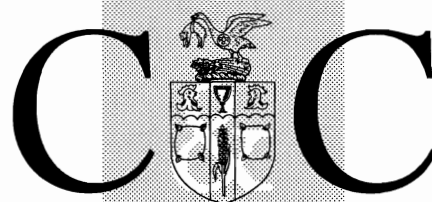


# R&D REPORT

## NO. 20

### Interaction Between Additives in Food Systems

June 1995



**Campden & Chorleywood**  
Food Research Association





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## Interaction Between Additives in Food Systems

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June 1995

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## SUMMARY

There has been much publicity given to the addition of chemicals to food. One area of concern is the interaction between additives and the possible health risk of the additive cocktail that is consumed each day. From the list of approved additives, sulphur dioxide, ascorbic acid and nitrite were selected for further study because of their known chemical reactivity, their frequent use in combination with other additives, and the possible formation of potentially toxic compounds. Of the published interactions, those between sulphur dioxide or ascorbic acid and the azo food dyes required further study, particularly with regard to the identification of dye breakdown products.

Kinetic studies of Amaranth, Black PN, Carmoisine, Ponceau 4R, Sunset Yellow and Tartrazine were carried out to determine the conditions necessary to obtain degradation products that could be identified by mass spectrometry.

- ♦ In the presence of bisulphite, dye degradations fell into two groups: those that produced secondary dyes, and those that lost colour without change in the wavelength of maximum absorbance of the visible spectrum. The rates of secondary dye formation were pH dependent.
- ♦ In the presence of ascorbic acid, the azo dyes were reduced to colourless compounds at rates that depended on pH.
- ♦ Sunset Yellow degradation in model systems and in a commercial soft drink under simulated retail conditions depended on bisulphite and ascorbic acid levels and also on exposure to light.
- ♦ Separation of Sunset Yellow from its degradation products was achieved by HPLC and capillary electrophoresis.
- ♦ Mass spectrometric identification of Sunset Yellow degradation products was attempted without prior separation. This was not a successful approach.

The results for Sunset Yellow suggest that current levels of sulphur dioxide and ascorbic acid in soft drinks do not cause significant azo dye degradation under normal storage conditions. However, this picture could change if sulphur dioxide or ascorbic acid levels are increased to meet market demands. Further work is required to identify the dye breakdown products that could potentially be formed.



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## 1. INTRODUCTION

In recent years there has been much publicity given to consumer concerns about the addition of chemicals to food. One area of concern is the interaction between additives and the possible health risks or joint effects of the additive cocktail that is consumed each day. Whilst food toxicologists acknowledge this potential, there is a lack of scientific data demonstrating adverse effects. In the absence of such data, the possibility of adverse interactions is taken into account by adopting substantial safety margins between no-effect levels observed in animals and the maximum levels of additives to which humans could be exposed.

Considering the large numbers of food additives permitted for use, a strategy for prioritisation of study of interactions on the basis of known chemistry was required. The strategy developed took into account the following factors:-

- (1) Known chemical reactivity of individual additives (food science literature).
- (2) Usage of additive combinations (MAFF Food Products Database).
- (3) Potential toxicity of reaction products (food toxicology literature).

From the list of additives approved for use in the UK, sulphur dioxide, ascorbic acid and nitrite were selected for further study because of their known chemical reactivity with other compounds, their frequent use in combination with other additives, and the formation of potentially toxic compounds from their reactions with other additives.

Of the known interactions, those between sulphur dioxide or ascorbic acid and the azo food dyes were considered to be the most suitable for laboratory studies in the first instance. The frequent use of combinations of these additives, especially in soft drinks, and the possible toxic properties of the dye degradation products so far identified, were strong arguments in favour of this proposal.

The present study therefore set out to determine some of the properties of the breakdown products of azo dyes in the presence of sulphur dioxide and ascorbic acid. Preliminary work was carried out on the rates of loss of six of the most frequently used azo dyes at elevated temperatures and in the presence of high sulphur dioxide and ascorbic acid levels. The loss of Sunset Yellow was then further investigated in a model soft drinks matrix and in a real commercial soft drink under realistic storage conditions. Finally, the degradation products of Sunset Yellow in the presence of sulphur dioxide were studied using high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and electrospray-mass spectrometry (ES-MS).

The following interactions have been reported in the literature and can potentially occur in foodstuffs containing additive combinations:

## **1.1 Sulphur Dioxide Interactions**

### **1.1.1 With azo dyes**

This is an important interaction because of the frequent combined use of sulphur dioxide and azo dyes, particularly in soft drinks, but also to a lesser extent in jams, sauces and other products.

The stability of the mono-azo dyes is variable (Wedzicha, 1984; Kroyer, 1986a). Reduction of the azo bond to the hydrazo product can occur as in the case of carmoisine (Wedzicha and Rumbelow, 1981). Early work had suggested that irreversible formation of potentially toxic amine could occur when the hydroxyl group was in the 2-position of one of the aromatic rings but this was not studied in this case.

Sulphur dioxide can also cause sulphonation of azo dyes as in the case of Sunset Yellow FCF in soft drinks (Damant *et al.*, 1989).

Disazo and other more complex azo dyes have fair to good stability to sulphur dioxide but the degradation products do not appear to have been studied in depth.

### **1.1.2 With other dyes**

Green S, a triarylmethane dye, has good stability to sulphur dioxide whereas Violet BNP, also a triarylmethane dye, has poor stability (Wedzicha, 1984). The products of decolorisation are probably sulphonates.

Indigo carmine has poor stability to sulphur dioxide although the products are unknown.

Quinoline Yellow, the only permitted Quinoline food colour, is reported to have excellent stability to sulphur dioxide.

The natural anthocyanin pigments form adducts with bisulphite ion. The ion adds to position 2 or 4 of the flavylum nucleus, decolorising the pigment and simultaneously conferring high heat stability on the glycosidic bond (Adams and Woodman, 1973).

### **1.1.3 With ascorbic acid degradation products**

In the presence of oxygen, ascorbic acid is oxidised to dehydroascorbic acid which can react with bisulphite ion to give the monohydroxysulphonate adduct. Under anaerobic conditions, dehydration and decarboxylation of ascorbic acid can cause the formation of 3,4-dideoxypentosulos-3-ene which can then react with bisulphite ion to give the corresponding 4-sulphopentosulose (Wedzicha, 1984).

### **1.1.4 With sorbic acid**

The bisulphite ion reacts slowly with sorbic acid at ambient temperature and acidic pH to give an adduct (Heintze, 1976; Khandelwal and Wedzicha, 1990).

### **1.1.5 With nitrite ion**

Nitrite and bisulphite ions can react to form products which are sulphonates of either hydroxylamine or ammonia (Wedzicha, 1984).

### **1.1.6 With B vitamins**

Sulphur dioxide can interact chemically with several B vitamins (Wedzicha, 1984). It can react readily with thiamin (vitamin B1), rendering it biologically inactive. One reason for restricting the use of sulphur dioxide in meat products is to prevent this interaction as meat is regarded as an important source of the vitamin.

Riboflavin (vitamin B2), pyridoxal phosphate (vitamin B6) and folic acid can form adducts with bisulphite although the effect on biological activity remains to be established.

## **1.2 Ascorbic Acid Interactions**

### **1.2.1 With azo dyes**

As with sulphur dioxide, the interaction of ascorbic acid with azo dyes is important because the combination is frequently used and also because of the possible toxic properties of the dye degradation products such as aniline and the sulphonated aromatic amines (Fogg and Summan, 1983a and 1983b, 1984; Kroyer, 1986b; Marovatsanga and Macrae, 1987).

### 1.2.2 With nitrite ion

Ascorbic acid reacts with nitrite ion, at pH values found in meat curing, to give dinitrosyl ascorbate (Fox *et al.*, 1981; Sebranek and Fox, 1985). Under anaerobic conditions, decarboxylation of ascorbic acid can lead to formation of 4,5-dihydroxy-2-oxopentanal which can then react with nitrite (Wedzicha *et al.*, 1982).

### 1.2.3 With aspartame

Cupric ion catalysed oxidation of ascorbic acid occurs more rapidly in the presence of aspartame. This is presumably due to the formation of a complex between the cupric ion and aspartame which is a more effective autoxidation catalyst than cupric ion alone (Hsieh and Harris, 1991). However, there appears to be no information on the degradation of aspartame under these conditions.

## 1.3 Nitrite Interactions

### 1.3.1 With chloride

Nitrite can react with chloride at pH values found in meat curing to give nitrosyl chloride (Sebranek and Fox, 1985).

### 1.3.2 With sorbic acid

Under acidic conditions, nitrite can react with sorbic acid or its methyl ester to give products which have mutagenic activity (Osawa and Namiki, 1982). In the case of the 1,4-dinitro-2-methyl pyrrole interaction product, addition of ascorbic acid abolished the mutagenicity, apparently as a result of reduction of the C-4 nitro group to a C-amino group.

### 1.3.3 With cysteine

Nitrite can react with cysteine under mild acid conditions to give S-nitrosocysteine (Byler *et al.*, 1983; Massey *et al.*, 1986). In many food products, cysteine would be naturally present rather than as an additive.

## **2. MATERIALS AND METHODS**

### **2.1 Kinetic Studies**

#### **2.1.1 Azo dye interactions with bisulphite**

Sunset Yellow, Amaranth, Ponceau 4R, Carmoisine, Black PN and Tartrazine were used as supplied by Unbar Rothon Ltd. without purification. Each dye was dissolved at 0.1mM in a 100mM citric acid solution containing 50mM sodium metabisulphite and the pH value adjusted to either 3.0 or 4.0, as appropriate, with citric acid. The solutions were then heated at 70°C in stoppered glass containers. Control samples of dyes were heated in the absence of metabisulphite. Visual colour changes were noted, and spectra measured in the 200-700nm region using a Unicam 8800 UV/VIS spectrophotometer with a 1mm pathlength quartz cuvette.

#### **2.1.2 Azo dye interactions with ascorbic acid**

The same dyes as used in the bisulphite studies were made up at 0.1mM in a 100mM citric acid solution containing 5.7mM (1000ppm) ascorbic acid and the pH value adjusted to either 3.0 or 4.0 with citric acid. The solutions were then heated at 70°C in capped plastic tubes. A control sample containing 5.7mM ascorbic acid in 100mM citric acid pH 3 (no azo dye) was also heated at 70°C. Visual colour changes were noted, and spectra measured in the 200-800nm region using a 1mm pathlength quartz cuvette.

#### **2.1.3 Sunset Yellow interactions with bisulphite and ascorbic acid**

##### **2.1.3.1 *Effect of temperature in the presence of bisulphite***

Sunset Yellow was dissolved at 0.17mM (70ppm) in a 100mM citric acid solution containing 50mM sodium metabisulphite and the pH value adjusted to 3.0 with citric acid. The solution was then held in stoppered glass containers at 20, 30, 50 and 70°C for up to 5h. Absorbance changes at 480nm were measured using a 1mm pathlength quartz cuvette.

##### **2.1.3.2 *Effect of bisulphite concentration***

Sunset Yellow was dissolved at 0.17mM (70ppm) in 100mM citric acid solutions containing 5, 30, 50 and 100mM sodium metabisulphite and the pH values adjusted to 3.0 with citric acid. The solutions were then heated at 70°C in stoppered glass containers. Absorbance changes at 480nm were measured using a 1mm pathlength quartz cuvette.

As a preliminary experiment on the preparation of the lemon yellow secondary dye, essentially salt-free for characterisation by mass spectrometry, the rate of Sunset Yellow degradation was studied in the presence of 100mM sulphurous acid (equivalent to 50mM sodium metabisulphite). The Sunset Yellow solution was adjusted to pH 3.0 with ammonia and heated at 70°C in a stoppered glass container. Absorbance changes at 480nm were measured.

#### **2.1.3.3 *Effect of bisulphite and ascorbic acid under aerobic and anaerobic conditions***

Sunset Yellow was dissolved at 0.17mM (70ppm) in 100mM citric acid solutions containing the following levels of sodium metabisulphite and ascorbic acid:-

1. 50mM sodium metabisulphite alone.
2. 50mM sodium metabisulphite and 71mM ascorbic acid.
3. 50mM sodium metabisulphite and 142mM ascorbic acid.
4. 142mM ascorbic acid alone.

After adjusting the pH of each solution to 3.0 with citric acid or sodium citrate, 3ml aliquots were heated at 70°C, in air and in vacuo, in a 1cm pathlength quartz anaerobic cuvette. Absorbance changes at 480nm and 414nm were measured during the heat treatment.

#### **2.1.3.4 *Degradation of lemon yellow secondary dye***

100ml of 0.17mM (70ppm) Sunset Yellow solution containing 100mM sulphurous acid was adjusted to pH 3.0 with ammonia and heated at 50-70°C. When near complete conversion to the lemon yellow dye had taken place, the pH was lowered to <1.0 with hydrochloric acid and the solution sparged with nitrogen to remove gaseous SO<sub>2</sub>. The solution was then freeze-dried.

The lemon yellow dye preparation was dissolved at 140ppm in 100mM citric acid solutions containing the following levels of sulphurous and ascorbic acids:-

1. 100mM sulphurous acid.
2. 71mM ascorbic acid.
3. 142mM ascorbic acid.

After adjusting the pH of each solution to 3.0 with ammonia, they were heated at 70°C in stoppered glass containers. Absorbance changes at 414nm were measured.

## 2.2 Sunset Yellow Storage Trials

The following additions were made to solutions containing 0.17mM (70ppm) Sunset Yellow and 1.8mM sodium benzoate in 100mM citric acid solution, pH 3.0:-

1. No addition control (Sunset Yellow and sodium benzoate in citric acid).
2. 0.45mM ascorbic acid (80ppm).
3. 0.91mM ascorbic acid (160ppm).
4. 2.27mM ascorbic acid (400ppm).
5. 0.39mM sodium metabisulphite (0.78mM bisulphite or 50ppm SO<sub>2</sub>).
6. 0.78mM sodium metabisulphite (1.56mM bisulphite or 100ppm SO<sub>2</sub>).
7. 3.12mM sodium metabisulphite (6.24mM bisulphite or 400ppm SO<sub>2</sub>).
8. 0.39mM sodium metabisulphite and 0.45mM ascorbic acid.
9. 0.39mM sodium metabisulphite and 2.27mM ascorbic acid.
10. Commercially available soft drink containing 50ppm SO<sub>2</sub> and 80ppm ascorbic acid.

Treatments 1-9 were sparged with nitrogen, aliquoted into glass universals and the headspace flushed with nitrogen before capping. Treatment 10 was used as supplied. Duplicate samples were stored for each treatment and for each sampling time. Samples were stored at 20°C and 50°C in light from Philips Colour 83 fluorescent tubes. The positions of the samples were adjusted in an attempt to obtain equal incident light intensities at both storage temperatures. Dark conditions were obtained by covering the sample bottles in aluminium foil.

At 20°C, absorbances at 414nm and 480nm were measured at zero time and after 3, 6, 9 and 12 months storage. At 50°C, absorbances were measured at zero time and after 4, 8 and 12 weeks storage. At the beginning and end of each storage trial, sulphur dioxide was determined by Monier-Williams distillation (CCFRA Reference TES-AC-094) and total ascorbic acid (Vitamin C) by autoanalysis (CCFRA Reference TES-AC-209).

## 2.3 Separation and Identification of Sunset Yellow Degradation Products

Sunset Yellow was degraded in the presence of either bisulphite or ascorbic acid. Separation of the degradation products from each other and from the original dye was then studied using HPLC and CE. Electrospray-mass spectrometry was investigated as a method of identifying the degradation products.



### 2.3.1 *HPLC studies*

A Gilson HPLC system was employed that involved connecting a Model 302 pump to a 150 x 406mm stainless steel column packed with Spherisorb ODS-2. Samples were introduced via a Rheodyne 7125 syringe loading port with a 100 $\mu$ l loop. Aqueous methanolic solvents, containing an ion-pair agent, were pumped through at 1.0ml.min<sup>-1</sup>. Eluted compounds were detected with a Holochrome variable wavelength detector set at either 237, 414 or 480nm (0.1 AUFS). Absorbance data was collected using a Datamaster. The following solvent systems were employed:-

System 1 - 40mM ammonium acetate (solvent A) and 40% methanol/60% 40mM ammonium acetate (solvent B). 0-8 minutes solvent A, 8-25 minutes solvent B, 26-30 minutes solvent A.

System 2 - Isocratic separation using 6mM tetrabutylammonium phosphate (TBAP) in methanol/water 42.5 : 57.5(v/v) as solvent.

The following samples were analysed:-

- 1) 0.17mM Sunset Yellow in deionised water (Milli-Q).
- 2) Lemon yellow dye solution prepared by heating 0.17mM Sunset Yellow and 50mM metabisulphite in deionised water at 70°C.
- 3) Sample 2 further heated at 70°C to a pale yellow solution.

Fractions were collected from the HPLC column to confirm the separation of peaks by UV/VIS spectrophotometry.

### 2.3.2 *Preparation of lemon yellow dye degradation products*

The following method was used to prepare degradation products of the lemon yellow dye for CE and ES-MS studies:-

100ml of 0.17mM Sunset Yellow was heated at 70°C under dark conditions in 100mM sulphurous acid adjusted to pH3 with ammonia. When the solution appeared very pale yellow in colour, the excess sulphur dioxide was removed by acidification to < pH 1.0, sparging with nitrogen and freeze-drying.

### 2.3.3 *Capillary electrophoresis studies*

CE was performed using a SpectraPHORESIS 1000 CE system with a SpectraPHORESIS 1000 V1.05 data system. The following conditions were used:-

Fused silica capillary (i.d. 50 $\mu$ m), 44cm total length and 37cm effective length.

UV/VIS detector for multiwavelength scanning.

Running voltage, 25kV (Pos).

Running buffer - 20mM ammonium acetate, pH 9.0.

Injection mode - hydrodynamic, 5 and 20 seconds.

The capillary was conditioned with 100mM sodium hydroxide for 15 minutes followed by buffer for 30 minutes. Between runs, the capillary was purged with 100mM sodium hydroxide for 5 minutes followed by buffer for 10 minutes. At the end of each day, the capillary was rinsed with Milli-Q water for 10 minutes.

The samples analysed were:-

- 1) 0.17mM (70ppm) Sunset Yellow in deionised water (Milli-Q).
- 2) Lemon yellow dye solution equivalent to a starting concentration of 0.34mM (140ppm) Sunset Yellow.
- 3) Degraded lemon yellow dye solution equivalent to a starting concentration of 0.85mM (350ppm) Sunset Yellow.
- 4) 0.58mM sulphanilic acid.

All samples were passed through 0.45 $\mu$ m membrane filters before analysis.

### 2.3.4 *Electrospray-mass spectrometry studies*

Analyses were performed using a VG Quattro mass spectrometer interfaced with an electrospray ionization source. All samples were analysed using the negative ionization mode of operation.

The operating parameters for the electrospray system were as follows:-

Solvent System - 50% Methanol (aq) + 0.1% ammonium acetate.

Nebulising gas flowrate	- 15 litre/hour
Drying gas flowrate	- 200 litre/hour
LC pump	- Gilson 303 analytical LC pump
Injection valve	- 7125 Rheodyne injection valve

The range of operating parameters for the mass spectrometer were as follows:-

Capillary potential	-	3,500V
High voltage lens potential	-	300V
Focusing lens	-	40 - 70V
Source temperature	-	50 - 70°C
Ion energy	-	0.3 - 1.0V
Energy ramp	-	0.0 - 0.2V
Low mass resolution	-	10.5 - 14.0
High mass resolution	-	12.5 - 16.0
Entrance filter	-	2.5 - 5V
Intermediate multiplier	-	650V

The focusing lens was tuned daily to achieve optimal response.

The samples analysed were:-

- 1). 0.17mM (70ppm) Sunset Yellow in deionised water (Milli-Q).
- 2). Lemon yellow dye solution, equivalent to a starting concentration of 0.34mM Sunset Yellow.
- 3). Degraded lemon yellow dye solution equivalent to a starting concentration of 0.34mM Sunset Yellow.
- 4). 0.17mM Sunset Yellow solution + 71mM ascorbic acid held for 7 days at 70°C.
- 5). 0.06mM sulphanilic acid.

All samples were passed through 0.45 $\mu$ m membrane filters before analysis.

### 3. RESULTS AND DISCUSSION

#### 3.1 Kinetic Studies

These studies were carried out to determine the conditions necessary to obtain degradation products of azo dyes that could be identified subsequently by mass spectrometry. Of the 9 azo dyes currently in use in the UK food industry, 6 were selected for study because of their frequent use in combination with sulphur dioxide or ascorbic acid (MAFF Food Products Database, 1992). These were Amaranth, Black PN, Carmoisine, Ponceau 4R, Sunset Yellow and Tartrazine (Figure 1).

##### 3.1.1 Azo dye interactions with bisulphite at pH 3.0 and pH 4.0

No colour changes were observed on heating the azo dyes for 6h at 70°C at pH 3.0 and pH 4.0 in the absence of bisulphite, and this was confirmed by spectral measurements.

The loss of dye absorbance on heating at 70°C in the presence of 50mM sodium metabisulphite (pH 3.0) is shown in Figure 2. After 1h heating, the order of dye stability (as % retention of  $A_{\max}$ ) was Tartrazine > Carmoisine > Ponceau 4R > Amaranth  $\approx$  Sunset Yellow > Black PN. However, this order was affected by heating time due to the formation of secondary dyes in the case of Sunset Yellow, Ponceau 4R and Amaranth (Figure 3; Table 1). The spectra of the new dyes overlapped significantly with those of the original azo dyes so that accurate rate constants for degradation could not be calculated.

At pH 4.0, loss of dye absorbance was associated with similar colour and spectral changes to those that occurred at pH 3.0. The apparent order of dye stability after 1h heating was different, however, being Tartrazine > Carmoisine > Amaranth > Black PN > Ponceau 4R > Sunset Yellow (Figure 4). This difference in stability order was due to the relatively rapid rates of conversion to the secondary dyes, particularly for Sunset Yellow and Ponceau 4R (Figure 5). As bisulphite ion is the predominant form of sulphurous acid at both pH values, the rapid conversion rates at pH 4.0 can best be explained by greater dye reactivity at the higher pH value.

Table 1

**Azo Dye Colour and Spectral Changes in the Visible Region on Treatment  
with Metabisulphite at pH 3.0**

Dye	Colour Change	$\lambda_{\text{max}}$ Change (nm)
Sunset Yellow	Orange → Yellow	480 → 414
Ponceau 4R	Red → Yellow	505 → 435
Amaranth	Red → Orange	520 → 455
Black PN	Blue/purple → Faded Blue/purple	None
Tartrazine	Yellow → Faded Yellow	None
Carmoisine	Red → Faded Red	None

The azo dyes in this study fell into two groups regarding their reactions with bisulphite, those that formed secondary dyes and showed a corresponding shift in  $\lambda_{\text{max}}$ , and those that lost colour (faded) without  $\lambda_{\text{max}}$  changing (Table 1). Sunset Yellow, Amaranth and Ponceau 4R all have hydroxyl groups *ortho* to the azo bond as well as unsubstituted *para* positions in the naphthalene nucleus. Such dyes exist predominantly as hydrazone tautomers rather than strictly azo compounds. This facilitates the addition of bisulphite ion to the *para* position, as demonstrated by Damant *et al* (1989) for the conversion of Sunset Yellow to a lemon yellow compound. Carmoisine and Tartrazine did not form secondary dyes on reaction with bisulphite. In the case of Carmoisine, a hydrazone tautomer is feasible but addition of bisulphite ion at the *para*-position appears not to take place probably as a result of charge delocalisation via the fused aromatic ring of the naphthalene system. Instead it has been suggested that one molecule of the dye reacts with one molecule of bisulphite to form an unstable complex that hydrolyses to a colourless hydrazo product (Wedzicha and Rumbelow, 1981). No structural evidence was presented for such a complex, however. In Tartrazine, the *ortho*-hydroxy group is a substituent of the pyrazoline ring that does not possess the  $\pi$ -electron mobility of a truly aromatic system. A hydrazone tautomer is therefore unlikely to form and the dye is consequently much more stable in the presence of bisulphite.

### 3.1.2 Azo dye interactions with ascorbic acid at pH 3.0 and pH 4.0

The loss of the original dye absorbance on heating at 70°C in the presence of 5.7mM (1000ppm) ascorbic acid (pH 3.0) is presented in Figure 6. After 1h heating, the order of dye stability (as % retention of Amax) was Tartrazine > Sunset Yellow  $\approx$  Amaranth  $\approx$  Ponceau 4R > Carmoisine > Black PN. This remained the same over 5h heating suggesting that no stable secondary dyes formed under these conditions. Confirmation of this was the visual fading of colour with no shift in the  $\lambda$  max. In the control sample containing ascorbic acid only, yellow-brown products formed that absorbed at 280 to 400nm, showing that oxidation occurred in the absence of the azo dyes.

Loss of dye absorbance and spectral changes at pH 4.0 were generally similar to those that occurred at pH 3.0 (Figure 7).

At pH 5.5, Fogg and Summan (1983a, 1984) found a similar order of dye stability in the presence of 1000ppm ascorbic acid to that found in the present study. Inhibiting the oxidation of ascorbate with EDTA caused a substantial reduction in dye degradation whilst exposure to tungsten light enhanced both dye and ascorbic acid degradation (Fogg and Summan, 1983b). At pH 7, Nursten and Williams (1969) found that Carmoisine lost colour more rapidly than at pH 3 on heating at 121°C in the presence of ascorbic acid, whilst Ponceau 4R was stable at this pH. They suggested that the sulphonic acid group *peri-* to the azo bond was the source of the increased stability of Ponceau to ascorbic acid. In the same study, the disazo dye Black PN was found to be particularly unstable in the presence of ascorbic acid. This was attributed to the reductive cleavage of both azo groups to yield 1,4-diamino-naphthalene-6-sulphonic acid, a compound that had been previously shown to be capable of catalysing the breakdown of the original dye (Eisenbrandt and Lang, 1966).

### 3.1.3 Sunset Yellow interactions with bisulphite and ascorbic acid

Combinations of Sunset Yellow, SO<sub>2</sub> and ascorbic acid are frequently used by the food industry, particularly in soft drinks, and so the kinetics of dye loss were investigated. High levels of SO<sub>2</sub> and ascorbic acid were employed in order to obtain measurable rates.

#### 3.1.3.1 *Effect of temperature on the bisulphite reaction*

The loss of dye on heating with 50mM sodium metabisulphite (pH 3.0) at temperatures between 20 and 70°C are shown in Figure 8. As the temperature was increased, the rate of conversion to the lemon yellow compound increased.

### 3.1.3.2 *Effect of bisulphite concentration*

The conversion of Sunset Yellow to the lemon yellow compound at 70°C in the presence of different concentrations of sodium metabisulphite (pH 3.0) is shown in Figure 9. As expected, the higher the level of bisulphite ion the faster Sunset Yellow degraded. Heating in the presence of either 50mM metabisulphite or 100mM sulphurous acid gave similar results showing that these were equivalent methods for producing lemon yellow dye.

### 3.1.3.3 *Effect of bisulphite and ascorbic acid under aerobic and anaerobic conditions*

As oxidation of bisulphite and ascorbic acid could lower the concentrations available to react with Sunset Yellow, a study was carried out to compare dye degradation kinetics under aerobic and anaerobic conditions.

#### 3.1.3.3.1 *Bisulphite alone*

The rate of conversion of Sunset Yellow to the lemon yellow secondary dye at pH 3.0 on heating at 70°C in the presence of 50mM sodium metabisulphite was similar whether air was present in the headspace or the reaction was carried out *in vacuo* (Figure 10). This suggests that any oxidation of bisulphite ion to bisulphate had no influence on the reaction rate. After about 3h at 70°C, the rate of conversion to lemon yellow was equal to its rate of degradation. All colour was completely lost after 20 days heating in air (Figure 11).

#### 3.1.3.3.2 *Ascorbic acid alone*

Sunset Yellow was previously shown to degrade only slowly in the presence of 5.7mM (1000ppm) ascorbic acid at pH 3.0 (Figure 6). Increasing the concentration to 142mM did not substantially alter the dye loss, about 20% decolorisation occurring after 5h at 70°C (Figure 12). The similar losses at different ascorbic acid concentrations may be presumed to be as a result of ascorbic acid oxidation reactions that occurred independently of azo dye reduction. This was confirmed by heating *in vacuo* when the dye loss increased to 32% after 5h.

### 3.1.3.3 *Bisulphite and ascorbic acid in combination*

In the presence of 50mM sodium metabisulphite and 71mM ascorbic acid (a concentration ratio used in some commercial soft drinks), the conversion of Sunset Yellow to the lemon yellow dye was affected by the presence of air in the headspace (Figure 13). The lowest conversion to the secondary dye was found *in vacuo* where the least oxidation of ascorbic acid would be expected. The loss of Sunset Yellow absorbance also occurred most rapidly *in vacuo*. These observations suggest that the ascorbic acid reduction of Sunset Yellow inhibits the bisulphite reaction. Further confirmation of this was obtained by the more pronounced effects found in the presence of 142mM ascorbic acid (Figure 14).

An alternative explanation of the findings is that the lemon yellow dye was readily reduced by ascorbic acid. However, the isolated lemon yellow dye was found to be stable in the presence of ascorbic acid (Figure 15). This implies that the maximum formation of the secondary dye should have occurred under these conditions. The fact that it did not occur suggests that ascorbic acid inhibited the reaction of bisulphite with Sunset Yellow.

The stability of the lemon yellow dye to ascorbic acid reduction may be due to the hydrazone structure proposed by Damant *et al* (1989). It may be hypothesised that, in the case of Sunset Yellow, ascorbic acid significantly reduces the azo form only. As Sunset Yellow exists mainly as the hydrazone tautomer, this would explain the low rate of reduction observed. The ready reduction of Black PN and Carmoisine would suggest that a higher proportion of these dyes is in the azo form whilst the stability of Tartrazine to ascorbic acid reduction implies that the azo bond is deactivated, perhaps by hydrogen bonding with the *ortho*-hydroxyl group in the adjacent pyrazoline ring.

Further degradation of the isolated lemon yellow dye took place in the presence of bisulphite (Figure 15), suggesting that the secondary dye can react further with bisulphite to yield colourless compounds.

## 3.2 Sunset Yellow Storage Trials

Sunset Yellow is frequently used in the soft drinks industry. Two storage trials were carried out, one at 20°C over 12 months and one at 50°C over 3 months, to determine the effect of bisulphite and ascorbic acid on the dye stability in a citric acid model system. The dye stability was compared with the stability in a commercial soft drink known to contain Sunset Yellow, bisulphite and ascorbic acid. Samples were exposed to lighting conditions similar to those found in retail stores, and also under conditions that excluded light.



### 3.2.1 20°C storage trial

Sunset Yellow, in the absence of bisulphite or ascorbic acid, remained stable over 12 months storage at 20°C in the light and in the dark (Figure 16). The samples retained the typical orange colour of Sunset Yellow.

#### 3.2.1.1 *Bisulphite effect at 20°C*

At 0.78mM bisulphite, only traces of lemon yellow secondary dye formation were observed. Exposure to light had a marked effect on the levels of the lemon yellow dye (Figure 17). Samples containing 1.56mM or 6.24mM bisulphite, stored in the dark, appeared strongly lemon yellow whilst the equivalent samples stored in the light were pale yellow. Light exposure also substantially reduced the levels of SO<sub>2</sub> detected (Table 2). These observations suggest that bisulphite interacts with the lemon yellow dye on exposure to light.

#### 3.2.1.2 *Ascorbic acid effect at 20°C*

In the presence of ascorbic acid, exposure to light increased Sunset Yellow degradation for all treatments, suggesting that the vitamin or the dye, or both, were photosensitive. In the light, a particularly variable loss was noted at 2.27mM ascorbic acid from 6 months onwards, with duplicate samples ranging from orange to pale orange (Figure 18). Ascorbic acid levels were reduced by 85-95%, after 12 months storage, irrespective of light exposure (Table 3).

No increase in absorbance at 414nm was observed, confirming that the lemon yellow dye was not formed under these conditions.

#### 3.2.1.3 *Effect of bisulphite and ascorbic acid together at 20°C*

Sunset Yellow showed only small losses during 12 months storage, both in the model system and in the commercial soft drink, each containing 0.78mM bisulphite and 0.45mM ascorbic acid (Figure 19). Larger losses were observed in model systems containing 0.78mM bisulphite and 2.27mM ascorbic acid, particularly in samples exposed to the light. As in the presence of 0.78mM bisulphite alone, only trace amounts of the lemon yellow dye were detected.

SO<sub>2</sub> levels were greatly reduced in the presence of ascorbic acid under both light and dark conditions (Table 2). Ascorbic acid levels were low in the model systems containing SO<sub>2</sub> but higher in the commercial soft drink (Table 3).

Table 2

**SO<sub>2</sub> Levels in Samples stored at 20°C**

Treatment	SO <sub>2</sub> level (mg/l)					
	Zero time		12 months (light)		12 months (dark)	
	Individual Samples	Mean	Individual Samples	Mean	Individual Samples	Mean
50ppm SO <sub>2</sub>	38 44	41	14 13	13	28 24	26
100ppm SO <sub>2</sub>	87 84	86	32 33	33	59 64	62
400ppm SO <sub>2</sub>	311 316	314	157 214	186	265 283	274
80ppm Ascorbic acid + 50ppm SO <sub>2</sub>	42 45	44	<5 <5	<5	<5 <5	<5
400ppm Ascorbic acid + 50ppm SO <sub>2</sub>	41 42	42	<5 <5	<5	<5 <5	<5
Commercial Soft Drink	51 60	56	29 27	28	29 28	28

Limit of detection = 5mg/l SO<sub>2</sub>.

Table 3

**Ascorbic Acid Levels in Samples stored at 20°C**

Treatment	Total Ascorbic Acid Level (mg/l)					
	Zero time		12 weeks (light)		12 weeks (dark)	
	Individual Samples	Mean	Individual Samples	Mean	Individual Samples	Mean
80ppm Ascorbic acid	70 67	68	9 8	9	4 4	4
160ppm Ascorbic acid	140 142	141	7	--	27 8	17
400ppm Ascorbic acid	314 317	315	42 18	30	20 20	20
80ppm Ascorbic acid + 50ppm SO <sub>2</sub>	68 67	67	3 3	3	<3 <3	<3
400ppm Ascorbic acid + 50ppm SO <sub>2</sub>	299 303	301	13 14	13	16 14	15
Commercial Soft Drink	63 71	67	7 16	11	7 18	12

Limit of detection = 3mg/l total ascorbic acid.

### 3.2.2 50°C storage trial

Sunset Yellow, in the absence of bisulphite or ascorbic acid, remained stable over 3 months storage at 50°C in the light and in the dark (Figure 20). The samples retained the typical orange colour of Sunset Yellow.

#### 3.2.2.1 *Bisulphite effect at 50°C*

About 20-25% loss of Sunset Yellow absorbance at 480nm occurred in samples containing 0.78mM and 1.56mM bisulphite whilst similar amounts of the lemon yellow dye were formed at both bisulphite levels (Figure 21). The samples still appeared orange. In the presence of 6.24mM bisulphite, however, approximately 85% loss of Sunset Yellow took place after 4 weeks storage and the samples appeared yellow due to the formation of the lemon yellow secondary dye. Unlike the results at 20°C, samples held in the light showed closely similar behaviour to those held in the dark. The difference between the 20°C and 50°C results could have been due to different light intensities at the two storage temperatures or to thermal effects overwhelming the photochemical reactions. Further work would be necessary to resolve this point.

The levels of SO<sub>2</sub> detected at zero time and after 12 weeks storage are given in Table 4. The higher the initial SO<sub>2</sub> level the greater was the proportion remaining on storage under both light and dark conditions.

#### 3.2.2.2 *Ascorbic acid effect at 50°C*

In the presence of 0.45mM ascorbic acid, the Sunset Yellow absorbance at 480nm decreased with storage time with 20% loss having taken place after 12 weeks (Figure 22). The higher the initial level of ascorbic acid, the more rapid was the absorbance loss, especially in samples stored in the light, and the greater was the variation between duplicates. After 12 weeks storage, colour fading was observed in the samples containing 0.91mM and 2.27mM ascorbic acid.

The levels of ascorbic acid were generally below the detection limit after 12 weeks storage (Table 5).

Table 4

**SO<sub>2</sub> Levels in Samples stored at 50°C**

Treatment	SO <sub>2</sub> level (mg/l)					
	Zero time		12 weeks (light)		12 weeks (dark)	
	Individual Samples	Mean	Individual Samples	Mean	Individual Samples	Mean
50ppm SO <sub>2</sub>	37 40	39	10	-	6 11	8
100ppm SO <sub>2</sub>	80 84	82	30	-	49 22	36
400ppm SO <sub>2</sub>	272 275	273	125 195	160	88 204	146
80ppm Ascorbic acid + 50ppm SO <sub>2</sub>	40 41	41	<5 <5	<5	19 <5	9.5
400ppm Ascorbic acid + 50ppm SO <sub>2</sub>	38 43	40	<5 <5	<5	<5 <5	<5
Commercial Soft Drink	38 42	40	<5 <5	<5	<5 <5	<5

Limit of detection = 5mg/l SO<sub>2</sub>.

Table 5

**Ascorbic Acid Levels in Samples stored at 50°C**

Treatment	Total Ascorbic Acid Level (mg/l)					
	Zero time		12 weeks (light)		12 weeks (dark)	
	Individual Samples	Mean	Individual Samples	Mean	Individual Samples	Mean
80ppm Ascorbic acid	79 79	79	<3 <3	<3	<3 <3	<3
160ppm Ascorbic acid	182 184	183	6 <3	-	<3 <3	<3
400ppm Ascorbic acid	455 455	455	100 <3	-	<3 <3	<3
80ppm Ascorbic acid + 50ppm SO <sub>2</sub>	86 86	86	<3 <3	<3	<3 <3	<3
400ppm Ascorbic acid + 50ppm SO <sub>2</sub>	455 461	458	37 28	32	41 14	27
Commercial Soft Drink	79 64	71	8 6	7	11 13	12

Limit of detection = 3mg/l total ascorbic acid.

### 3.2.2.3 *Effect of bisulphite and ascorbic acid together at 50°C*

In the model system and commercial soft drink, each containing 0.78mM bisulphite and 0.45mM ascorbic acid, Sunset Yellow degraded to only a minor extent, under light and dark conditions, and little or no lemon yellow dye was produced (Figure 23). No visible colour change occurred in the model system or soft drink.

In the presence of 0.78mM bisulphite and 2.27mM ascorbic acid, Sunset Yellow degraded to only a minor extent up to 8 weeks storage, but after 12 weeks a large amount of breakdown had occurred in samples exposed to light and marked colour loss was observed. Under both light and dark conditions, the 12 weeks storage results were also much more variable than those obtained at shorter storage times. The breakdown did not correspond with an increase in lemon yellow dye formation as seen from the sharp decrease in the absorbance at 414nm. Under dark conditions, bisulphite slowed down the rate of Sunset Yellow loss compared with ascorbic acid alone. This suggests that bisulphite inhibited the ascorbic acid reduction of Sunset Yellow.

As at 20°C, SO<sub>2</sub> levels were lower in the presence of ascorbic acid (Table 4). However, in contrast to the results at 20°C, total ascorbic acid levels were generally higher in samples containing bisulphite (Table 5). This may be explained in terms of bisulphite inhibition of ascorbic acid browning reactions. Both SO<sub>2</sub> and ascorbic acid were retained at higher levels in the commercial soft drink than in the model systems.

The practical value of the storage trials at 20°C and 50°C can be summarised as follows:-

1. Little degradation of Sunset Yellow occurred in a commercial soft drink even when stored at 50°C. This behaviour was similar to that found for a model system containing 50ppm SO<sub>2</sub> and 80ppm ascorbic acid.
2. Bisulphite and ascorbic acid were more stable in the commercial soft drink than in the comparable model system.
3. At 50°C, bisulphite inhibited the ascorbic acid degradation of Sunset Yellow in model systems.
4. Substantial amounts of Sunset Yellow degradation occurred in model systems containing elevated levels of bisulphite or ascorbic acid especially on exposure to light.



### 3.3 Separation and Identification of Sunset Yellow Degradation Products

Potential pathways of Sunset Yellow degradation are shown in Figure 24. The structure of the lemon yellow secondary dye determined by Damant *et al* (1989) is shown in the *enol* form only.

#### 3.3.1 HPLC studies

Using reversed-phase ion-pair HPLC with tetrabutylammonium phosphate (TBAP) as ion pair agent, Sunset Yellow was well separated from the lemon yellow secondary dye formed by reaction with bisulphite ion (Figures 25 and 26). The retention time for Sunset Yellow was 14.0min ( $\pm 0.3$ min), whilst the retention time for the lemon yellow dye was 8.3min ( $\pm 0.3$ min). In a Sunset Yellow sample completely converted to the lemon yellow form, a major component with retention time relative to lemon yellow ( $R_{Ly} = 0.48$ ) was observed along with a minor component at  $R_{Ly} = 0.75$ . On further treatment with bisulphite, to almost complete colour loss, the latter component ran with  $R_{Ly} = 0.80$  along with significant amounts of components with  $R_{Ly} = 0.51, 0.57$  and  $0.67$  (Figure 27). None of the original Sunset Yellow and only traces of the lemon yellow dye were detected.

Using TBAP in a similar solvent to that used in the present study, Damant *et al* (1989) found shorter retention times for both Sunset Yellow and the lemon yellow dye. This could feasibly be due to their reversed phase packing (ChromSpherC<sub>18</sub>) having a lower affinity for the dyes than Spherisorb ODS-2. However, the retention time of the lemon yellow dye relative to Sunset Yellow (0.54) was similar to that found in the present work (0.59), suggesting that the chromatographic separations were based on the same underlying mechanisms in both cases. Damant's finding of a Sunset Yellow degradation product at  $R_{Ly} = 0.85$  was therefore probably in agreement with the present finding of a degradation product at  $R_{Ly} = 0.80$ . The fact that this was a relatively minor component in the present study whilst apparently being a major degradation product in Damant's study may feasibly be attributed to differences in the model system conditions. For instance, citric acid and sodium benzoate were used by Damant which, though stable in themselves, could have had a catalytic influence on Sunset Yellow degradation. In this part of the present study, these additives were not employed as they were considered as potential interferences in the HPLC analysis of degradation products. Three other compounds found by Damant were present in small concentrations at  $R_{Ly}$  values that did not correspond with values found in the present study. These compounds may not therefore have been Sunset Yellow degradation products.

Due to its relatively high molecular weight and involatility, TBAP is incompatible with ES-MS and ammonium acetate was considered as an alternative ion pair agent. Ammonium acetate is volatile and known to be compatible in negative ion applications of ES-MS (Bruins *et al*, 1987; Lee *et al*, 1989). However, two major problems were encountered:

1. The precision of Sunset Yellow retention times was poor, differing by as much as 25% between runs.
2. The ammonium acetate appeared to contain some impurities that eluted at around the same retention times as expected for the Sunset Yellow degradation products. Ammonium acetate was therefore not evaluated any further.

### 3.3.2 Capillary electrophoresis studies

As a result of the problems of selecting a satisfactory ion pair agent for HPLC separation of the Sunset Yellow degradation products, some preliminary CE studies were performed. It was considered that defining the conditions for CE separation would be valuable in subsequent studies using linked CE-MS for the identification of breakdown products.

Using the visible  $\lambda_{\text{max}}$  for detection (480nm), Sunset Yellow was found to migrate as a single species with a migration time of 3.5 minutes (Figure 28). A similar result was obtained using 215nm detection, suggesting that the dye was essentially a pure compound. After treatment with bisulphite, the resultant lemon yellow dye was detected at its  $\lambda_{\text{max}}$  (415nm) with a migration time of 4.9 minutes (Figure 29). The slower migration time was consistent with the presence of three sulphononic acid groups compared with the two sulphonates of the parent dye. Detection at 215nm confirmed the presence of the lemon yellow dye at 4.9 minutes. The azo bond had apparently not cleaved on degradation as no sulphanilic acid was detected in an almost completely decolourised sample (Figure 30). A commercial sample of sulphanilic acid gave a single component with a migration time of 2.8 minutes (Figure 31).

Whilst azo dyes have been determined in wastewater by capillary zone electrophoresis linked to tandem mass spectrometry (Lee *et al*, 1989), no studies appear to have been carried out on identifying the dye degradation products. The above work therefore provides the basis for future studies using linked CE-MS to identify azo dye degradation products.

### 3.3.3 Electrospray-mass spectrometry studies

ES-MS of Sunset Yellow (MW=408) in water at a concentration similar to that found in soft drinks showed the presence of a strong peak at  $m/z$  202.9 corresponding to the doubly-charged species  $(MW-2H)^{2+}/2$  (Figure 32). The singly-charged species  $(MW-H)^+$  at 407 was relatively weak. Confirmation that the 203 peak was doubly-charged was obtained by the detection of a peak 0.5 units higher at  $m/z$  203.4 due to the presence of the  $C^{13}$  isotope in Sunset Yellow (Figure 33).

Comparisons of mass spectra from the lemon yellow secondary dye and the degraded lemon yellow preparations were made with spectra from similarly treated control samples containing all components of the model system except the azo dye (Figure 34). As a result of these comparisons, many peaks were shown to be present in the controls and were therefore not linked with the degradation of Sunset Yellow. It is feasible that many of the species detected were formed through polymerisation reactions involving forms of  $SO_2$  not removed by the acidification and degassing procedure. For example, it may be speculated that the peak at  $m/z$  97, probably due to  $HSO_4^-$ , could add on  $SO_2$  ( $H_2SO_3$ ) and then, by elimination of water or hydrogen peroxide, could produce new species with  $m/z$  values in the observed range.

A single minor peak at  $m/z$  244 was detected, that was feasibly associated with the lemon yellow dye (MW = 490) (Figure 35). This was assigned as a doubly-charged ion because of the presence of a peak at  $m/z$  244.5 (Figure 36). As a triply-charged species was not detected at  $m/z$  162.3, this implies that the newly acquired  $H_2SO_3$  had not ionised in the same way as the sulphonic acid groups in the original Sunset Yellow. This could be due to the addition reaction proceeding through the oxygen of the bisulphite rather than the sulphur. Such an O-bonded group may not be resonance stabilised as effectively as a sulphonate group and therefore would not be expected to ionise as readily.

No peaks were found in the colourless, degraded lemon yellow sample that could be related to the azo dye (Figure 37). Sulphanilic acid, readily detected in standard solutions through its singly-charged ion at  $m/z$  172 (Figure 38) was not found in the degraded lemon yellow sample. It may be concluded that the loss of colour in the presence of bisulphite did not involve cleavage of the azo bond.

The mass spectra for decolorised samples containing ascorbic acid were dominated by a peak at  $m/z$  175 corresponding to  $(MW-H)^+$  of ascorbic acid (Figure 39). The absence of a peak at  $m/z$  172 may be due to sulphanilic acid being present at too low a level for detection.

## 4. CONCLUSIONS

### 4.1 Kinetic Studies

In the presence of a large excess of bisulphite ion, the six azo dyes used in this study degraded over a period of several hours at 70°C. The dyes fell into two groups: those that produced secondary dyes on reaction with bisulphite, viz Amaranth, Ponceau 4R and Sunset Yellow, and those that lost colour without any change in the visible region of the spectrum, viz Black PN, Carmoisine and Tartrazine. The order of dye stability at pH 4.0 was different to that at pH 3.0 mainly due to a more rapid rate of secondary dye formation at the higher pH. Secondary dye formation was attributed to the predominance of hydrazone tautomers in the original dye that facilitated bisulphite addition to the unsubstituted *para* position in the naphthalene nucleus.

In the presence of a large excess of ascorbic acid, the azo dyes were reduced to colourless compounds on heating for several hours at 70°C. The dye degradation rates were higher at pH4.0 than at pH3.0 though the relative rates were largely unaffected as secondary dyes were not formed on reduction.

In mixtures containing Sunset Yellow, bisulphite and ascorbic acid, it was concluded that ascorbic acid reduction of the dye inhibited the bisulphite addition reaction.

### 4.2 Sunset Yellow Storage Trials

Sunset Yellow did not break down in model systems at pH 3.0 in the absence of bisulphite or ascorbic acid. In the presence of bisulphite alone, at 20°C, a lemon yellow secondary dye formed at the higher bisulphite levels which then broke down. The latter degradation was particularly marked in samples stored in the light and it corresponded with low levels of SO<sub>2</sub> detected at the end of the storage trial. At 50°C, the loss of Sunset Yellow correlated with the production of lemon yellow dye which did not break down significantly. Exposure to light at 50°C apparently had no effect on this reaction nor on the SO<sub>2</sub> levels detected.

In the presence of ascorbic acid alone, the rate of Sunset Yellow loss was dependent on ascorbic acid concentration and on exposure to light. Light enhanced the dye loss at the higher ascorbic acid concentrations and it had a greater effect at 20°C than at 50°C. No ascorbic acid was detected in most of the samples at the end of the 50°C trial.

In the presence of bisulphite and ascorbic acid together, little Sunset Yellow degradation occurred at the lower ascorbic acid level in the model system or commercial soft drink at either storage temperature. At the higher ascorbic acid concentration in model systems, more dye breakdown occurred especially in samples exposed to the light. Little or no lemon yellow dye was formed in any case. At both temperatures,  $\text{SO}_2$  levels were lower in the presence of ascorbic acid whilst ascorbic acid levels were generally higher in samples containing bisulphite. Both  $\text{SO}_2$  and ascorbic acid were retained better in the commercial soft drink than in the model systems.

The results suggest that current levels of sulphur dioxide and ascorbic acid in soft drinks do not cause significant dye degradation under normal storage conditions. However, this picture could change if  $\text{SO}_2$  and ascorbic acid levels are increased to meet market demands.

#### 4.3 Separation and Identification of Sunset Yellow Degradation Products

Reversed-phase ion-pair HPLC gave a good separation of the lemon yellow secondary dye from the original Sunset Yellow dye. Colourless degradation products were detected by UV absorbance and found to be well separated from the lemon yellow dye. Impurities in ammonium acetate interfered with the detection of Sunset Yellow degradation products, ruling out the ammonium salt as an ion pair reagent to replace tetrabutylammonium phosphate.

Capillary electrophoresis also gave a good separation of the lemon yellow dye from the original Sunset Yellow and separated the colourless degradation products from the dyes. No sulphanilic acid was detected in Sunset Yellow samples degraded in the presence of bisulphite.

Electrospray-mass spectrometry was used to characterise Sunset Yellow, at concentrations similar to those found in soft drinks, by the presence of a singly-charged negative ion at  $m/z$  407 and a doubly-charged species at  $m/z$  203. Using crude preparations of the lemon yellow dye, from which most of the free  $\text{SO}_2$  had been removed, a minor component with  $m/z$  244 was detected that was assigned as a doubly-charged ion. This was interpreted by suggesting that the newly acquired bisulphite in the lemon yellow dye had not ionised in the same way as the Sunset Yellow sulphonate groups. No species were found in the colourless, degraded lemon yellow that could be related to the azo dye. In agreement with the capillary electrophoresis finding, sulphanilic acid was not detected, implying that the azo bond had not cleaved in the presence of bisulphite.

The mass spectra for Sunset Yellow samples decolorised in the presence of ascorbic acid were dominated by a peak at  $m/z$  175 due to the ascorbate monoanion. The intensity of ions arising from  $\text{SO}_2$  and ascorbic acid suggests that their prior removal, or separation by HPLC or capillary electrophoresis, is required before detection of Sunset Yellow degradation products will be possible.

Further work is required to identify dye degradation products purified by chromatography or electrophoresis. This would involve mass spectrometry and then confirmation of proposed structures by nuclear magnetic resonance spectrometry. Such unambiguous identification is essential to determine whether or not compounds are formed that could potentially be toxic.

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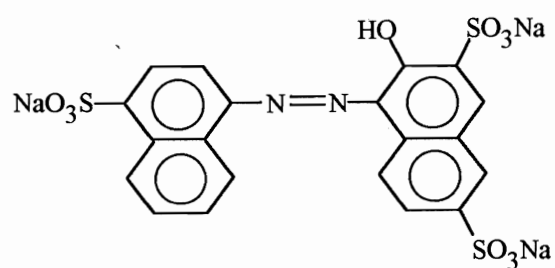
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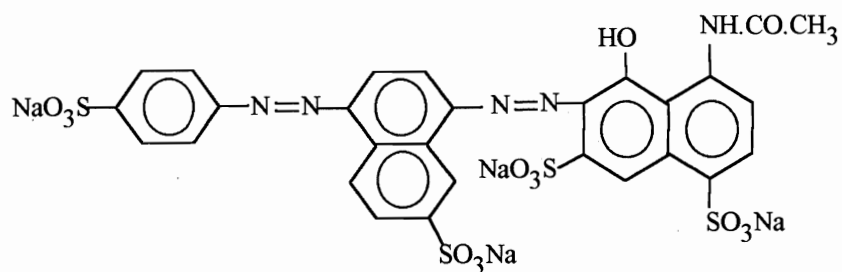
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Figure 1

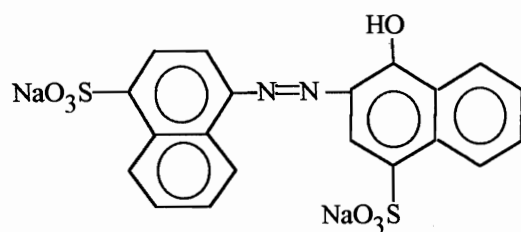
Structure of the azo dyes under investigation



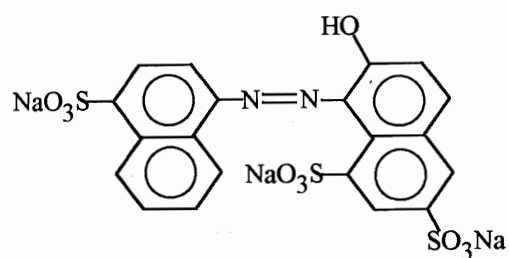
Amaranth



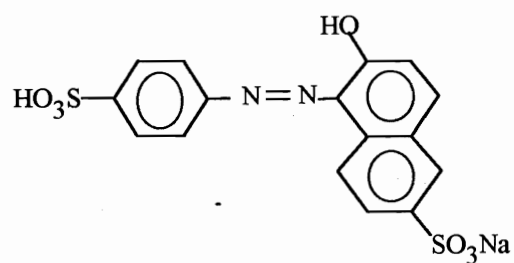
Black PN



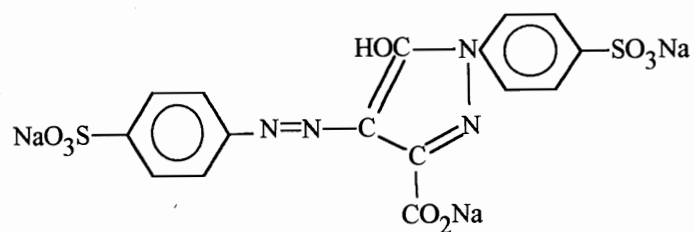
Carmoisine



Ponceau 4R



Sunset Yellow



Tartrazine

Figure 2

Loss of absorbance on heating azo dyes at 70°C with 50mM metabisulphite (pH3.0)

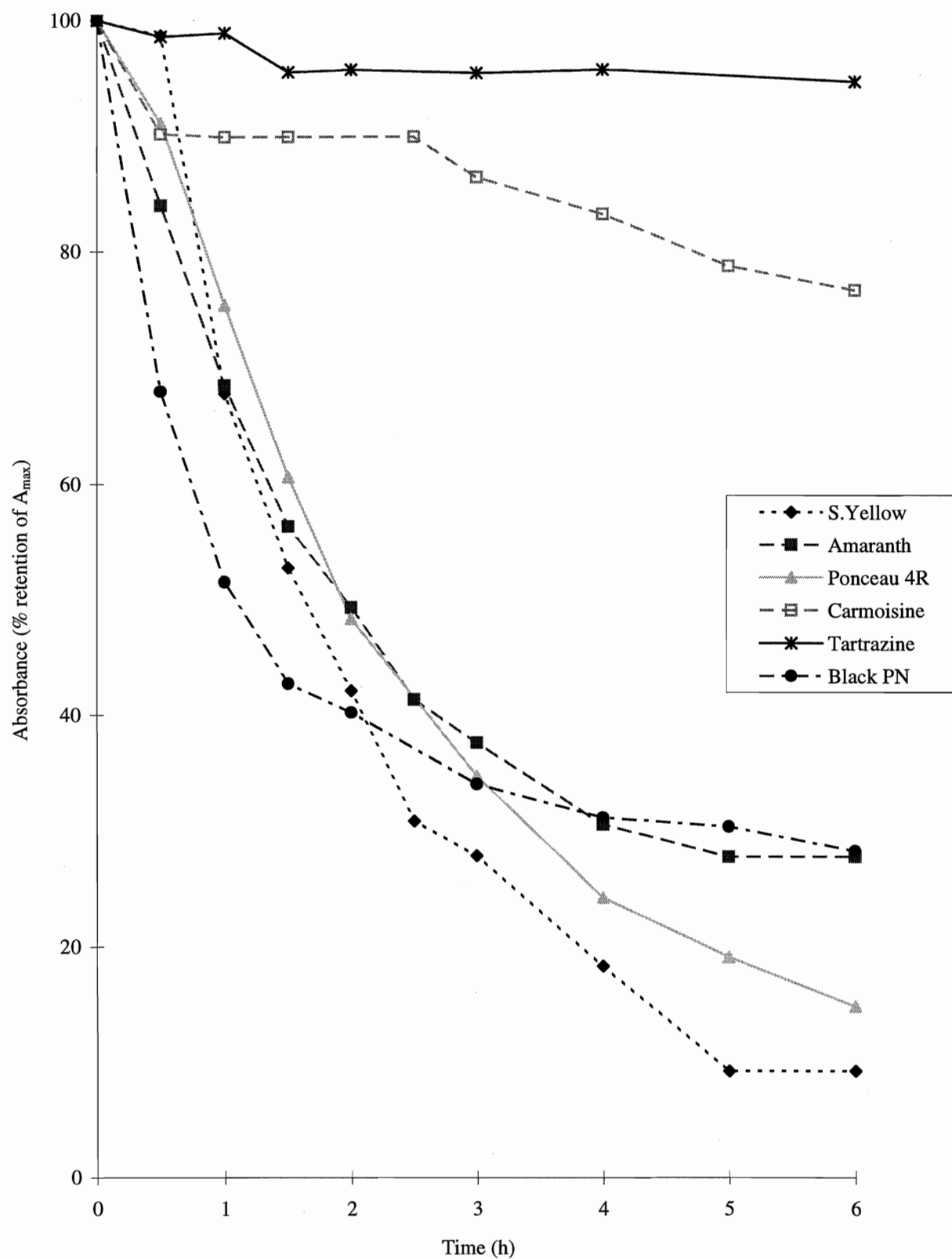


Figure 3

Formation of secondary dyes on heating Sunset Yellow, Amaranth and Ponceau 4R at 70°C with sodium metabisulphite (pH3.0)

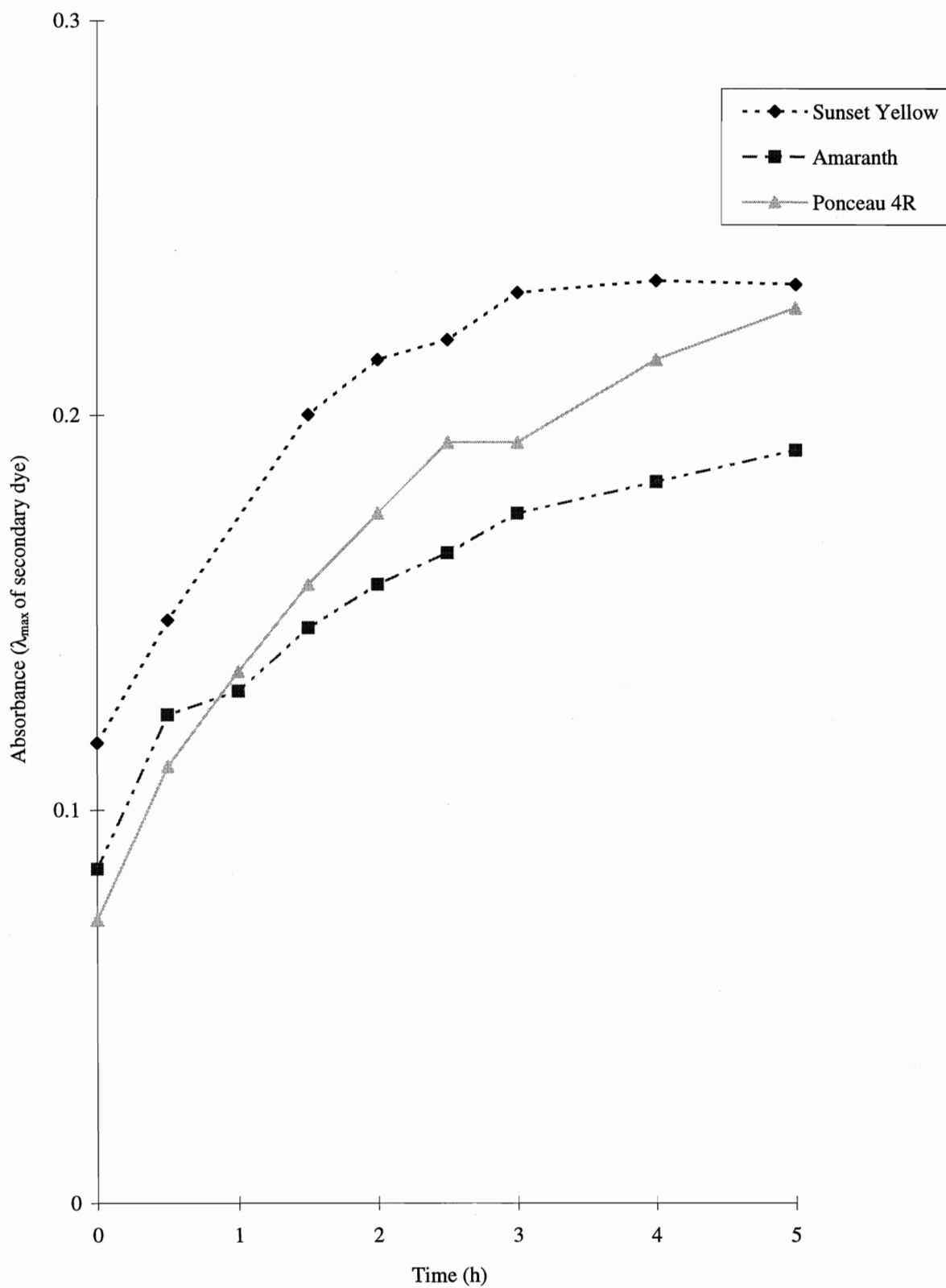


Figure 4

Loss of absorbance on heating azo dyes at 70°C with 50mM metabisulphite  
(pH4.0)

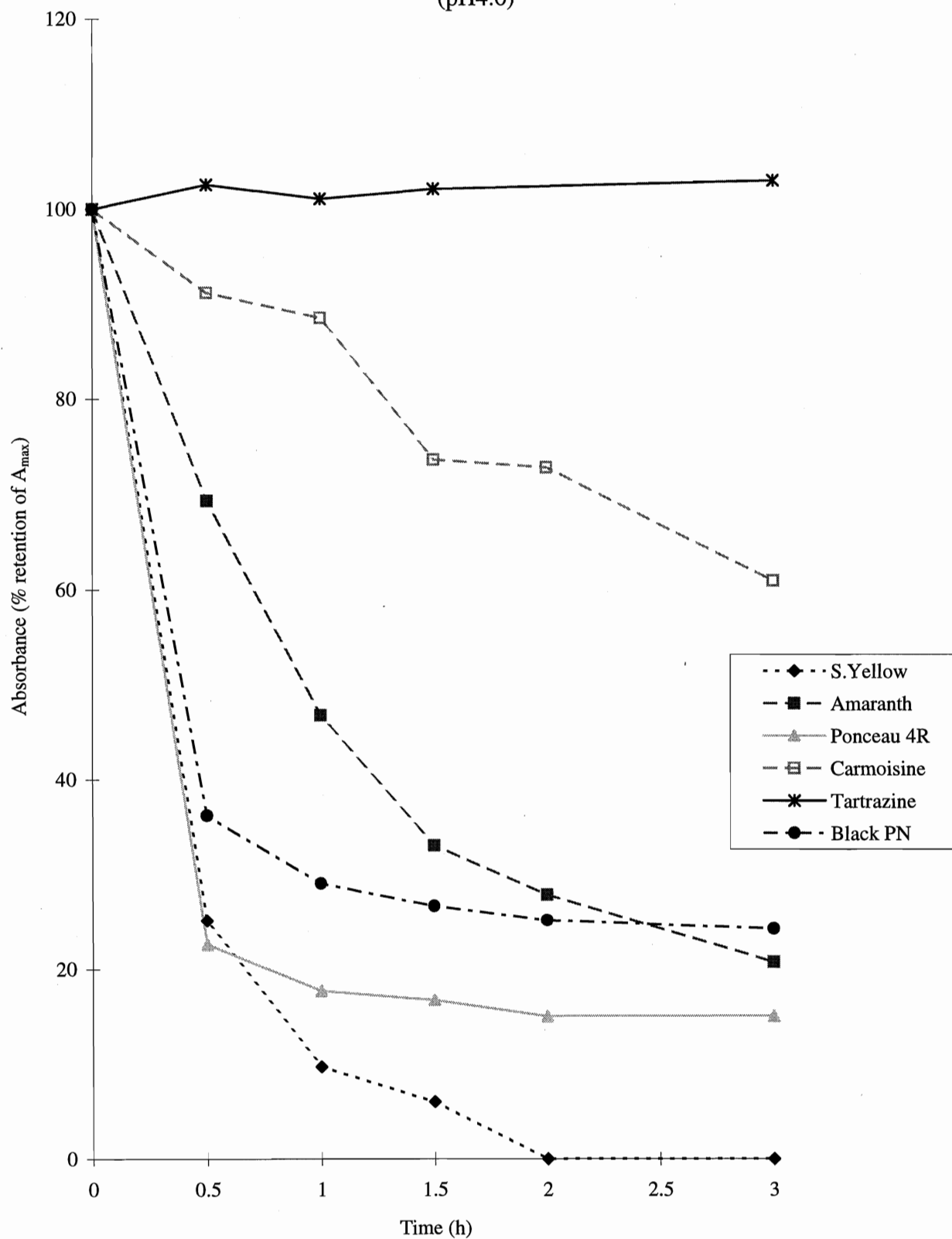


Figure 5

Formation of secondary dyes on heating Sunset Yellow, Amaranth and Ponceau 4R at 70°C with sodium metabisulphite ( pH 4.0)

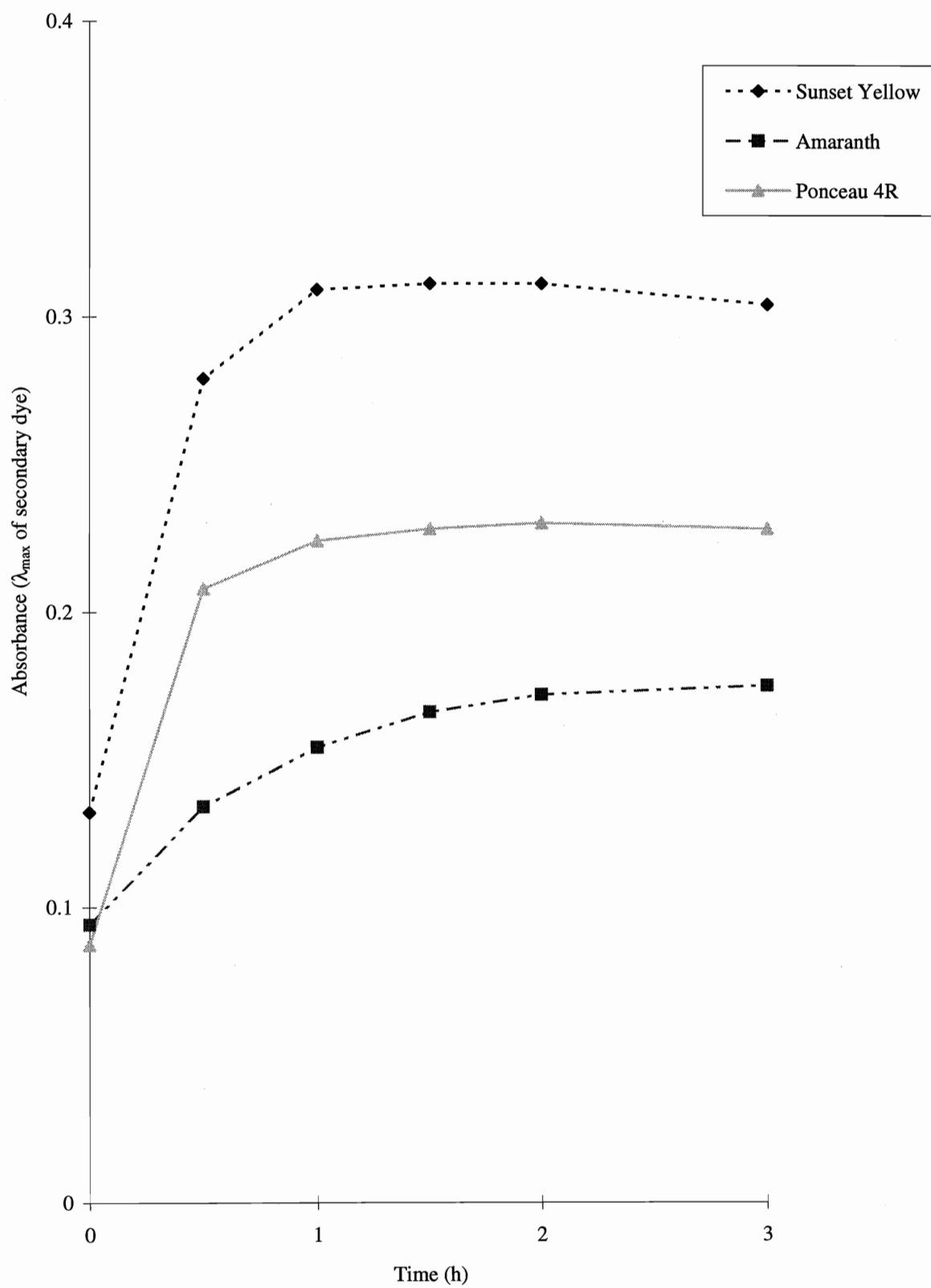


Figure 6

Loss of absorbance on heating azo dyes at 70°C with 5.7mM (1000ppm)  
ascorbic acid (pH3.0)

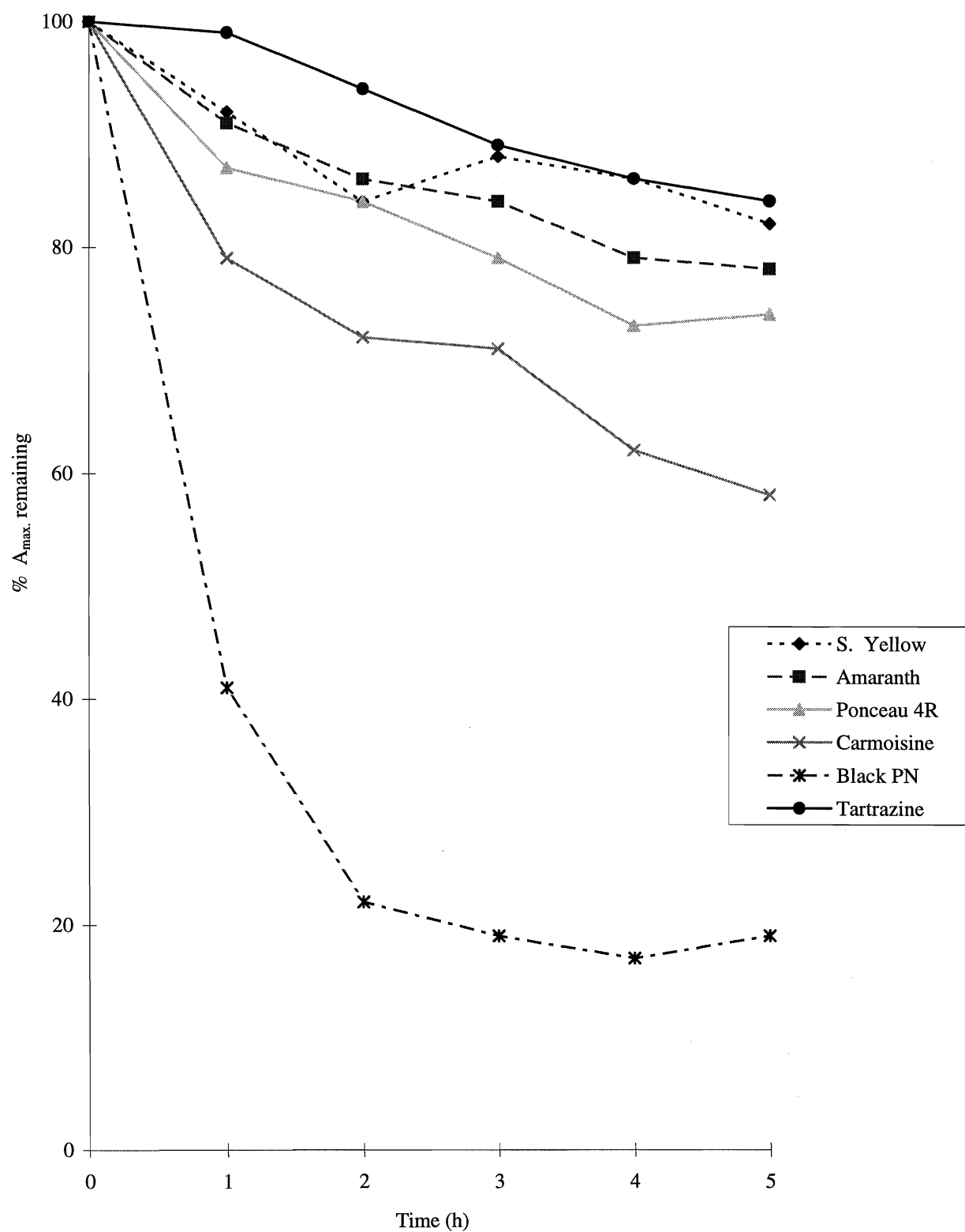




Figure 7

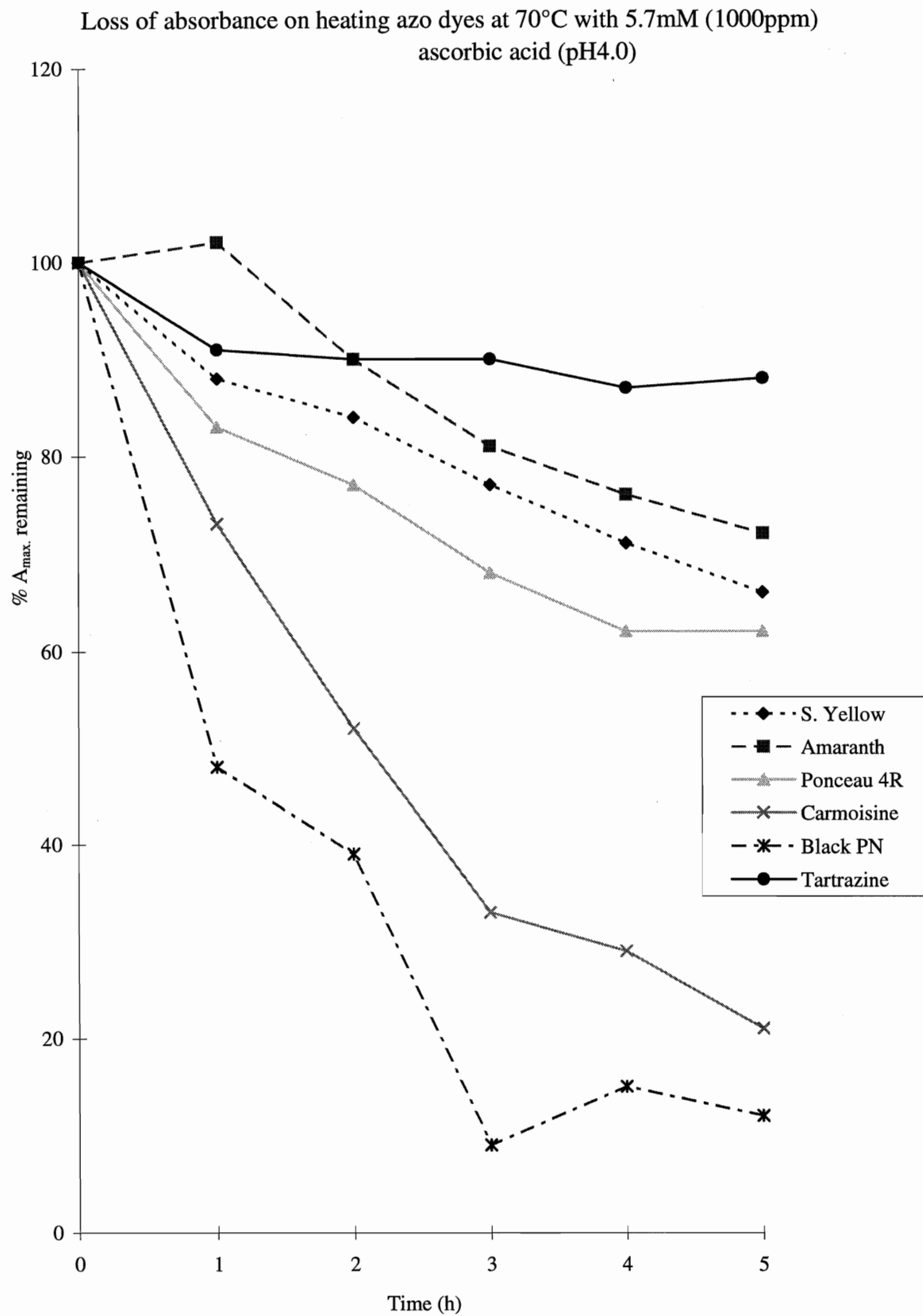


Figure 8

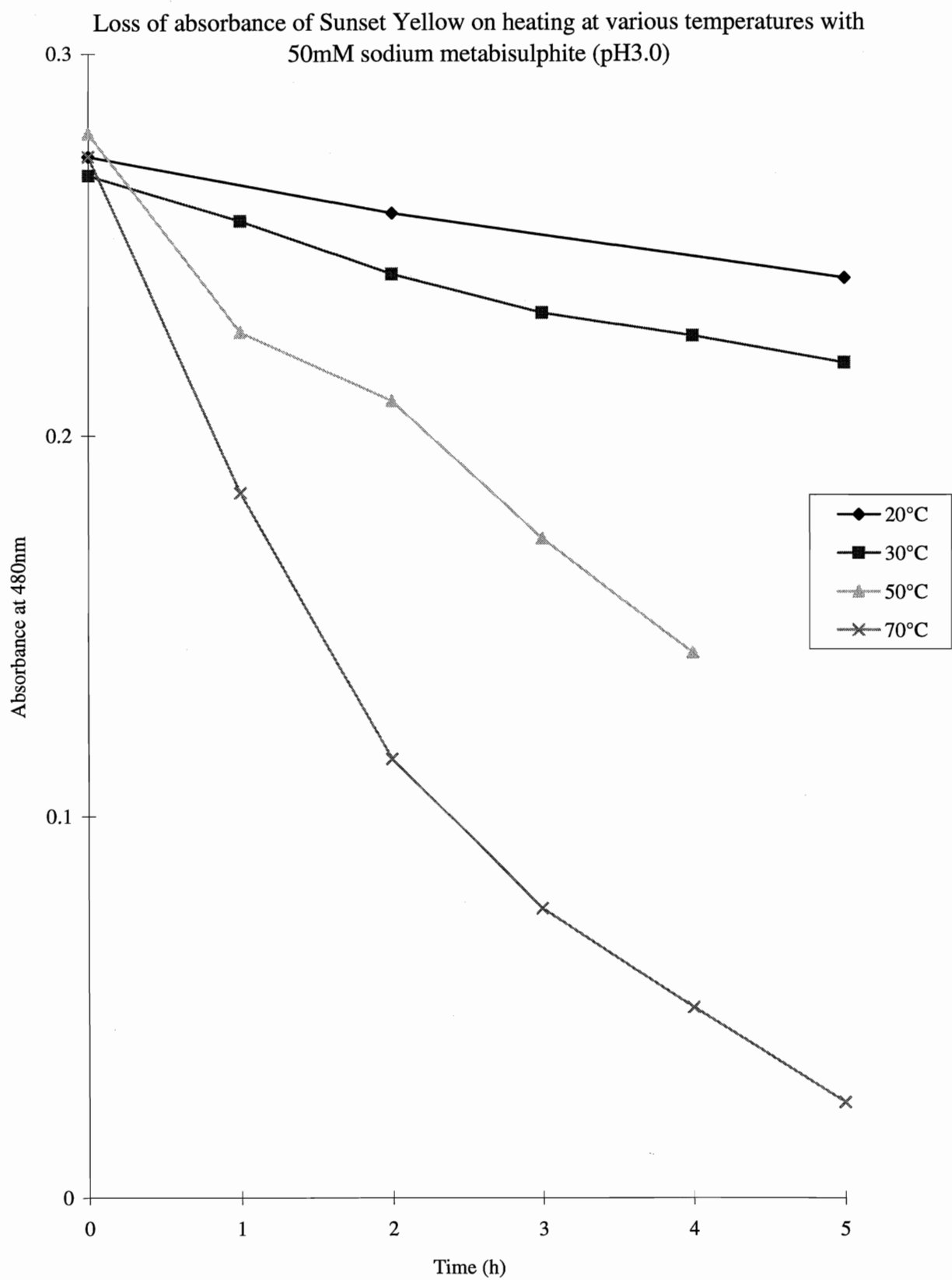


Figure 9

Loss of absorbance of Sunset Yellow on heating in the presence of various concentrations of sodium metabisulphite ( pH 3.0)

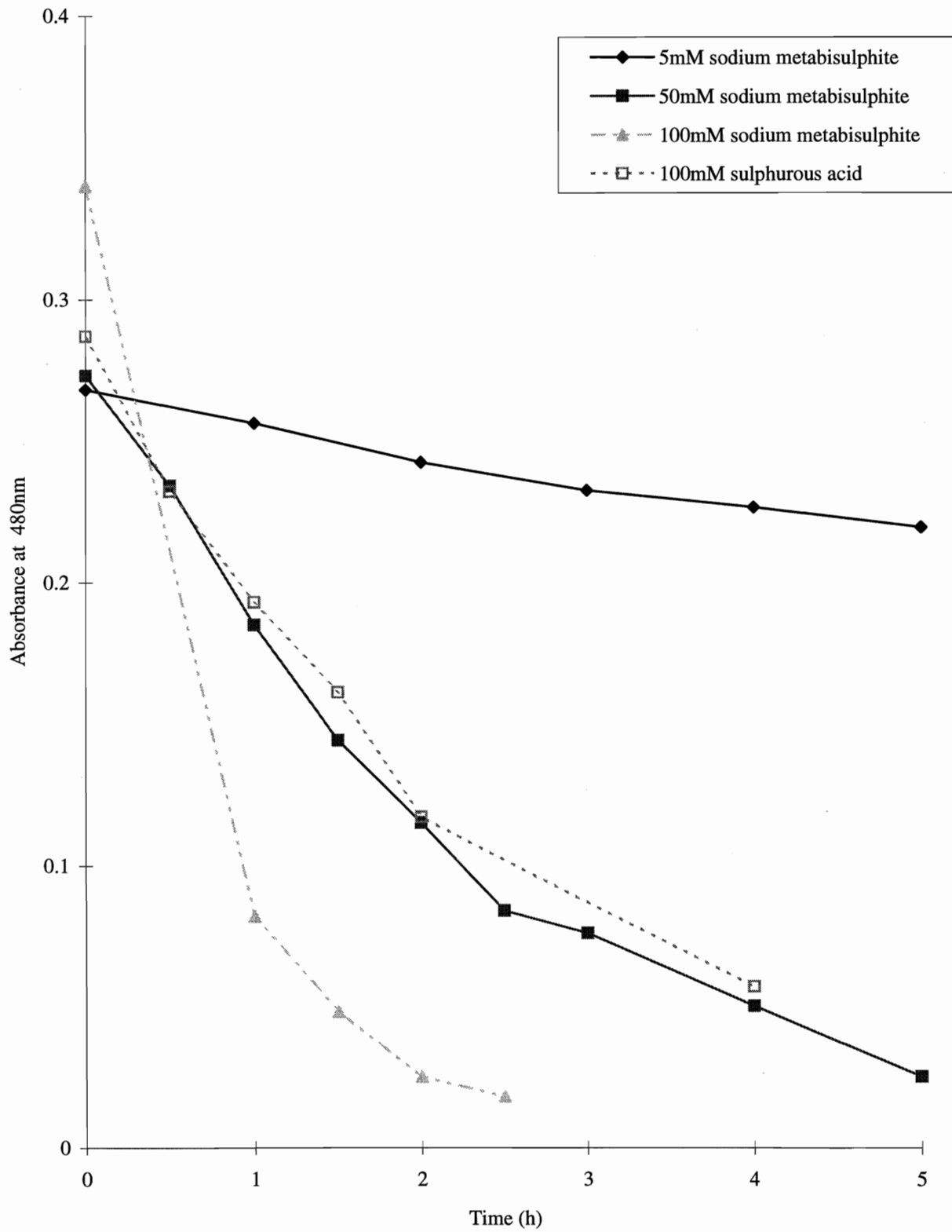


Figure 10

Changes in absorbance of Sunset Yellow on heating at 70°C with 50mM metabisulphite (pH3.0)

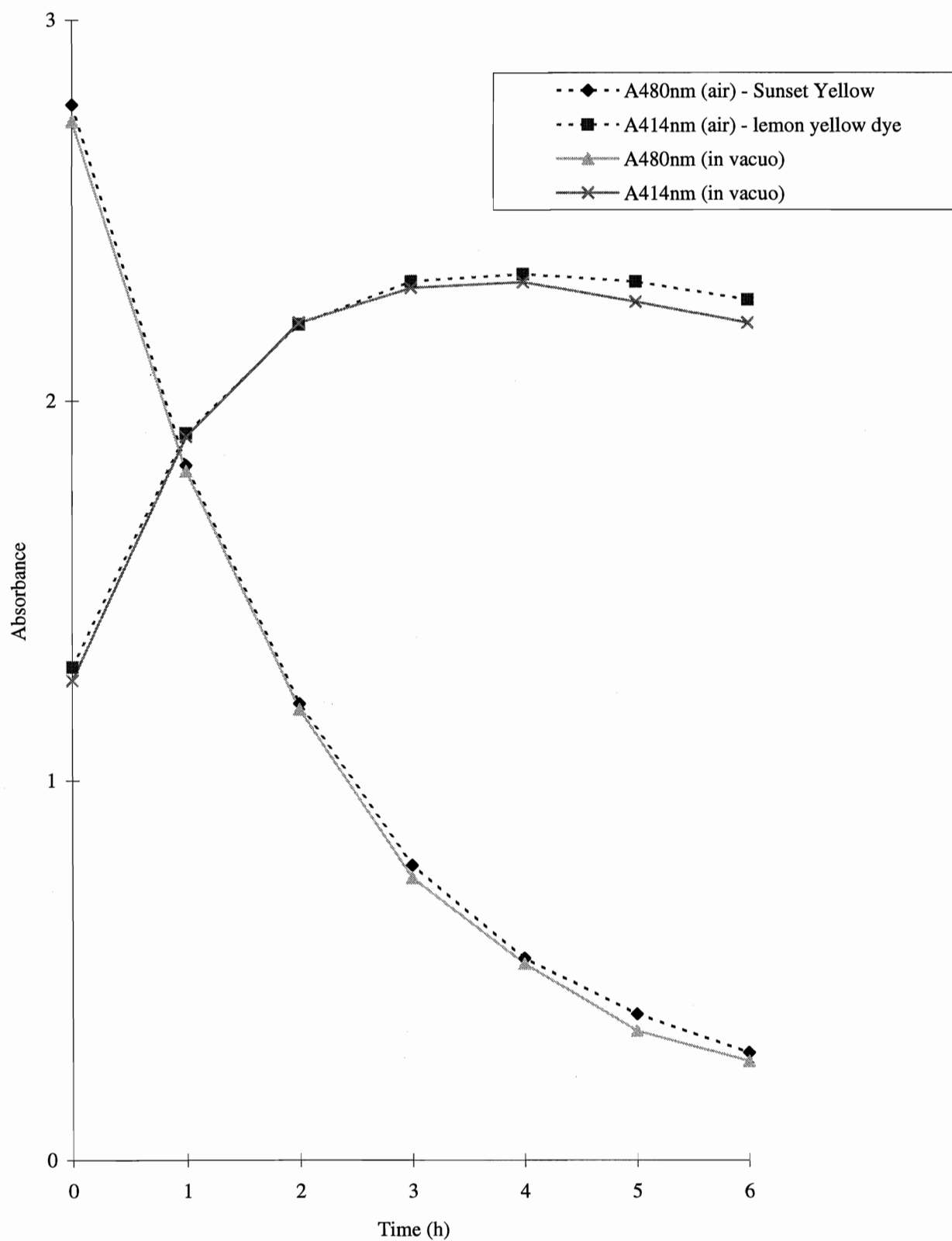


Figure 11

Changes in absorbance of Sunset Yellow on extended heating at 70°C in air  
with 100mM sulphurous acid (pH3.0)

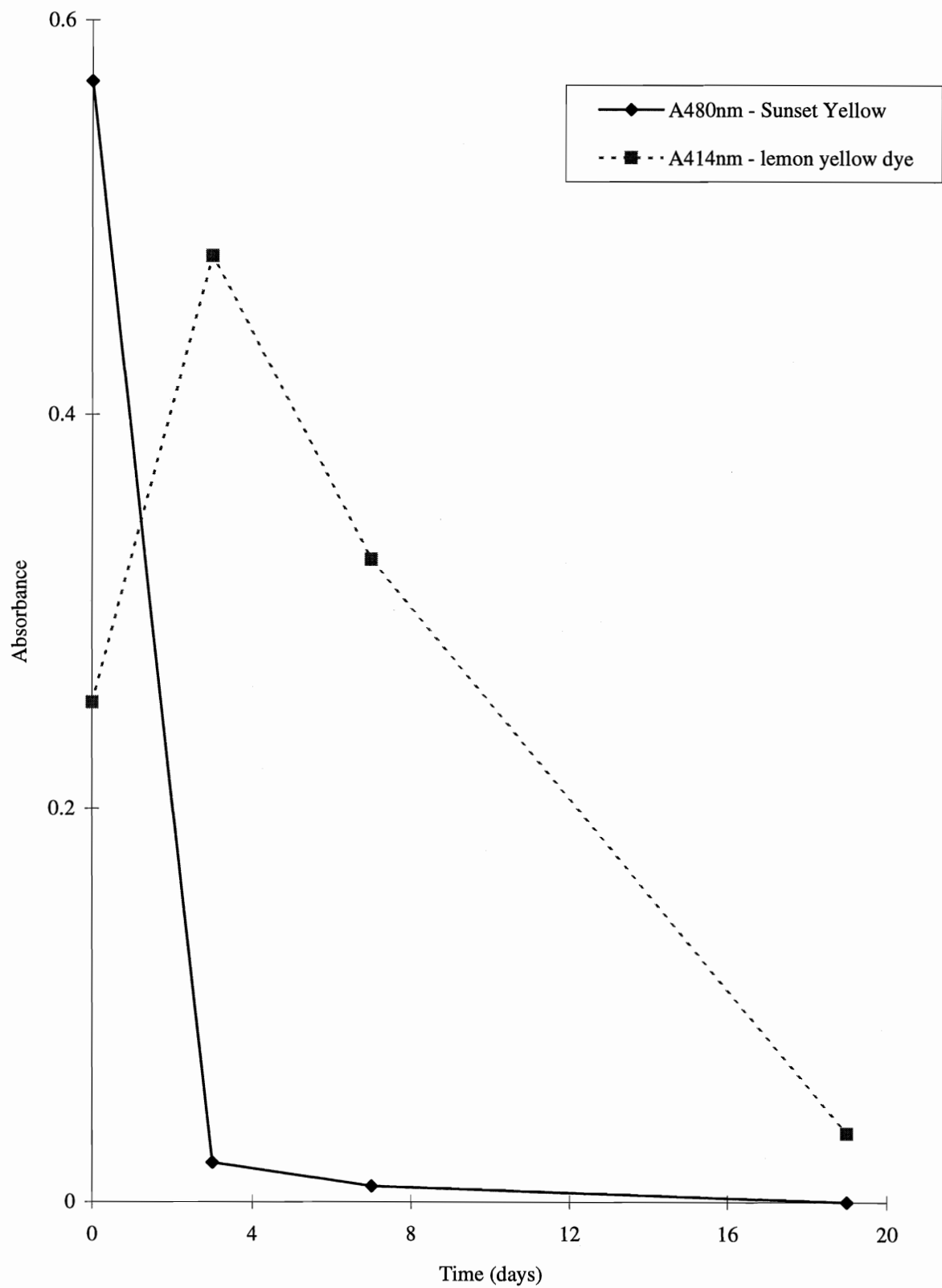


Figure 12

Changes in absorbance of Sunset Yellow on heating at 70°C with 142mM ascorbic acid (pH3.0)

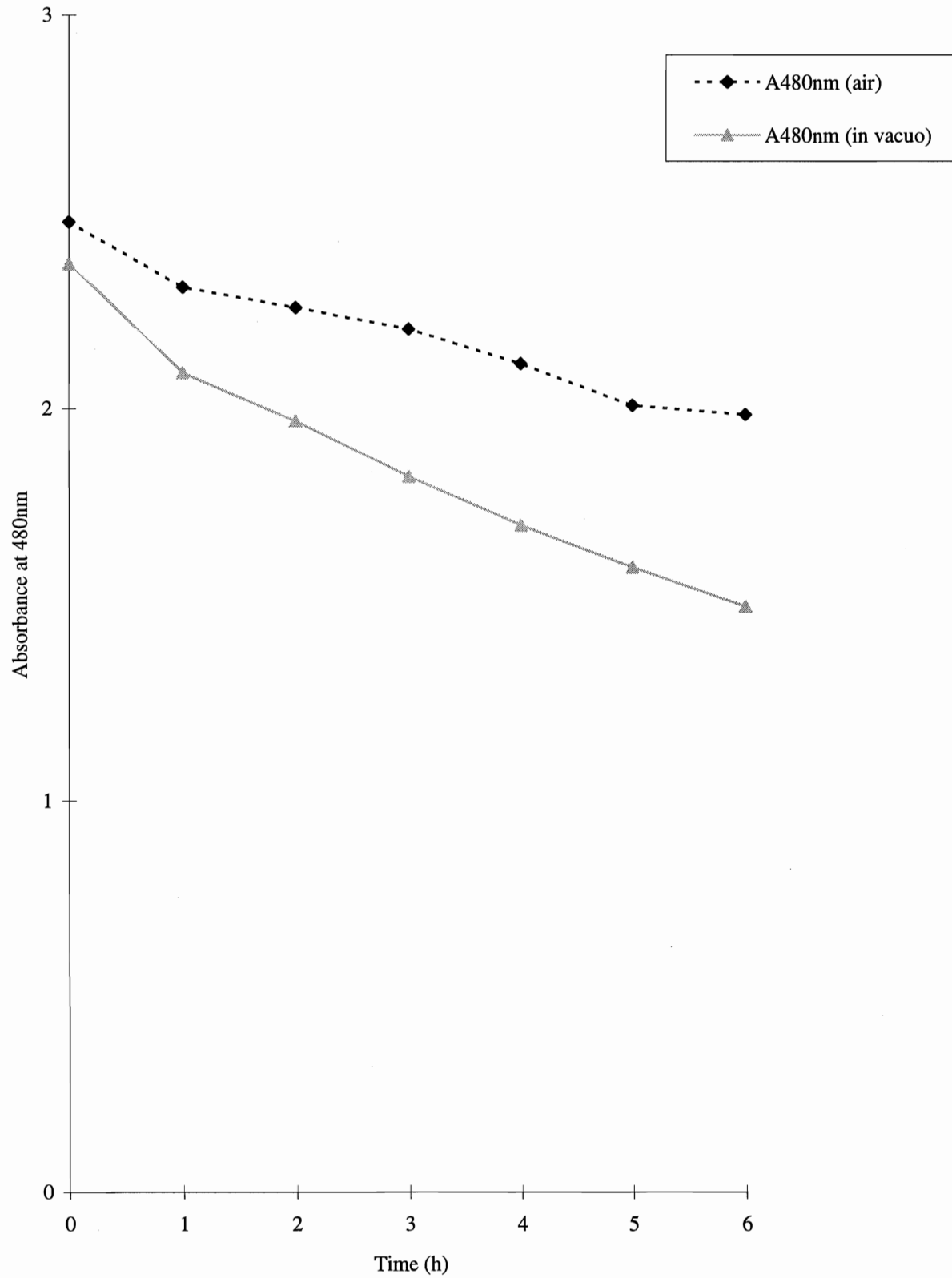


Figure 13

Changes in absorbance of Sunset Yellow on heating at 70°C with 50mM metabisulphite and 71mM ascorbic acid (pH3.0)

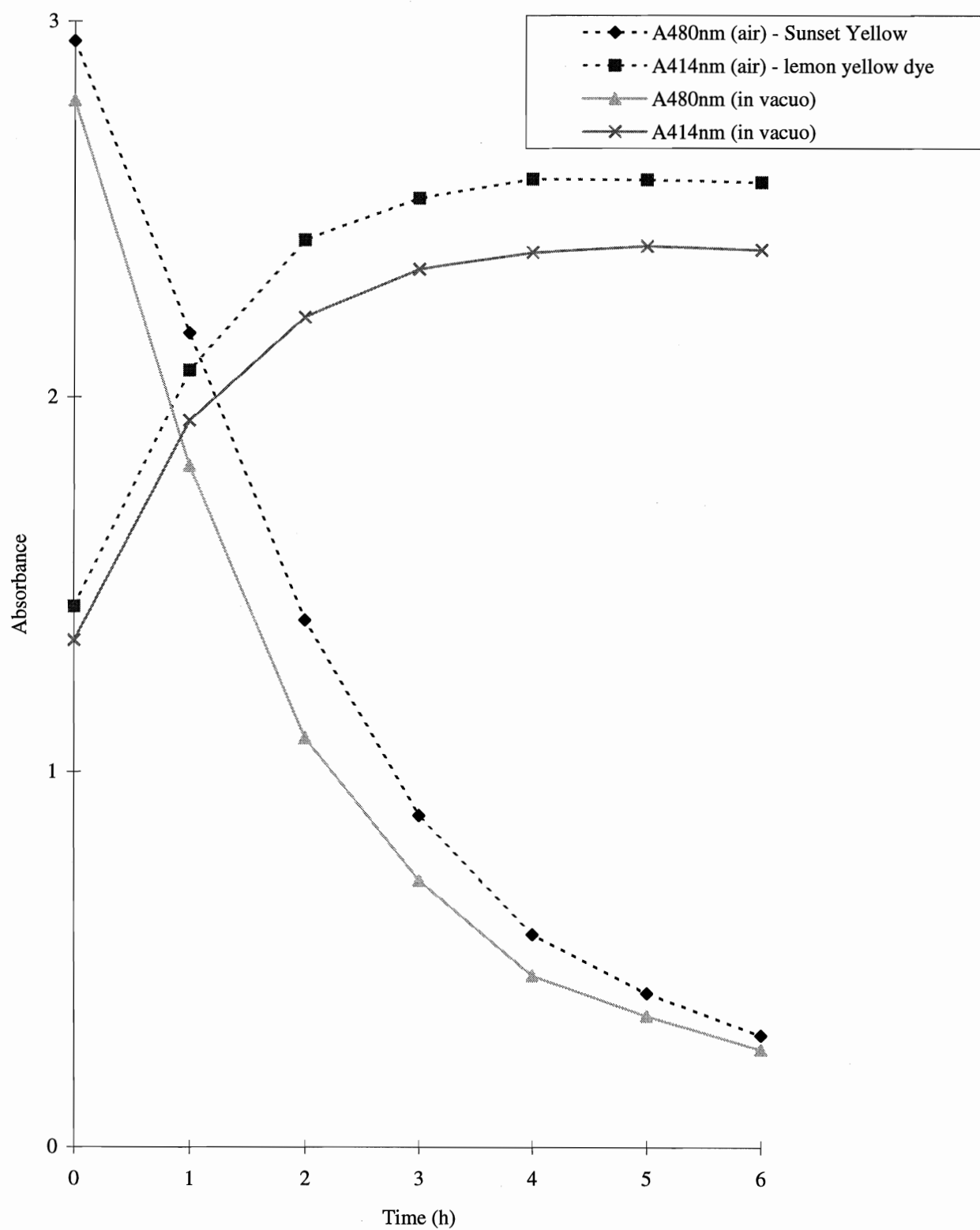


Figure 14

Changes in absorbance of Sunset Yellow on heating at 70°C with 50mM metabisulphite and 142mM ascorbic acid (pH3.0)

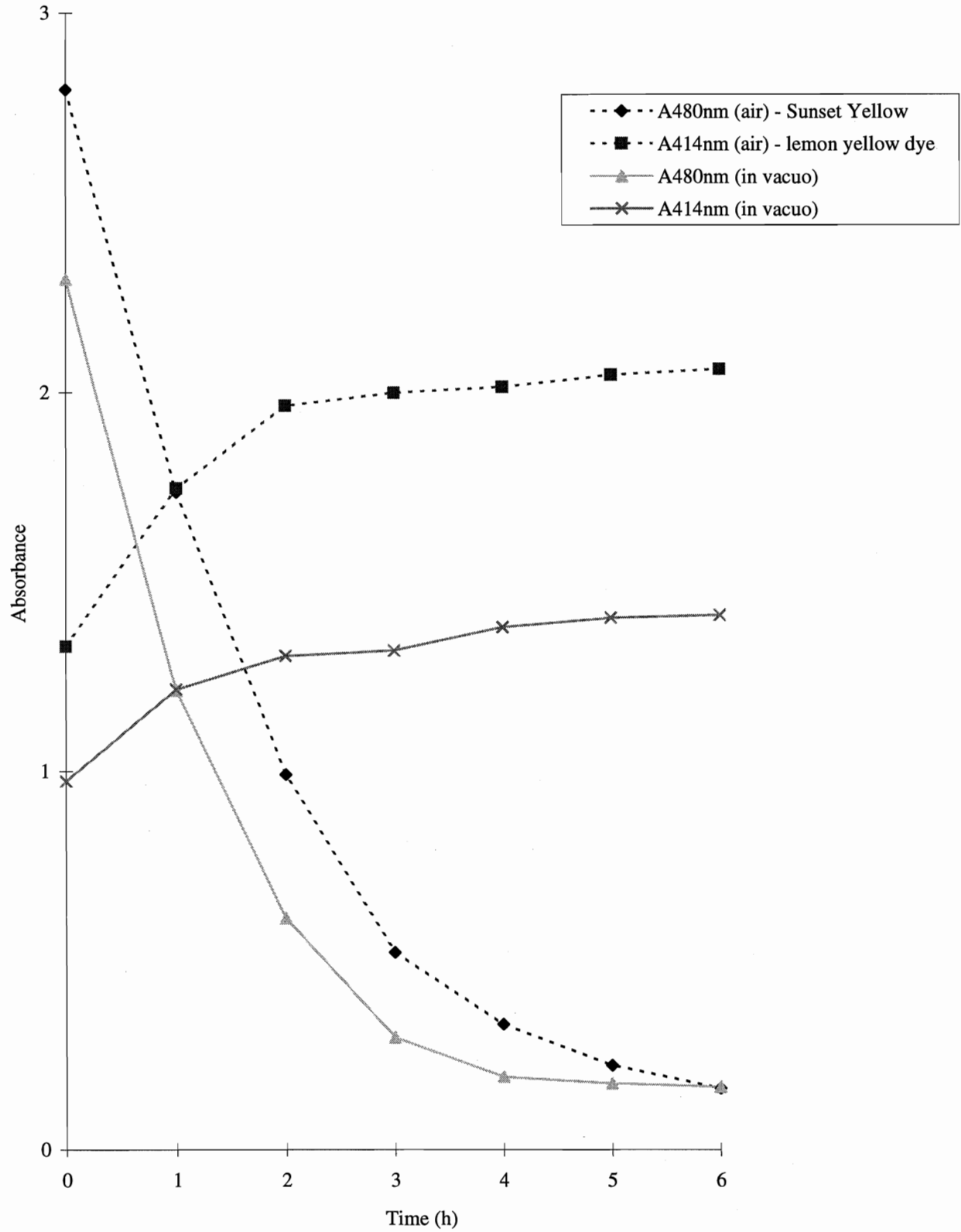




Figure 15

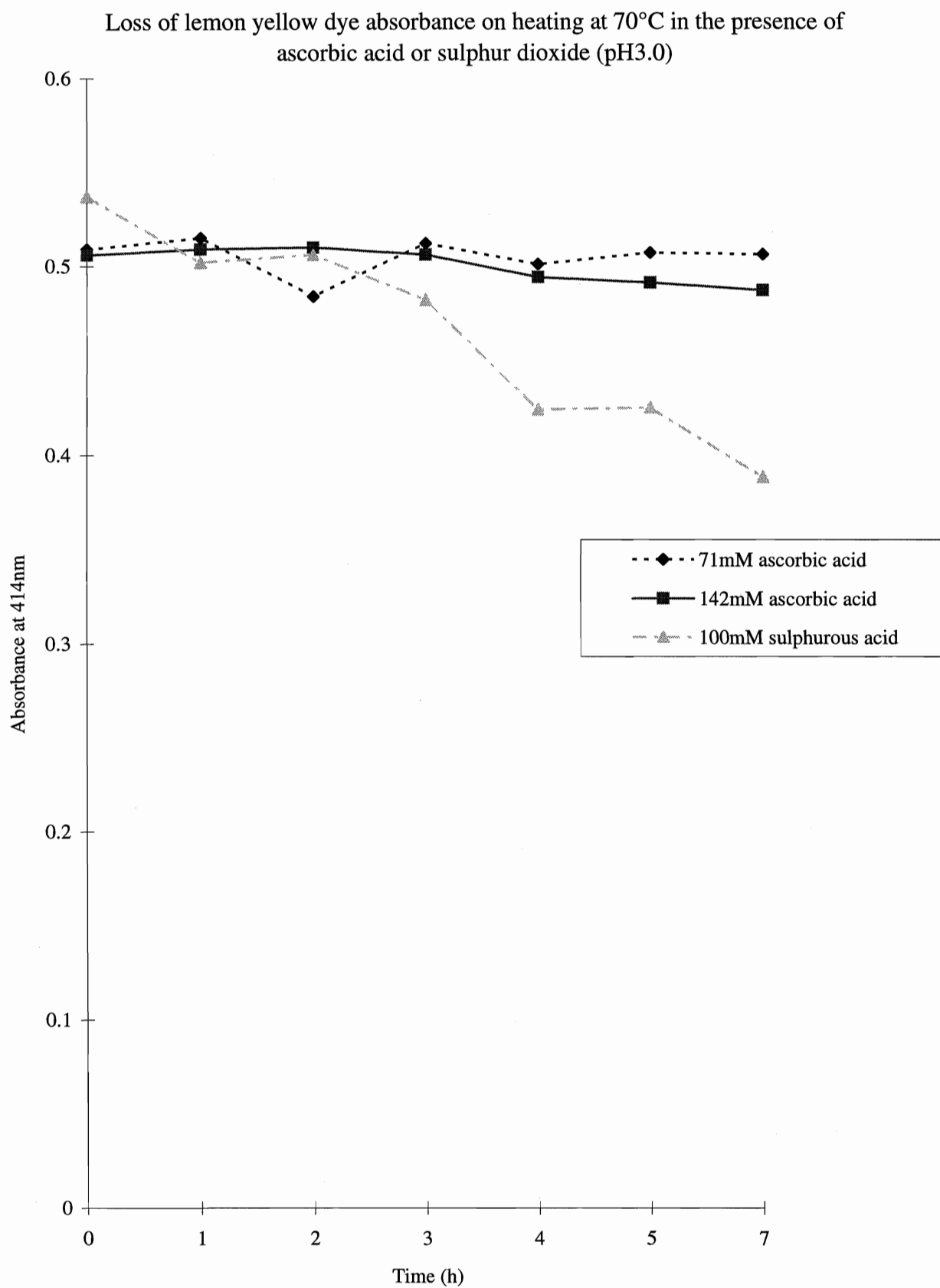
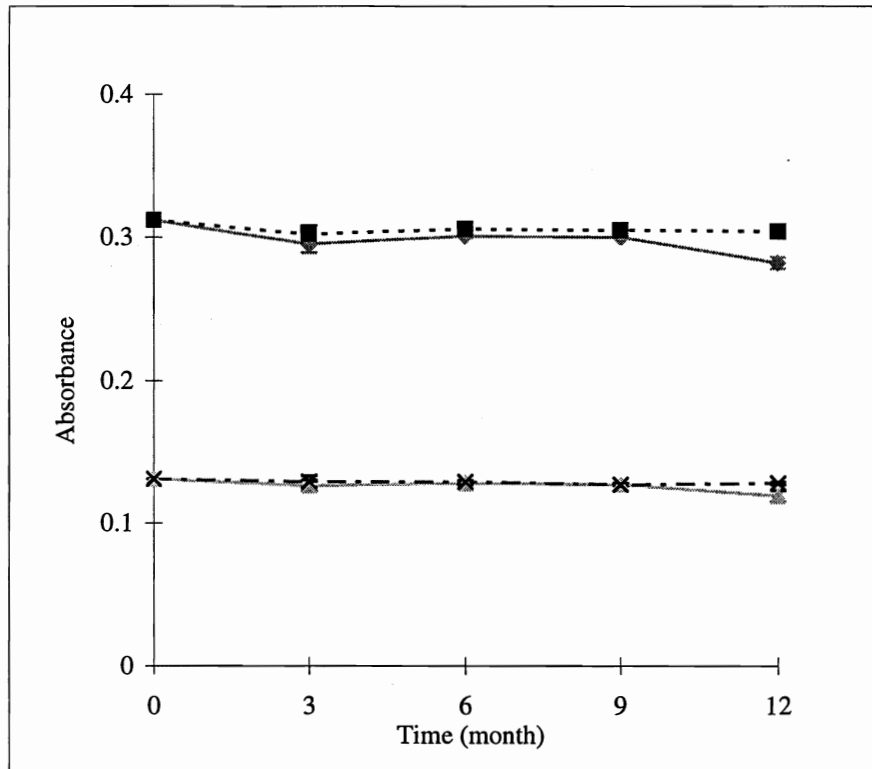


Figure 16

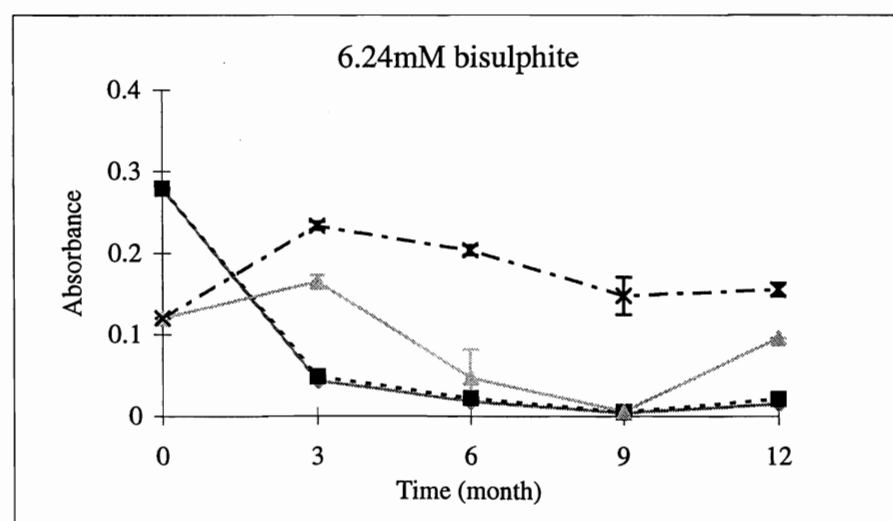
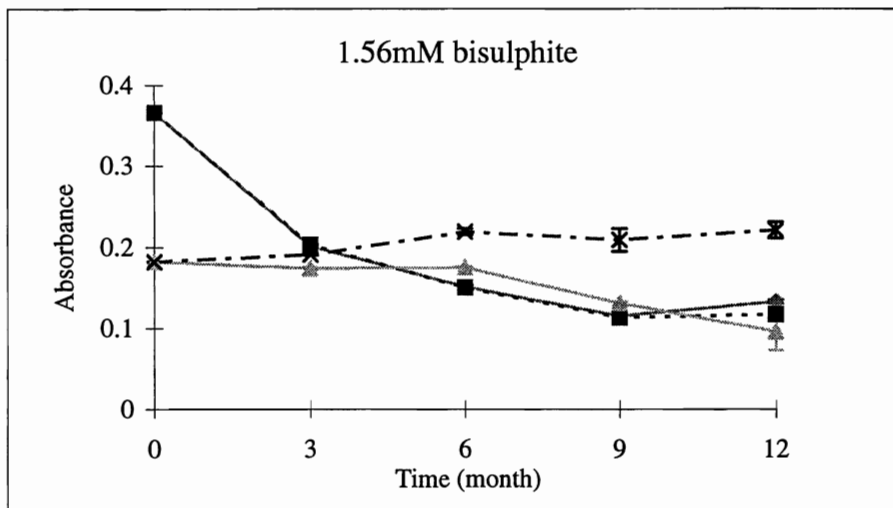
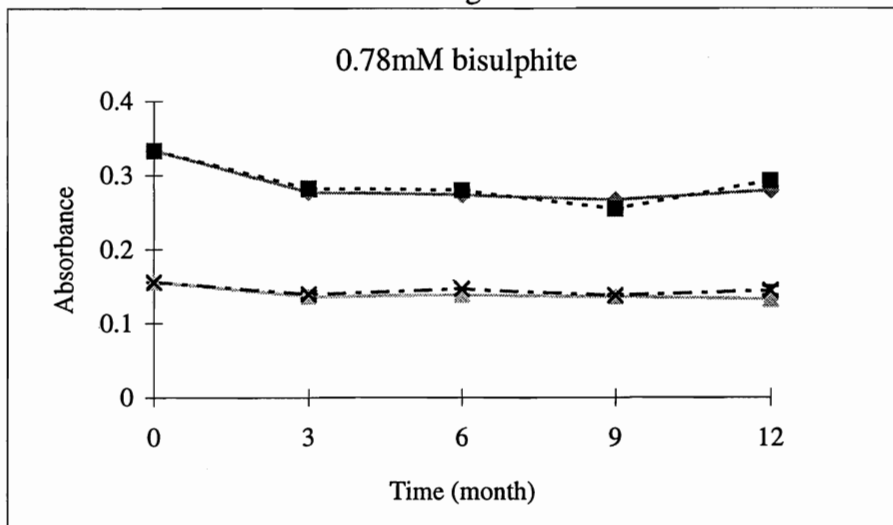
Change in Sunset Yellow absorbance in the absence of bisulphite or ascorbic acid during model system storage at 20°C



—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - × - - A414nm (dark)  
Error bars show range of duplicates

Figure 17

The effect of bisulphite on Sunset Yellow absorbance during model system storage at 20°C

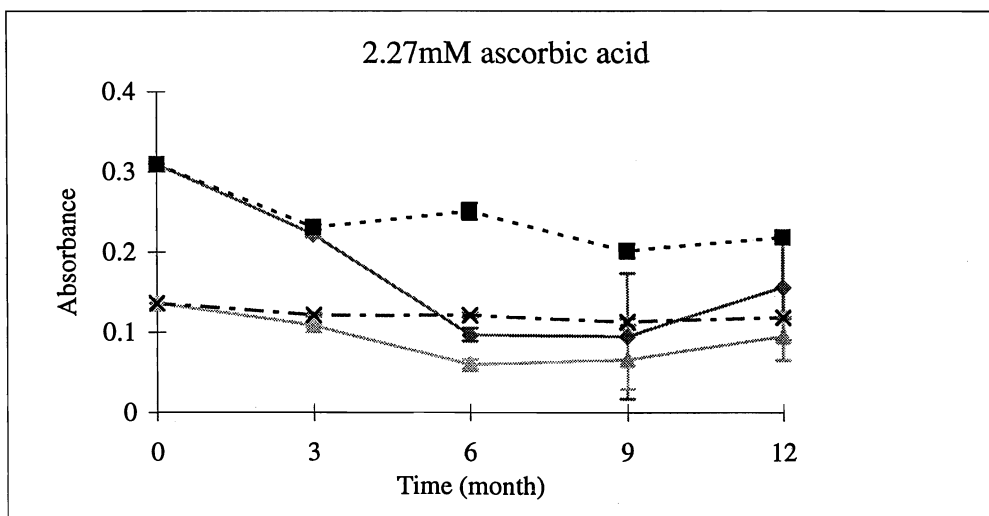
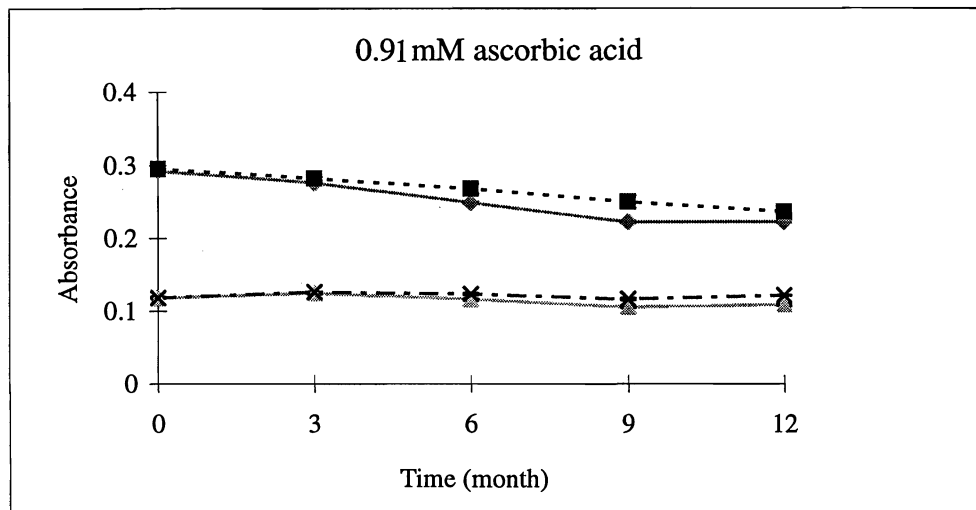
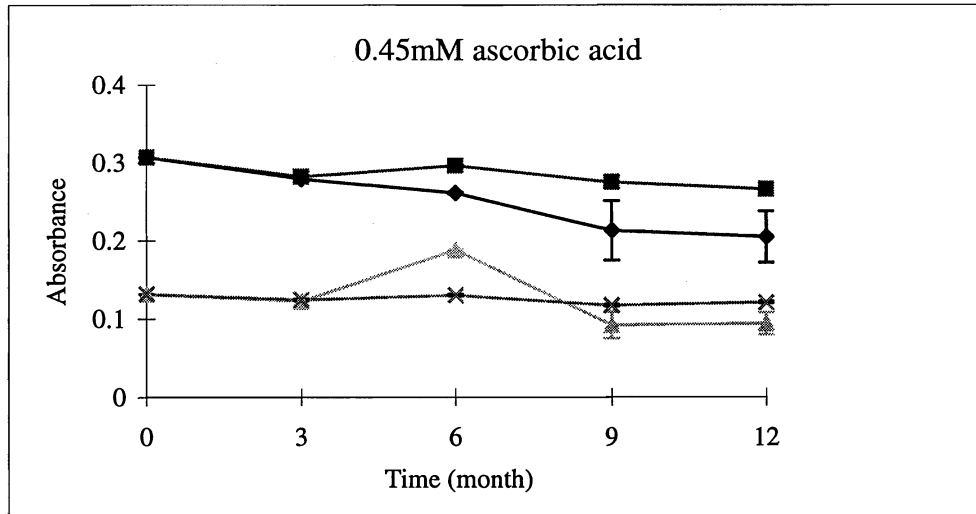


—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - ✕ - - A414nm (dark)

Error bars show range of duplicates

Figure 18

The effect of ascorbic acid on Sunset Yellow absorbance during model system storage at 20°C

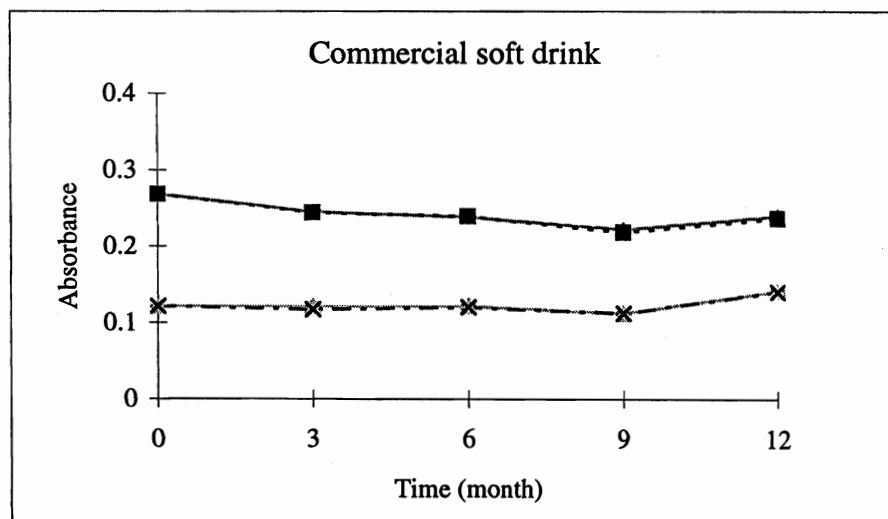
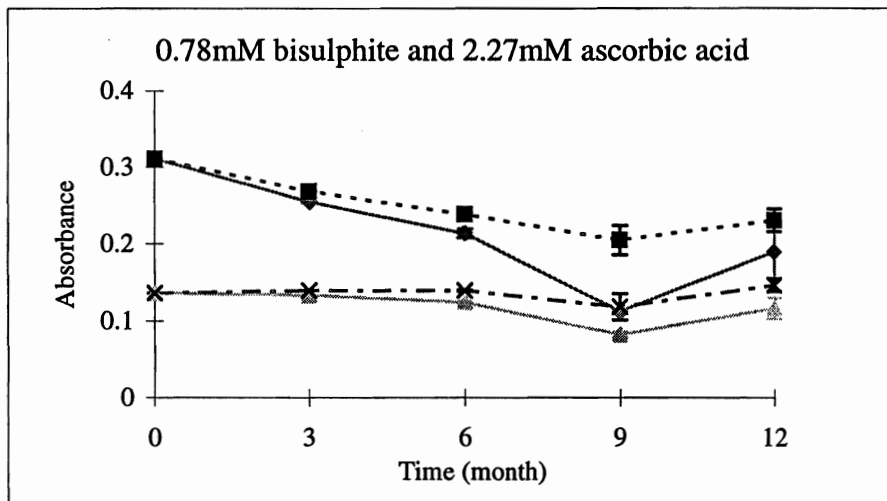
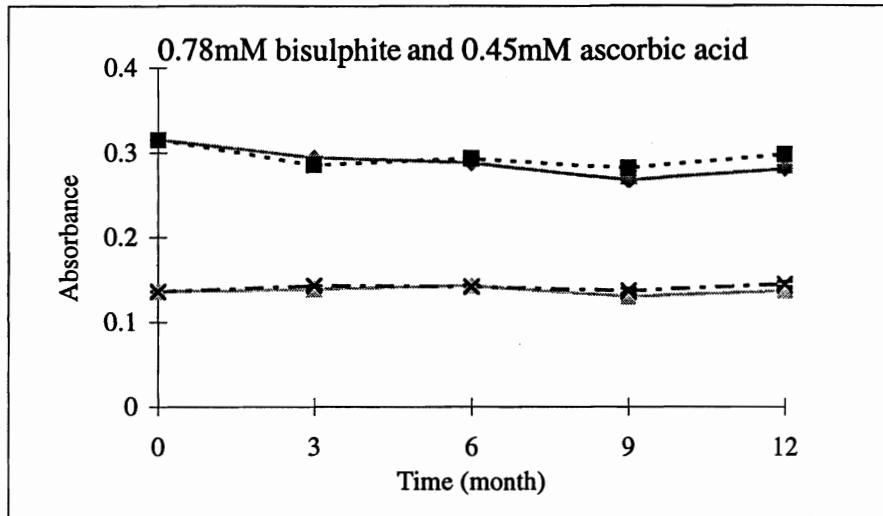


—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - × - - A414nm (dark)

Error bars show range of duplicates

Figure 19

The effect of bisulphite and ascorbic acid combinations on Sunset Yellow absorbance during storage of model systems and a commercial soft drink at 20°C

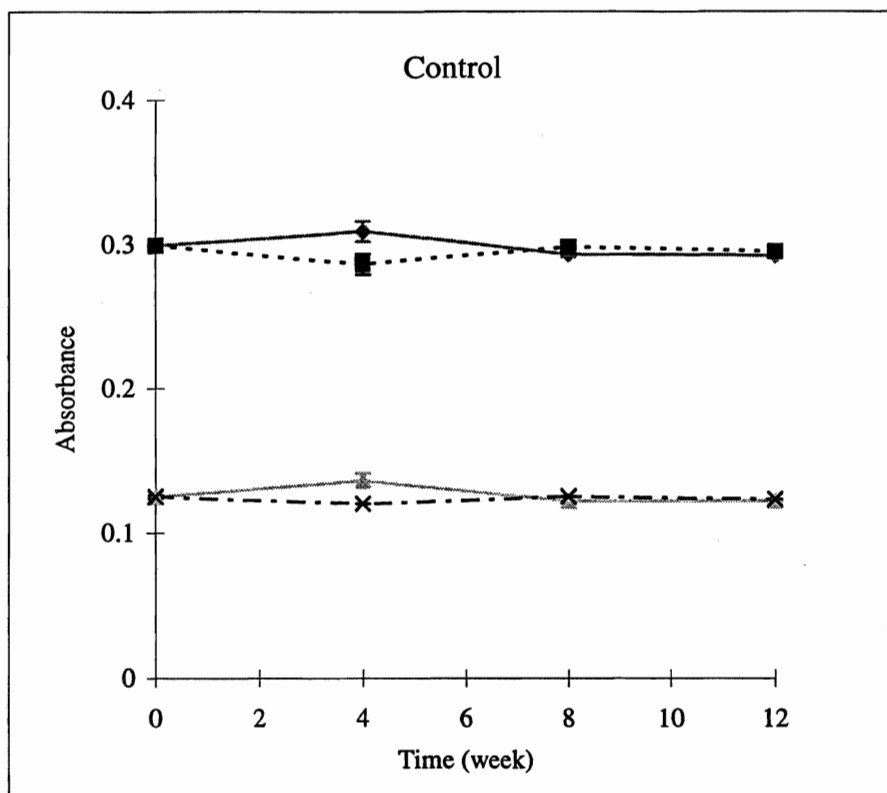


—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - × - - A414nm (dark)

Error bars show range of duplicates

Figure 20

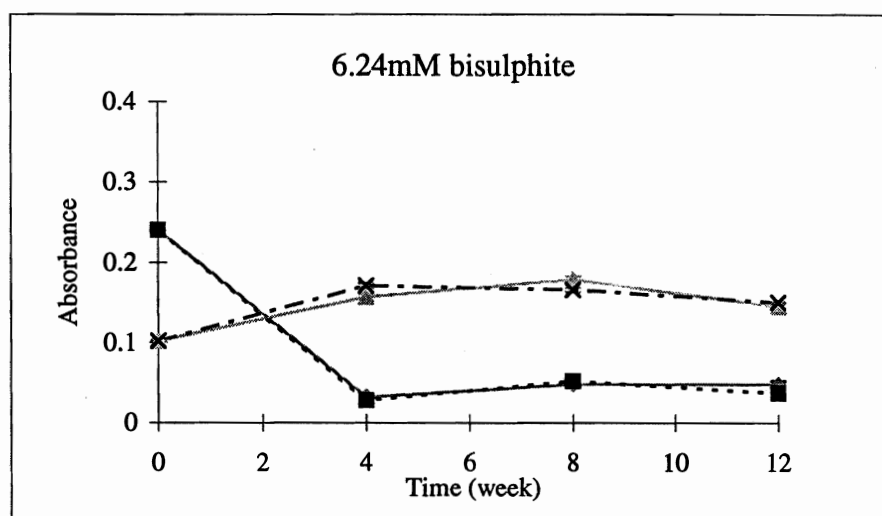
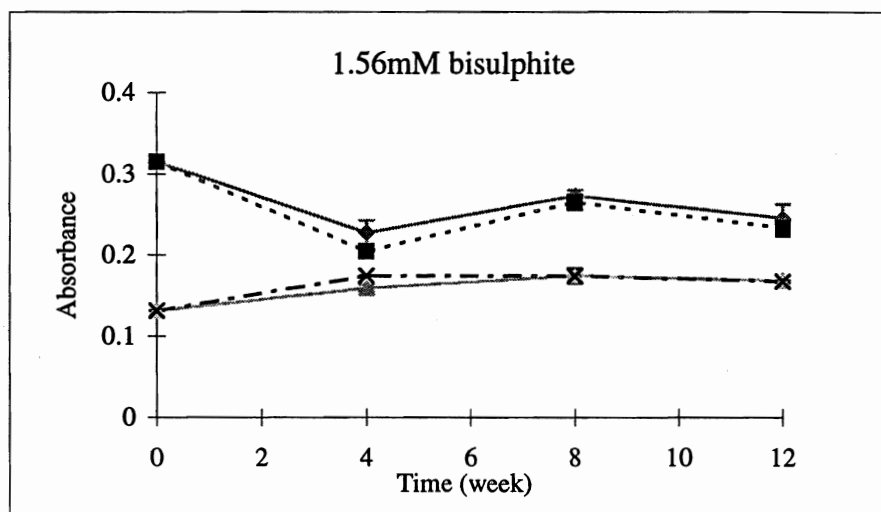
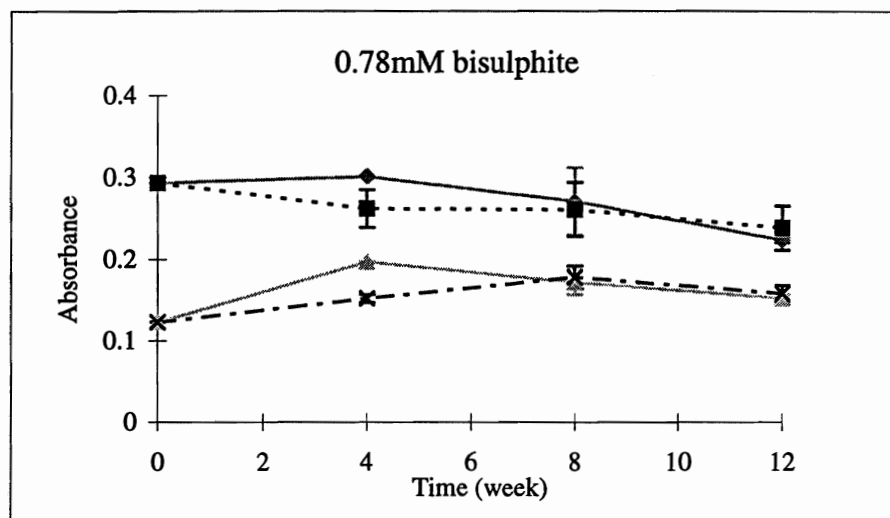
Change in Sunset Yellow absorbance in the absence of bisulphite or ascorbic acid during model system storage at 50°C



—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - ✕ - - A414nm (dark)

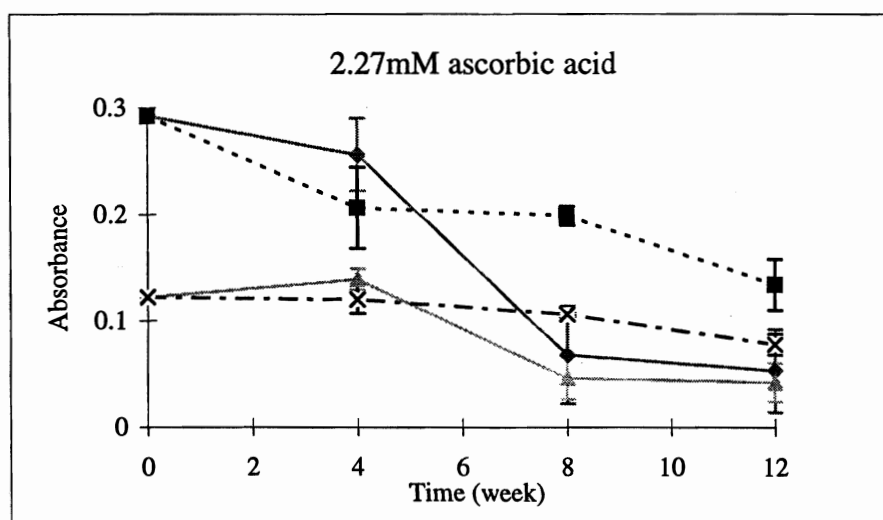
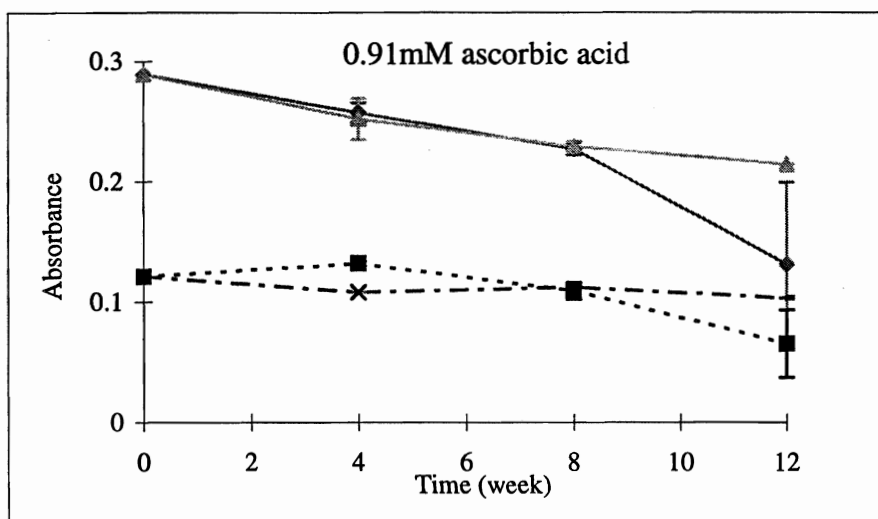
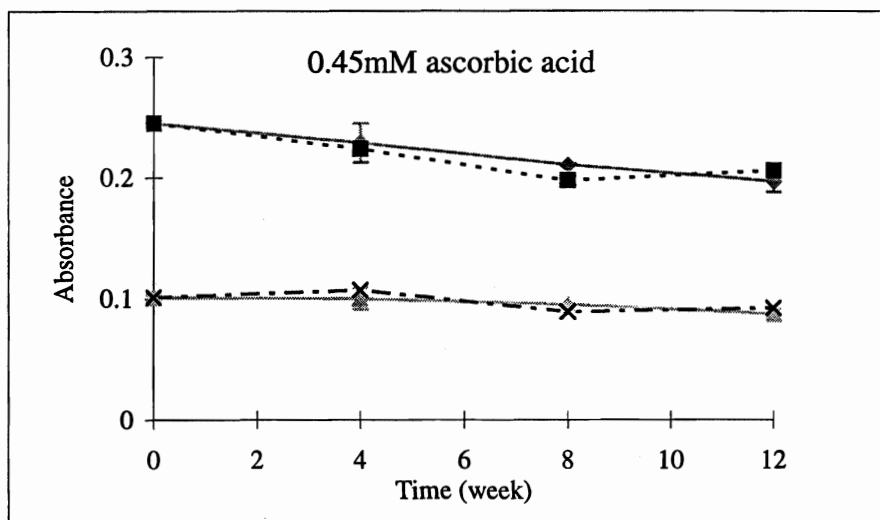
Error bars show range of duplicates

Figure 21  
The effect of bisulphite on Sunset Yellow absorbance during model system storage at 50°C



—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - × - - A414nm (dark)  
Error bars show range of duplicates

Figure 22  
The effect of ascorbic acid on Sunset Yellow absorbance during  
model system storage at 50°C

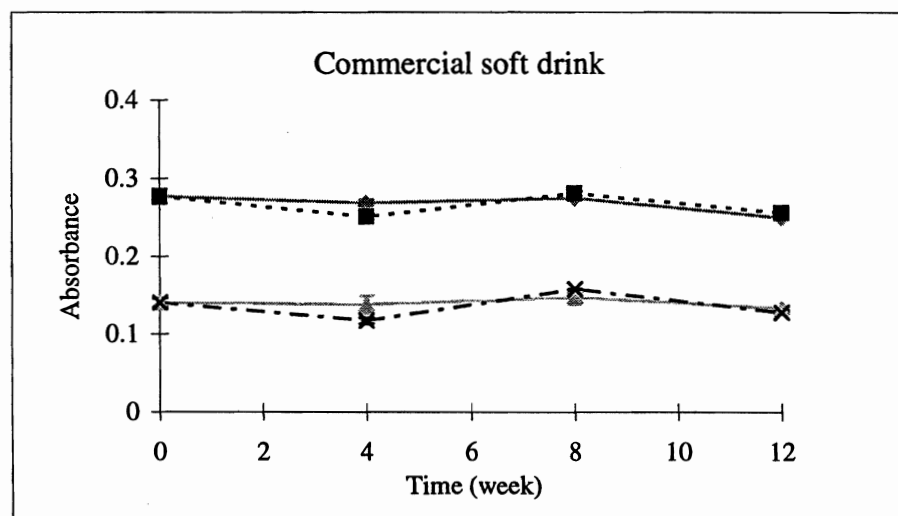
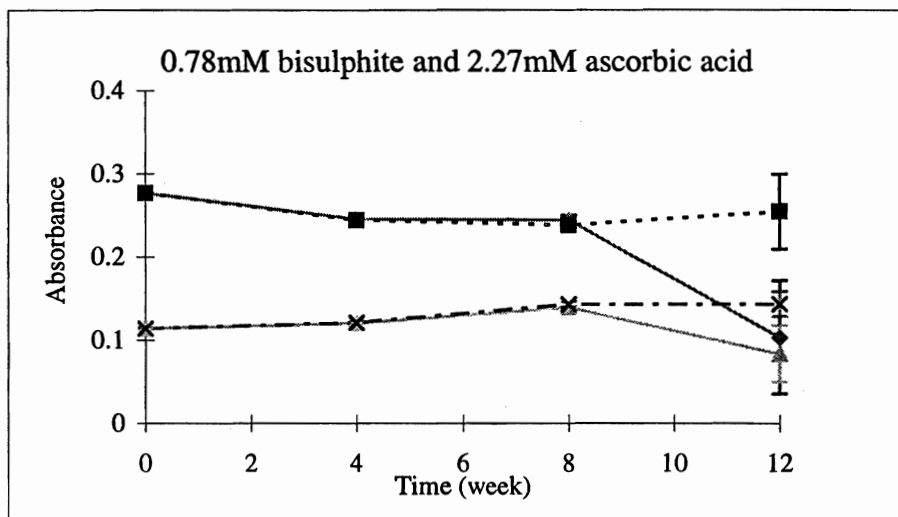
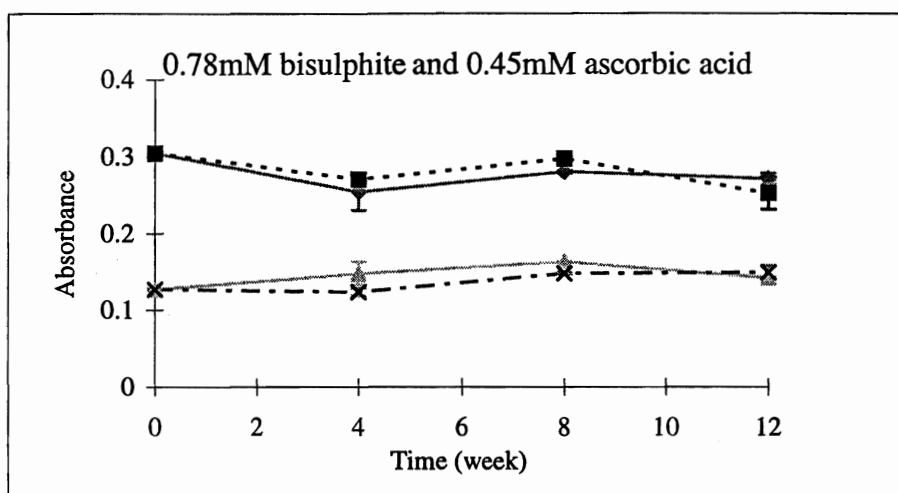


—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - × - - A414nm (dark)  
Error bars show range of duplicates



Figure 23

The effect of bisulphite and ascorbic acid combinations on Sunset Yellow absorbance during storage of model systems and a commercial drink at 50°C



—◆— A480nm (light)    - - ■ - - A480nm (dark)    —▲— A414nm (light)    - - × - - A414nm (dark)

Error bars show range of duplicates

Figure 24

# Sunset Yellow degradation by sulphur dioxide and ascorbic acid

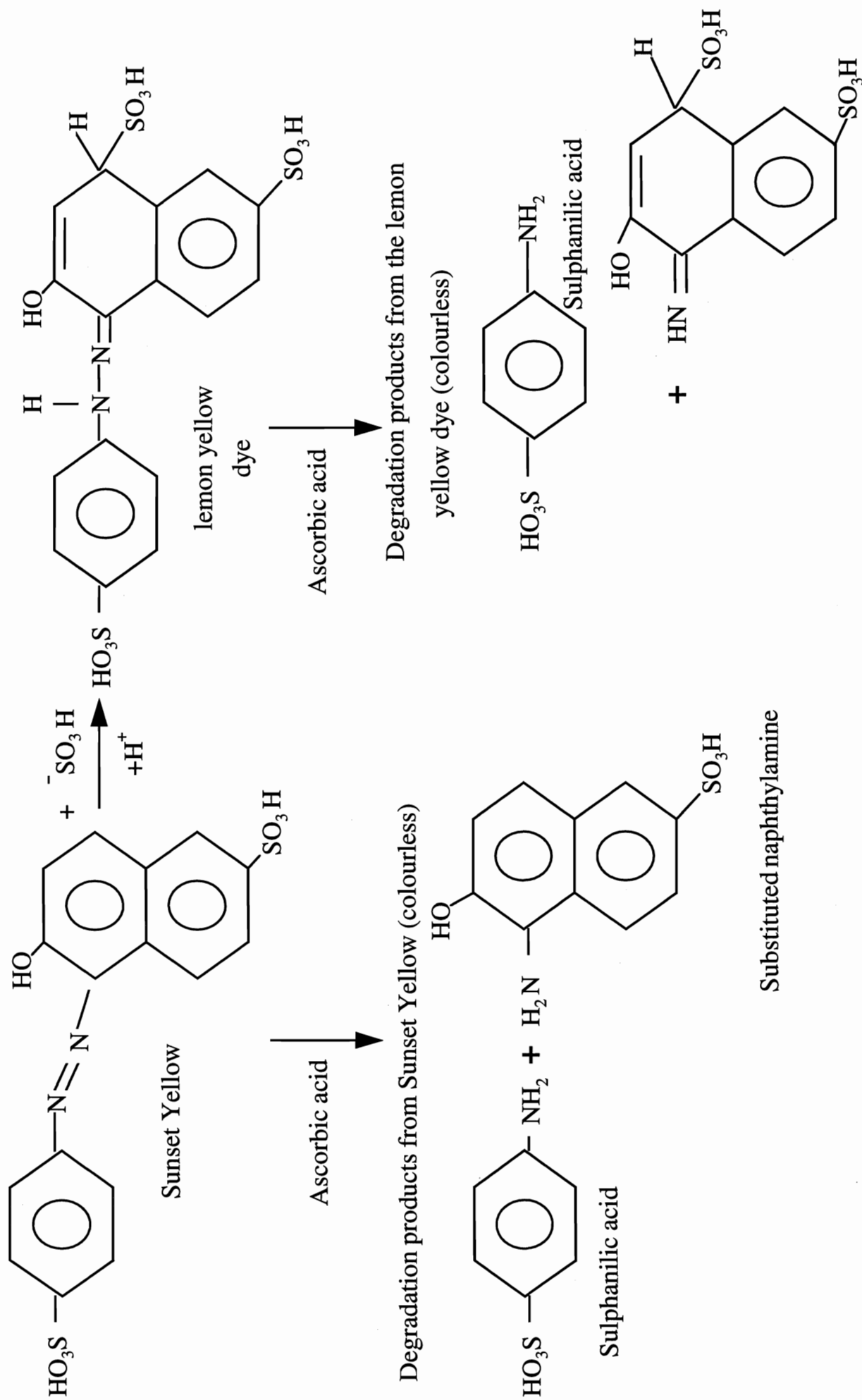


Figure 25  
HPLC of Sunset Yellow

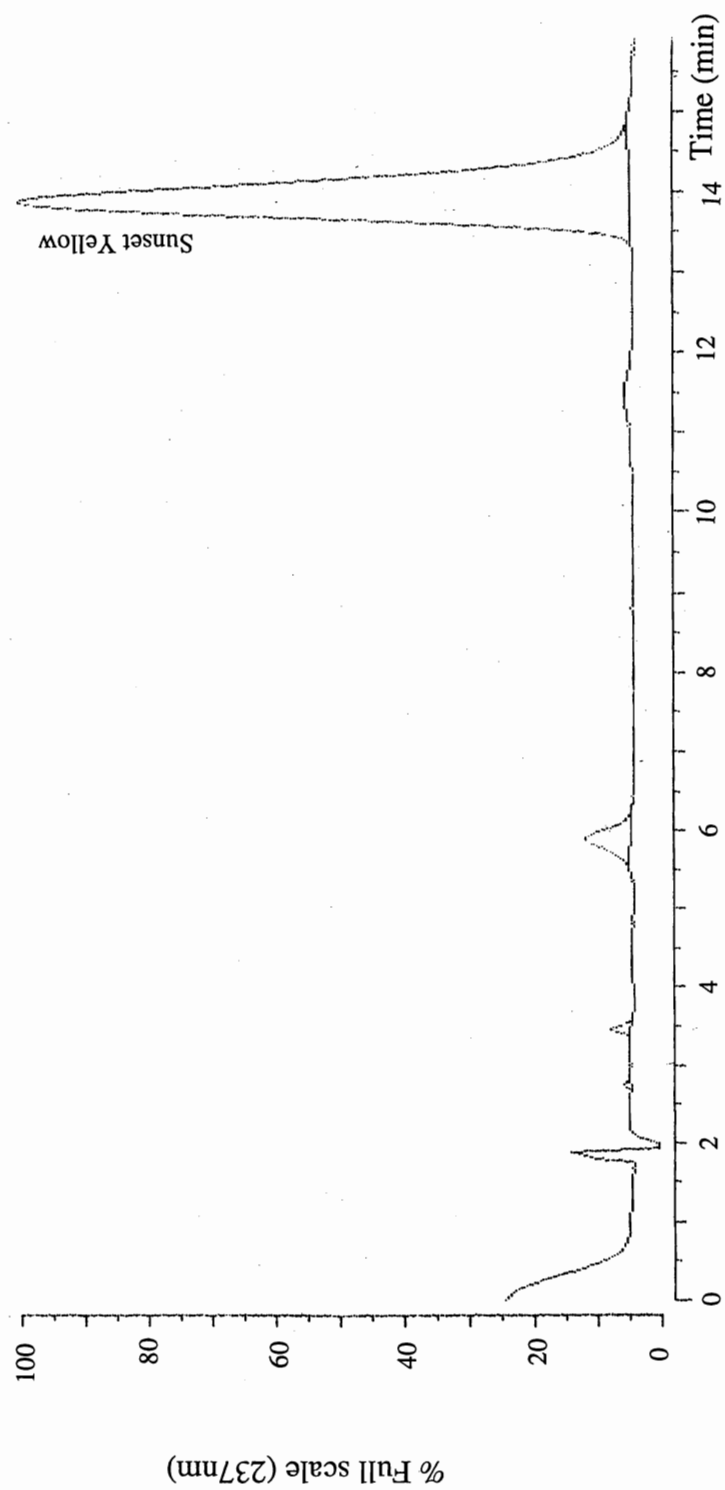


Figure 26

HPLC of the lemon yellow secondary dye

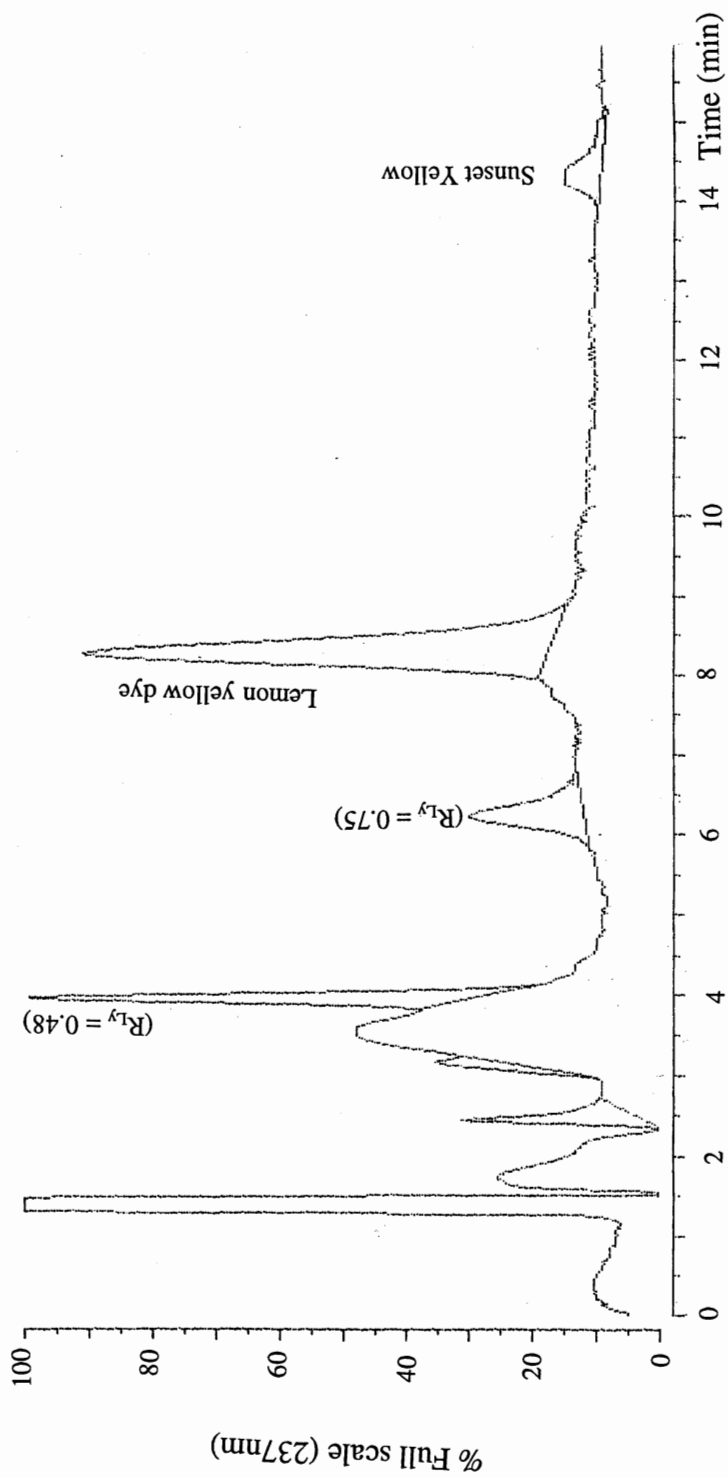


Figure 27

HPLC of the degraded lemon yellow dye

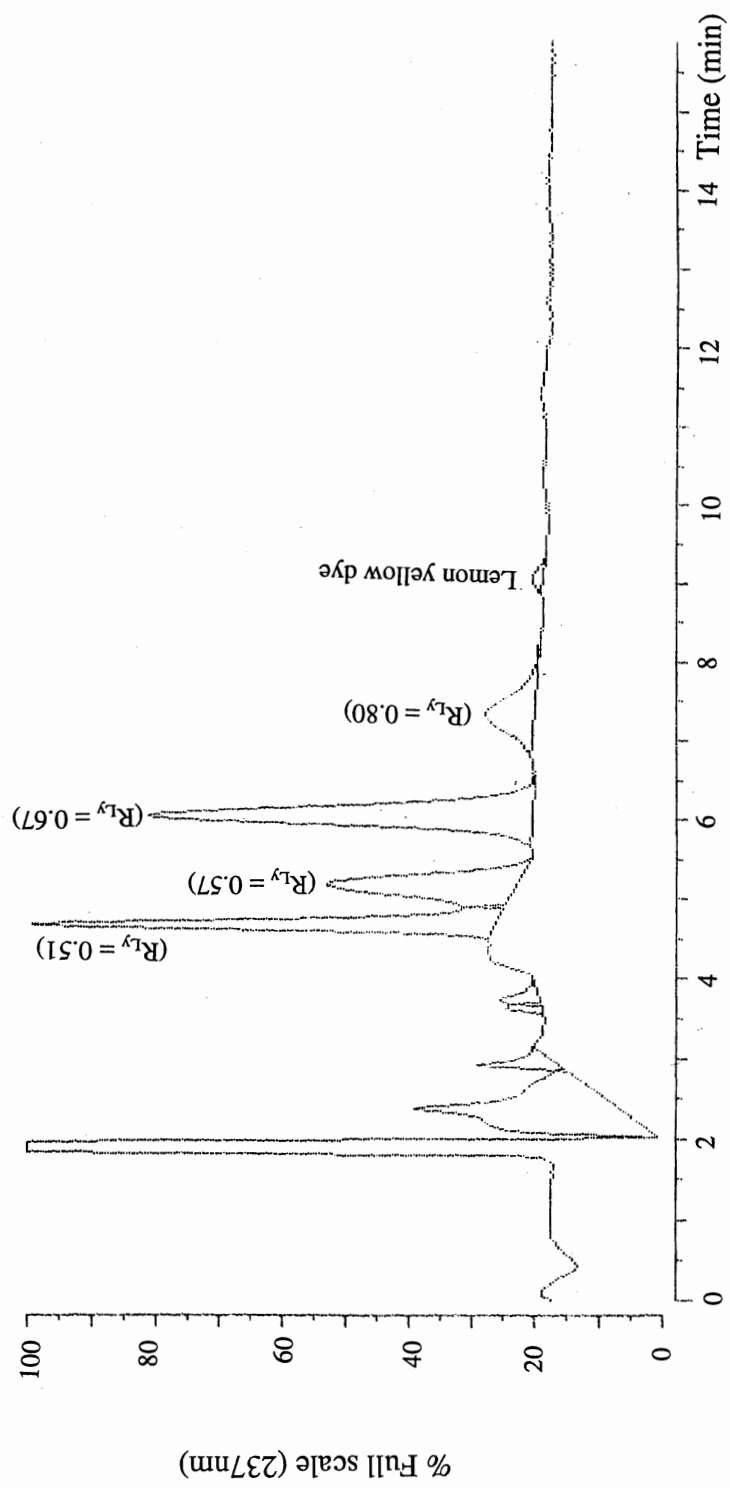


Figure 28

Capillary electropherogram of Sunset Yellow

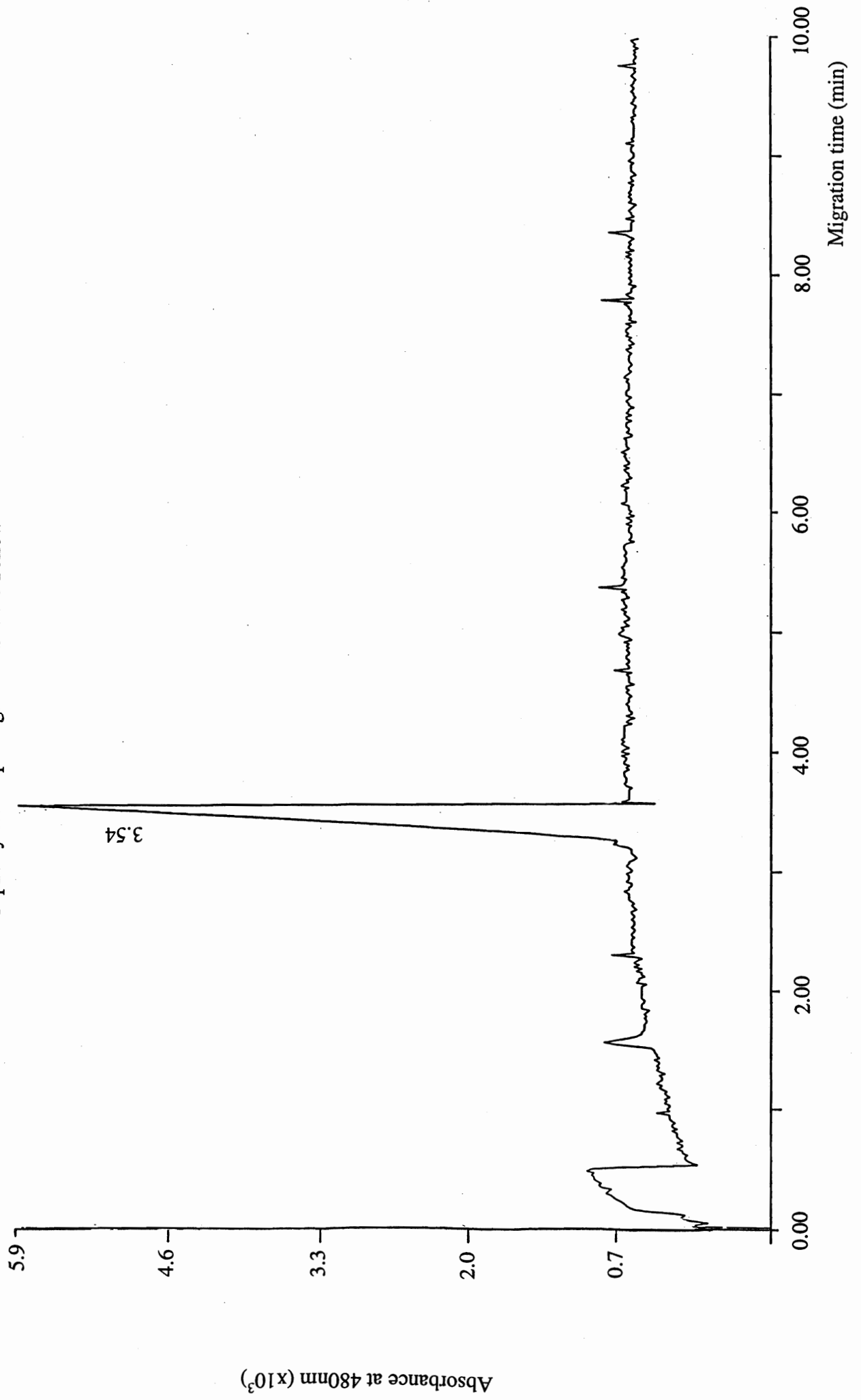


Figure 29

Capillary electropherogram of the lemon yellow secondary dye

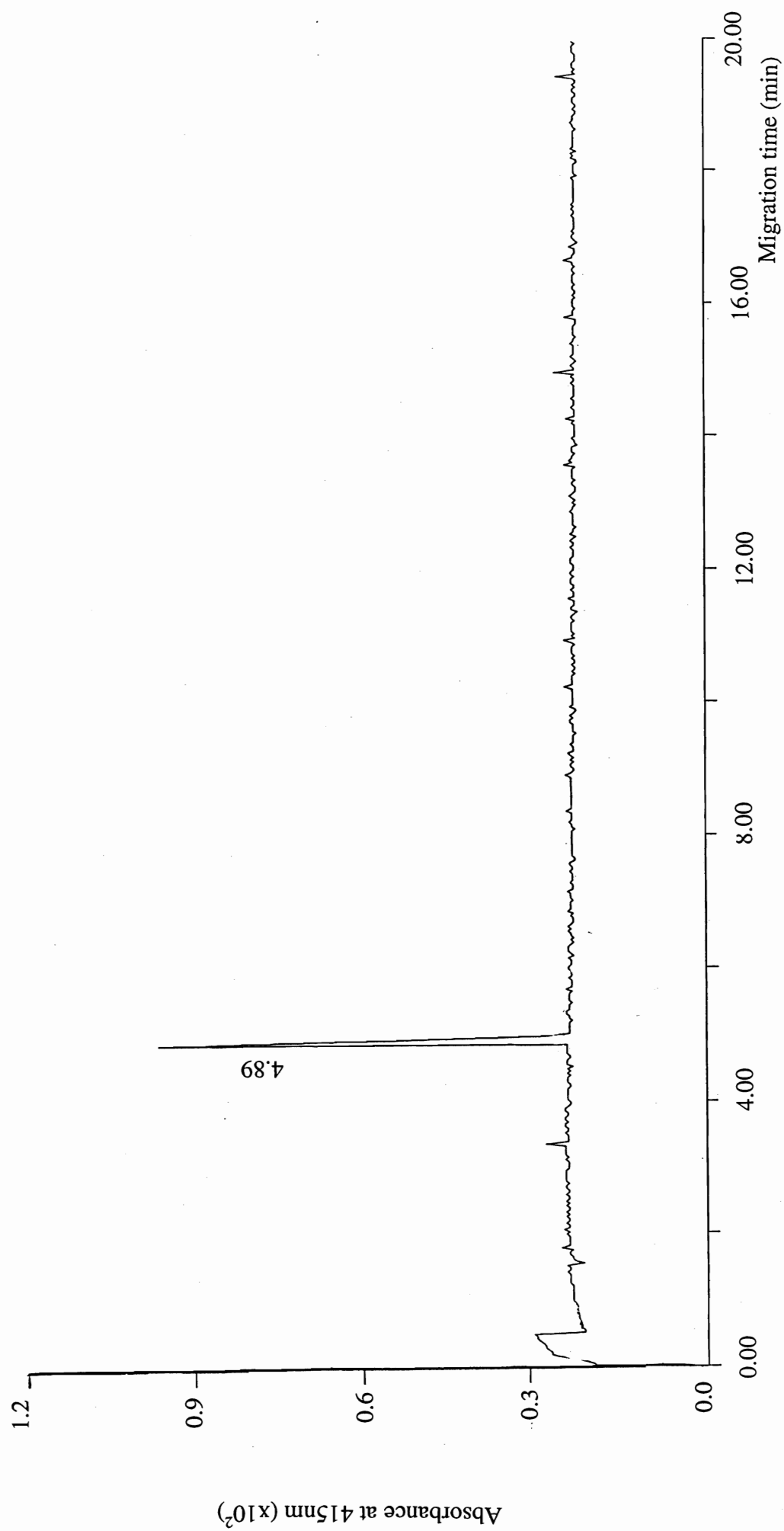


Figure 30

Capillary electropherogram of degraded lemon yellow secondary dye

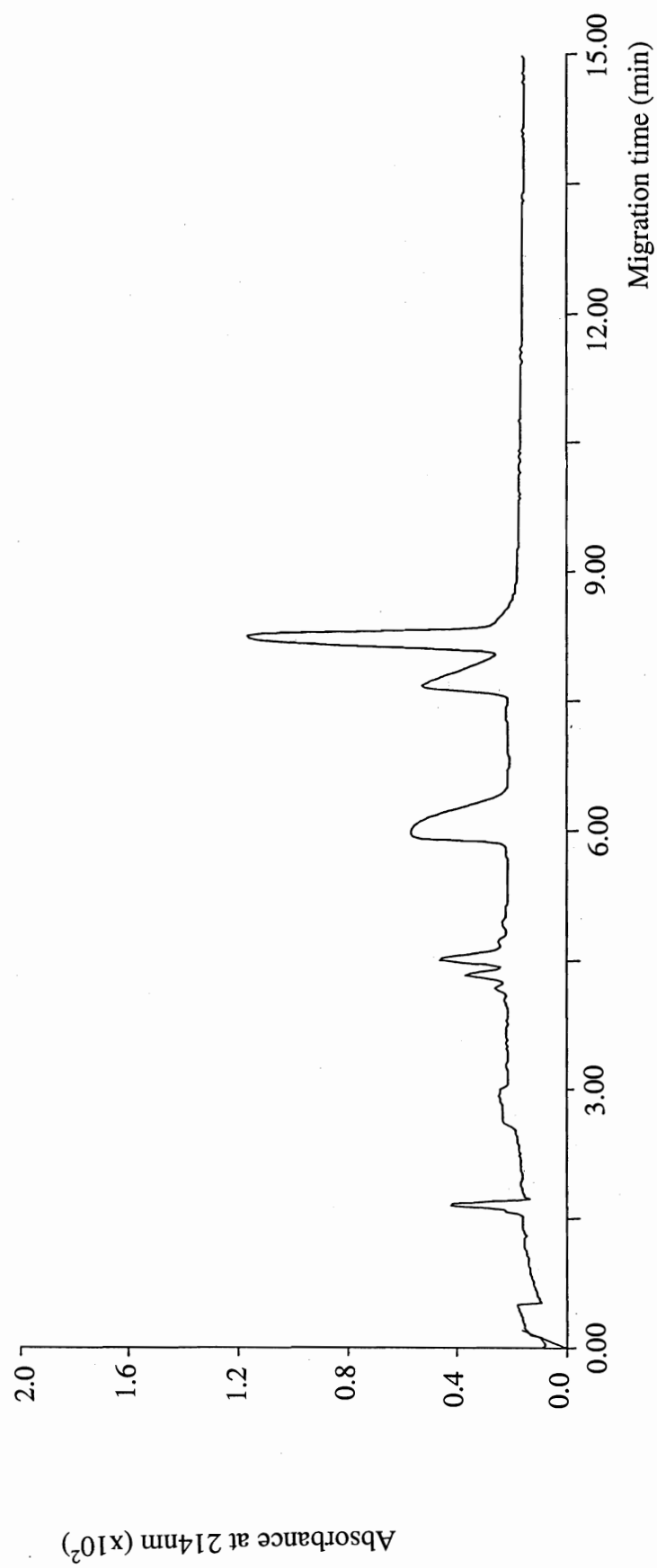




Figure 31

Capillary electropherogram of sulphanilic acid

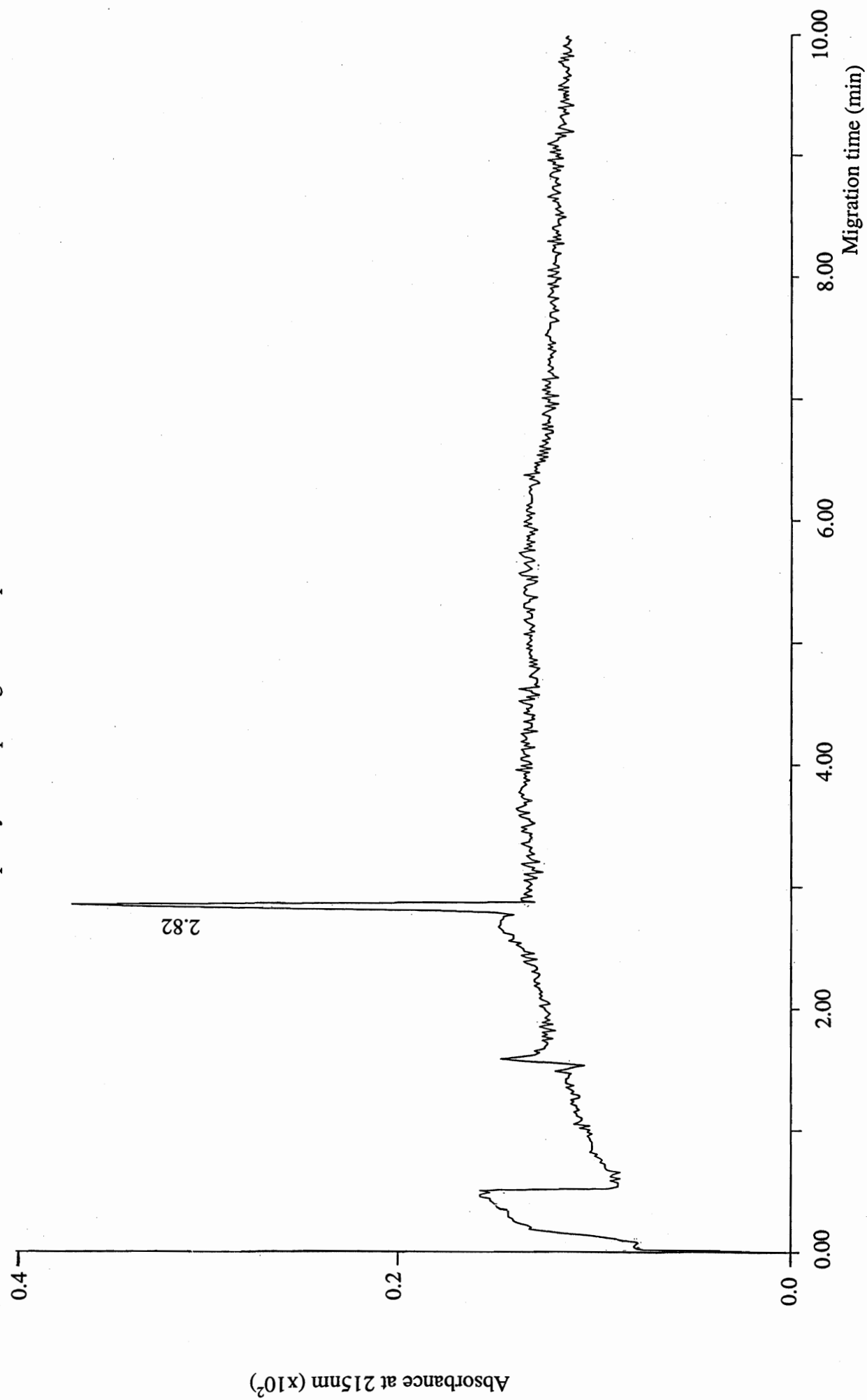


Figure 32

Electrospray-mass spectrum of Sunset Yellow

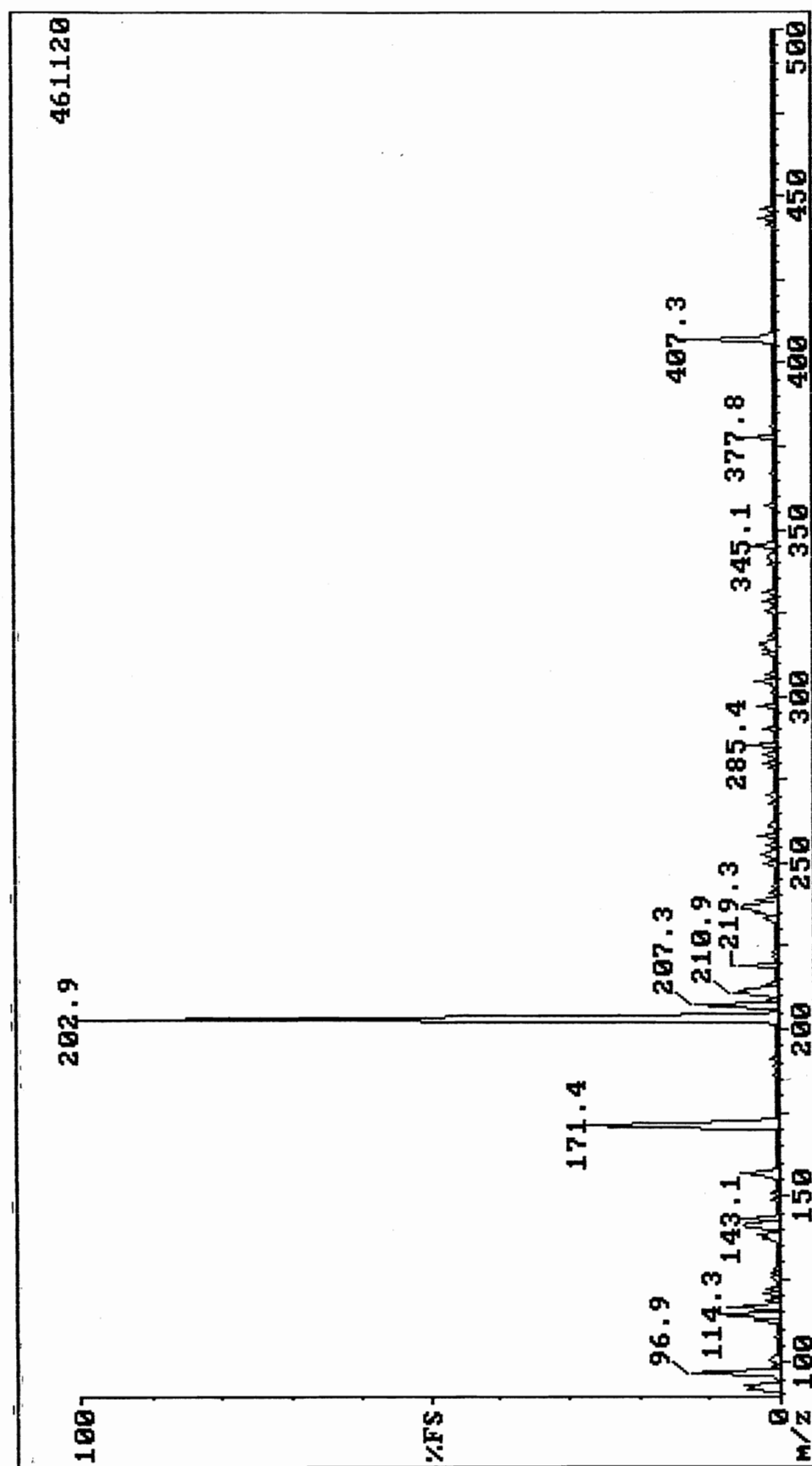


Figure 33

High resolution electrospray-mass spectrum of Sunset Yellow

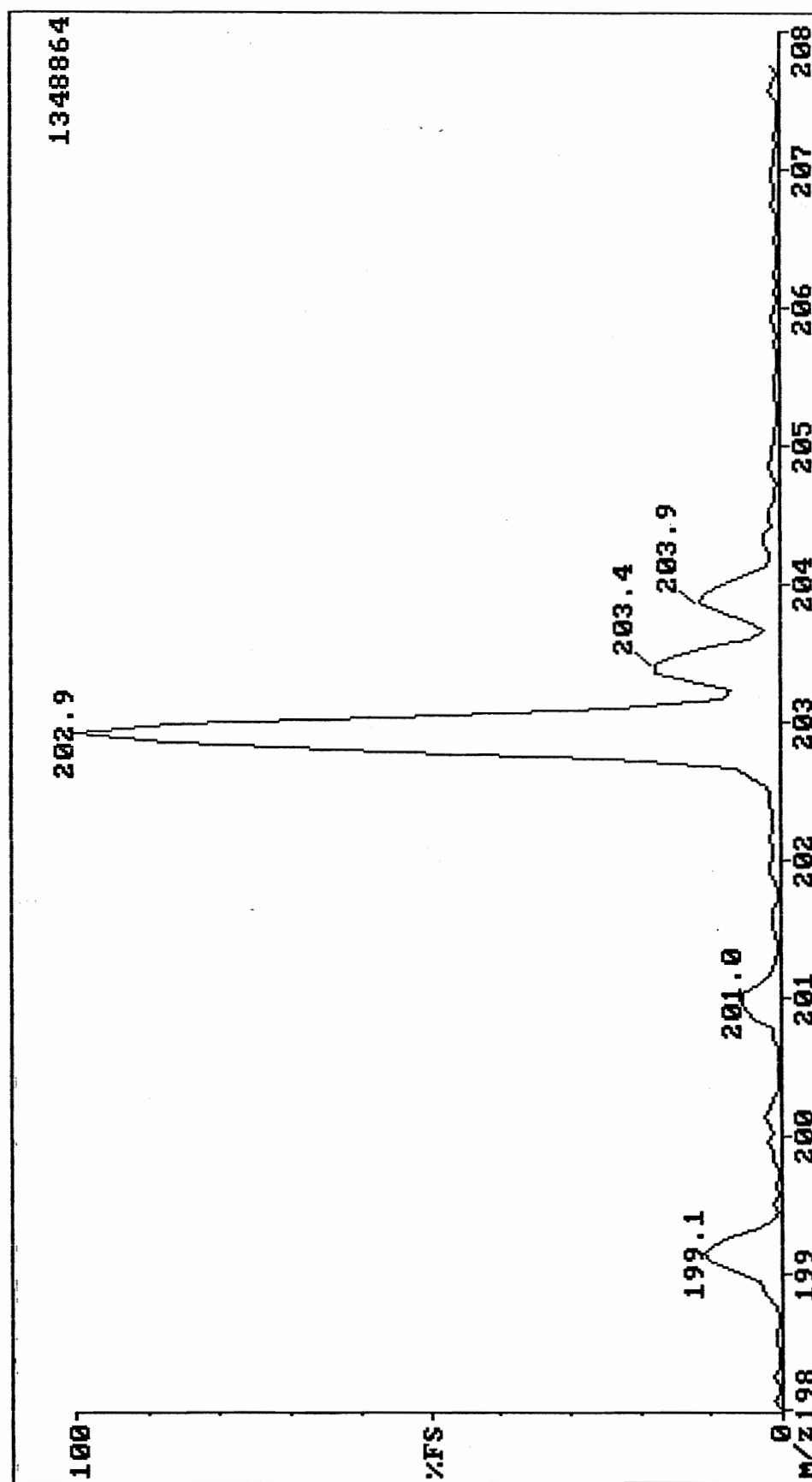


Figure 34

Electrospray-mass spectrum of the sulphurous acid control containing no Sunset Yellow

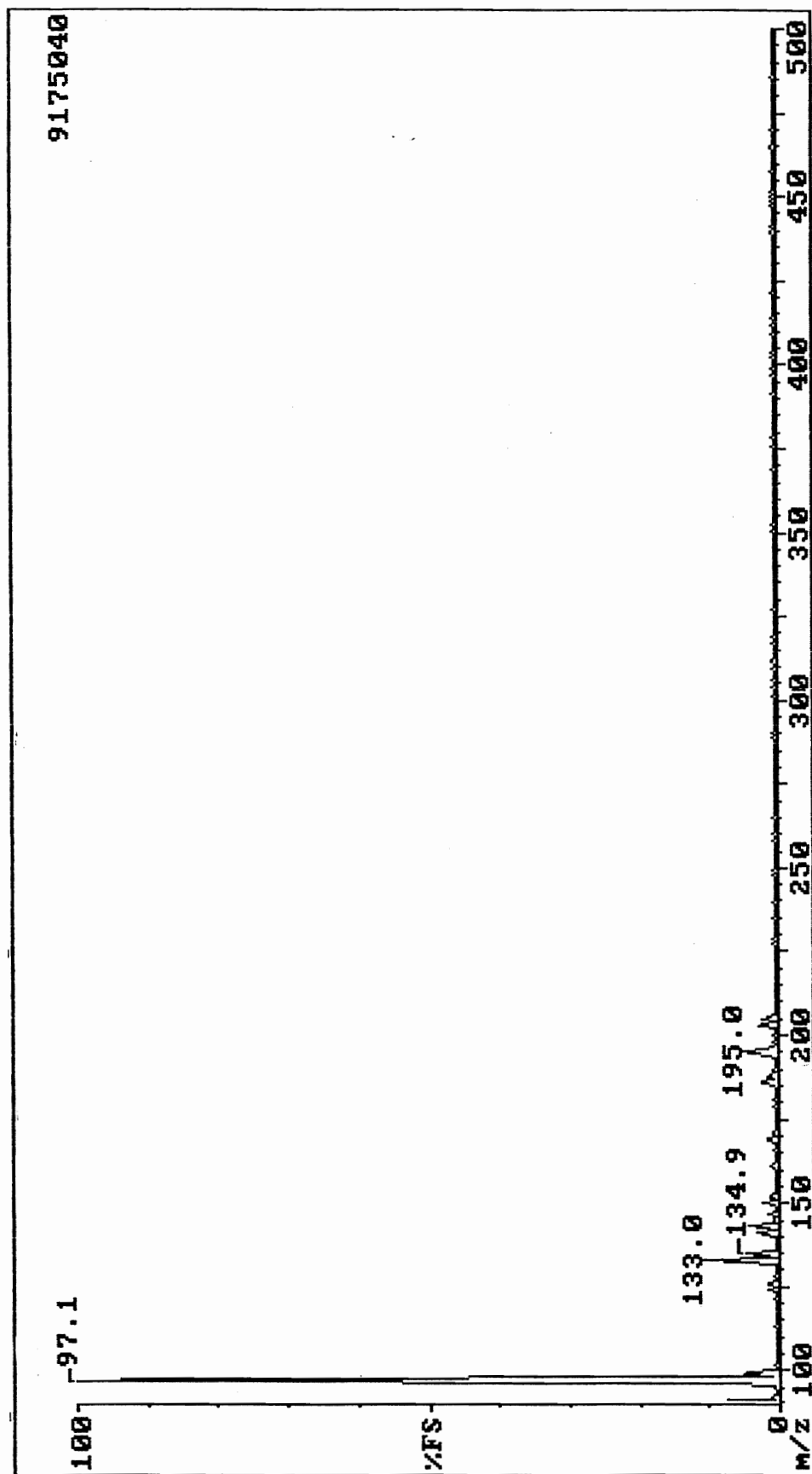


Figure 35

Electrospray-mass spectrum of the lemon yellow dye preparation

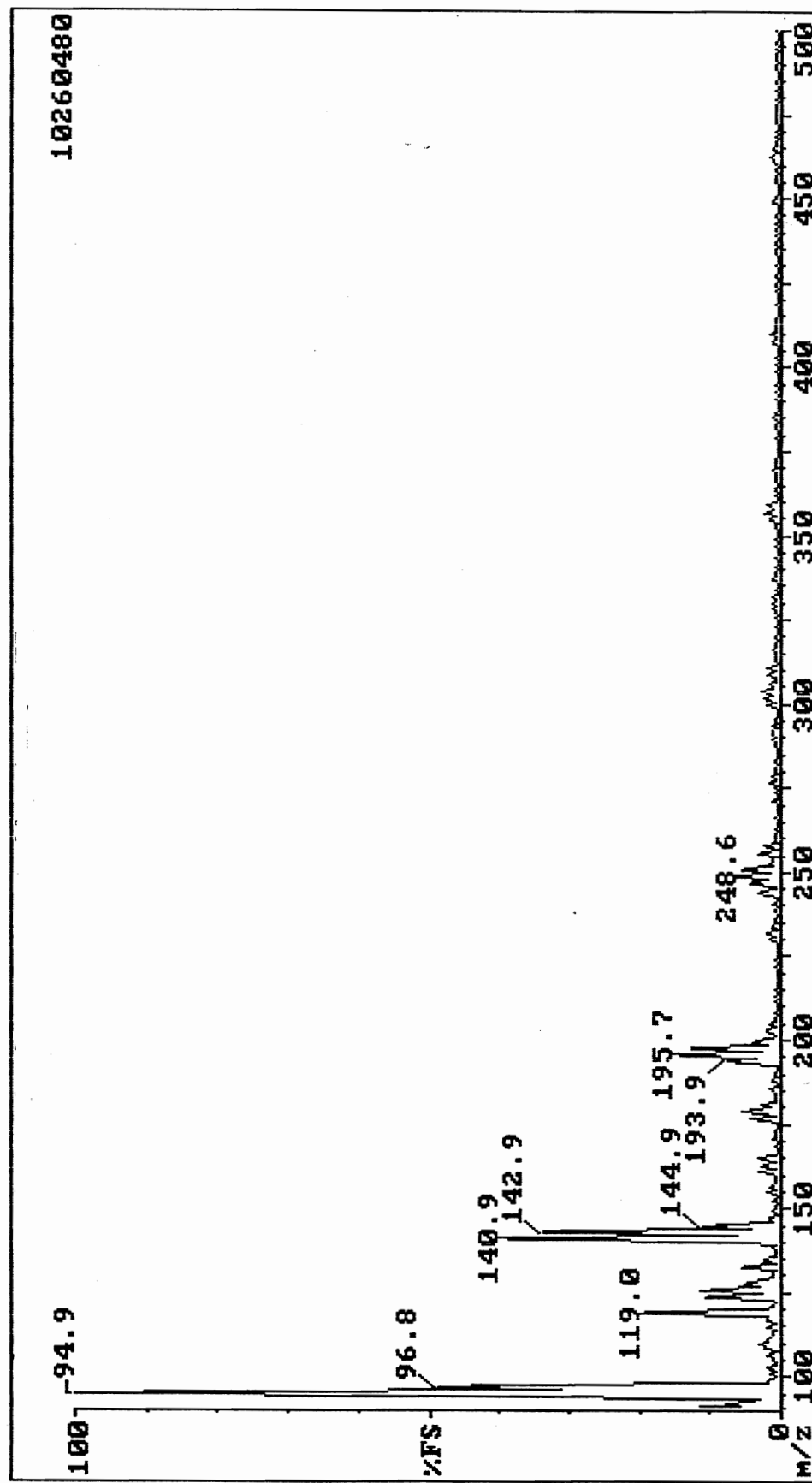


Figure 36

High resolution electrospray-mass spectrum of the lemon yellow dye preparation

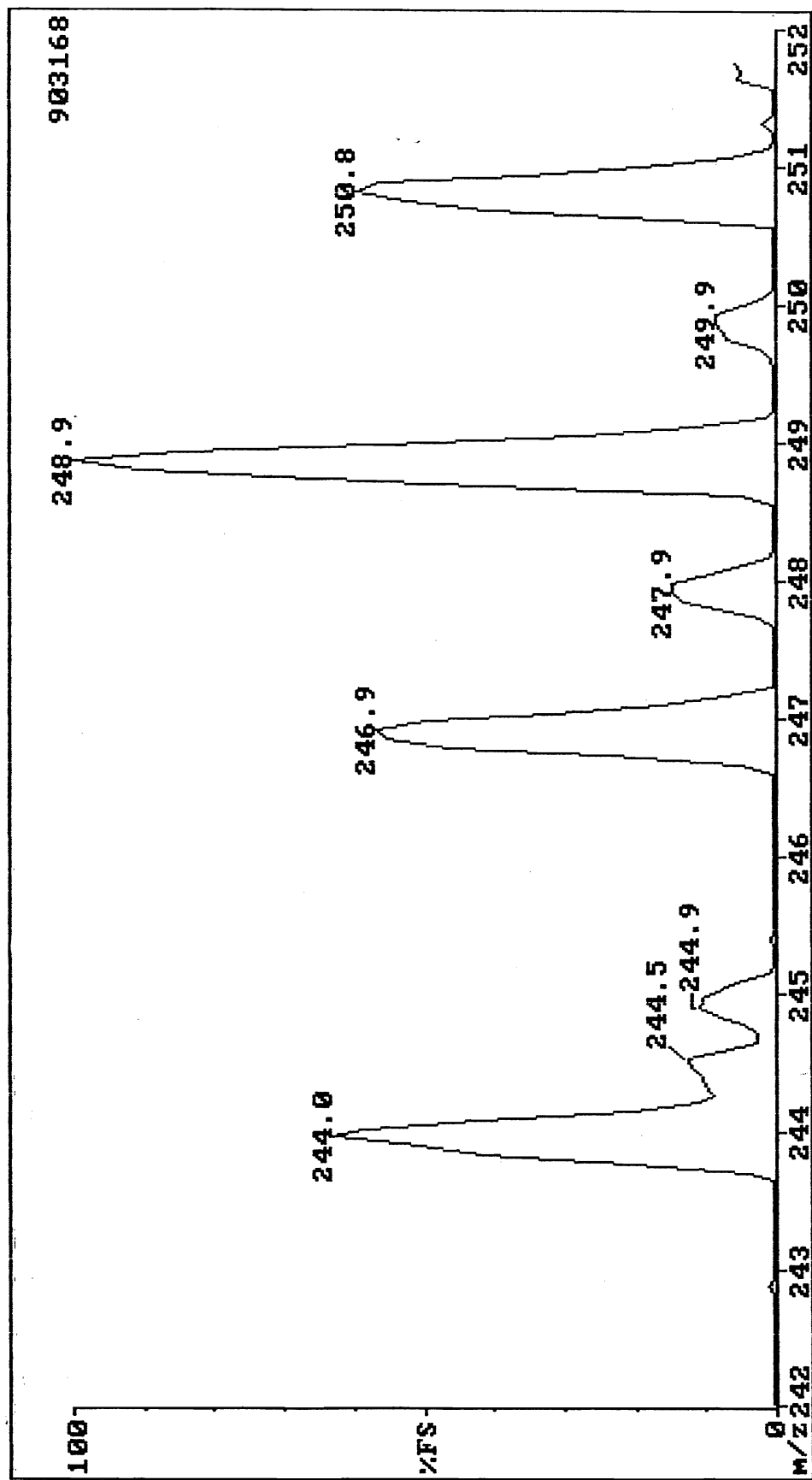


Figure 37

Electrospray-mass spectrum of the degraded lemon yellow dye preparation

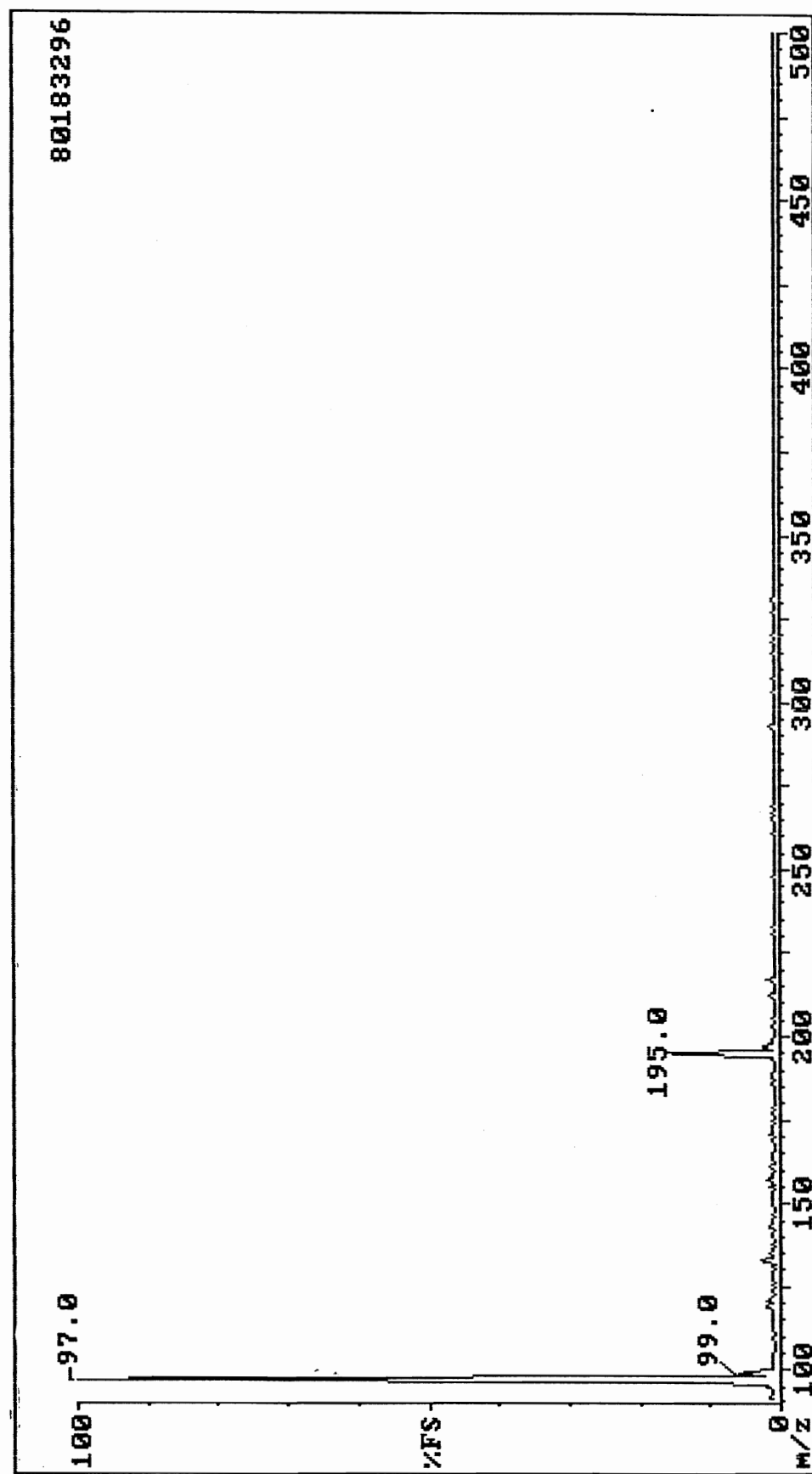


Figure 38

Electrospray-mass spectrum of sulphanilic acid

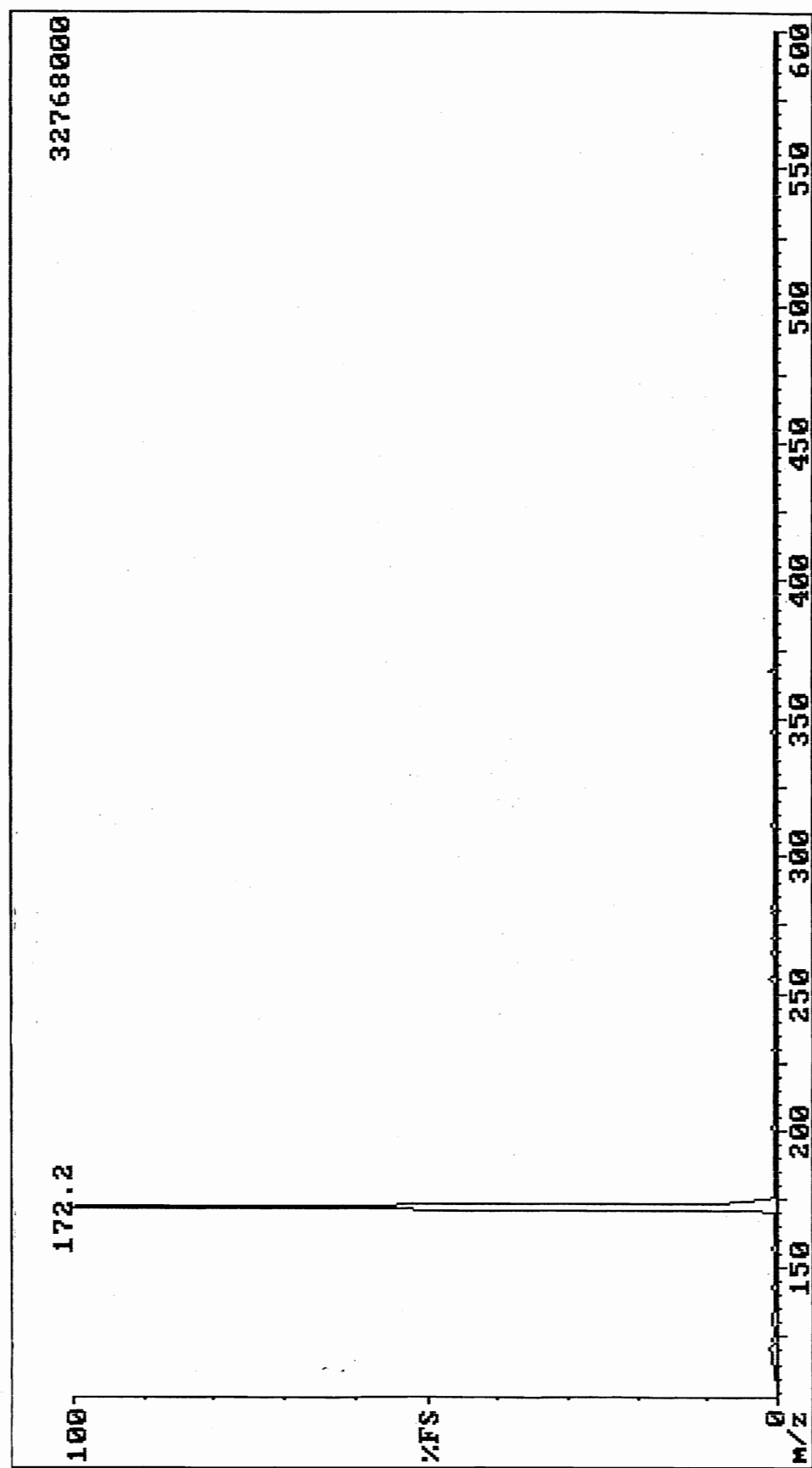




Figure 39

Electrospray-mass spectrum of Sunset Yellow partially degraded in the presence of ascorbic acid

