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Kinetics of decomposition of hydroperoxides formed during the oxidation of soya phosphatidylcholine by analysis of chemiluminescence generated

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Abstract

Chemiluminescence (CL) can be used to measure the extent of oxidation of soya phosphatidylcholine (S-PC) as has been shown previously, utilizing a mixture of the S-PC with medium chain monoacylglycerol. The CL data obtained from such a measurement was examined in this work to estimate the kinetics of decomposition of hydroperoxides that are generated by oxidation. CL intensity has been counted in an inert atmosphere at various temperatures and the data analyzed by non-isothermal and isothermal reaction kinetic analysis, primarily based on isoconversion methods. The isothermal data has also been analyzed to obtain an estimate of the order of reaction. The apparent activation energy for the hydroperoxide decomposition steps in this system is obtained as 74 and 62 kJ/mol; the reaction order changes from 2 to 1 as the reaction proceeds and the hydroperoxides are consumed. Possible mechanisms of hydroperoxide decomposition have been discussed.

Keywords: Chemiluminescence; Hydroperoxide decomposition; Isoconversion methods; Oxidation kinetics; Phosphatidylcholine oxidation

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1. Introduction

Chemiluminescence (CL) is a virtually universal property of oxidizable organic substances. With the development of sensitive photomultipliers and single-photon counting devices, interest in the use of this technique has grown. Among the areas in which CL measurement has shown a lot of promise is the study of oxidation of lipids (see

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Usuki et al., 1979; Miyazawa et al., 1994; Singh et al., 1996) and the oxidative degradation of polymers (see George et al., 1983; Billingham et al., 1991; Forsström et al., 1994). These fields are of interest to the pharmaceutical (and foods) industry; the question of oxidation being relevant to both the packaging and the contents thereof.

Of the many reactions involved in the oxidation of lipids or polymers (simplified schemes in Vassil'ev, 1967; George et al., 1983; George, 1989), only the termination stages are sufficiently exoenergetic to produce a product in an excited state. The most accepted theory is the Russel mechanism in which two alkyl-peroxy radicals (at least one of them being secondary) combine via an unstable tetraoxide intermediate to produce an excited carbonyl (triplet state in ketone), an alcohol, and molecular oxygen (Scheme 1). This excited carbonyl (indicated by the *) then emits light as it returns to the ground state.

Various figures have been given for the energetics of this reaction ranging from 170 to 420 kJ/mol (Vassil'ev, 1967; Gundermann and McCapra, 1987; George et al., 1983; Pei et al., 1989).

The alkyl-peroxy radicals required for the generation of CL via the Russel mechanism arise from the hydroperoxides present/generated in the sample. This is the basis for the use of CL to measure the extent of oxidation in a sample (Mendenhall, 1990). Billingham et al. (1991) support this by stating that the rate determining step for CL in an inert atmosphere (from polypropylene) is the decomposition of peroxides, the luminescent step being the recombination of product radicals.

A number of values have been presented for the energetics and kinetics of hydroperoxide decomposition as measured by CL. Cash et al. (1987) obtained a value of 98 kJ/mol and first order kinetics for the decomposition of methyl linoleate hydroperoxides. Values calculated by examining more 'complex' oxidizing systems by CL lead to numbers such as 106 kJ/mol (oxidized polypropylene, Matisova–Rychla et al., 1981), 63 kJ/mol and first order kinetics (peroxidized polyethylene, Zlatkevich, 1987), 88 kJ/mol and mixed-order kinetics (peroxidized polypropylene, Zlatkevich, 1989). Other values obtained by analyzing CL data as an indicator of overall oxidization kinetics include 67 kJ/mol for nylon-6,6 (George et al., 1983), 113 kJ/mol for polypropylene (Celina and George, 1993), and 85 kJ/mol for nylon-6,6 (Forsström et al., 1994). The activation energy parameter must however be defined as 'apparent' since we are not dealing with clearly defined elementary reactions in these cases.

Other reports on the value of oxidation enthalpy are 84 kJ/mol calculated from data on methyl linoleate autoxidation in Labuza et al. (1969), a value of 70 kJ/mol for oxidation of edible oils using isothermal calorimetry data by Kasprzycka–Guttman and Odenziak (1994), and Chien (1968) who reported 109 kJ/mol for decomposition of polyethylene hydroperoxide. These values agree well with those obtained by CL for the 'complex' systems above.

The apparent activation energies (E_A) calculated by the researchers reviewed above, have been obtained by using certain parts of the CL data or by assuming certain mechanisms. George et al. (1983) and Cash et al. (1987) have used the initial CL intensity at different temperatures to calculate E_A. Matisova–Rychla et al. (1981) fit first-order reaction rate equations to the last part of their CL curves to calculate rate constants at various temperatures. Zlatkevich (1989) fit CL data from both isothermal and non-isothermal measurements to first and second-order rate equations to obtain E_A. Forsström et al. (1994) plotted steady-state value of CL intensity at different operating temperatures against inverse temperatures. Kasprzycka–Guttman and Odenziak (1994) fit their isothermal calorimetry data to a kinetic equation describing autooxidation. These methods for the analysis of thermoluminescence kinetics are summarized by Mahesh et al. (1989).

Our group has recently completed an evaluation of direct CL measurements for determining the oxidation status of (soya) phosphatidylcholine (Singh et al., 1996). We decided to calculate the (apparent) energetics of phospholipid hydroperoxide decomposition from CL data and compare the

$$R_1R_2HCOO^{\cdot} + {}^{\cdot}OOCR_1R_2R_3 \longrightarrow R_1R_2C{=}O^{*} + O_2 + R_1R_2R_3COH$$

Scheme 1. Reaction.

values obtained for polymers reported above. No comparative data is available in the literature. Furthermore, a novel approach has been taken to the calculation of E_A from CL data in this report based on a non-isothermal reaction kinetics analysis whereby no a priori assumption is required as to the mechanism involved.

Non-isothermal kinetic analysis is an area of active research since the 1960s and numerous schemes have been put forward (Brown et al., 1980; Flynn et al., 1987). The most successful are the so-called isoconversion methods (see reviews in Ozawa, 1992; Chen et al., 1993). The data analyzed by these methods in the literature are almost exclusively obtained from thermogravimetric analysis, i.e. data is in the form of weight loss/gain as a function of time and a constant heating rate. We have however utilized the generality in the isoconversion method of Friedman (1964) to analyze our CL data, and obtain an apparent activation energy and propose a probable mechanism.

2. Materials

Chromatographically purified soya phosphatiydylcholine (S-PC) ($\geq 98\%$; batch no. E 40302) and medium-chain monoacylglycerol (MCM) ($\geq 99\%$; approx. 20% C10:0 and 80% C8:0 fatty acids; batch no. K 40921) were obtained from Karlshamns LipidTeknik AB, Stockholm, Sweden. Samples for the CL studies were prepared by dissolving S-PC in molten MCM (melting point 32–35°C) to a concentration of 50 wt.%. Appropriate amounts of the components were weighed and sealed in a glass bottle under nitrogen purge. The mixture was stirred with a magnet and held between 50–60°C for 2–4 h to obtain a uniform solution. This material was then used in the measurements below. No further oxidation was induced in the sample which was kept at -20°C in a vial filled with dry nitrogen. All measurements in this report were made on the same batch of 50/50 w/w S-PC/MCM mixture.

3. Methods

3.1. Chemiluminescence

The procedure for CL measurements on phosphatidylcholine has been described by Singh et al. (1996). CL measurements were made with a CLD 100 chemiluminescence detector containing a CLC-10 photon counter (detection range 300–800 nm) from Tohoku Electronic Industrial, Miyagi, Japan. The apparatus has a compartment in which an open sample container can be placed. The whole compartment can be heated; a shutter isolates the photomultiplier tube from this compartment when measurements are not being made.

HPLC sample vials were cut to obtain small glass cups (13 mm ø; 10 mm high) which were used to hold the lipid sample (~ 200 mg) in the CL apparatus. These were introduced into the sample compartment only when the compartment was at 26°C; it was always placed at the same position. Prior to starting measurements, the compartment was flushed with nitrogen for 10 min and the nitrogen flush was continued throughout the measurement period. At time $t = 0$, the nominal temperature setting of the compartment was raised to the desired operating temperature (73, 83, 93, 98 and 103°C). CL counting was started simultaneously and continued for the desired length of time after which the sample was removed and the compartment cooled to room temperature before the next run. Two sets of experiments were carried out: one in which CL was measured for 5000 s and the second where counting was continued for a period of 50 000 s.

Data is obtained as CL counts/10 s as a function of time (see Fig. 1). The instrument also simultaneously reports a sample compartment temperature. However, this temperature does not correspond with the actual sample temperature. Actual sample temperature was measured in separate experiments by placing a temperature probe in the lipid sample and repeating the above CL measurement procedure for 5000 s but with the shutter closed. A correlation between the sample compartment temperature versus time data reported by the instrument and the actual sample temperature was thus obtained (Fig. 2). This cor-

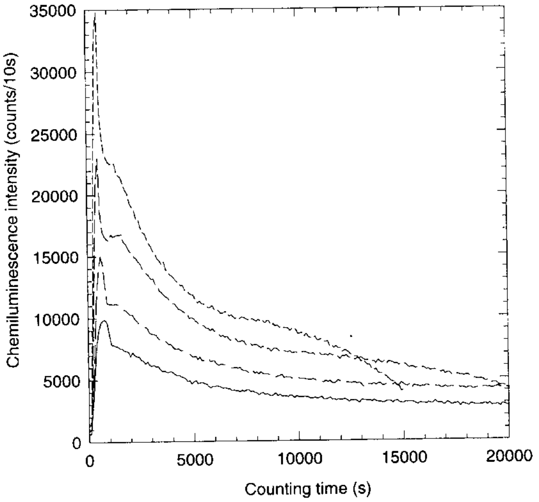


Fig. 1. Chemiluminescence intensity curves obtained from the 50/50 S-PC/MCM sample on heating in an inert atmosphere to 73, 83, 93 and 103°C (ascending order of curves).

relation was very reproducible; the procedure was performed for each of the above nominal operating temperatures. This analysis showed that non-isothermal conditions prevailed between 0 and 5000 s after which the sample temperature was very nearly constant.

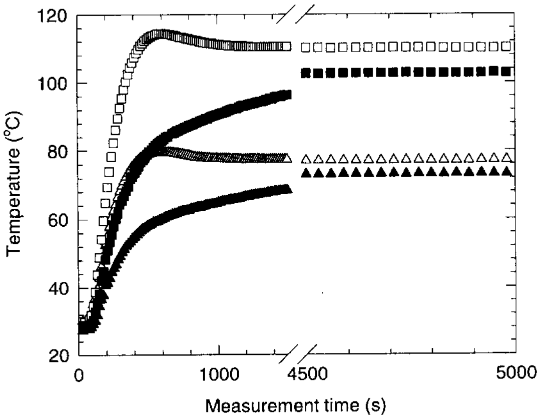


Fig. 2. Correlation between actual sample temperature (filled symbols) and temperature shown by apparatus (open symbols) for set temperature = 73°C (triangles), and 103°C (squares).

Table 1
Oxidation parameters of 50/50 S-PC/MCM w/w sample

Method	Parameter	Measured value
UV spectroscopy[a]	$A_{233\ nm}-A_{300\ nm}$	0.237 (AUs/0.02 g S-PC)
	$A_{270\ nm}-A_{300\ nm}$	0.053 (AUs/0.02 g S-PC)
HPLC-ELS-UV detection[a]	S-PC content	45.4 ± 1.4%
	Index 1	0.71
	Index 2	not detected
TBARS assay[a]	Malondialdehyde	117.2 μg/g S-PC
Other aldehydes[b]	Formaldehyde	300.2 μg/g S-PC
	Capronaldehyde	520.7 μg/g S-PC

[a] Singh et al. (1996).
[b] Coutrim et al. (1993).

3.2. Other methods of oxidation characterization

The lipid samples used in the above CL studies were characterized by UV absorption 233 nm and 270 nm, TBARS assay, HPLC with Evaporative light scattering-UV detection (Singh et al., 1996), and an HPLC method for aldehyde determination based on the reaction of carbonyl group with the amino group in 2,4-dinitrophenyl hydrazine (see Coutrim et al., 1993). Results of these analyses are summarized in Table 1. Comparision with the data presented in Singh et al. (1996) shows that the samples analyzed here correspond to relatively low levels of oxidation; no cyclic peroxides should be involved.

4. Kinetic analysis of chemiluminescence data

4.1. Method 1: differential analysis

A generalized differential form of reaction rate equation may be used to define the effective hydroperoxide degradation rate.

$$-\frac{d\alpha}{dt} = k(T)f(\alpha) \tag{1}$$

where α is the fraction of reactant left, $f(\alpha)$ is a rate function representing the reaction mechanism, and $k(T)$ is the rate constant at temperature T. If we assume that the luminescence reaction(s) go to completion (at the highest experimental set temperature, $T_{\max}$) producing a final total count of I_{tot}, then the initial hydroperoxide concentration P_0 is proportional to I_{tot}, while the hydroperoxide content at any time t, $P(t)$, is proportional to $[I_{\text{tot}} - \Sigma_{t=0}^{t} I(t)]$, $\Sigma_{t=0}^{t} I(t)$ being the total counts at time t (Zlatkevich, 1989). Thus,

$$\alpha = \frac{P(t)}{P_0} \equiv \frac{I_{\text{tot}} - \sum_{t=0}^{t} I(t)}{I_{\text{tot}}} \qquad (2)$$

where

$$I_{\text{tot}} = \sum_{t=0}^{t_{\max}} I(T_{\max}; t) \equiv P_0 \qquad (3)$$

The rate constant can be represented by the Arrhenius relationship

$$\mathrm{k}(T) = A \cdot \mathrm{e}^{-E_A/RT} \qquad (4)$$

where A is the pre-exponential factor, R is the gas constant (8.3144 J/K mol), and T is the reaction temperature in Kelvin. Substituting Eqs. (2) and (4) into Eq. (1) and taking the logarithm on both sides gives

$$\ln\left[-\frac{1}{P_0}\frac{\mathrm{d}P}{\mathrm{d}t}\right] = \ln A + \ln\left[f\left(\frac{P}{P_0}\right)\right] - \frac{E_A}{RT} \qquad (5)$$

For constant values of (P/P_0), the function $f(P/P_0)$ is assumed to be a constant (at all temperatures values). This is equivalent to assuming that the underlying chemistry of the luminescence process is independent of the temperature. Eq. (5) can then be used to calculate E_A by the procedure described below.

The cumulative CL intensity versus time curve, for each nominal operating temperature, is first converted into a fractional hydroperoxide content (P/P_0)–time curve by Eq. (2). I_{tot} is obtained by summing the counts for the highest operating temperature. The implicit assumption is that all of the hydroperoxides are decomposed in such an experiment. The assumption is supported by the observation that the CL intensity is almost down to background levels by 15 000 s for an operating temperature of 103°C. Several (P/P_0) values over the non-isothermal and isothermal portions of the experiment (between 0.99 and 0.40 in steps of 0.01) are selected and the corresponding $[-(1/P_0)(\mathrm{d}P/\mathrm{d}t)]$ and t determined by interpolation. The corresponding actual T values at time t are also obtained from the sample compartment-sample cup temperature correlation data as discussed earlier (Fig. 2). This procedure is repeated for each of the nominal operating temperatures. For each chosen value of (P/P_0), the corresponding $\ln[-(1/P_0)(\mathrm{d}P/\mathrm{d}t)]$ from each nominal operating temperature is then plotted against the corresponding $(1/T)$. E_A is then obtained from the slope of the regression line according to Eq. (5) (examples shown in Fig. 3a). The results are summarized in Fig. 4a.

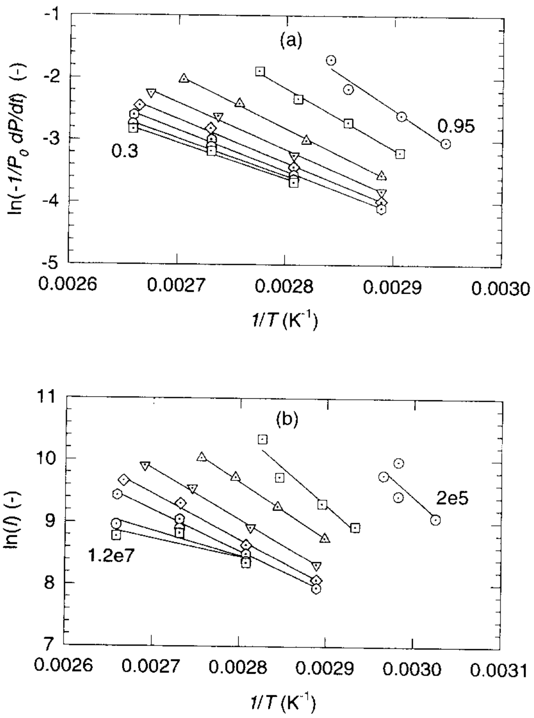


Fig. 3. Examples of graphical expression of (a) Eq. (5) for various (P/P_0) (= 0.95, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3), and (b) Eq. (7) for $\Sigma_{t=0}^{t} I(t)$ (= 2e5, 1e6, 2e6, 4e6, 6e6, 8e6, 1e7, and 1.2e7).

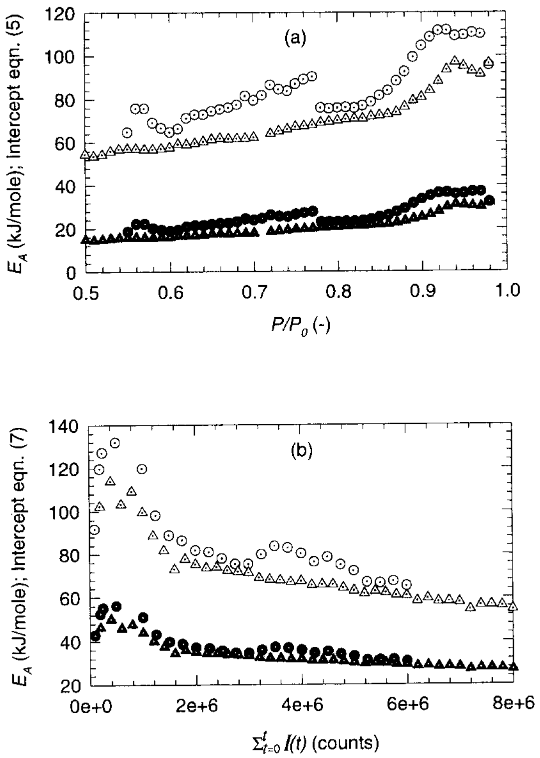


Fig. 4. Values of E_A (open symbols) and intercepts (filled symbols) calculated from (a) Eq. (5), and (b) Eq. (7) as functions of (P/P_0) and $\Sigma_{t=0}^{t} I(t)$ (cumulative counts) values respectively. Circles and triangles represent different CL data sets.

4.2. Method 2: integral analysis

The observed CL intensity at time t, $I(t)$, (counts/time) can be expressed as (Billingham et al., 1991)

$$I(t) = G\Phi L \tag{6}$$

where L is the rate of the luminescent reaction, G is a geometric term and Φ is the quantum efficiency of the reaction. G is determined by the photon detection efficiency of the photomultiplier tube, the geometry of the detector and that of the sample itself; this can be reasonably taken to be a constant in this work. Φ is a product of the fraction of potentially luminescent reactions which produce excited states and the fraction of such states which emit light rather than decaying by a radiationless transition. For the purpose of this work, Φ is also taken to be an invariant since the samples analyzed are similar.

L can be related to the hydroperoxide degradation rate defined above in Eq. (1). Substituting from Eqs. (1) and (3) for L gives

$$\ln[I(t)] = \ln G\Phi A + \ln\left[f\left(\frac{I_{\text{tot}} - \sum_{t=0}^{t} I(t)}{I_{\text{tot}}}\right)\right] - \frac{E_A}{RT} \tag{7}$$

Eq. (7) is solved for E_A. The assumption again is that for constant values of α, the function $f(\alpha)$ is constant. Values of $\Sigma_{t=0}^{t} I(t)$ are selected over the non-isothermal and isothermal parts of the runs (between 10^4 and 10^7 counts). The corresponding t and $I(t)$ are obtained for each of the nominal operating temperatures from the raw data curves. The actual sample temperature T at time t in each of the cases is also determined as indicated above from Fig. 2. $\ln[I(t)]$ and $(1/T)$ for each nominal operating temperature are plotted for each chosen value of $\Sigma_{t=0}^{t} I(t)$, and E_A obtained from the slope of the line fitted according to Eq. (7) (examples shown in Fig. 3b). The results from this analysis are summarized in Fig. 4b.

4.3. Method 3: reaction order analysis of isothermal data

The general luminescent reaction can be described as an nth order reaction producing light

$$nP \xrightarrow{k_n(T)} h\nu + \text{products} \tag{8}$$

The kinetic Eq. (1) can then be written as

$$-\frac{1}{P_0}\left(\frac{dP}{dT}\right) = k_n(T) f\left(\frac{P}{P_0}\right) = k_n(T)\left(\frac{P}{P_0}\right)^n \tag{9}$$

Under isothermal conditions, this equation can be integrated for various values of n to obtain

$$n = 0\text{: } P_0 - P(t) = k_0(T)t \tag{10}$$

$$n = 1\text{: } \frac{P_0 - P(t)}{P_0} = 1 - e^{-k_1(T)t} \tag{11}$$

and

for $0 < n < 1$ and $n > 1$

Table 2
Isothermal data analysis by Method 4: extent-of-reaction analysis, Eq. (16)

Nominal set temperature pairs (T_a, T_b)	73, 83°C	73, 93°C	83, 93°C	83, 103°C	93, 103°C
For α ranges between	0.725 and 0.500	0.575 and 0.500	0.575 and 0.300	0.475 and 0.300	0.475 and 0.300
Calculated E_A (kJ/mol)	56.8 ± 0.2	59.3 ± 0.2	58.7 ± 0.3	49.0 ± 0.2	40.4 ± 0.1

$$\frac{1}{(n-1)P^{(n-1)}} - \frac{1}{(n-1)P_0^{(n-1)}} = \mathrm{k}_n(T)t \qquad (12)$$

while P_0 is defined as in Eq. (3).

The above equations can be used to determine reaction order and rate constant. One approach is to fit the isothermal part of CL data to the above equations for a series of different n's. The value of n which gives the best fit is taken to be the estimate for the reaction order.

4.4. Method 4: extent-of-reaction analysis of isothermal data

For an isothermal reaction, the rate Eq. (1) can be integrated

$$-\int_{\alpha_1}^{\alpha_2} \frac{\mathrm{d}\alpha}{f(\alpha)} = \mathrm{k}(T) \int_{t_1}^{t_2} \mathrm{d}t \qquad (13)$$

to give, without loss of generality

$$F(\alpha_1) - F(\alpha_2) = \mathrm{k}(T)(t_2 - t_1) \qquad (14)$$

where F is the integral function of $1/f$. This relationship must hold for all pairs of α's at temperatures for which the kinetic function f is valid. Thus, for a pair of α's, α_1 and α_2, at two temperatures, say T_a and T_b, the ratio of rate constants from Eq. (14) is obtained as

$$\frac{\mathrm{k}(T_a)}{\mathrm{k}(T_b)} = \frac{t_{b2} - t_{b1}}{t_{a2} - t_{a1}} \qquad (15)$$

Substituting from Eq. (4) into Eq. (15) gives

$$\frac{E_A}{R}\left(\frac{1}{T_b} - \frac{1}{T_a}\right) = \ln \frac{t_{b2} - t_{b1}}{t_{a2} - t_{a1}} \qquad (16)$$

Eq. (16) can be used to obtain E_A by employing pairs of (isothermal) temperatures and α's. All combinations of α values, 0.025 units apart, over the isothermal reaction range have been used for each nominal operating temperature pair to calculate E_A. A summary of the results is presented in Table 2.

5. Results and discussion

The CL curves for the set of experiments run for 50 000 s is shown in Fig. 1. The CL intensity passes through a maximum due to two competing processes as the sample is heated: the increase in rate of hydroperoxide decomposition and the resulting decrease in concentration of available hydroperoxide species. The position of the peak is therefore related both to the concentration of hydroperoxide and the heating rate. Such peaks have also been reported for preoxidized polymers heated in an inert atmosphere, e.g. Billingham et al. (1991) and Zlatkevich (1989)]. Another observation that can be made is that the curves for 93 and 103°C show a relatively rapid decrease in CL counts near the end of the experiment.

The non-isothermal and isothermal CL data from each of the experiments has been analyzed according to Friedman (1964) as described above in Methods 1 and 2. The two methods are complementary to each other as can be seen in Figs. 3 and 4. The calculated E_A values vary with the parameter (P/P_0) or $\Sigma^{t}_{t=0} I(t)$ used (Fig. 4). However, the major variation occurs in the analysis of the initial part of the CL measurements which occur under heating rates of the order of 5–10°C/min. A normal non-isothermal reaction process should not give rise to this variation implying that other phenomenon are influencing the reaction being observed. Possible explanations include a reduction in the viscosity of the lipid sample with heating leading to more effective diffusion and recombination of radicals. This effect would lead to a decrease in the E_A with increasing temperature. Another phenomenon that is likely to occur is that the oxygen dissolved in the lipid leads to an initial spurt of extra oxidation as the temperature starts to rise. The effect is a concentration of hydroperoxide that is higher at each point in time than would have been the case if all the hydroperoxide had been found only at the start of the measurement. The effective reaction rate is thereby slower or the calculated E_A higher. This effect is obviously only seen during the initial part of the measurement before all the oxygen is used up. These phenomena can explain the initial decrease in E_A at high (P/P_0) (>0.9) or low $\Sigma_{t=0}^{t} I(t)$ $(<1\times10^6$ counts) in Fig. 4a and Fig. 4b. The change in E_A for (P/P_0) values between 0.9 and 0.55 is smaller but a downward trend is apparent (Fig. 4a); an average E_A has however been calculated between these limits. Similarly, the limits of $\Sigma_{t=0}^{t} I(t)$ used are 1×10^6 to 6×10^6 and to 8×10^6 counts in Fig. 4b; the lower limits are determined by the need to have at least three points to fit Eq. (7). The results are given in Table 3 with the values lying in the range 60–80 kJ/mol. An average value of 74 kJ/mol is obtained from the two sets of experiments. This value compares very well with the activation energies in the literature reviewed earlier. The numbers obtained from polymers are in general slightly higher which can be ascribed to differences in material density, diffusivity of radicals, and light transmitivity, as well as differences in methods of evaluation.

Table 3
Summary of E_A and n values obtained by different methods

Method of data analysis	E_A (kJ/mol)	n
Differential analysis Eq. (5) (Method 1)		
Data set 1 (5 set temperatures)	79.1 ± 9.0	2
Data set 2 (4 set temperatures)	68.8 ± 10.3	
Average	74	
Integral analysis Eq. (7) (Method 2)		
Data set 1 (5 set temperatures)	76.9 ± 6.9	NA
Data set 2 (4 set temperatures)	66.3 ± 9.9	
Average	72	
Isothermal reaction order analysis (Method 3)		
Best data fit (Data set 2)	NA	1 and 2
Eq. (11), First order fit (Data set 2)	62	1[a]
Isothermal extent of reaction analysis (Method 4)		
(Data set 2)	NA[b]	NA

[a] Forced fit of first order reaction equation to isothermal data down to $\alpha \geq 0.25$ as shown in Fig. 6.

[b] Due to the proposed shift in reaction mechanisms; this method of analysis is not strictly valid; calculated values are shown in Table 2.

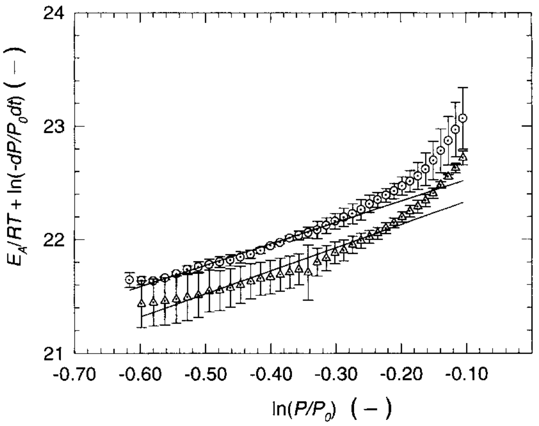


Fig. 5. Plot according to Eq. (17) for estimation of reaction order by the Friedman method. Circles and triangles represent different CL data sets.

Once a value has been obtained for E_A (74 kJ/mol), the analysis can be extended to obtain an estimate of the reaction order after Friedman (1964). For a reaction of order n, Eq. (5) can be rewritten as

$$\frac{E_A}{RT} + \ln\left[-\frac{1}{P_0}\frac{dP}{dt}\right] = \ln A + n\ln\left(\frac{P}{P_0}\right) \qquad (17)$$

Substituting for E_A, the left-hand side can be evaluated for various values of (P/P_0) for each T. The average value of the left-hand side can then be calculated for each (P/P_0) and plotted against $\ln(P/P_0)$ to obtain n by regression. The results are plotted in Fig. 5 and show that second order kinetics can be used to approximate the data for (P/P_0) values from 0.9 to 0.55. No attempt has been made to calculate the pre-exponential factor A for the reaction from this plot, since it is likely to be confounded with geometric factors influencing measurement and luminescent efficiency terms as indicated in connection with Eq. (6).

Analysis of the isothermal portion of the data by Method 3 requires an estimate of the value of

P_0 when fitting the CL curves by the reaction Eq. (10)–Eq. (12). Ideally, P_0 should be obtained from the cumulative intensity at infinite time; however, the value at 15 000 s for the experimental run at 103°C has been used here. This, combined with the rapid decrease in CL counts near the end of the experiment as discussed earlier, gives (P/P_0) values that approach zero abruptly. Normal reaction kinetics except for zero-order reactions have a much more gradual approach to completion. In order to avoid this effect, the isothermal data has been analyzed down to a (P/P_0) value of 0.25 only. (This restriction applies to data for 93 and 103°C only.) Results of this analysis are presented in Fig. 6 which shows that good fits are obtainable with $n = 1$ (Eq. (11)) for all the temperatures. The k values obtained are 3.16×10^{-5}, 5.91×10^{-5}, 1.11×10^{-4} and $1.72 \times 10^{-4}\,\mathrm{s}^{-1}$ for 73, 83, 93 and 103°C, respectively, which gives an activation energy of 62 kJ/mol. However, using a nonlinear regression fit with variable n (Eq. (12)) gives best fits with $n \cong 1.74$, 0.99, 0.83 and 0.75 for 73, 83, 93 and 103°C, respectively. No particular reason can be given for this discrepancy except that there may be more than one set of parameters that fit the data; the actual data fit is only marginally better in terms of sums of squares residuals (data not shown). The value of $n \cong 1.74$ for 73°C however departs sufficiently from $n = 1$ to lead to a test of hypothesis that two reaction processes are underway simultaneously. The following equation representing simultaneous first and second order reactions was fit to the isothermal data

$$\left(\frac{P}{P_0}\right) = \frac{\dfrac{k_1}{k_1 + k_2} e^{-k_1 t}}{1 - \dfrac{k_2}{k_1 + k_2} e^{-k_1 t}} \qquad (18)$$

The analysis shows that a significant second order reaction effect is present in the data obtained at 73°C. For the data at 83°C, the second order term improves the fit slightly but is not significant. For the other temperatures, the equation does not converge.

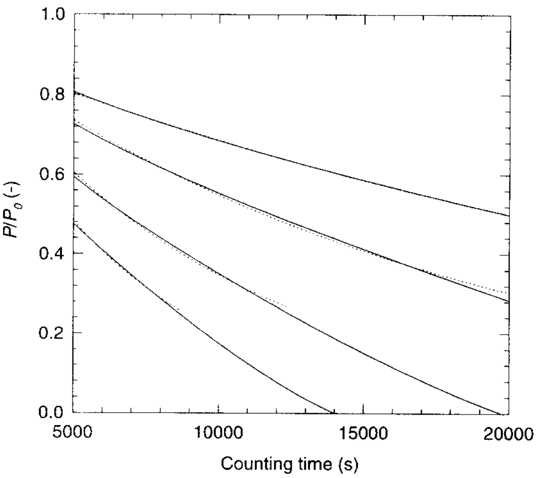


Fig. 6. Fit of Eq. (11) to isothermal portion of CL data. The full lines represent original data; the regression curves are dotted.

$$2\,R_1R_2HCOOH \longrightarrow R_1R_2HCOO^{\bullet} + R_1R_2HCO^{\bullet} + H_2O$$

Scheme 2. Reaction.

The above data analyses indicates a probable shift in the order of reaction, and thereby in the mechanism, with increase in extent of reaction. Examples of shift in mechanisms are available at least for polymers (Chien and Jabloner, 1968; George, 1989; Zlatkevich, 1989). No comparable discussion is available for fats and oils but Slawson and Adamson (1976) ascribe the origin of CL from their study of autoxidation of linolenic acid to a bimolecular mechanism. Cash et al. (1987) ascribe first order kinetics to the decomposition of methyl linoleate hydroperoxides in the bulk as compared to second order kinetics when adsorbed on silica gel.

It is possible that the first stage of CL emission is a result of the alkyl-peroxy radicals generated by the bimolecular decomposition of hydroperoxides present, as indicated in Scheme 2, with any dissolved oxygen also taking part through the oxidative cascade (Singh et al., 1996). As the CL emission continues, the concentration of hydroperoxides drops, and the unimolecular decomposition (homolysis) of hydroperoxides becomes the predominant mechanism for the generation of alkoxy and hydroxy radicals (Scheme 3).

$$R_1R_2HCOOH \longrightarrow R_1R_2HCO^{\bullet} + {}^{\bullet}OH$$

Scheme 3. Reaction.

$$R_1R_2HCO^{\cdot} + {}^{\cdot}OH + 2\,R_1R_2HCOOH \longrightarrow 2\,R_1R_2HCOO^{\cdot} + R_1R_2HCOH + H_2$$

Scheme 4. Reaction.

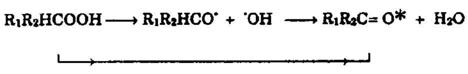
CL may then be emitted from the alkoxy radicals via the free radical induced decomposition of hydroperoxides that leads to the production of alkyl-peroxy radicals shown in Scheme 4. Thus the reaction in Scheme 3 is followed by the fast reaction 4 (Cash et al., 1987), and subsequently, the Russel mechanism in Scheme 1.

An alternative reaction (to Russell mechanism) is the generation of CL by a disproportionation of the alkoxy radicals or a molecular rearrangement reaction of hydroperoxides as indicated in Scheme 5. The mechanism requires that the peroxide be secondary.

The reactions in Scheme 3 or Scheme 5 are favoured in inert atmospheres and could well become dominant as the dissolved oxygen is used up and the hydroperoxide concentration in the sample drops. Thus, for the study made at 73°C, the radicals are generated by both the bimolecular and unimolecular decomposition of hydroperoxides. With increasing temperature of experiment and the increasing degrees of conversion achieved, the unimolecular decomposition becomes dominant at the later stages in the process as the hydroperoxides are consumed.

Method 4 for analysis of isothermal data gives estimates of E_A (Table 2) that are somewhat lower than that obtained by the Friedman analysis methods (Methods 1 and 2) in Table 3. The analysis in Method 4 is made on the assumption of unchanging kinetics which does not hold true here in the isothermal data range.

The possible reasons for the sudden dip seen in the CL curves for 93 and 103°C are worth speculation. Can the radical recombination reaction change to a non-luminescent pathway or does the hydroperoxide decomposition follow pathways that do not generate free radicals? Is it related to the concentration of other products from oxidation, some of which can function as quenchers? More work with samples of differing extents of oxidation is required to clarify the process occuring here.



$$R_1R_2HCOOH \longrightarrow R_1R_2HCO^{\cdot} + {}^{\cdot}OH \longrightarrow R_1R_2C{=}O^{*} + H_2O$$

Scheme 5. Reaction.

The methods of kinetic data evaluation employed in this work have a number of advantages. The isoconversion data analysis technique (Methods 1 and 2) used here does not require the assumption of any reaction mechanism; reaction order obtained by combining results from non-isothermal and isothermal data is considered to be more reliable than that from any single measurement technique (Zuru et al., 1990). While it is advantageous to have experiments in which the temperature has been raised at a controlled rate, it is not a neccessity as has been demonstrated in this work; however, experiments with (various) controlled heating rates are required to clarify the mechanism and to obtain a better estimate of E_A than could be obtained here.

6. Conclusions

Data obtained from measurement of chemiluminescence from soya phosphatidylcholine under inert atmosphere has been analyzed. Both non-isothermal and isothermal kinetic analysis have been carried out. An isoconversion method has been utilized for the first time on CL data. Results suggest a complex mechanism for the hydroperoxide decompostion process that leads to chemiluminescence. The apparent activation energy obtained for the decompositon of hydroperoxides is 74 kJ/mol, with an estimated reaction order of 2 at the early stages. The decomposition likely proceeds by both bimolecular and unimolecular reactions. The dominant reaction order changes to 1 as the concentration of hydroperoxides drops. An apparent activation energy calculated for this reaction is 62 kJ/mol. The values of E_A are of the same order of magnitude as those in the literature.

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