Influence of Inorganic Pigments on the Formation of *N*-Nitrosodiethanolamine

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It was found that *N*-nitrosodiethanolamine (NDELA) was formed when diethanolamine (DELA) was heated in the presence of some inorganic pigments. Reactions were carried out as follows: a mixture of 30% (w/w) aqueous solution of DELA (1 mL) and pigment (0.3 g) was heated at 90 °C for 1 h. Carbon black, activated carbon, ferric ferrocyanide, and yellow iron oxide were shown to nitrosate DELA to form 180, 160, 35, and 1.0 μg of NDELA/1 g of pigment. NDELA was determined by high-performance liquid chromatography–thermal energy analysis (HPLC–TEA). The formation of NDELA was studied as a function of time, temperature, and pH. As to the effect of pH on the NDELA formation, different results were obtained between the case of some pigments and that of nitrite. In the gas desorbed from pigments with heating at 300 °C, nitrogen oxides (NOx) were detected and determined by the continuous Saltzman procedure. It was observed that NDELA was present after the reaction of NOx gas with DELA. On the basis of the data obtained, it was elucidated that NOx adsorbed on the pigments played the major role in the NDELA formation.

N-Nitrosodiethanolamine (NDELA), a known animal carcinogen (Druckrey et al., 1967; Hilfrich et al., 1978; Lijinsky et al., 1980; Preussmann et al., 1981), has been detected recently in cutting fluids (Fan et al., 1977b), in certain cosmetics (Fan et al., 1977a), in pesticides (Zweig et al., 1980), and in tobacco (Schmeltz et al., 1977). For some of these cases the origin of this contaminant is not completely understood. Some plausible explanations are that diethanolamine (DELA) reacts with a nitrosating agent such as adventitious nitrogen oxides (Challis and Kyrtopoulos, 1978, 1979), nitrite (Lijinsky et al., 1972) or *C*-nitro compounds (Fan et al., 1978; Schmeltz and Wenger, 1979). After we reviewed the results of the NDELA finding in some cosmetic products reported by Food and Drug Administration in Feb 1978, we noticed that inorganic pigments were frequently contained in the cosmetic products that were contaminated with NDELA. So we began a study of the nitrosating potential of the inorganic pigment. No papers has been published on this subject.

EXPERIMENTAL SECTION

Materials. The following materials were used as received. Carbon black (Misubishi Kasei Kogyo Co., Japan; Degussa Co., BRD) and activated carbon (Hitachi Tanso Kogyo Co., Japan; Takeda Yakuhin Kogyo Co., Japan) were commercial grade and the other inorganic pigments were commercial cosmetic grade. NO gas and NO₂ gas used were obtained from Nippon Sanso Co., Japan. Reagent NDELA (Tokyo Chemical Industry Co., Japan) was purified by elution from a silica gel column and by using preparative HPLC. All other materials, unless otherwise specified, were of reagent grade (Wako Pure Chemical Industry Co., Japan).

Apparatus. An HPLC was constructed with a Model LC-3A high-pressure pump (Shimazu Co., Japan) and a Model SIL-1A injector (Shimazu Co., Japan) equipped with a 200-μL sample loop and a column, 4.6 mm i.d. × 25 cm long Nucleosil 5 NH₂ (Nagel Co., BRD), connected in series. The mobile phase was a mixture of *n*-hexane, dichloromethane, and methanol (60:35:5 v/v). It's flow rate was 2 mL/min. This system was connected to a thermal energy analyzer detector, 502 (Thermo Electron Co.), which was set at a pyrolyzer temperature of 550 °C, a vaccum of 1.5 torr, and an attenuation factor of 16 or 8.

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The cooling materials of the cold traps were dry ice–ethanol (−70 °C) for the first trap and liquid nitrogen–ethanol (−100 °C) for the second trap.

A JEOL JGC-20KFP GLC was connected to a JEOL JMS-D300 mass spectrometer equipped with a JMA-2000 data processing system. The conditions were as follows: a glass column (1 m × 2 mm i.d.) packed with 1% silicone OV-1 on Chromosorb W (AW-DMCS); helium flow rate, 30 mL/min; the column temperature, 180 °C; filament current, 300 μA; electron voltage, 70 eV.

A Hitachi 303 spectrophotometer was used for nitrile analysis and NOx analysis.

Reaction of DELA with Inorganic Pigment. One milliliter of 30% (w/w) aqueous solution of DELA and 0.3 g of the pigment were added to a vial, and the mixture was ultrasonicated. Then they were heated at 90 °C for 1 h. The pH of the reaction solution was 10.5 because of the alkalinity of DELA. When the pH dependence of the NDELA formation was examined, the pH of the reaction solution was adjusted at pH 7.0 and at pH 4.5 by adding acetic acid. In order to examine the effect of the reaction time and the temperature on the NDELA formation, the reaction time and the temperature were changed as follows: 1, 3, and 5 h at 25, 45, 70, and 90 °C.

Reaction of DELA with NOx Gas. One milliliter of the NOx gas diluted with nitrogen gas at atmospheric pressure was injected with a syringe into a sealed vial, containing 1 mL of 30% (w/w) aqueous solution of DELA. The dead space above the solution was 4 mL. The vial contents were shaken vigorously for 2 min, and it was heated at 90 °C for 1 h.

Determination of NDELA in the Reaction Solution. After the reaction was finished, the reaction solution was filtered and the residue pigment was washed with ethanol (4.5 mL × 5 times). The filtrate was evaporated under vaccum at 35 °C, and the volume was reduced to 1 mL. A total of 10 μL of the sample solution was injected to HPLC–TEA system. The NDELA peak was detected at a retention time of 6 min. The standard NDELA solution (50 μg/mL of ethanol) was stored in a refrigerator. Quantitation of the sample solution was made by comparing the peak area of the sample with that of the standard NDELA. The limit of detection of NDELA by TEA was 0.15 ng, which corresponds to 15 ng/mL in the reaction solution. The confirmation of NDELA formed after the reaction of DELA with carbon black was performed by the GC–MS method. The material eluting at

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Table I. Formation of NDELA in Aqueous DELA Solution in the Presence of Pigments

	NDELA, μg/g of pigment	
inorganic pigment	pH 4.5	pH 10.5
carbon black (1)	340	180
carbon black (2)	100	85
carbon black (3)	70	50
activated carbon (1)	70	160
activated carbon (2)	75	43
ferric ferrocyanide (1)	81	76
ferric ferrocyanide (2)	32	35
yellow iron oxide (1)	12	1.0
yellow iron oxide (2)	7.6	0.7
red iron oxide	3.0	0.27
black iron oxide	5.8	0.17
hydrated aluminum silicate	3.7	0.17
ultramarine blue	1.2	0.17
talc	4.5	0.09
mica	5.0	0.06
titanium dioxide	2.2	>0.05

the retention time of NDELA was collected from the HPLC, which was operated at the same condition as mentioned before. The solvent was evaporated under the stream of nitrogen, and the residue was derivatized to the trimethylsilyl ether by BSTFA. This sample was analyzed by GC–MS and was identified as NDELA by comparison of mass spectra with that of the authenitc sample.

Determination of Nitrite in the Pigments. A total of 25 mL of water was added to 1 g of pigment and ultrasonicated for 5 min and centrifuged. A total of 10 mL of the supernatant was pipetted into the flask. A 1-mL aliquot of a 1% (w/v) solution of sulfanilamide [prepared in 10% (v/v) hydrochloric acid] was pipetted into the flask and mixed well. After a few minutes, 1 mL of a 0.12% (w/v) aqueous solution of N-(1-naphthyl)ethylenediamine dihydrochloride was pipetted into the flask and immediately mixed well. Absorbance at 540 nm was read, and the nitrite content of the sampes were calculated from comparative standard and sample absorbances. The limit of detection of nitrite in this procedure is about 200 ng/g (pigment).

Determination of NOx Gas in the Pigments. Inorganic pigment, from 0.5 to 8 g, was added into the glass tube, and it was heated at 300 °C for 3 h under a stream of Ar gas. NOx in the gas desorbed from the pigments was determined by the Saltzman procedure (Saltzman, 1954). The Saltzman reagent formulation was 5 g of sulfanilamide, 0.05 g of N-(1-naphthyl)ethylenediamine dihydrochloride, 5 mL of glacial acetic acid, and 10 mL of hydrochloric acid dissolved in 1000 mL of distilled water. The reagent formulation in an oxidation vessel consisted of potassium permanganate (25 g) and sulfuric acid (25 g) dissolved in 1000 mL of distilled water. This $KMnO_4$–H_2SO_4 trap was used to oxidize NO to NO_2. The limit of detection of NOx in this procedure was about 0.4 μg. This value corresponds to 0.05 μg/g (pigment) when 8 g of sample is subjected to this method.

RESULTS AND DISCUSSION

The amount of NDELA formed in aqueous DELA solution in the presence of pigments is shown in Table I. Consierable amounts of NDELA were formed both at pH 4.5 and at pH 10.5, when carbon black, activated carbon, or ferric ferrocyanide was used. On the other hand, relatively small amounts of NDELA were formed when the other pigments were used, and the amounts of NDELA formed at pH 4.5 were about 10-fold larger than the amounts of NDELA formed at pH 10.5. It is new phenomenon and interesting that NDELA is formed when
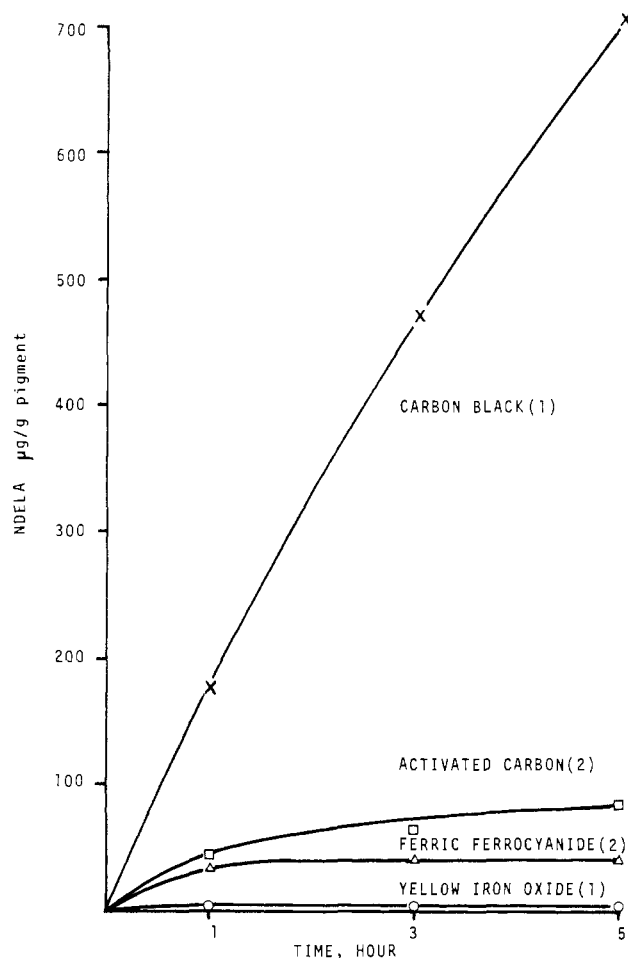

Figure 1. Formation of NDELA as a function of reaction time: 30% (w/w) DELA, 1 mL; pigment, 0.3 g; at 90 °C and at pH 10.5.

DELA is heated in the presence of some inorganic pigments. So we examined this NDELA formation as a function of reaction time, temperature, and pH using representative pigments.

The time dependence of NDELA formation is shown in Figure 1. As for carbon black, the amounts of NDELA formed increased in proportion to the reaction time. In contrast, saturation of NDELA formation was observed within 1 or 2 h when ferric ferrocyanide or yellow iron oxide was used. The effect of temperature on the NDELA formation is shown in Figure 2. Generally in these pigments, the amounts of NDELA formed increased as the reaction temperature increased. The pH dependence of NDELA formation is shown in Figure 3. When 3.5 μg of sodium nitrite was used, the amounts of NDELA formed decreased as the pH increased. A similar result was obtained when yellow iron oxide was used. On the other hand, carbon black, activated carbon, and ferric ferrocyanide were different from nitrite in the point of the pH dependence of NDELA formation.

The amounts of nitrite in some pigments were determined, and the following data were obtained: activated carbon, 3.4; yellow iron oxide, 1.1; ferric ferrocyanide, 0.2 (units of μg/1 g of pigment). The value of NDELA calculated was computed by the amount of nitrite found in the pigments by assuming that nitrite reacted with DELA stoichiometrically. When we compare the calculated value with the value of NDELA found, which was the experimental result after the reaction of DELA with the pigments at pH 4.5, the calculated value of NDELA formed is much smaller than the found value. This means that nitrites found in these pigments played only a small role
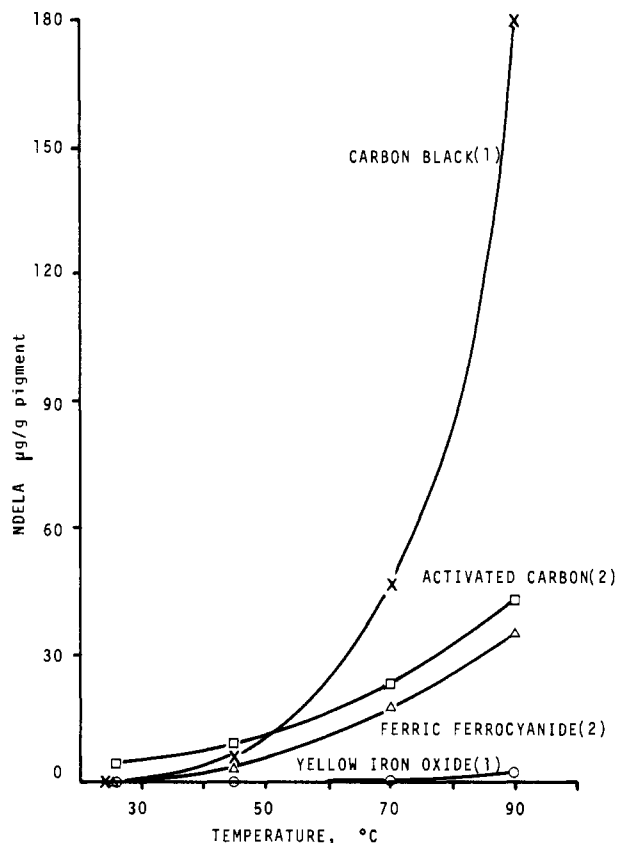
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Figure 2. Formation of NDELA as a function of reaction temperature: 30% (w/w) DELA, 1 mL; pigment, 0.3 g; at pH 10.5 and for 1 h.
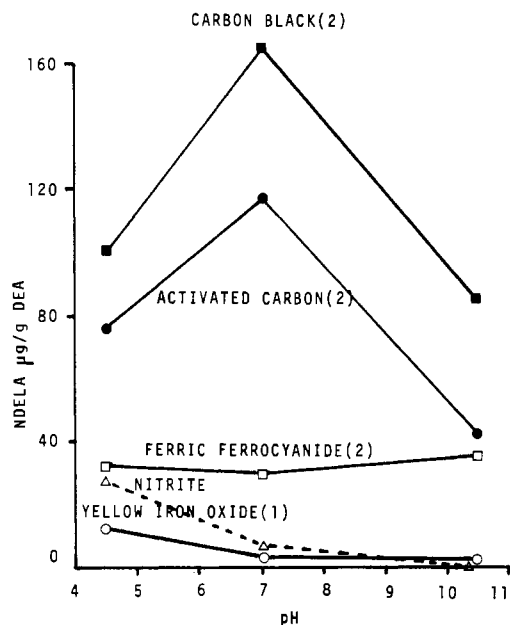

Figure 3. Effect of pH on the formation of NDELA: 30% (w/w) DELA, 1 mL; pigment, 0.3 g; at 90 °C and for 1 h.

in the nitrosating reactions. Therefore, there might be some factors other than nitrite that played a main role in the NDELA formation in these pigments.

NOx gas easily nitrosated morpholine and piperidine in the alkaline solution (Challis and Kyrtopoulos, 1977). So NDELA formation from DELA and NOx gas was examined. The effect of pH on the NDELA formation is shown in Figure 4. NDELA was formed almost equally at pH 4.5 and at pH 10.5. These results were very different from the case of nitrite and were similar to that of carbon black
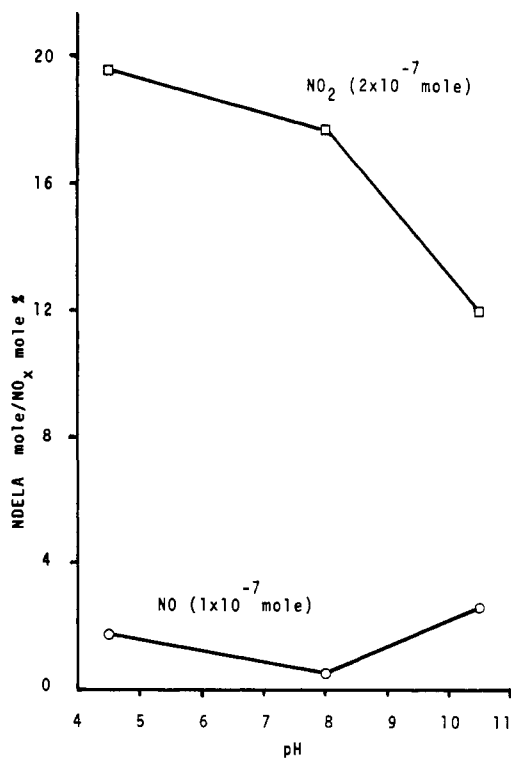

Figure 4. Effect of pH on the formation of NDELA from DELA and NO_x: 30% (w/w) DELA, 1 mL; NO_x/N_2, 1 mL; at 90 °C and for 1 h.

Table II. Amount of NOx Found in Pigments and That of NDELA Formed

pigment	NOx, $\mu g/g$ of pigment, found	NDELA, $\mu g/g$ of pigment calcd	found
carbon black (1)	60	270	180
activated carbon (2)	17	76	41
ferric ferrocyanide (2)	0.53	2.4	35
yellow iron oxide (1)	0.33	1.5	0.9

and activated carbon. The amounts of NOx gas in some pigments were determined, and the results are shown in Table II. The value of NDELA calculated was computed by the amount of NOx found in the pigments by assuming that NOx reacted with DELA stoichiometrically. The value of NDELA found was the experimental result after the reaction of DELA with the pigments at pH 10.5. Comparing the two values, for example, 270 with 180 and so on, we found the calculated value corresponded with the found value, except for the ferric ferrocyanide. On the basis of the data obtained, it was concluded that NOx adsorbed on the pigments (carbon black, activated carbon, and yellow iron oxide) played a greater role in the NDELA formation than nitrite did. As for ferric ferrocyanide, it is speculated that the oxidation reaction plays the major role in the NDELA formation. Oxidation of DELA would lead to the formation of glycolaldoxime and subsequently hydroxylamine. Upon further oxidation, hydroxylamine could yield either nitrite or other oxides of nitrogen (Ong et al., 1981). We will report on this at a later date.

By the way, these pigments are widely used in many kinds of industry, so these pigments would have some relation to the nitrosamine contaminations in the environment. For example, carbon black will be in contact with amines in the tire industry.

In conclusion, this study demonstrates that NDELA was formed when DELA was heated in the presence of some inorganic pigments and that NOx gas adsorbed on the

pigments played a greater role in the nitrosating reaction than nitrite did. We hope our study will be useful in the prevention of the nitrosamine contaminations.

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Adsorption onto an Oil Surface and Emulsifying Properties of Bovine α_{s1}-Casein in Relation to Its Molecular Structure

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When coconut oil is emulsified with an α_{s1}-casein solution, the α_{s1}-casein is adsorbed onto the surface of the oil globules and stabilizes the emulsion. This adsorption on the emulsified oil, as well as the emulsifying properties of α_{s1}-casein, was investigated by using some of the large peptides derived from α_{s1}-casein by limited proteolysis. The adsorbability of α_{s1}-casein onto the emulsified oil surface was reduced by removal of the N-terminal segment (1–23 residues) with pepsin but not by removing the C-terminal segment (145–199 residues) with papain. The emulsifying properties (emulsifying activity, emulsion capacity, creaming stability) were changed by the removal of the N-terminal segment (1–23 residues). α_{s1}-Casein adsorbed on the emulsified oil was barely extracted from the oil surface with 0–5 M urea and was not hydrolyzed by pepsin. These results suggest that α_{s1}-casein is tightly adsorbed onto the oil globule surfaces, principally by its hydrophobic N-terminal region, and that it stabilizes the oil globules.

Basic studies on protein structure and functionality are essential for the utilization of new protein sources into many functional food systems. The various properties of proteins, namely, molecular size, shape, conformation, net charge, hydrophobicity, and protein–protein interactions, have been noted as factors that influence such functional properties as emulsification and foamability of these proteins (Horiuchi et al., 1978; Chou and Morr, 1979; Kinsella, 1981).

In previous papers (Yamauchi et al., 1980; Shimizu et al., 1981), we have studied the emulsifying properties of bovine whey proteins in relation to their structures. During these investigations we found that the adsorption of proteins onto an oil surface during emulsification depends on various structural properties of the proteins, including the hydrophobicity and such conformational factors as the rigidity of the molecule. Since the adsorption of proteins is essential for stabilizing the oil globules and affects the properties of the emulsion (Tornberg, 1979), the adsorption mechanism is of great interest. However, the general relationship between the structure and the adsorbability of proteins is still obscure.

Caseins, the major proteins of bovine milk, are known to be flexible proteins without a rigid conformation and their primary structures have already been established (Mercier et al., 1971; Ribadeau-dumas et al., 1972; Jollès et al., 1972; Mercier et al., 1973). Furthermore, caseins have amphiphilic properties in their primary structures. The hydrophobic and hydrophilic areas are distinguishable. Studies using caseins, therefore, may provide valuable information on lipid–protein interactions. Such studies can be expected to elucidate the relationship between the

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