30 as stationary phase, and the detection unit was a flame ionization detector (FID). In principle, kitols can be analyzed using photometry or other chromatographic methods such as gel permeation chromatography (GPC). However, quantitation becomes a difficult task because of different responses of the individual components using UV detectors. This obstacle can be overcome by using SFC with a flame ionization detector, which yields approximately mass proportional signals.

In Figure 2, typical SFC diagrams are shown for a fresh VAA laboratory sample (a), the same sample after storage for 5 days at 80 °C (b), a fresh VAPr (c), and a fresh VAPal laboratory sample (d). SFC is a powerful analytical tool, which allows not only the separation of retinyl ester from kitol ester but also a detailed separation of retinol isomers (VAA and VAPr, 21−23 min retention time; VAPal, 27.5−30 min retention time) and different kitols (VAA and VAPr, >28 min retention time; VAPal, >39 min retention time). Please note that the intensity scale is cut off at about one-third to half of the total all-E peak height to magnify the isomer and kitol peaks. The big peak at 15 min in all chromatograms is due to butylated hydroxytoluene (BHT), which was added as an anti-

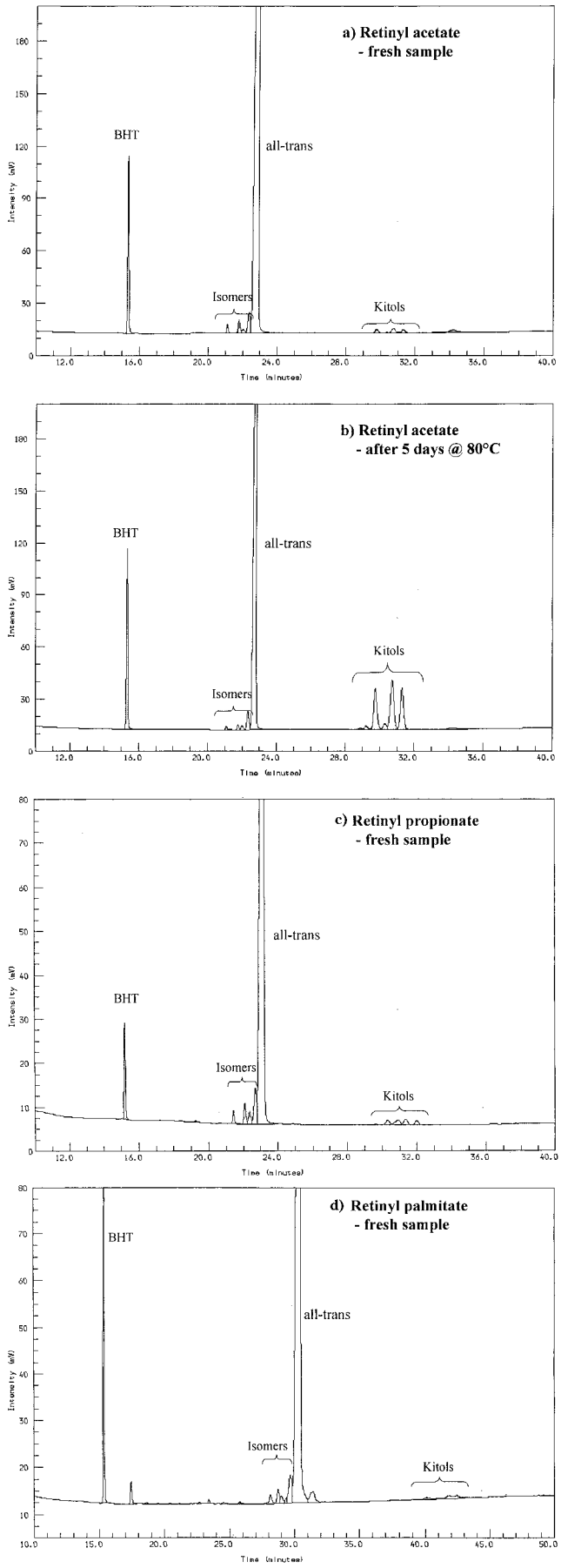

Figure 2. SFC diagrams for a fresh VAA sample (a), the same VAA sample after storing for 5 days at 80 °C (b), a fresh VAPr sample (c), and a fresh VAPal sample (d).

oxidant. As the FID used in our SFC apparatus is approximately mass proportional, the overall mass balance can be determined from the peak areas with a very small experimental error.

The characterization and peak identification were performed by preparative GPC followed by analytical investigation of the fractions obtained by UV−vis, ^{1}H and ^{13}C NMR, and mass spectroscopy (B. Nowakowsky, BASF Aktiengesellschaft, unpublished results). The collected fractions were re-injected into the SFC system to ensure the proper peak identification.

Microcalorimetry. Calorimetric measurements were performed with a 2277 TAM thermal activity monitor by ThermoMetric AB, Sweden (Suurkuusk and Wadsö, 1982), under isothermal conditions (temperature stability better than $\pm 10^{-4}$ °C). The signal from the microcalorimeter represents the heat flow (thermal power) of the investigated samples as a function of time. The power signal has a sensitivity better than ±0.1 μW and a baseline stability over 24 h of ±0.2 μW.

For sample preparations, 3 mL glass ampules were filled in a glovebox with ∼1 g of retinyl ester under inert conditions (N_2 atmosphere) and closed with a crimp top. It is extremely important to avoid oxygen contamination within the ampules, because the power signal from oxidative retinyl ester degradation is ∼2 orders of magnitude higher than the nonoxidative retinyl ester degradation.

The retinyl ester-filled ampules were equilibrated for 15−20 min at the measurement temperature together with water-filled reference ampules before they were placed in the measuring position. After a further 3−5 h, the system was equilibrated satisfactorily and the true power signal could be detected. The measurements were carried out for 2−3 days at different temperatures in the range from 40 to 80 °C.

Theory. *Kinetic Models.* The decrease of educt A with time t during a chemical reaction is given by

$$-\mathrm{d}c_\mathrm{A}/\mathrm{d}t = v = k_a c_\mathrm{A}^a \tag{1}$$

with v the reaction rate, a the reaction order, and k_a rate constant for a reaction of ath order.

The reaction order can, for instance, be determined by using the method of the initial reaction rates $v_0 = v(t \rightarrow 0)$. Transforming eq 1 gives

$$\ln(|\mathrm{d}c_\mathrm{A}/\mathrm{d}t|)_{t\rightarrow 0} = \ln(k_\mathrm{a}) + a \ln(c_{\mathrm{A}0}) \tag{2}$$

The initial reaction rates have to be measured for several known initial concentrations of educt A. The slope of a linear plot according to eq 2 gives the wanted reaction order a.

The kitol formation is known to be a bimolecular Diels−Alder reaction. It can be described by a second-order rate law:

$$2A \rightleftarrows K \tag{3}$$

mass balance

$$c_0 = c_{\mathrm{A}0} + 2c_{\mathrm{K}0} = c_\mathrm{A}(t) + 2c_\mathrm{K}(t) \tag{4}$$

reaction rate

$$-\mathrm{d}c_\mathrm{A}/\mathrm{d}t = 2\mathrm{d}c_\mathrm{K}/\mathrm{d}t = k_2 c_\mathrm{A}^2 = k_2[c_{\mathrm{A}0} - 2(c_\mathrm{K} - c_{\mathrm{K}0})]^2 \tag{5}$$

In the case of a second-order rate law according to eq 5, the reaction rate depends on the initial educt concentration $c_{\mathrm{A}0}$: a decrease in $c_{\mathrm{A}0}$ yields a slower educt conversion. The integrated rate law describes the changes of the educt concentration as a function of time:

$$\frac{c_{\mathrm{A}0}}{c_\mathrm{A}(t)} = 1 + c_{\mathrm{A}0}k_2 t = \frac{1}{1 - 2\dfrac{c_\mathrm{K}(t) - c_{\mathrm{K}0}}{c_{\mathrm{A}0}}} \tag{6}$$

Kinetic Methods: SFC. The educt conversion into kitols was analytically followed by SFC (method described above). The analytical results are given in relative peak areas, which are approximately proportional to the mass content of the respective component. Therefore, it is more meaningful to use molal concentrations $[m]$ = (mol/kg) instead of molar concentrations $[c]$ = (mol/L). Equation 6 can be transformed to give

retinyl ester conversion

$$F_{\mathrm{A}0}/F_\mathrm{A}(t) = 1 + m_{\mathrm{A}0}kt \tag{7}$$

kitol formation

$$1 - \frac{F_\mathrm{K}(t) - F_{\mathrm{K}0}}{F_{\mathrm{A}0}} = \frac{1}{1 + m_{\mathrm{A}0}kt} \tag{8}$$

with $F_i(t)$ the area percent of component i at time t, $m_{\mathrm{A}0}$ the initial ($t = 0$) educt molal concentration (in mol/kg), and k the second-order rate constant [in kg/(mol·s)]. The half-life $t_{1/2}$ (time at 50% educt conversion) is given by

$$t_{1/2} = 1/m_{\mathrm{A}0}k \tag{9}$$

Rate constants usually show a strong temperature dependence, which often follows the Arrhenius law

$$k(T) = A \exp[-E_\mathrm{a}/RT] \tag{10}$$

respectively

$$1/t_{1/2} = Am_{\mathrm{A}0} \exp[-E_\mathrm{a}/RT] \tag{11}$$

with A the pre-exponential factor and E_a the activation energy.

This relation allows the estimation of rate constants at temperatures not accessible experimentally. Moreover, it is possible to determine activation energies.

Kinetic Methods: Microcalorimetry. The educt conversion into kitols was also investigated microcalorimetrically. The thermal power measured with this method is directly proportional to the reaction enthalpies and the reaction rates of all processes taking place within the sample ampule. In the case of one defined reaction the thermal power is given by

$$P = \mathrm{d}Q/\mathrm{d}t = \Delta_\mathrm{r}H(\mathrm{d}m_\mathrm{A}/\mathrm{d}t) \tag{12}$$

with $\Delta_\mathrm{r}H$ the enthalpy of reaction (in kJ/mol) and m_A the molal concentration = amount of educt A per total sample mass (in mol/kg).

If the reaction law is known, $(\mathrm{d}m_\mathrm{A}/\mathrm{d}t) \approx (\Delta m_\mathrm{A}/\Delta t)$ can be substituted by the appropriate equations. In the case of the kitol formation reaction the second-order rate law gives according to eq 5

$$P = \Delta_\mathrm{r}Hkm_\mathrm{A}^2 \tag{13}$$

If $\mathrm{d}m_\mathrm{A}/\mathrm{d}t$ as well as k is known from independent analytical experiments, reaction enthalpies will be determined using eqs 12 and 13. By measuring the initial thermal power P_0, one can quickly obtain the reaction enthalpy using the following equation:

$$\Delta_\mathrm{r}H = P_0/km_{\mathrm{A}0}^2 \tag{14}$$

Substituting eq 13 into eq 10 gives the equation

$$P_0(T) = P_0^* \exp[-E_\mathrm{a}/RT] \tag{15}$$

with

$$P_0^* = \Delta_\mathrm{r}Hm_{\mathrm{A}0}^2A$$

If P_0 is measured at several temperatures, the slope of a plot

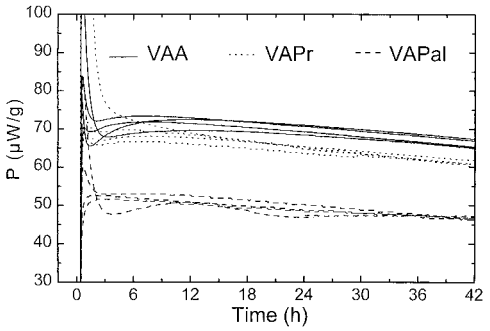

Figure 3. Microcalorimetry: thermal heat flow of VAA, VAPr, and VAPal sealed under nitrogen atmosphere measured at 80 °C.

of $\ln(P_0)$ versus $1/T$ will give the activation energy of the investigated reaction as well.

RESULTS AND DISCUSSION

Comparison of Analytical Data and Microcalorimetry. In this section we want to compare the experimental results from SFC and microcalorimetry on a qualitative basis before going into the quantitative analysis.

A conclusive microcalorimetry study will be possible only if the detected thermal heat flow correlates with the degree of conversion of the corresponding chemical reaction.

In Figure 3 experimental $P-t$ curves are shown for the three retinyl esters at 80 °C measured by microcalorimetry. The initial peak found in all curves is due to thermal equilibration effects after the vials have been put into the measurement compartment. Over the observation time of 2−3 days a slowly decreasing exothermic heat flow between 40 and 80 μW/g is detected in all cases. The thermal heat developed per gram of ester increases in the order of VAPal < VAPr < VAA.

The reproducibility of the $P-t$ curves is a crucial issue, particularly for these experiments, as it has to be assured that oxygen is excluded from the vials. In independent experiments it has been shown that the heat flow for the oxidation reaction in the presence of oxygen is roughly 100 times higher than for the kitol formation under ideally inert conditions. Even a very slow diffusion of oxygen into the vials can cause a distinct, positive deviation from the thermal heat flow caused by the kitol formation. Keeping that in mind, we find very good reproducibility of the $P-t$ curves in the order of ±5−10%.

From the almost constant P value over the first couple of days of the experiment performed at 80 °C, it is expected that there is a constant rate of reaction. In Figure 4, where the thermal power and the kitol content for VAPal at 80 °C are plotted as a function of t over 3−15 days, it can be seen that this is indeed the case. The kitol content is linearly increasing with time up to 10% kitols. For higher degrees of conversion the slope of the kitol content curve is decreasing, corresponding to decreasing P values.

At lower temperatures a much smaller rate of conversion and accordingly a much smaller heat flow are found. The temperature dependence of the kitol formation and the kinetic analysis will be discussed in detail later.

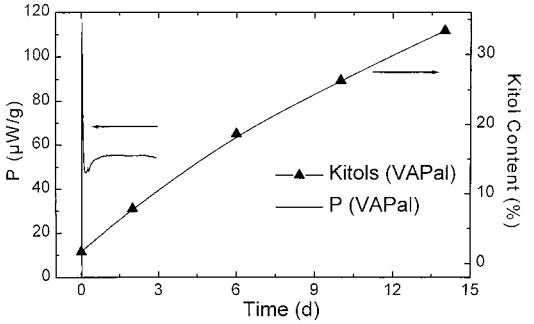

Figure 4. Correlation between kitol formation (SFC) and thermal heat flow (microcalorimetry) of VAPal sealed under nitrogen atmosphere at 80 °C.

Kinetic Analysis of Kitol Formation Using Analytical Data. In this section, a quantitative analysis of the chromatographic data leading to the order of reaction, rate constants, and temperature dependence (Arrhenius activation energies) is presented. These basic data sets have to be determined once by time-consuming, conventional analytical experiments, in order to perform all later shelf life tests by microcalorimetric investigations within several days.

From the literature (Mousseron-Canet et al., 1968; Burger and Garbers, 1973; Pfoertner et al., 1988) it is known that different kitol isomers do exist, which can be formed from all-E and Z-isomers of the retinyl esters. The exact mechanism of kitol formation with respect to isomer distribution and exact structure determination of the reaction products will not be investigated in this study. On the basis of our analytical data for the stability testing, we find in general that the all-E isomers of the retinyl esters are decreasing and the kitols are increasing with time, whereas the Z-isomers of the retinyl esters are essentially unchanged. Hence, for our analysis we assume that the kitols are formed in a reaction between two all-E retinyl ester molecules only (overall net reaction). That is, we do not take any isomerization pre-equilibria and reactions between all-E and Z-isomers into account. For high degrees of conversion, the formation of higher oligomers of the retinyl esters becomes more and more likely. The upper limit, at which the dimerization is the only major mechanism for the kitol formation, can be estimated to be ∼30−40% kitol content (see later). For even higher conversions, deviations from our expectations are found. Due to the same degradation mechanism (kitol formation) for all three vitamin A esters, only two of them (VAA and VAPal) are investigated in detail by chromatographic experiments. It is assumed that the VAPr kitol formation will follow the same reaction order.

The missing kinetic data will be provided by rapid microcalorimetric experiments later on (based on the reaction order determined for VAA and VAPal).

Determination of the Order of Reaction. The rate law of the kitol formation was determined from the concentration dependency using the method of initial rates (Atkins, 1990). A concentration series of VAA dissolved in both tetradecane and paraffin oil (10, 25, 50, and 100% VAA) was stored at 80 °C, and the all-E VAA content and the kitol content were analyzed every day. From these analytical data the initial reaction rate was calculated. As mentioned earlier, it is known from the literature (Giannotti et al., 1966; Mousseron-Canet et al., 1968; Burger and Garbers, 1973) that kitols are formed in a bimolecular Diels−Alder reaction (cf. eq 3).

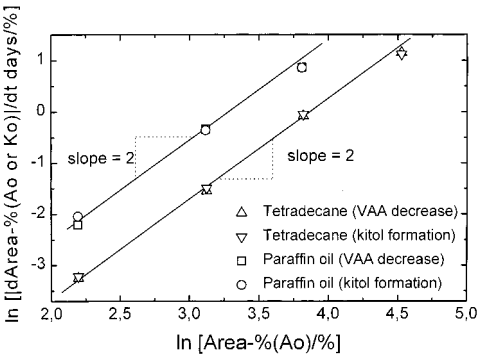

Figure 5. Determination of the reaction order of the kitol formation reaction at 80 °C from the initial rates with respect to the initial concentrations (determined by SFC).

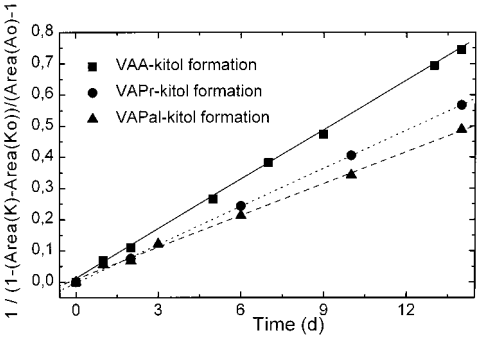

Figure 6. Analysis according to second-order kinetics of the kitol formation reaction at 80 °C for VAA, VAPr, and VAPal. The correlation coefficients for the linear regressions are 0.9997, 0.9999, and 0.9986, respectively.

Therefore, the kitol formation is expected to follow a second-order rate law. Figure 5 is a double-logarithmic plot of the initial reaction rate of both all-E VAA decrease and kitol formation as a function of initial concentration of all-E VAA. The slope of this type of plot is identical with the order of reaction. All analytical data are in excellent agreement with second-order kinetics for both kitol formation and all-E VAA decrease.

The data for tetradecane as the solvent give an excellent linear relation over the whole range of 10−100% VAA. In comparison, the rate of reaction in paraffin oil shows positive deviations, still showing a second-order rate law. Apparently, tetradecane behaves like a perfectly inert solvent, not changing the reaction rates valid for molten VAA. The paraffin oil has a specific effect on the rate of kitol formation, which could be due to viscosity or polarity effects or interaction with side components and impurities such as traces of acid.

Second-Order Rate Constants. The second-order rate constants k were determined through linear regression using eq 7 for the retinyl ester conversion and eq 8 for the kitol formation. In Figure 6, typical data and fitting curves are shown for VAA, VAPr, and VAPal at 80 °C. Over a time period of 14 days at 80 °C corresponding to a conversion of 30−40% of the initial retinyl ester to kitols, we find that the data are in excellent agreement with second-order kinetics; that is, the data are following a straight line in plots of the type of Figure 6.

The reciprocal of the slopes determined from linear regression is identical with the half-life (cf. eq 9) from which the k data can be calculated. In Figure 7, the reciprocal half-lives are plotted versus the initial mass fraction of all-E VAPr. For solutions of VAPr in tetradecane and peanut oil the same rate constant [$k = 1.9 \cdot 10^{-4}$ g/(mol·s)] is found in the entire concentration range from 10 to 100% VAPr; that is, the kinetics in oily solution are identical to the kinetics in the pure VAPr melt. In contrast, a much faster rate of kitol propionate formation [$k = 5.3 \cdot 10^{-4}$ g/(mol·s)] is found for paraffin oil as a solvent. The origin of this specific solvent effect is not absolutely clear (cf. last section).

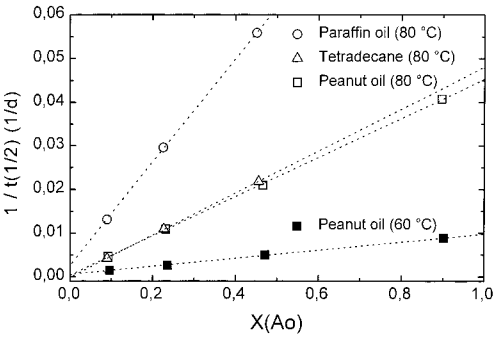

Figure 7. Plot of the reciprocal half-life of the kitol formation for VAPr solutions in different oils as a function of the initial mass fraction of all-E VAPr.

Table 2. Half-Lives and Bimolecular Rate Constants for the Kitol Formation Reaction of Retinyl Esters

	VAA		VAPr		VAPal	
T (°C)	$t_{1/2}$ (days)	$k \times 10^4$ [g/(mol·s)]	$t_{1/2}$ (days)	$k \times 10^4$ [g/(mol·s)]	$t_{1/2}$ (days)	$k \times 10^4$ [g/(mol·s)]
80	19	2.15	22	1.90	25	2.75
75	29	1.44			38	1.77
70	44	0.94			56	1.19
65	63	0.65			85	0.79
60	96	0.43	104	0.43	130	0.51
55	1200	0.034			260	0.26
50	4600	0.009			405	0.16
40	3700	0.012			1030	0.065
35			1400	0.031		
22			6700	0.007		

In Table 2, the summary of the half-lives and bimolecular rate constants at different temperatures determined from analytical data is given for the three retinyl esters. The experimental error of $t_{1/2}$ and k can be estimated to be ±5−10%.

From the data in Table 2 it can be seen that the rate of reaction is clearly increasing with increasing temperature for all esters. When going from high temperatures to temperatures below 60 °C, a distinct step toward slower kinetics is observed for VAA but not for VAPr and VAPal (see also next section). This effect is due to the different melting points (mp) of the investigated substances: VAA (mp = 60 °C) is a crystalline solid material at temperatures below 60 °C, whereas VAPr (mp < 20 °C) and VAPal (mp = 26 °C) are oily liquids for the whole investigated temperature range (all mp data are from BASF safety data sheets).

Finally, we mention that rate constants calculated from the all-E retinyl ester content of the samples are systematically higher by 5−10% than the rate constants given in Table 2 calculated from the kitol contents. This means the vitamin A ester degradation is slightly faster than the kitol ester formation. Possible explanations for this finding are side reactions such as hydrolysis due to traces of water and acids as well as oxidation due to traces of oxygen taking place in parallel to the dimerization reaction. Also, a small difference in the mass proportionality of two retinyl ester molecules versus one kitol ester molecule stemming from our detector could be possible.

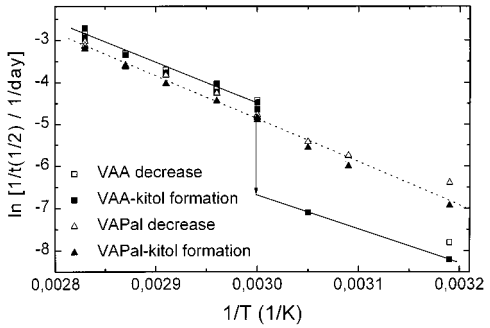

Figure 8. Arrhenius plot from analytical data for VAA and VAPal.

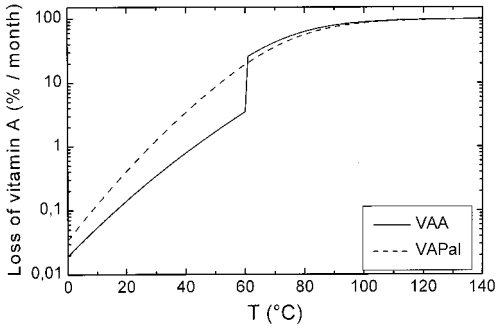

Figure 9. Calculated loss of vitamin A due to kitol formation for different storing temperatures (the data are valid for pure vitamin A esters as starting material).

Temperature Dependence (Arrhenius Plots). In general, the rate of reaction following first- or second-order kinetics is significantly increasing with temperature. This was investigated in detail for VAA and VAPal. For the rate constant an exponential relation according to the Arrhenius law, eq 11, is valid in most of the cases. In Figure 8 the analytical data for VAA and VAPal are included in an Arrhenius plot for temperatures between 40 and 80 °C.

For VAPal the Arrhenius law is valid for the whole temperature range; that is, the data are following a straight line. From the slope of the linear relation the activation energy can be calculated. In the lower temperature regime (40−55 °C) we find a higher scattering of the data due to the fact that smaller degrees of conversion at a given uncertainty of the analytical data are causing higher relative deviations. Hence, the data analysis with linear regression was performed using the data for 60−80 °C. An excellent agreement with the Arrhenius law is found for VAA in this temperature range, too. The following activation energies for the kitol formation reaction are obtained:

$$\text{VAA:} \quad E_a = 81\ (\pm 3)\ \text{kJ/mol}$$

$$\text{VAPal:} \quad E_a = 83\ (\pm 2)\ \text{kJ/mol}$$

As mentioned above, the activation energy for the VAPr will be determined by more rapid microcalorimetric experiments.

When going from the high-temperature to the low-temperature regime, clear deviations from the initial curve are found for VAA. As already mentioned in the last section, this is due to the crystallization of VAA at 60 °C. At this temperature a distinct step toward smaller ordinate values is taking place, meaning that the kinetics of kitol formation is slowed significantly when the phase transition from liquid to solid takes place. As kitol formation is a bimolecular process, this effect is easily understood in terms of reduced mobility and steric hindrance. In the solid state it is more difficult for the two reaction partners to get into the right position to allow the cycloaddition. We obtained only a few VAA results for temperatures below 60 °C. The Arrhenius plot still looks linear; however, due to the small conversion rates at these temperatures (leading to large experimental errors), we did not analyze the slope.

The validity of the Arrhenius law allows the prediction of rates of reaction for lower temperatures, where measurements are very time-consuming due to slow conversion rates, as well as for higher temperatures. The extrapolation of the high-temperature behavior of VAPal down to low temperatures such as room temperature can easily be done. For VAA we have to keep in mind that this extrapolation is not feasible because of the phase transition at 60 °C. Without the experimental data points at 40−60 °C for VAA we would have overestimated the predicted conversion rate of VAA below its melting point.

Table 3. Calculated Loss of Vitamin A Acetate Due to Kitol Formation at Elevated Temperatures (Valid for Pure VAA as Starting Material)

T (°C)	loss of VAA (%/h)	*T* (°C)	loss of VAA (%/h)
80	0.23	120	3.7
90	0.50	130	6.7
100	1.0	140	11.4
110	2.0		

Quantitative Prediction of Vitamin A Stability. In Figure 9 and Table 3 data for the loss of vitamin A potency are given for VAA and VAPal at different temperatures. The data (percent loss per time) were calculated for the pure vitamin A esters. This is the "worst case" for the vitamin A loss, as the rate of conversions can be severely slowed by dilution with an inert oil (second-order kinetics). For VAA we have the same step at 60 °C as seen in the Arrhenius plot (Figure 8)—the loss per time becomes smaller in the crystalline state below 60 °C. For VAPal we did not perform any studies below the melting point (26 °C), so we extended the predicted curve for molten VAPal down to 0 °C, still knowing that a step comparable to the VAA curve should show up in the VAPal curve at 26 °C. At typical refrigerator temperatures (4−8 °C) the vitamin A loss is ∼0.05−0.1% per month, at room temperature ∼0.5% per month. The data in Table 4 cover the temperature range that can be achieved during processing of foods or other products containing vitamin A derivatives.

At very high temperatures (>200 °C) kitols are separated into retinyl esters in a retro-Diels−Alder reaction. The data in Table 4 were calculated for a temperature range at which this reverse reaction does not have to be taken into account.

Microcalorimetric Results. Isothermal microcalorimetry is a well-established technique to study the stability of different chemical substances. It is widely used in pharmaceutical (Buckton, 1995; Koenigbauer, 1991) and material science (Lundgren, 1989) to obtain thermodynamic (heat of reaction) and kinetic (rate constants, rate laws) information of the appropriate investigated process. The knowledge of these parameters can be used to predict the degradation rates under storage conditions. Therefore, microcalorimetry has the potential to substitute for conventional stability tests

Table 4. Reaction Enthalpies for the Kitol Formation Reaction of Retinyl Esters

T (°C)	P_0[a] (μW/g)	$k \times 10^4$ [g/(mol·s)]	$\Delta_r H$ (J/g)	$\Delta_r H$ (kJ/mol)
		VAA		
80	−68 ± 0.7	2.15	−129	−42
75	−51 ± 0.7	1.44	−144	−47
70	−35 ± 1.2	0.94	−152	−50
65	−24 ± 1.5	0.65	−149	−49
60	−14 ± 1.3	0.43	−130	−43
		VAPal		
80	−47 ± 1.2	2.75	−112	−59
75	−45 ± 1.1	1.77	−165	−87
70	−31 ± 1.4	1.19	−171	−90
65	−15 ± 1.3	0.79	−123	−65
60	−12 ± 0.9	0.51	−160	−85
		VAPr		
80	−62 ± 1.0	1.90	−137	−50
60	−16 ± 1.4	0.43	−157	−54

[a] Standard deviations are given for the initial thermal power P_0.

to a certain extent (effects such as permeability of containers have to be tested separately).

Microcalorimetry alone is not sufficient to give all thermodynamic and kinetic parameters. Therefore, a basic data set of kinetic information has to be determined through classical analytical experiments once for any unknown system. As soon as these data are known, all further stability tests might be performed by microcalorimetry alone. Within days, shelf life predictions can be made from the initial thermal power of these experiments. There is no need for further time-consuming (~6−12 months) conventional shelf life tests.

Using the vitamin A esters as model systems, the rate constants derived from the chromatographic data were combined with the microcalorimetric measurements to calculate the heat of reaction (see eq 14). Additionally, the activation energies of the kitol formation were determined from the temperature dependence of the specific thermal power (see eq 15).

Determination of the Reaction Enthalpies. The reaction enthalpies for the kitol formation of the 3 retinyl esters were determined using eq 14. The initial thermal power of the kitol formation (average of at least three measurements, error ±5−10%) and the appropriate bimolecular rate constants are shown in Table 4. For the determination of the initial molal retinyl ester concentration an all-E content of 90% was taken into account. The calculated specific and molar reaction enthalpies are also summarized in Table 4 for a temperature range of 60−80 °C. The detected microcalorimetric data for temperatures <60 °C scatter up to 30% and were therefore not used to calculate reaction enthalpies.

Within the accuracy of the data there is no indication for a temperature dependence in the reaction enthalpy between 60 and 80 °C. Therefore, average values were formed to give

VAA: $\Delta_r H = -46\ (\pm 4)$ kJ/mol VAA ⇔ $-141\ (\pm 11)$ J/g VAA

VAPr: $\Delta_r H = -51\ (\pm 5)$ kJ/mol VAPr ⇔ $-147\ (\pm 14)$ J/g VAPr

VAPal: $\Delta_r H = -77\ (\pm 14)$ kJ/mol VAPal ⇔ $-146\ (\pm 27)$ J/g VAPal

The molar reaction enthalpies of VAA and VAPr are nearly equal, whereas the enthalpy for VAPal is ~60−65% higher. A satisfactory reason for this behavior could not be found. Most probably these differences are caused by the differences of the chemical structure of the retinyl esters (i.e., palmitate versus acetate and propionate).

Figure 10. Scheme of the Diels−Alder reaction.

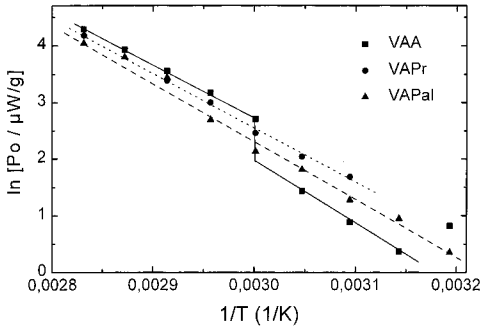

Figure 11. Arrhenius plot from microcalorimetric data.

To our knowledge there are no published reaction enthalpies available for the kitol formation of retinyl esters. For a rough estimate of the reaction enthalpy of the kitol formation, mean bond energies of the involved single and double bonds were taken into account. The kitol formation is a Diels−Alder reaction, where according to Figure 10 two new C−C single bonds were formed and two C=C double bonds were converted into single bonds.

Using known mean bond energies $E(\text{C}-\text{C}) = -348$ kJ/mol and $E(\text{C}=\text{C}) = -612$ kJ/mol (Atkins, 1990), the calculated reaction enthalpy for the kitol formation is given by

$$\Delta_r H_{\text{calcd}} = 2E(\text{C}-\text{C}) - 2[E(\text{C}=\text{C}) - E(\text{C}-\text{C})] \quad (16)$$

$$\approx -170 \text{ kJ/mol kitol}$$

$$\approx -85 \text{ kJ/mol retinyl ester}$$

The experimentally determined reaction enthalpies are therefore consistent with these rough estimates and lie in a chemically reasonable regime. Especially for VAPal there is an excellent agreement between the theoretical and experimental values.

Temperature Dependence (Arrhenius Plots). According to eq 15 the initial thermal power of the kitol formation (measured by microcalorimetry) can be used to determine the activation energy of this process. In contrast to the thermodynamic investigation, this determination can be conducted completely independently from the analytical data and is therefore a good proof of consistency between both methods.

In analogy to Figure 8, Figure 11 shows the microcalorimetric data for the three retinyl esters (initial thermal power) in an Arrhenius plot for the temperature range from 40 to 80 °C.

In the case of VAPal and VAPr a linear behavior can be found for the whole investigated temperature range. In accordance with the analytical VAA data, the microcalorimetric data show a big step toward higher ordinate values at the VAA melting point (~60 °C). This corresponds to a dramatic acceleration of the kitol formation after the phase transition.

The activation energies of the kitol formation, using the microcalorimetric data between 60 and 80 °C, were found to be

$$\text{VAA:} \quad E_a = 77\ (\pm 2)\ \text{kJ/mol}$$

$$\text{VAPr:} \quad E_a = 81\ (\pm 3)\ \text{kJ/mol}$$

$$\text{VAPal:} \quad E_a = 87\ (\pm 3)\ \text{kJ/mol}$$

Within the experimental error these microcalorimetrically determined activation energies are in accordance with the results obtained from the analytical experiments. Using microcalorimetry, these data can be observed more rapidly than by chromatographic experiments. Therefore, the data of VAPr determined by microcalorimetry are given additionally.

Theoretically it is possible to calculate reaction enthalpies from the ordinate intersection of the Arrhenius plot as well (see eq 15). Due to the exponential relation, the experimentally caused uncertainties of the ordinate intersection (1−3%) lead to errors in the reaction enthalpy of between 40 and 75%. A meaningful investigation of the reaction enthalpies using the Arrhenius plot is therefore not possible.

It was mentioned several times in this paper that after having completed all of these basic kinetic investigations, a lot of time may be saved for further shelf life tests, using microcalorimetry. This will be explained in the following paragraph.

Due to quality control regulations, industry has to perform a lot of shelf life stability tests of their products on a regular basis. Usually several hundred product samples of different batches or different production sites are tested per year and typically stored for 6−12 months under defined conditions. Later on, analytical work has to be done additionally, which makes this conventional procedure very time- and money-consuming.

Having the knowledge of the kinetic parameters, which we determined here for the vitamin A esters, the shelf lives of these products can be predicted within a few days. Here, the initial thermal power (measuring time < 3 days) of vitamin A esters has to be measured, for example, at 60 °C. Using the Arrhenius equation and the appropriate activation energy one can calculate the theoretical initial thermal power at room temperature. According to eq 12, the degradation rate can then be calculated by considering the appropriate reaction enthalpy. Therefore, without additional analytical work, a reasonable shelf life prediction can be made within several days.

Microcalorimetry was proven to be a powerful tool capable of giving both qualitative and quantitative information. The degradation reaction of retinyl esters under inert atmosphere is known to be kitol formation. Having that information, a quantitative kinetic and thermodynamic analysis could be performed using microcalorimetric data. With the quantitative knowledge about the kitol reaction the shelf life stability (in perfectly sealed containers) can be predicted by simply using the initial thermal power of vitamin A ester formulations within a few days. This offers an enormous saving of money and time compared to conventional stability tests. Obviously, this microcalorimetric method is not limited to vitamin A products.

ABBREVIATIONS USED

mp, melting point; VAA, vitamin A acetate (retinyl acetate); VAPr, retinyl propionate; VAPal, retinyl palmitate; SFC, supercritical fluid chromatography.

SYMBOLS

a = reaction order
A = pre-exponential factor [g/(mol·s)]
c_0 = initial concentration of educt and product (mol/L)
c_A = educt concentration (mol/L)
c_{A0} = initial educt concentration (mol/L)
c_K = kitol concentration (mol/L)
c_{K0} = initial kitol concentration (mol/L)
E_a = activation energy (kJ/mol)
$F_i(t)$ = area % of component i at time t (%)
$\Delta_r H$ = enthalpy of reaction (kJ/mol)
k_a = rate constant for a reaction of ath order
k_2 = second-order rate constant [g/(mol·s)]
m = molality (mol/kg)
m_A = educt molality (mol/kg)
m_{A0} = initial ($t = 0$) educt molal concentration (mol/kg)
P = thermal power (μW)
P_0 = initial thermal power (μW)
Q = heat (kJ)
R = gas constant [8.314 J/(K·mol)]
t = time (h)
$t_{1/2}$ = half-life (days)
T = thermodynamic temperature (K)
v = reaction rate [mol/(L·s)]

ACKNOWLEDGMENT

We thank Brigitte Nowakowsky and Günter Schulz (both BASF Analytical Department) for performing the SFC analyses. We greatly appreciate preliminary microcalorimetry work done by Lutz End and Georg Zwissler (both BASF Fine Chemicals Division).

LITERATURE CITED

Albert, K.; Schlotterbeck, G.; Braumann, U.; Haendel, H.; Spraul, M.; Krack, G. Strukturbestimmung von Vitamin-A Acetat-Isomeren mit HPLC-^{1}H NMR-Kopplung. *Angew. Chem.* **1995**, *107*, 1102−1104.

Ammo, T.; Washitake, M.; Takashima, Y.; Sato, S. Stability of vitamin A. II. Change rate and its process of vitamin A acetate in aqueous ethanolic solution. *Bitamin* **1968**, *37*, 19−24.

Atkins, P. W. The rates of chemical reactions. In *Physical Chemistry*, 4th ed.; Freeman, Oxford University Press: New York, 1990.

Buckton, G. Applications of isothermal microcalorimetry in the pharmaceutical sciences. *Thermochim. Acta* **1995**, *248*, 117−129.

Burger, B. V.; Garbers, C. F. Diels−Alder reactions. Part III. Condensation of Methyl *trans*-β-formylcrotonate with retinol acetate, with a note on the structure and stereochemistry of kitol. *J. Chem. Soc., Perkin Trans. 1* **1973**, 590−595.

Chaundy, F. K.; Bower, D. K.; Kilbride, T. K. Process for incorporation a material in a cross-linked gelatin, and product therefrom. U.S. Patent 5153177, 1991.

Das, K. K. Preparation of kitol acetate from retinyl acetate by the action of indirect sunlight. *Indian J. Chem.* **1974**, *12*, 1216.

Fukamachi, C.; Schumacher, H.; Bewert, W.; Schneider, J. Stabiles Gemisch mit einem Gehalt oxidationsempfindlicher Verbindungen. Eur. Patent 0326026, 1989.

Giannotti, C.; Das, B. C.; Lederer, E. Sur la constitution chimique du kitol, dimère de la vitamine A. *Bull. Soc. Chim. Fr.* **1966**, 3299−3303.

Koenigbauer, M. J.; Brooks, S. H.; Rullo, G.; Couch, R. A. Solid-state stability testing of drugs by isothermal calorimetry. *Pharm. Res.* **1992**, *9*, 939−944.

Laohasongkram, K.; Thamjariyapan, S. Kinetics of vitamin A degradation in pork liver puree during heat processing. *Cell Biol. Int.* **1995**, *19*, 229−236.

Lundgren, R.; Sandén, R. A facile method for the study of slow physical and chemical processes in polymeric systems. *J. Appl. Polym. Sci.* **1989**, *37*, 1221−1231.

Mousseron-Canet, M.; Lerner, D.; Mani, J.-C. Structure d'un photodimère de la vitamine A. *Bull. Soc. Chim. Fr.* **1968**, 4639−4645.

Ottaway, P. B. Stability of vitamins in food. In *The Technology of Vitamins in Food*; Ottaway, P. B., Ed.; Blackie Academic & Professional: London, U.K., 1993.

Paquette, G.; Kanaan, A. M. Degradation of retinyl acetate in simple solvent systems. *Food Chem.* **1985**, *18*, 211−231.

Pfoertner, K.-H.; Englert, G.; Schoenholzer, P. Photosensitized [4+2] cyclodimerizations of aromatic retinoids. *Tetrahedron* **1988**, *44*, 1039−1052.

Schlotterbeck, G.; Tseng, L.-H.; Händel, H.; Braumann, U.; Albert, K. Direct on-line coupling of capillary HPLC with 1H NMR spectroscopy for the structural determination of retinyl acetate dimers: 2D NMR spectroscopy in the nanoliter scale. *Anal. Chem.* **1997**, *69*, 1421−1425.

Schumacher, H.; Grafen, P. Verfahren zur Herstellung von Trockenpulvern oxidationsempfindlicher Substanzen. Eur. Patent 0074050, 1982.

Shah, R. C.; Raman, P. V.; Shah, B. M.; Vora, H. H. Accelerated tests in relation to shelf life of multivitamin liquid preparations. *Drug Dev. Commun.* **1976**, *2*, 393−403.

Suurkuusk, J.; Wadsö, I. A multichannel microcalorimetry system. *Chem. Scr.* **1982**, *20*, 155−163.

Yoshioka, S.; Aso, Y.; Takeda, Y. Isothermal and nonisothermal kinetics in the stability prediction of vitamin A preparations. *Pharm. Res.* **1990**, *7*, 388−391.

Received for review October 21, 1998. Revised manuscript received September 23, 1999. Accepted November 2, 1999.

JF981163Y