Spectrophotometric Determination of Saturated and Alpha, Beta-Unsaturated Carbonyl in Complex Systems at a Single Wavelength

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Abstract

The simultaneous determination of trace concentrations of saturated and α,β-unsaturated carbonyl compounds in simple or complex systems at a single wavelength is described. From the wavelength of maximum absorbance of the alkaline carbonyl-2,4-dinitrophenylhydrazone determined at a precise time-interval, the total carbonyl, percentage of saturated carbonyl, and μg C = O/Absorbance unit are then subsequently calculated from previously determined trilinear parameters. These parameters are calculated from the relative response of known concentrations of pure (99.6+%) saturated and α,β-unsaturated carbonyl compounds prepared by large-scale high resolution GLC. The method is applicable in simple or complex systems, such as hydrocarbon, aromatic or aliphatic, oxygenated ester, acid, alcohol or ether systems as well as kerosenes and petroleum distillates.

Introduction

THE EARLY LITERATURE describes many methods for the determination of carbonyl compounds or their 2,4-dinitrophenylhydrazones (DNPH). Only a few of them are useful for low concentrations of carbonyl compounds and, almost without exception, they neglect the possible suppression or enhancement from the presence of α,β-unsaturated carbonyl compounds. The applicable early methods have been adequately reviewed (8) and, with the exception of the work by Henick et al. (5), require only brief mention.

Henick, Benca, and Mitchell (5) first described a procedure for determining both saturated and $\alpha\beta$US carbonyl compounds in a mixed system. Their procedure however is quite specific for fats and is based on the formation of the DNPH in benzene solvent by using trichloracetic acid catalyst, followed by spectrophotometric determination of an alkaline solution. Work in this laboratory shows trichloracetic acid to be a poor but acceptable catalyst under carefully controlled conditions. Day and Lillard (1) and Horikx (6) however reported decomposition of hydroperoxides in fats with the subsequent formation of spurious carbonyls, using trichloracetic acid at 60C. Fioriti (2) confirmed their findings. He also observed a marked reduction, but not prevention, of spurious carbonyl formation with reduced temperature and/or the addition of sodium bisulfite or hydrogen iodide to the system. Mizuno and Chipault (11) also described interferences of peroxides in the total carbonyl content of autoxidized fats and oils, using the trichloroacetic acid catalyst of Henick et al.

Gaddis et al. (3,4) separated monocarbonyls as the DNPH from rancid fats with paper chromatographic procedures, followed by spectrophotometric determination of the separate DNPH. More recently they describe the isolation of mono carbonyls by vacuum distillation and testing with Girard T reagent. Mizuno, McMeans, and Chipault (12) separated volatile carbonyls by GLC instead of paper chromatography. They obtained much better separation. By appropriate choice of the stationary phase and adequate standards they were able to identify certain carbonyls from the GLC trace. Jones and Monroe (7) quantitized GLC analysis of mixtures of pure carbonyl-DNPH; Mason, Johnson, and Hamming (10) regenerated specific carbonyls from their DNPH derivatives and identified the carbonyl by mass spectrometer techniques. None of the above separation techniques are useful for practical analytical differentiation of saturated and $\alpha\beta$US carbonyl compounds in complex oxygenated and/or hydrocarbon matrices normally encountered in this laboratory.

A general method for the simultaneous determination of either low or high concentration of carbonyl compounds in complex oxygenated systems at two wavelengths was recently described by Jordan (8). This method is an extension of the earlier work described by Jordan and Veatch (9), who observed anomalous behavior in the absorption spectrum of certain complex systems. The chromaphoric shift to longer wavelengths for maximum absorption of DNPH was erroneously attributed to possible aromatic behavior. The later work showed the chromaphoric shift to be caused by delocalization of the π electrons of the conjugated $\alpha\beta$US carbonyl in the system and not by any aromatic contamination. The present work describes a method whereby both saturated and $\alpha\beta$US carbonyl can be obtained at a single wavelength. Basically the absorbance per μg carbonyl (as $>C=O$) changes linearly with the wavelength for maximum absorption as the ratio of saturated to $\alpha\beta$US carbonyl in the sample changes. From three known parameters which are plotted trilinearly, the saturated and $\alpha\beta$US carbonyl in the samples are easily calculated.

Experimental Procedure

Apparatus and Reagents

Absorbance measurements were made on a Beckman DB Spectrophotometer, with a suitable variable speed recorder, using 1-cm. matched quartz cells.

Carbonyl-free Formula 30 alcohol (95% ethanol, 5% methanol). Two grams of 2,4-dinitrophenylhydrazine and 5 milliliters of concentrated hydrochloric were added to 5 liters of alcohol. The mixture was refluxed for 1 hr, and the alcohol was distilled from an all-glass distillation column.

n-Hexane (95 mole % minimum). This was purified as described for the alcohol except it was refluxed for 12 hr.

2,4-dinitrophenylhydrazine. This was Eastman Kodak Company No. 284, a saturated solution in carbonyl-free Formula 30 alcohol.

2-n-Hexenal. It was purified to better than 99.6

mole % by preparative GLC fractionation, using 4-in. diameter sectioned columns.

2-Ethyl-2-hexenal. This was purified to better than 99.6 mole %, as described for 2-n-hexenal.

n-Hexanal. This was purified to better than 99.8 mole %, as described for 2-n-hexenal.

n-Heptanal. This was purified to better than 99.7 mole %, as described for 2-n-hexanal.

Solution I. This was 3:7 carbonyl-free n-hexane: Formula 30 alcohol.

Recommended Procedure

Accurately weigh an appropriate size of sample into a graduated 25-ml mixing cylinder. (For extremely high carbonyl, blend an appropriate sample in solution I and pipette 5 ml into mixing cylinder.) Add 5 ml of solution I (unless it is a blended sample), 2 ml of 2,4-dinitrophenylhydrazine, and 0.1 ml of concentrated hydrochloric acid to each cylinder. Prepare a reference for each sample exactly the same way but without any sample. Heat in a water bath so that the cylinder contents are immersed below the water level, at 55 ± 1C for 30 min. Remove and cool to room temperature. Since the alkaline DNPH color of saturated aldehydes fades more rapidly with time than for either ketones or $a\beta$US carbonyls, a consistent sequence such as the following should be used.

Dilute one sample and one reference at a time with alcoholic potassium hydroxide solution (29.5 g of potassium hydroxide, 90 ml of water, and 415 ml of Formula 30 alcohol), and mix well. Let the color for each diluted sample and reference develop exactly 6 min before the spectral scan is started. During this time the spectrometer should be balanced with the reference and the sample placed in the sample compartment. (Changes in color will have no effect on balancing a double-beam instrument.) At exactly 6 min the spectral scan is started at 460 mμ and continued to 400 mμ at a rate of 40 mμ/min, using 1-cm matched quartz cells and recorded at a rate of 4 to 6 in./min. Each sample and reference are handled in the same way so that the 6-min color-development time can be maintained.

Calculation

From each spectrum, determine the wavelength of maximum absorbance (λ_{max}) and the absorbance (A) of λ_{max}.

The carbonyl concentration is now calculated by utilizing the three calibration parameters (Figure III), obtained from standards as follows:

determine μg C = O/A unit (μg C = O/A) for λ_{max}.

determine the % saturated carbonyl corresponding to the μg C = O/A for λ_{max}.

Then

$$\frac{\mu\text{g saturated C=O}}{\text{gram sample}} = \frac{(\text{A})\left(\frac{\mu\text{g C=O}}{\text{A}}\right)(\%\text{ saturated})}{\text{sa. wt in grams}}$$

$$\frac{\mu\text{g } a\beta\text{US C=O}}{\text{gram sample}} = \frac{(\text{A})\left(\frac{\mu\text{g C=O}}{\text{A}}\right)(100-\%\text{ saturated})}{\text{sa. wt in grams}}$$

The dotted lines in Figure III represent the way data may be obtained from the trilinear plot for any typical sample.

Preparation of Calibration Parameters

Prepare separate standard solutions of n-hexanal and 2-n-hexenal. The n-hexanal standards should contain from 0.2 to 1.0 μg C = O/ml, and 2-n-hexenal standards should contain from 0.1 to 0.6 μg C = O/ml in solution I. Pipette a 5-ml aliquot of each standard solution into a separate graduated 25-ml mixing cylinder. Add 2 ml of saturated 2,4-dinitrophenylhydrazine and 0.1 ml of concentrated hydrochloric acid to each mixing cylinder. Prepare a reference for each sample, containing reagents but no sample, and proceed as outlined in the procedure above. Determine the wavelength of maximum absorbance (λ_{max}) and the absorbance A for each set of standards. From known weights of standards calculate the μg C = O/A. Both λ_{max} and μg C = O/A should be constant for saturated and $a\beta$US pure carbonyl standards. These results set the limits for parameters of wavelength and μg C = O/A.

TABLE I

Calibration Data from Mixtures of Pure Saturated and a,β-Unsaturated[a] Carbonyl Compounds

Sample	μg C = O/25 ml				Abs. max.	λ max	μg C = O/A
	Sat.	a,β Unsat.	Total	% Sat.			
A	26.11	0	26.11	100	0.755	426.1	34.5
B	13.06	6.14	19.20	68	0.644	432.4	29.8
C	13.06	12.28	25.34	51.5	0.896	438.9	28.3
D	6.53	12.28	18.81	34.7	0.695	442.4	27.0
E	0	12.28	12.28	0	0.502	448.9	24.5

[a] In this case, n-hexanal and 2-n-hexenal.

Prepare a series of mixed saturated and $a\beta$US carbonyl standards from the previously prepared pure standard solutions and ranging from 90% to 10% saturated carbonyl. Analyze these as previously described. Determine λ_{max} and absorbance at λ_{max} for each one. Calculate μg C = O/A for each mixed standard. Plotting μg C = O/A *vs.* λ_{max} from 0 to 100% saturated carbonyl should result in a linear line of constant slope.

Plot the observed parameters on trilinear coordinates shown in Figure III.

Data in Table I show typical results for mixtures of pure saturated and $a\beta$US carbonyls in solvents. These results are a few of the many which were used to develop the trilinear coordinates basic to this work. These data show values for pure saturated and pure $a\beta$US carbonyl slightly different from those described by Figure III. This points out that some error in the method is inherent and is caused by the uncertainly of instrument response. Earlier work (8) was hampered by malalignment of the instrument and a slightly impure $a\beta$US carbonyl standard. Both have been corrected, and the correct wavelength for maximum absorbance is 426 ± 0.4 and 449 ± 0.6 mμ for pure saturated and pure $a\beta$US respectively. Thus the data are within the prescribed uncertainty observed in calibration.

Determination of saturated and $a\beta$US carbonyls which are added to pure solvents is relatively easy after calibration. The practical application and utility of a procedure however is demonstrated only when

TABLE II

Recovery of Carbonyl Blended in a Complex Oxygenated System

Sample	Added μg C = O		Recovered μg C = O		% Recovered	
	Saturated	$a\beta$US	Saturated	$a\beta$US	Saturated	$a\beta$US
A	0	0	115	0	.....	.....
A + B	279	0	397	0	101	.....
A + C	93	0	202	2	97	.....
A + D	93	10	209	9	102	90
A + E	0	118	104	116	91	98
A + F	20	118	128	128	95	108
A + G	0	50	113	50	98	100
A + H	60	50	180	50	103	100

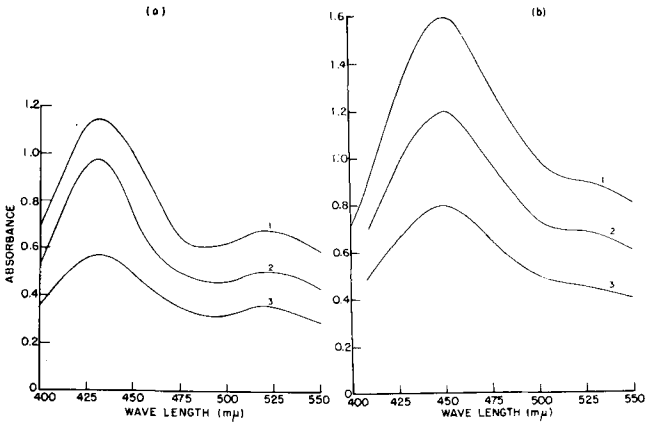

FIG. 1. Spectra of (a) 0.533, 1.066, 1.600; (b) 0.86, 1.29, 1.72; μg C = O/ml of standard n-heptaldehyde and 2-ethyl-2-hexenal respectively, obtained 10 min after addition of alcoholic potassium hydroxide solution.

the same carbonyls are added to and recovered from complex systems that already contain carbonyl in addition to a multitude of other trace contaminants. Table II represents the recovery of both n-hexanal and 2-n-hexenal from a complex oxygenated system.

The recovery is quite good in all cases ranging from 90 to 108%. These additions range up to 51% $a\beta$US carbonyl as 2-n-hexenal. The range of results is in part attributable to the inherent limitation of the instrument. At best, the absorption near λ_{max} is poorly resolved, and this poses a slight uncertainty in correctly defining the wavelength for maximum absorption. Instrumental errors have been minimized, but not eliminated, by expanding the spectrum in the critical area with a variable speed recorder.

An extensive study describing the recovery of added saturated and $a\beta$US carbonyls from complex systems, which contained up to 40% of their carbonyl impurity as $a\beta$US carbonyl prior to addition has been previously described (8). Since the procedure described here is a modification of the earlier work, it is unnecessary again to present the voluminous recovery data. Rather a comparison of results on typical samples obtained by the two similar procedures will show the recovery potential of the procedure described by this work, provided the results are equivalent. Data in Table III describe results by both the differential wavelength and single wavelength procedures for several typical samples ranging from zero to 30% $a\beta$US carbonyl. The data show both procedures to yield essentially equivalent results. There is no significant difference in results by the two procedures. However there appears to be a slight negative bias on the low $a\beta$US carbonyl results. This trend reverses for high values. This is the expected trend since instrument limitation becomes relatively more significant as the limits of either pure saturated or $a\beta$US carbonyl are approached. All the data however are based on similar instrument response except that in the differential procedure two constant wavelengths are used instead of a continuously variable wavelength between the two narrow limits described by this procedure.

Figure 1 shows comparative absorption spectra for typical DNPH of saturated and $a\beta$US carbonyls. Aldehydes and ketones, unsaturated but not conjugated, also have the characteristic spectrum of saturated carbonyls. The spectra in a) and b) represent three different concentrations of each carbonyl. These spectra were obtained six minutes after the formation of the alkaline hydrazone and were used

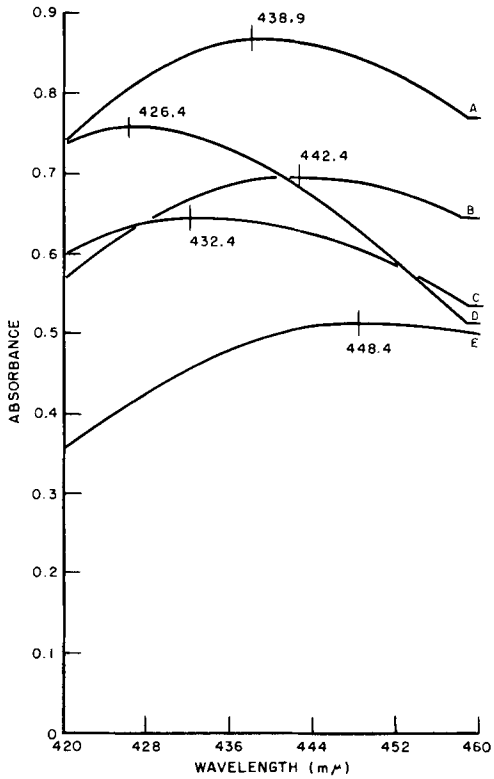

FIG. 2. Expanded spectra of absorbance *vs.* wavelength, showing λ_{max} for mixtures (A) 51, 49% n-hexanal, 2-n-hexanal; (B) 35, 65% n-hexanal, 2-n-hexanal; (C) 68, 32% n-hexanal, 2-n-hexanal; (D) pure n-hexanal; (E) pure 2-n-hexanal respectively.

to develop the wavelength and μg C = O/A limits for the procedure covered by this work. Part a) demonstrates typical spectra for saturated carbonyls with a primary and secondary maximum at 426 and 520 mμ respectively whereas part b) represents typical $a\beta$US carbonyl with maxima at 449 and near 520 mμ respectively. From the representative curves in part a) and b) it becomes readily apparent that qualitatively saturated ketones, as well as aldehydes, may be differentiated from $a\beta$US carbonyl compounds. Saturated aldehyde and ketone DNPH show peak absorbance near 426 mμ and a secondary maximum near 520 mμ; $a\beta$US carbonyls peak near 450 mμ with a secondary near 520 mμ. The saturated aldehyde-DNPH alkaline color fades rapidly both at 426 and near 525 mμ; neither the saturated ketone

TABLE III

Comparative Results Obtained Between the Proposed Single and the Differential Wavelength Procedures

Sample No.	μg Saturated C = O/g		μg $a\beta$US C = O/g	
	Diff. procedure	Single λ	Diff. procedure	Single λ
19873	126	127	1	1
19874	132	138	7	6
19875	123	130	10	6
19876	124	130	10	5
19877	77	78	14	10
19878	96	102	8	5
48	74	77	23	21
19879	66	67	1	0
19880	60	62	0	0
19881	94	95	6	6
19882	114	120	4	0
19883	63	70	20	15
19884	51	59	25	21
55	74	74	11	10
18010	3000	2945	1137	1145
18479[a]	3408	3353	969	1002
1039	189	193	2	2
9072	447	414	32	57
615B	630	633	27	37

[a] Average of five determinations.

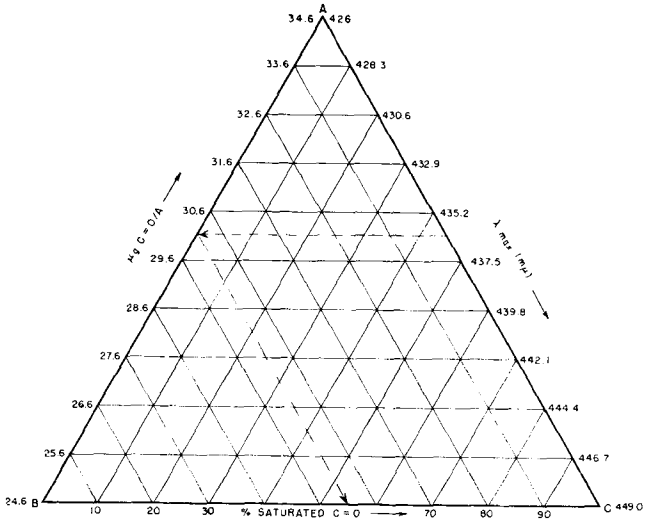

FIG. 3. Trilinear coordinates, showing relationship between μg C = O/A, λ_{max}, and % saturated carbonyl. Dotted line simulates the use of these trilinear coordinates.

or $\alpha\beta$US carbonyl-DNPH fades rapidly. Thus, by wavelength for primary and secondary maximum absorbance, general types can be suggested.

Figure 2 shows a portion of the spectrum covering the narrow wavelength as it is expanded to show the range of maximum absorption observed in this work. Curves D and E represent pure n-hexanal and 2-n-hexenal respectively. The other curves, *i.e.*, A, B, C, represent different blends of the two pure components and show how the wavelength for maximum absorption shifts as the amount of either component changes. For example, as 2-n-hexenal compared with n-hexanal decreases, the λ_{max} shifts closer to that for pure n-hexanal and is described by Curve C. Likewise, as 2-n-hexenal compared with n-hexanal increases, λ_{max} shifts toward that for pure 2-n-hexenal and is described by Curve B. Of general interest, too, is the great change in the shape of the curves over this expanded region in going from pure saturated to pure $\alpha\beta$US carbonyl. This change graphically amplifies the visual general change observed in Figure 1.

Figure 3 graphically shows the trilinear coordinates which are obtained from calibration data and used to calculate saturated and $\alpha\beta$US carbonyl at a single wavelength. Essentially this shows that the μg C = O/A decreases and wavelength of maximum absorption increases as the percentage of saturated carbonyl decreases and that these parameters change linearly. The dotted lines describe a typical interpretation for calculating a sample composition from an observed λ_{max}.

Figure 4 shows expanded spectral scans over the narrow wavelength limits previously described for some typical samples shown in Table III. Again the great difference in spectral shape is apparent and represents samples ranging from 100% saturated in curves 6 and 3 to about 50% saturated in curve 1. The other curves are similar to those between 100 and 50% saturated carbonyl. The reason for the steep slope observed for curve 6, compared with that for curve 3, is not known except that increasing the sample concentration did partially correct the difference. The similarity is however quite apparent. Curve 5 is quite similar to curves 6 and 3, but a distinct

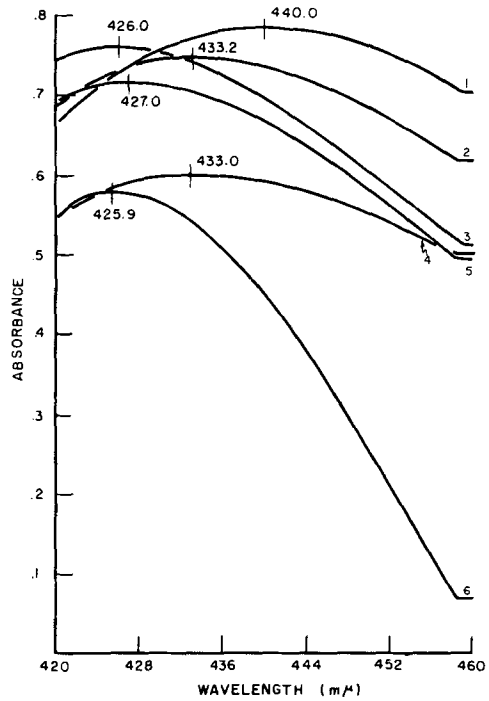

FIG. 4. Expanded spectra of typical samples, showing absorbance *vs.* wavelength. Samples range from pure saturated curve 6, and 3 up to about 50% α,β-unsaturated curve 1.

flattening of curve 5 is noted even though the maximum absorbance has shifted only 1 mμ. This flattening and gradual parabolic change becomes more pronounced with curves 4 and 2 until the trend of the curve shape shows a marked reverse change with curve 1.

Discussion

The work was done on a Beckman DB Spectrophotometer. Both the Cary Model 14 and BL 505 spectrophotometers recorded slightly different wavelengths for absorbance maximum and different sensitivity from the Beckman DB. Thus the limiting wavelength and μg C = O/A parameters will, to a minor degree, depend on the instrument used.

Normally the high quality reagents used to prepare solution I require no further treatment. However both Formula 30 alcohol and n-hexane contain minute $\alpha\beta$US carbonyl impurities. Further purification of these two reagents is necessary in order to obtain optimum results.

With the appearance of large-scale high resolution preparative GLC, sample purity is no longer difficult. The 2-n-hexenal purchased contained only 60–70% of the desired component. This was purified to near 99.9% in just minutes. The other standards used were also purified the same way. All the samples except 2-ethyl-2-n-hexenal were stable. It started degrading after a few weeks on the shelf and is not recommended as a standard unless purification equipment is readily available. Any other carbonyl standards can be as easily used, provided adequate purification is carried out.

A six-minute time-interval after the addition of alcoholic potassium hydroxide solution was chosen because this allows the color to develop adequately and the color reduction because of the time of the reference and sample are still nearly equivalent. The reduction in color with time becomes important because of the greater color stability of $\alpha\beta$US and

aliphatic ketone carbonyl-DNPH compared with saturated aldehydic carbonyl-DNPH. A separate reference for each sample is necessary so that all the results will be on a uniform basis since the color reduction for samples and a reference will eventually exceed the usable 15-minute time limit discussed in a previous work (9), whereby significant errors in the comparative absorbance between saturated and $\alpha\beta$US carbonyl-DNPH systems and the reference occur. Additionally, unless an exact time-interval is used, spurious variable limiting parameters, especially for the saturated aldehydes carbonyl-DNPH, will be observed. Other time intervals could also be chosen, provided they fall within the 5- to 10-minute range. Of greater importance however is the absolute necessity for maintaining a constant time-interval for all samples since even a deviation of a few seconds from the selected interval will have a noticeable effect on the results.

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