Table III. Effect of Addition of Fe^{2+}, MetMb, and Ascorbic Acid to Pork Muscle Residue and Length of Storage at 4° upon TBA Numbers[a]

Sample	TBA no.'s[b]			
	0 hr	24 hr	48 hr	72 hr
Control, muscle residue and water	0.30	0.40	0.60	0.70
Muscle residue and Fe^{2+} [c]	0.35	0.65	1.05	1.35
Muscle residue and Fe^{2+} and ascorbic acid[c]	0.35	1.55	1.85	2.35
Muscle residue and MetMb[d]	0.20	0.30	0.40	0.60

[a] The reactants were mixed and heated and TBA numbers were measured at the times specified. [b] TBA numbers = mg of malonaldehyde produced per 1000 g of meat. [c] Fe^{2+} = 1 ppm; ascorbic acid = 5 ppm. [d] Concentration of MetMb = 5 mg/g.

Liu and Watts (1970) have reported that both heme and nonheme iron were capable of accelerating oxidation of lipids in cooked meat. Rather than attempting to remove prooxidant substances and add back heme and nonheme iron, as was done in the present investigation, Liu and Watts (1970) treated meat with H_2O_2 to destroy the heme compounds. Since the H_2O_2-treated samples had lower TBA values, they concluded that the myoglobin also served as a prooxidant in cooked meat. Sato and Hegarty (1971) concluded that the inhibiting activity of H_2O_2 is associated with its activity as an oxidizing agent. Presumably, the oxidation of ascorbic acid, which enhances the effect of Fe^{2+}, accounts for the inhibition of lipid oxidation in H_2O_2-treated samples.

There is indirect evidence that nonheme iron plays a major role in accelerating lipid oxidation in muscle tissue, since lipid oxidation in meat has been observed to proceed more rapidly at lower pH values (Keskinel *et al.*, 1964). Liu (1970) pointed out that the prooxidant activity of Fe^{2+} is at a maximum in the range of pH 5.0–5.5. The effectiveness of EDTA and polyphosphates as inhibitors of lipid oxidation in meat (Sato and Hegarty, 1971; Timms and Watts, 1958) can be easily explained if nonheme iron is a catalyst of lipid oxidation.

In studies on cooked meat pigments, Ledward (1971) reported that porphyrins in the denatured heme compounds may possess some low-spin characteristics. Low-spin compounds are known to be less effective as catalysts of lipid oxidation. These observations may help explain why MetMb did not act as a prooxidant in cooked meat systems.

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Determination of Lead in Organic Coloring Dyes by Atomic Absorption Spectroscopy

Allan Ford,[1] Barbara Young,[2] and Clifton Meloan*

A fairly rapid method has been developed for determining Pb contaminants in food coloring dyes. The sample is digested in HNO_3-$HClO_4$ and taken to dryness, the salts are dissolved, and a Pb-diethyldithiocarbamate chelate is formed and extracted with xylene. The lead content is determined by atomic absorption spectroscopy. The detection limit with respect to the dyes is about 0.4 ppm on the average.

The lead content of food coloring dyes should not exceed 10 ppm as required by the CODE of Federal Regulations–Title 21. A faster method than the Official Methods of Analysis of the AOAC (1970a–c) is desired. These methods, while sensitive enough, require 1–2 days for the dry ashing and the multiple extractions required to remove the interferences. The proposed procedure can be accomplished in 1–2 hr.

EXPERIMENTAL SECTION

Equipment. Perkin-Elmer Models 290 and 303 atomic absorption spectrophotometers with a Boling burner and a Jarrell-Ash unit consisting of a 0.5-m monochromator mounted on an optical rail and a Beckman total consumption burner were used. The lamp was a Westinghouse high spectral output lamp, type pF 290 with an 80-μF damping capacitor.

Chemicals. Food, Drug, and Cosmetic (1960) food coloring dyes used were: FD&C Red 2 (trisodium salt of 1-

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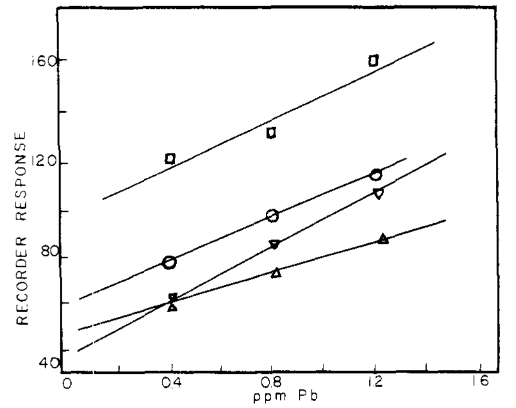


Figure 1. Water-soluble dyes: (○) FD&C Blue 1; (▽) FD&C Green 5; (□) FD&C Red 2; (△) FD&C Red 3.

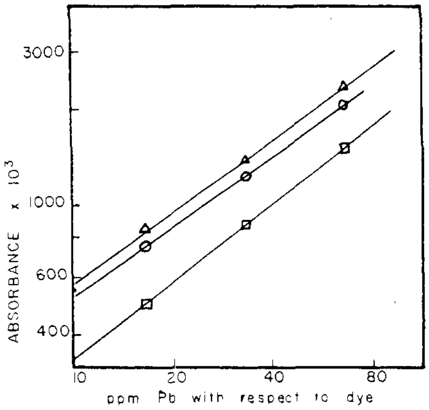


Figure 2. Typical dye analyses results: (△) Rhodamine; (□) FD&C Blue 1; (○) FD&C Green 5.

(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid), also known as amaranth; FD&C Red 3 (disodium salt of 9-*O*-carboxylphenyl-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone), also known as erythrosine; FD&C Red 17 (1-*p*-phenylazophenylazo-2-naphthol); FD&C Red 19 (3-ethochloride of 9-*O*-carboxylphenyl-6-diethylamino-3-ethylimino-3-isozanthene); FD&C Blue 1 (disodium salt of 4-{[4-(ethyl-*p*-sulfophenzylamino)phenyl]-(2-sulfoniumphenyl)-methylene}-[1-(*N*-ethyl-*N*-*p*-sulfobenzyl)-$\Delta^{2,5}$-cyclohexadienimine]); FD&C Blue 2 (disodium salt of 5,5′-indigotindisulfonic acid), also known as Indigo Carmine; FD&C Green 5 (disodium salt of 1,4-bis(*o*-sulfo-*p*-toluino)anthraquinone); FD&C Green 7 (monosodium salt of 4-{[4-(*N*-ethyl-*p*-sulfobenzylamino)phenyl]-(*o*-chlorophenyl)-methylene}-1-(*N*-ethyl-*N*-*p*-sulfoniumbenzyl-$\Delta^{2,5}$-cyclohexadienimine). Lead cyclohexane butyrate was used as a Pb standard, 500 μg/ml in xylene, and lead nitrate was used as a lead standard, 500 μg/ml in 0.05 *M* HCl.

Procedure. Three grams of dye was weighed into a 200-ml Berzelius beaker and standard additions of 10, 20, or 25 ppm of Pb as $Pb(NO_3)_2$ in 0.005 *M* HCl were added. $HClO_4$-HNO_3 (3:1) (20 ml) was added and the samples digested (see below) and taken to dryness. Immediately after removal from the hot plate a small quantity of H_2O was added to wash down the sides and aid in dissolution of the warm sample. The samples were then transferred to 125-ml erlenmeyer flasks and 5 ml of 1% ascorbic acid was added to reduce interference during extraction.

The samples are shaken with 5 ml of 1% diethyl dithiocarbamate, diethylammonium salt (DDDC) in xylene, separated using a silicone treated phase separating paper (Whatman 1-PS), and run immediately upon extraction using the 2833-Å line. The 2170 Å was used with the PE 303 or Jarrell-Ash instruments. Calibration curves or standard additions were made using either lead nitrate or lead cyclohexane butyrate. Readout was on a Honeywell chart recorder.

Caution. Before ashing an untried dye, especially a dye with several diazo groups, it should be checked with a small sample. Quite often, especially with dyes not wetted by the acid mixture, the initial reaction with the nitric acid was so vigorous that it foamed out of the beaker. When this happens the acid mixture should be added more slowly. Some samples tend to foam heavily during digestion; this may be stopped by removing them from the hot plate intermittently until foaming stops. The foam will generally explode, much like gasoline, if it comes in contact with the hot plate, but we have never experienced a detonation. With new samples, scaled down experimentation is best.

DISCUSSION

Water-Soluble Dyes. Four dyes (Figure 1) were run in concentrations of 1 g/25 ml and aspirated directly. The dyes produced fairly linear curves as shown in Figure 1 but there was a large amount of flame noise as would be expected as these low levels. Green 5 initially contained 0.82 ppm of Pb and Blue 1 1.45 ppm of Pb as verified by a dithizone colorimetric method. Since the curves are linear and both sample size and dilutions are of convenient size, standard addition techniques can be used.

Dyes, General. Straight perchloric acid digestion and aspiration of this perchlorate digest were attempted. While it was found that $HClO_4$ could be safely aspirated into a Desicote covered burner in an air-C_2H_2 flame without an explosion, the method was discontinued. The final viscosity varied so much from sample to sample and there was such a high salt content that the final results were quite erratic and the recoveries were poor. In addition, straight $HClO_4$ solutions reacted too rapidly with some dyes. HNO_3 was added to reduce the possibility of forming explosive perchlorate esters before the $HClO_4$ becomes an oxidizing agent. A HNO_3-$HClO_4$ mixture of 1 and 3 was found to be the most effective.

An extraction procedure was then developed. A 1% solution of DDDC was used to chelate the Pb. This ligand was chosen because it forms a chelate that can be extracted from highly acid solutions (pH < 0.1-6) as shown by Jordan (1968) and because both the diethylammonium salt and the sodium salt have been used previously by Bode and Tusche (1957), Bode and Neumann (1960), Chernikhov and Dobkin (1949), Hessel (1968), Jordon (1968), Strafford *et al.* (1945), and Tati *et al.* (1966) in nonaqueous atomic absorptions.

Although the diethyldithiocarbamic acid decomposes rapidly to CS_2 and the corresponding amine, as shown by Joris *et al.* (1970), the diethylammonium salt is stable enough to be extracted from acid solution as long as no relatively good oxidizing agents are present (Grand and Tamus, 1968). Ascorbic acid is used to reduce any potential interferences, in this case Fe and I_2 (Jordon, 1968).

$CHCl_3$, CCl_4, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), and xylene were evaluated as extraction solvents. The gaseous HCl formed in the flame from $CHCl_3$ and CCl_4 proved to be a serious limitation. DDDC is extractable into MEK and MIBK but both tend to form intrafacial emulsions and both are rather soluble in water. Moreover, the solubility of MIBK changes somewhat with ionic strength changes in the aqueous layer.

Xylene was chosen because of its low water solubility, high solubility for the DDDC-Pb chelate, and its lack of emulsion forming characteristics. DDDC in xylene will also remove Pb from $PbSO_4$ which is formed in several cases. However, it produces a rather noisy flame and for best results a recorder should be used for the analyses.

Ascorbic acid is used to remove the I_2 interference. Iodine in the dye is oxidized to I_2 which is extractable into xylene. I_2 reacts stoichrometrically with $Pb(DDDC)_2$ to

Table I. Effect of Salts on the Extraction of Pb into Xylene Containing DDDC

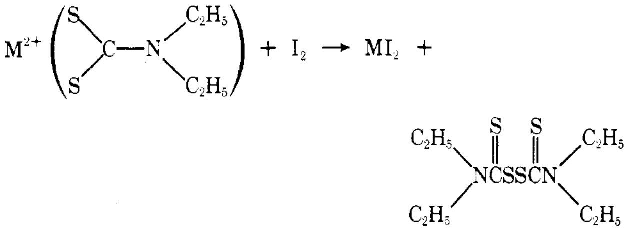
Sample	Reading (recorder signal in mV)
Standard	23.4
Standard	23.4
Standard	23.4
K_2SO_4 A[a]	23.1
K_2SO_4 B	23.0
KNO_3 A	23.1
KNO_3 B	20.8
NaH_2PO_4 A	23.1
NaH_2PO_4 B	23.1
NaCl A	23.2
NaCl B	22.3
0.2% DDC in xylene	0.0

[a] Flask A in each case was 0.23 *M* in $HClO_4$.

Table II. Recovery Studies

	ppm added	ppm recovered
Blue 1	10.0	10.1
Blue 1	20.0	20.4
Blue 1	25.0	25.3
Red 3	10.0	10.3
Red 3	20.0	20.5
Red 3	25.0	25.6
Green 5	10.0	9.7
Green 5	20.0	20.0
Green 5	25.0	24.5
Red 19	10.0	9.3
Red 19	20.0	19.7
Red 2	10.0	10.1
Red 2	20.0	20.7
Red 2	25.0	25.4
Blue 2	10.0	10.1
Blue 2	20.0	20.0
Blue 2	25.0	25.2

give the metal iodide and the corresponding bis(diethylthiocarbamoyl) disulfide as shown by Grand and Tamus (1968).



Formation of $PbSO_4$. The k_{sp} of $PbSO_4$ is 1.06 × 10^{-8} (mol/l.)^2 at 18°. Some dyes contain S which is oxidized to SO_4^{2-} during the digestion and $PbSO_4$ will form. Various salt solutions of Pb and $HClO_4$ were extracted with 0.2% DDDC in an effort to determine their effect on the extraction. The results are shown in Table I.

RESULTS

Figure 2 shows the results for three of the dyes with three standard additions to each one. Table II shows the results of recovery studies for six of the dyes. Each value is the average of three determinations.

The proposed procedure requires about 1-2 hr. It was tested on nine dyes with an average recovery of 100.0%. It was found that if the dye was water soluble that a direct aspiration of this solution could be made and the Pb determined by atomic absorption. If the dye was not water soluble, a HNO_3-$HClO_4$ (1 + 3) digestion mixture rapidly destroyed the dye.

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