# The thermotropic phase behavior of ascorbyl palmitate: an infrared spectroscopic study[1]

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The infrared spectra of aqueous potassium ascorbyl palmitate were studied as a function of temperature using Fourier transform infrared techniques. From a light scattering experiment the Krafft point of 0.1 *M* potassium ascorbyl palmitate was determined to be 48°C. The temperature-induced changes in infrared spectral parameters such as frequency and bandwidth characterize this Krafft point as a phase transition from a conformationally ordered, poorly hydrated solid phase, to an isotropic micellar phase. The phase transition of this "pseudosoap" occurs over a temperature range of about 10°C, reflecting the progressive hydration of the solid upon micellization, a behavior typical of surfactants such as soaps.

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En utilisant les techniques infrarouges de la transformation de Fourier, on a étudié les spectres infrarouges des solutions aqueuses de palmitateascorbyle de potassium en fonction de la température. A partir d'un expérience de dispersion de la lumière, on a déterminé que le point Krafft pour une solution 0,1 *M* de palmitateascorbyle de potassium est de 48°C. La température provoque des changements dans les paramètres spectraux tels la fréquence et la largeur des bandes qui caractérisent ce point Krafft en tant que transition de phase à partir d'une conformation ordonnée, mal hydratée dans la phase solide, jusqu'à une phase micellaire isotropique. La transition de phase de ce "pseudo" savon se produit au dessus d'un intervalle de température de 10°C environ réflétant ainsi l'hydratation progressive du solide lors de la formation des micelles, c'est un comportement typique des savons.
[Traduit par le journal]

## Introduction

Ascorbyl palmitate, the C-6 monoester of vitamin C with palmitic acid, was first synthesized by Swern *et al*. in 1943 (1) with the intention in mind to combine the reductive properties of ascorbic acid with the lipid solubility of a fatty acid. This derivative of ascorbic acid is actually a lipophilic vitamin C and has been shown to be as biologically active as its original hydrophilic counterpart (2, 3). Since both vitamin C and palmitate esters are natural food ingredients, ascorbyl palmitate is now widely used in the food industry as a natural preserver of oils and fats (4, 5).

The effectiveness of ascorbyl palmitate in protecting against oxidation is similar to that of other commercial lipid antioxidants, such as α-tocopherol (vitamin E). In fact, it has been shown that tocopherols become more powerful antioxidants when used in combination with ascorbyl palmitate (5, 6) and this effect can be further enhanced by addition of lecithin (3, 6). The synergism between vitamin C and vitamin E when used as antioxidants in biomembranes has been recently confirmed by pulse radiolysis measurements (7). Thus, the chemical behavior of ascorbyl palmitate as a lipophilic analogue of vitamin C is of interest not only in view of its value as a food additive, but also with respect to its possible biological role as a lipophilic vehicle of vitamin C.

Despite its importance to the food industry, there are few data in the literature regarding the structure and physicochemical properties of ascorbyl palmitate or its salts. In principle, one would expect these properties to reflect both those of vitamin C and those of a fatty acid ester. In addition, the salts of ascorbyl palmitate (at neutral pH ascorbyl palmitate is always a salt) are amphiphilic molecules and structurally similar to soaps such as the *n*-alkanoates or the *n*-alkyl sulphates. A common property of such surfactants is that above a certain temperature, referred to as the Krafft point (8–14), the solubility of the surfactant in water increases dramatically. The salts of ascorbyl palmitate exhibit the same behavior. Using a light scattering technique and Fourier transform infrared spectroscopy we have studied the Krafft point transition, and report on the data herein.

## Experimental

*Materials and Sample Preparation*

Ethyl palmitate was from Sigma, St. Louis, MO and sodium ascorbate from Merck, Darmstadt, Germany. Ascorbyl palmitate (O$^6$-palmitoyl-L-ascorbic acid) was a commercial product from the U.S. Biochemical Corporation, Cleveland, OH. The potassium (or sodium) salts were prepared from equimolar amounts of ascorbyl palmitate and KOH (or NaOH), each dissolved in the minimal amount of absolute ethanol. The salts which precipitated upon mixing the two ethanolic solutions were filtered off, washed with absolute ethanol, and dried under vacuum.

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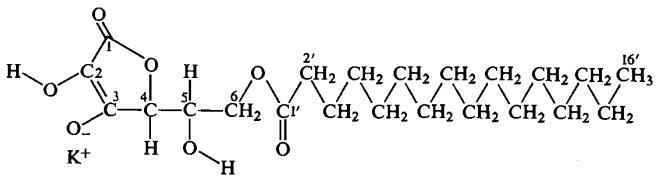

Samples of aqueous 0.1 *M* (4.5 wt.%) potassium or sodium ascorbyl palmitate were obtained by warming the solid salt in the corresponding amount of $D_2O$ to about 50°C in a closed flask; above 40°C the opaque suspension becomes a clear solution which upon cooling turns into a uniform curd at room temperature. The pD (meter reading) of the clear solution was 8.1. Thin samples (15 μm thick in $CaF_2$ cells) of the curd were prepared for infrared spectroscopy according to the detailed methods reported elsewhere (15, 16).

*Spectra and Data Processing*

Infrared spectra were recorded with a Digilab FTS-11 Fourier transform infrared spectrometer equipped with a mercury cadmium telluride detector (Infrared Associates, New Brunswick, NJ). Typically, six hundred scans were accumulated and co-added using a maximal optical retardation of 0.5 cm and a moving mirror velocity of 0.6 cm/s. The resultant interferograms were triangularly apodized and Fourier transformed with one level of zero filling to yield a resolution of ~2 $cm^{-1}$. The spectra of solid samples were recorded as KBr pellets (1 mg sample in 100 mg KBr) of 1 $cm^2$ surface area. Temperature control was achieved by using a hollow cell mount thermostated by a flow of ethanol–water which gives a temperature stability of better than ±0.1°C (17). The temperature was monitored with a thermocouple located against the cell window, and temperatures were continuously recorded by a Newport digital pyrometer equipped with a printer. The spectrometer computer controlled the complete operation of recording a spectrum, printing and incrementing the temperature, waiting for temperature equilibration, and then repeating the sequence (18). Frequencies were measured by determining the center of area of the top five data points of a peak. Bandwidths were obtained by digitally subtracting a linearly interpolated baseline extending from 3000 to 2800 $cm^{-1}$ and computing the widths at ¾ of the peak height. The precision (and reproducibility) of measuring temperature-induced changes in frequency and bandwidth is better than ±0.05 $cm^{-1}$ (19).

The light scattering experiment was performed by measuring the light transmitted at 580 nm through a 1 mm quartz cell containing the system under study. A Cary 219 uv–vis spectrophotometer equipped with the automated temperature reactant accessory and a coupled chart drive was employed.

## Results and Discussion

*General Thermal Properties*

At room temperature potassium ascorbyl palmitate (APK) and sodium ascorbyl palmitate (APNa) are insoluble in water except at extremely low concentrations. However, on heating a water–APK (or water–APNa) mixture, the solubility increases rapidly above a certain temperature, and a clear solution is formed. If the solution is then cooled it solidifies as an opaque curd.

This behavior is well known in ionic surfactant–water systems (8–15). The curd is a semicrystalline mesophase in which the surfactant molecules form lamellar structures. According to the degree of hydration and three dimensional ordering it may be referred to as a gel (well hydrated and unilamellar) or a coagel (poorly hydrated and multilamellar) (20). While X-ray measurements are required to unequivocally determine if at a given temperature a system forms a gel or a coagel, the extreme opacity observed in the aqueous ascorbyl palmitate salts is indicative of a coagel system.

The temperature at which the transition occurs from the coagel (or gel) to the micellar phase is generally referred to as the Krafft point or as the "melting" point of the hydrated solid surfactant. However, the physical meaning of the solubility curve is different from that of ordinary solid–solute equilibrium curves and a stricter definition of the Krafft point is that of the concentration at which the coagel, monomers, and micelles are in equilibrium (10).

The Krafft point is dependent on the concentration and the type of counterion. In the case of 0.1 *M* potassium and sodium palmitate soaps, Krafft points of 30°C and 60°C respectively have been reported (21). Using light scattering techniques we find Krafft points of 48°C and 44°C for 0.1 *M* APK and APNa respectively. That is, the influence of the counterion is much smaller and, contrary to the behavior of the corresponding palmitate soaps, that of sodium ascorbyl palmitate is lower than that of potassium ascorbyl palmitate.

*Infrared Spectra*

The thermotropic properties of potassium ascorbyl palmitate were investigated in detail by infrared spectroscopy. The 1800–1300 $cm^{-1}$ region of the spectra of the anhydrous solid, the ordered coagel, and the isotropic micellar phase of APK are shown in the bottom segment of Fig. 1. The top segments show spectra of sodium ascorbate and ethyl palmitate, accurate frequencies are given in Table 1. It can be seen that, in general, the spectrum of APK is comprised of the summation of the palmitate and ascorbate spectra, particularly in the micellar phase.

Of more significance to this study are the differences between the spectra of the solid, the coagel, and the micellar phases of APK. Considering first the spectra of the solid and the coagel (Fig. 1, C and B respectively), it can be seen that the C=O stretching bands at 1744 and 1735 $cm^{-1}$ are broad-

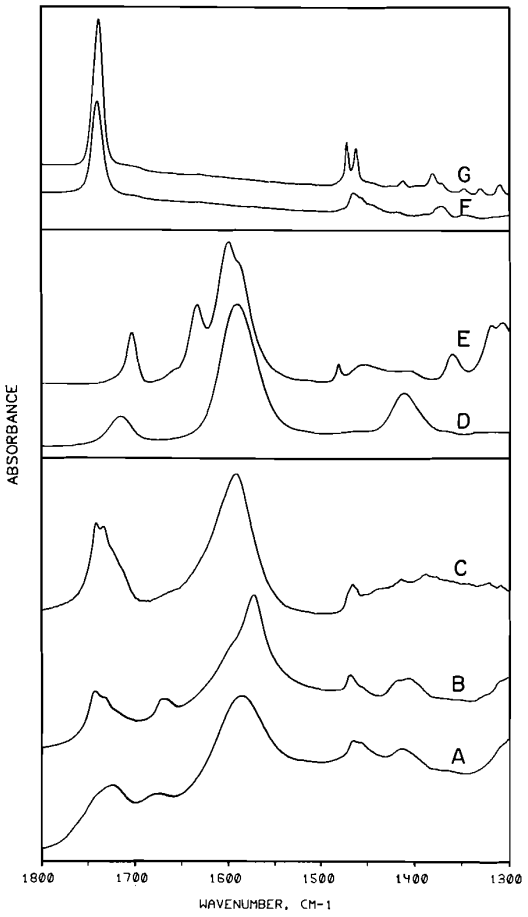


FIG. 1. Infrared spectra in the 1800–1300 $cm^{-1}$ region of 0.1 *M* potassium ascorbyl palmitate in $D_2O$ at 56°C (A) and at 20°C (B), polycrystalline APK at 20°C (C), sodium ascorbate in $D_2O$ (D) and as polycrystalline solid (E), and ethyl palmitate in KBr at 30°C (F) and at 8°C (G).

ened on formation of the coagel, suggesting some degree of hydrogen bond formation with water of hydration. Rather more dramatic changes are evident in the C=C stretching bands. The weak broad band at ~1660 $cm^{-1}$ in the spectrum of the solid gains intensity and has two components in the coagel (see Table 1). Even greater changes are observed in the strong C=C stretching band near 1600 $cm^{-1}$. Formation of the coagel results in a strong narrow band at 1574 $cm^{-1}$, while a weak band near 1600 $cm^{-1}$ is retained. The shift to lower frequency of the main C=C stretching band suggests a reduction of the double bond character of the C(2)=C(3) bond. The sensitivity of this bond to the state of the ascorbate moiety has already been demonstrated. Formation of the sodium salt shifts the C=O stretching band from 1753 to 1702 $cm^{-1}$, and the C(2)=C(3) stretching band from 1670 to 1593 $cm^{-1}$ (23), and increases the C=O and C(2)=C(3) double bond lengths by 0.017 Å and 0.035 Å respectively (24).

The interaction resulting in the shift of the C(2)=C(3) stretching band to 1574 $cm^{-1}$ is specific to the coagel and must result from a particular intra- or intermolecular interaction, possibly involving partial hydration. At this point, we are unable to assign the individual C=C stretching bands, other than to give them the general classification of double bond stretching vibrations associated with the ascorbyl moiety.

The spectrum of the micellar phase is somewhat simpler than that of the coagel. The broad bands in this region are typical of those encountered in solution spectra of materials capable of engaging in extensive hydrogen bond formation, while the bandshapes indicate an enhanced mobility of the particular functional groups. Nonetheless, discrete absorptions are still present, as suggested by the asymmetry of the C=O stretching band contour, and demonstrated by the distinct frequency maxima found after Fourier self-deconvolution (see Table 1).

Another vibrational mode of interest is the $CH_2$ scissoring band around 1468 $cm^{-1}$, which is used extensively for the characterization of the packing of acyl chains in solid hydrocarbons, lipids, and surfactants (25, 26). Its observation at 1471.5 $cm^{-1}$ in the coagel is indicative of a triclinic packing of

TABLE 1. Characteristic group frequencies (in $cm^{-1}$) of potassium ascorbyl palmitate and of selected model compounds[a]

| | Acyl chain vibrations | | Head group vibrations | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\nu_{as}(CH_2)$ | $\nu_s(CH_2)$ | $\nu$(C(1)=O) | | $\nu$(C(1')=O) | | $\nu$(C=C) | | |
| APK, solid (KBr) | 2917.8 | 2850.3 | 1744* | 1735* | 1725* | | ~1660 | 1609* | 1594* |
| APK, coagel ($D_2O$) | 2917.8 | 2850.3 | 1745* | 1733* | 1723* | 1673* | 1663* | 1599* | 1574* |
| APK, micellar ($D_2O$) | 2923.6 | 2853.2 | 1744* | — | 1724* | 1678 | | | 1590 |
| ANa, solid (KBr) | | | — | — | 1702 | | ~1660 | 1602* | 1593* |
| ANa, solution ($D_2O$) | | | — | — | 1717 | | | | 1591 |
| EP solid (KBr) | 2917.0 | 2849.0 | 1744* | 1738* | — | | | | |
| EP melt (KBr) | 2924.9 | 2854.1 | 1742* | 1738* | — | | | | |

[a]Abbreviations: APK, potassium ascorbyl palmitate; ANa, sodium ascorbate; EP, ethyl palmitate. The asterisks indicate frequencies obtained by Fourier self-deconvolution (22), a technique which reduces the spectral linewidth of component bands at the expense of the line shape and the signal-to-noise ratio.

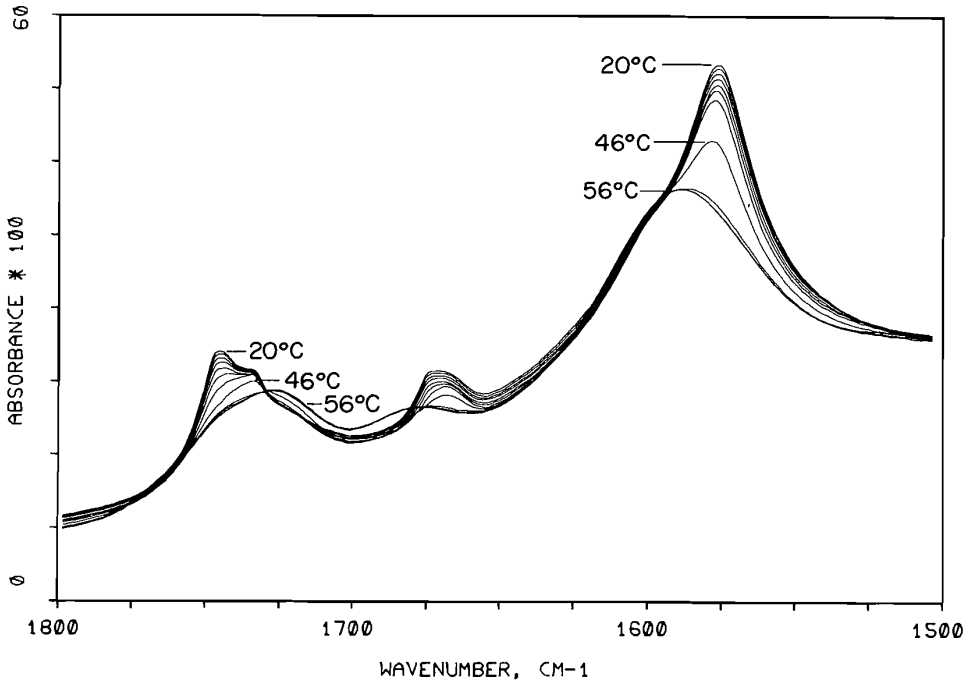


FIG. 2. Infrared spectra in the 1800–1500 $cm^{-1}$ region of 0.1 *M* APK in $D_2O$ as a function of temperature. There are 11 individual spectra taken in 3.6°C intervals; the top spectrum (with the largest peak height) is at 20°C, and the bottom spectrum (with the smallest peak height) at 56°C.

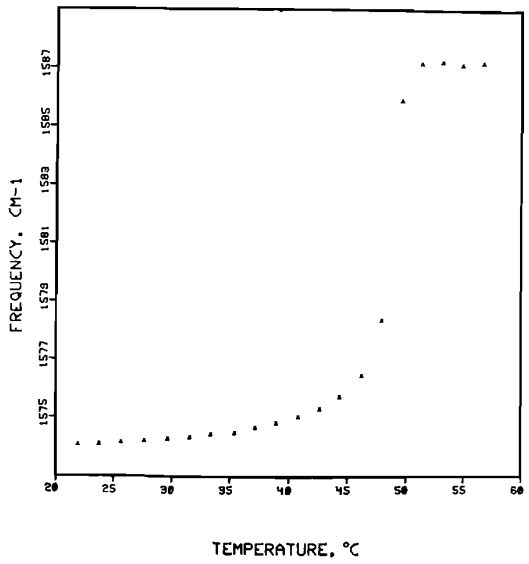


FIG. 3. Temperature-dependence of the frequency (band maximum) of the C=C stretching vibration of APK in $D_2O$.

this surfactant below the Krafft point. Such packing has previously been observed in solid *n*-alkanoates (27).

The temperature dependence of the 1800–1500 $cm^{-1}$ region of the infrared spectrum of 0.1 *M* APK is shown in Fig. 2. The abrupt changes on transition from the coagel to the micellar phase are clearly evident. Also demonstrated are continuous spectral changes prior to micellization. As the temperature is raised from 20°C to ~40°C these changes are evident as reductions in the heights of the bands at 1745, 1673, and 1574 $cm^{-1}$. Other bands characteristic of the coagel (1733 and 1663 $cm^{-1}$) do not change as rapidly. Although there will be a slight increase in the monomer concentration in this range, these spectral changes are too large to be simply accounted for in terms of a decrease in the coagel concentration. Therefore we believe that these changes result from progressive hydration of the palmitate C=O group and of the ascorbate moiety prior to micellication.

In the range 40 to 51°C, the rate of change is much greater. This is illustrated in Fig. 3, which shows a

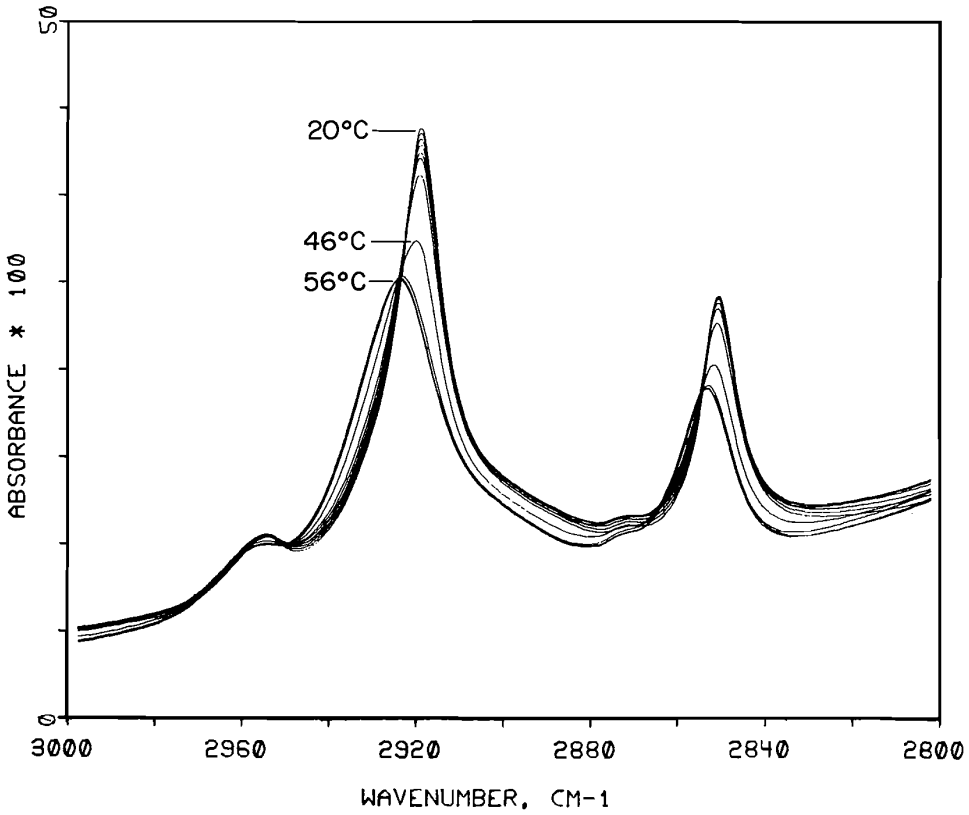


FIG. 4. Infrared spectra in the C—H stretching region of 0.1 *M* APK in $D_2O$. For further details, see caption to Fig. 2.

plot of the temperature dependence of the position of the maximum of the C=C stretching band contour at 1574 $cm^{-1}$. Although the parameter reflects the sum of the changes in the two component bands, it does provide an excellent monitor of the rate of change of the bands. It can be seen that the frequency varies throughout the entire temperature range with a maximal rate of change (>90% of the entire change) between 45 and 51°C (48 ± 3°C). Within this range the changes can be attributed to the continuously changing concentrations of the coagel and micellar phases.

The C—H stretching region of the infrared spectrum is shown in Fig. 4. There are four distinct groups of absorption bands in this region, the strong antisymmetric and symmetric $CH_2$ stretching bands around 2920 and 2850 $cm^{-1}$, respectively, and the weaker asymmetric and symmetric $CH_3$ stretching bands at 2955 and 2873 $cm^{-1}$, respectively. Also evident is a weak Fermi resonance band near 2900 $cm^{-1}$. These bands exhibit a thermotropic behavior similar to that of the vibrational modes originating in the head group.

Two distinct types of spectra are observed in Fig. 4. The spectra at temperatures below 40°C are narrow, indicating relatively low acyl chain mobility and the absolute frequencies are characteristic of fully extended all-*trans* acyl chains. As the temperature is raised, continuous changes are observed prior to the transition to the micellar phase. These changes were monitored by plotting the temperature dependence of the frequency and bandwidth of the symmetric $CH_2$ stretching band, and are shown in Fig. 5. Identical plots (not shown here) were obtained from the temperature-dependence of the antisymmetric $CH_2$ stretching mode.

Below 40°C the frequency indicating all-*trans* acyl chains in the ordered coagel is almost invariant, whereas the bandwidth shows continuous changes between 20 and 40°C. This demonstrates an increase in the acyl chain mobility within this

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