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Instability of Amaranth in the presence of some polyoxyethylene nonionic surface-active agents

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INSTABILITY OF AMARANTH IN THE PRESENCE OF SOME POLYOXYETHYLENE NONIONIC SURFACE-ACTIVE AGENTS

by

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D. R. G.

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HISTORY OF AMARANTH

Coloring of pharmaceuticals to improve their appearance has been practiced since early times. Although their use in this respect increased slowly until the middle of the nineteenth century, it became much more prevalent after that time. With the discovery of mauveine in 1856 by Sir William Perkin (1) the door was opened to a whole new field of synthetic dyestuffs providing for a wide array of colorants.

For many years the toxicity of these color additives received little attention. By 1900, Congress was ready to appropriate funds to enable the Secretary of Agriculture to investigate these "coloring matters" in order to establish the criteria for their safe use in foods, drugs, and cosmetics. Previous to the passage of the Federal Food and Drug Act of 1906, seven colors were accepted as harmless. During the interim between this act and the Food, Drug and Cosmetic Act of 1938, fifteen additional agents were certified for food, drug, and cosmetic use (2).

This new act of 1938 divided certification into three classes: F D & C colors for food, drugs, and cosmetics; D & C colors for drugs and cosmetics, and Ext. D & C colors for external use preparations only. This act provided for more controls over the use of color additives with tests for purity, stability, toxicity, and local

irritation. It further provided that the structure of the dye be known and purity be determined by suitable analytical procedures.

In recent years the FDA has decertified a number of formerly approved colorants based upon more extensive pharmacological testing. Increasing concern over the effects of these agents led to the passage of the Color Additive Amendments of 1960. By the provisions of this bill all colors will become subject to uniform statutory rules, following a predetermined transitional period. Although an extension of time may be granted, the law provides that all D & C colors will be automatically decertified, unless the pharmaceutical industry can demonstrate their safety, by 1963. Since the number of F D & C dyes is limited, this provision has caused some concern among many members of the industry (2).

Colorants are added to pharmaceuticals for several reasons. From the standpoint of the consumer the esthetic value of a preparation can be greatly enhanced by the addition of a coloring agent. Since the association of color and taste is significant, due consideration of such psychological factors are important in producing acceptable medicinals.

Artificial coloring also enables the production of reproducible, uniform preparations, particularly when such medicines contain crude drugs or crude drug extractions.

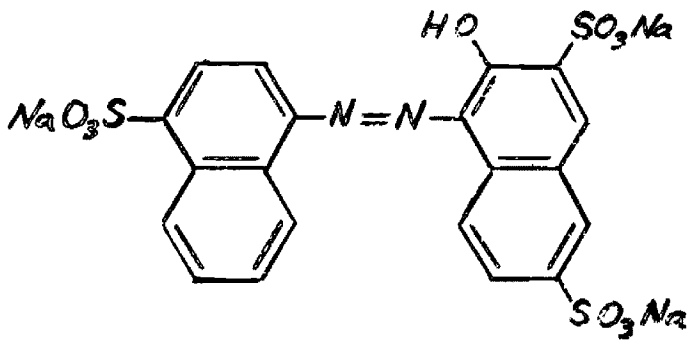
Normal lot to lot variations in crude drugs can lead to differences in color in the finished product. With tablets, slight variations in manufacturing procedures or physical character of the ingredients will yield varying degrees of whiteness. Syrups and elixirs containing natural volatile oils may also exhibit batch to batch variation in color. Thus, the addition of a colorant masks these undesirable differences and provides a method for producing and maintaining uniformity of color (2).

One of the greatest benefits achieved from the application of dyes for coloring pharmaceuticals is product identification. With the limited shapes and sizes of tablets available as well as the number of monograms, the practice of differentiating products by color has proved very helpful to all concerned with the dispensing and administration of medicines. These visual determinations are not restricted just to tablets, but include capsules and in many cases can be extended to liquids. In fact, with the exception of a few drugs, almost all tablets and capsules can be identified through size, shape, markings, and color.

Amaranth was first synthesized in 1886 by Knecht, by coupling diazotized α-naphthylamino-4-sulfonic acid with β-naphthol-3,6-disulfonic acid (3). Although used as a textile dye, when certified it is F D & C Red #2 and can be incorporated into foods, drugs, and cosmetics. It first became official in the sixth edition of the National

Formulary as the coloring agent for Three Bromides Elixir (4). In 1942, it was admitted to the United States Pharmacopeia and was the colorant for Phenobarbital Elixir, which status it still maintains (5). When cudbear became critical to obtain during World War II, the N.F. Committee authorized the substitution of amaranth in all N.F. preparations containing the former (6).

Amaranth is a dark red-brown powder, soluble in water (1 in 15) and slightly soluble in alcohol. The aqueous solution is vivid red and is stable towards light (3). A one per cent solution is also official in the U.S.P. Amaranth has the following structural formula:



HISTORY OF THE NONIONIC SURFACTANTS

Economic Considerations

In order to appreciate more fully the history of the nonionic surfactants, it might be well to consider briefly the economic background of these agents and the status which they have achieved in the surfactant industry as a whole. Production and sales figures, per se, create little interest, but they do serve to exemplify the rapid rate of growth and expansion which this segment of the industry has experienced.

The so-called "boom" of the surfactant industry occurred after World War II and the total production of these agents has been on a remarkable increase since that time (7). The nonionics, although secondary to the anionics in total production, have experienced a very rapid rate of growth over the last few years. This is due primarily to a reduction in the cost of their production and a growing appreciation of their applicability to many fields (8). Almost any hydrophobic compound with a carboxy, hydroxy, amido or amino group possessing replaceable hydrogen on the nitrogen can be reacted with ethylene oxide to yield a nonionic surfactant. Varying the amount of ethylene oxide added and, hence, the length of the polyoxyethylene chain can lead to a large number of related products. Since each of these products differs sufficiently in its

properties, a whole class of compounds can be created which can be adapted to almost any set of end-use requirements (9).

Table I lists the total production and sales of all surfactants from 1945 through 1960 and was compiled from the data found in the U. S. Tariff Commission's annual Census of Organic Chemicals (10). The surface-active agents covered in these reports include synthetic organic detergents, wetting, emulsifying, and dispersing agents. Soap, waxes, and plasticizers are omitted. The data are reported in terms of 100% active material and exclude all inorganic salts, water, and diluents.

Table II was compiled from this same source and represents the total production and sales of non-sulfonated ester and ether nonionic surface-active agents for the years 1949 through 1960. The figures include only the cyclic and acyclic non-sulfonated esters and ethers and exclude certain non-sulfonated nitrogen containing compounds generally considered as nonionic and, thus, tend to produce a slightly diminished picture of their status. In compiling these data from the Tariff Commission Reports it was not feasible to segregate all the nonionics produced, since these reports show some figures which include both nonionic and cationic agents in the same total. Only those figures which clearly accounted for the nonionics were utilized.

The discrepancy which exists between the production and sales figures in these tables stems from two sources.

The total production of a company reporting to the commission represents both that which it intends to market plus that which it retains for in-plant use and which is not made available for sale. The second contributing factor is explicable on the basis of annually changing inventories.

These tables clearly exemplify the rapid growth of the surfactant industry and especially that segment concerned with nonionic production. Nonionics, in 1949, accounted for less than 10% of the total. In 1960, they occupied better than 20%. In the twelve-year span from 1949 through 1960, total surfactant production increased a little over three and one-half times. During this same period nonionic production of these two classes experienced an increment of over eleven and one-half times. In other words, growth of the nonionics exceeded that of the total field by a factor of three for comparable time periods.

Although the introduction of the nonionics into detergent formulations has accounted greatly for their growth (11), certainly their utilization for pharmaceutical purposes has been an important aspect of their consumption. However, there appears to be no reliable figure available concerning the expenditures of the pharmaceutical industry for the nonionics (12).

TABLE I

TOTAL SURFACE-ACTIVE AGENT PRODUCTION AND SALES 1945-1960

Year	Production		Sales			
	Million Pounds	% over or under previous year	Million Pounds	% over or under previous year	Million Dollars	% over or under previous year
1945	184	21.1	158	17.0	41	36.7
1946	242	31.5	214	35.4	58	41.5
1947	291	20.2	234	9.4	61	5.2
1948	375	12.9	275	17.6	88	44.3
1949	430	14.7	339	23.4	84	-4.4
1950	676	57.3	555	63.4	126	49.8
1951	693	2.5	590	6.3	126	0.1
1952	741	7.0	612	3.8	120	-5.0
1953	921	24.3	732	19.6	145	21.1
1954	1,026	11.3	913	24.7	196	35.5
1955	1,153	12.4	1,066	16.7	241	22.9
1956	1,148[a]	11.4[b]	1,047[a]	8.9[b]	208	-9.2
1957	1,206	5.1	1,123	7.3	217	4.3
1958	1,355	12.4	1,202	7.1	235	8.6
1959	1,504	11.0	1,372	12.5	271	11.1
1960	1,532	1.9	1,399	2.0	278	2.6

Data: U. S. Tariff Commission Reports.

a. excludes oil-soluble petroleum sulfonates previously included in this group, now under another classification.

b. adjusted figure derived by deducting the compounds under a from 1955 totals, since these are included in 1955 totals but not in 1956.

TABLE II

PRODUCTION AND SALES OF NONSULFONATED ESTER AND ETHER NONIONIC SURFACE-ACTIVE AGENTS 1949-1960[a]

		Production	Sales		
Year	Type	1000 Pounds	1000 Pounds	1000 Dollars	Unit Value[b]
1949	cyclic	15,038	13,702	5,812	0.42
	acyclic	14,547	13,666	5,165	0.38
1950	cyclic	30,540	30,683	8,773	0.29
	acyclic	20,363	17,103	6,286	0.37
1951	cyclic	25,885	23,167	9,769	0.42
	acyclic	20,821	18,240	6,827	0.37
1952	cyclic	26,894	20,525	9,314	0.45
	acyclic	36,342	21,297	7,893	0.37
1953	cyclic	41,991	30,923	11,800	0.38
	acyclic	69,885	65,649	20,082	0.31
1954	cyclic	42,089	35,001	11,998	0.34
	acyclic[c]	88,478	81,205	20,226	0.25
1955	cyclic	71,819	48,858	15,012	0.31
	acyclic	101,227	95,151	24,190	0.25
1956	cyclic	79,479	64,633	18,726	0.29
	acyclic	114,200	103,487	27,165	0.26
1957	cyclic	84,310	68,802	20,248	0.29
	acyclic	133,691	107,690	28,473	0.26
1958	cyclic	102,347	84,846	22,853	0.27
	acyclic	166,702	119,452	28,159	0.24
1960	cyclic	166,436	133,821	33,233	0.25
	acyclic	177,430	108,694	32,382	0.30

Data: U. S. Tariff Commission Reports.

a. excludes certain nonsulfonated nitrogen-containing compounds generally considered as nonionic.

b. calculated from rounded figures.

c. includes certain lauric, oleic and stearic acid esters reported as plasticizers prior to 1953.

Pharmaceutical and Medicinal Applications

The unique properties of the nonionic surfactants and their relative lack of incompatibilities renders these products quite amenable to pharmaceutical applications and their use in this respect has increased appreciably in recent years. The development of the use of the polymeric glycols in pharmacy is a result of their unusual properties, especially water solubility, low toxicity, and wide range of compatibilities (13). The use of these compounds has been extended to almost every phase of pharmacy and form of medication.

Bird (14) recommended the use of propylene glycol monostearate as a suppository base which he felt was superior to cocoa butter in many ways. Inclusion of glyceryl monostearate in cocoa butter bestows self-emulsifying properties to the suppository base leading to increased absorption of the incorporated medicaments (15).

The utility and versatility of the Tweens,* particularly Tween 61, as suppository bases has been described (16). These agents are nontoxic, non-irritating, and quite adaptable to suppository formulation. Introduction of other members of the Tween series, such as Tween 60, yields a faster melting base possessing more water soluble

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*Trademark of Atlas Powder Co., Wilmington, Delaware for the polyoxyethylene sorbitan esters of the common fatty acids.

properties and a more rapid emulsification of the base. Bases prepared with these materials congeal more rapidly.

The solubilizing power of these surfactants is widely used and recommended. Steroidal hormones have been rendered soluble by the use of Tween 20 (17). Likewise, vitamin A palmitate and menadione have been rendered soluble by the polyethenoxy nonionics (18,19). The ability of Tween 20 to perform this function on lipophilic vitamins and hormones increases sharply above 65% of surfactant concentration. This sudden increase in solubilizing power is due to some abrupt change in the internal structure of the surfactant solution (20).

Monte-Bovi highly recommends the use of Tween 20 in the preparation of elixirs and aromatic waters in which long filtration processes are necessary (21,22). Along this same line Steen, Marcus, and Benton (23) prepared several aromatic waters using varying percentages of the water soluble Tweens, concluding that Tween 20 at a concentration of 2% produces the most favorable products. Elixirs prepared by the use of this agent compare favorably with those prepared by official methods and are more versatile and stable as vehicles (24).

Swafford and Nobles (25) extended the use of Tween 20 to the preparation of several syrups. This agent solubilizes the active ingredients and reduces the time necessary to prepare such syrups as Tolu Balsam, Orange, and

Aromatic Eriodictyon. The resulting products compare favorably with official preparations.

Tween 20 solubilized flavoring oils have been recommended for masking the taste of unpleasant antacid suspensions. Their employment for this purpose seems to exert no deleterious effect upon the medication (26). Myrj 51,* when used as a solubilizer for the oils in Aromatic Elixir, is judged to yield a product superior to the official one except for foaming (27). This modified elixir can be more quickly prepared with the aid of the nonionic than the official preparation. It does not cloud even upon infinite dilution with water.

Iodine dissolves in many nonionic solutions. Rendered soluble in this manner, it is an effective antibacterial agent possessing properties more desirable than the tincture (28). Nonionic solubilized iodine solutions produce less irritation on intact skin and lose less iodine by sublimation from the treated area. The non-staining properties of these solutions is a further advantage over iodine tincture.

More thorough studies into the solubilizing properties of the Tweens have been conducted. O'Malley, et al. (29), investigated the effects of the dispersion of peppermint oil in water through Tween 20 and polyethylene glycol

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*Trademark Atlas Powder Co., Wilmington, Delaware for polyoxyethylene stearate.

400 (PEG 400). Results indicated that with Tween, the Tween-oil-water mixture may be clear, but on dilution with water may cloud. Further dilution could lead to a clear solution again. Their conclusions are based upon ternary phase diagrams of the three component system. Similar plots of PEG 400, peppermint oil, and water indicate that this substance acts as a solubilizer by co-solvency rather than through micelle formation as indicated by the Tween. Von I. Ellö (30) in his work showed that the various Tweens studied have different abilities to solubilize the same oil and the same Tween does not possess the same solubilizing capacity on different oils.

Certainly one phase of pharmacy to benefit by the nonionics is that concerned with ointments. As is well known, there is no universal ointment base amenable to use as a vehicle for all medications. The older oleaginous bases not only were often poor from a medicinal standpoint, but their esthetic value was also quite low. The introduction of the nonionics has led to a wider array of bases which are superior in almost all respects to the older types. Because there is a greater variety of base ingredients available, problems of incompatibility can be more easily resolved (31). The absorption of medication from ointment bases is very much a function of the base. Incorporation of surfactants can often lead to more efficient and effective treatment (32,33). Phenolic ointments

prepared in petrolatum were shown to be devoid of antiseptic value until concentrations of 10% and above were reached. This same lack of activity was also noted for other antiseptic phenolic substances prepared in oleaginous bases (34). Subsequent incorporation of phenol into emulsion bases showed activity at a concentration of 2%.

Dodd, Hartmann, and Ward (35) studied nine different surface-active agents as possible ingredients in ointment bases and concluded that, of those studied, only the nonionics were preferable for this use. These compounds produced no irritation on intact skin and interfered little or not at all with the process of wound healing. A water-washable base of the emulsion type containing PEG 4000 was compatible with a wide range of medicaments including a Whitfield's mixture (36).

Different base ingredients, such as petrolatum, PEG, etc., have varying crystalline nature and this is an important consideration in the preparation of good ointments. For PEG compounds the average crystal size decreases with increasing molecular weight of the polyglycol. Addition of other components can modify these structural characteristics as illustrated by the ability of surface-active agents to reduce the average crystal size of PEG 4000 (37). Another aspect of PEG bases, often neglected, is their ability to promote the efficiency of incorporated medicinals (13,38). Since many compounds exhibit a

higher activity in these ointment bases as compared to the oil type bases, their concentration may be and often should be reduced. This point is important, particularly in extemporaneous compounding.

The addition of a Span* to a PEG yields a hydrophilic base which can absorb up to 10% water (38). Normally anhydrous bases of PEG can take up only 3% water. Higher amounts of water tend to soften the base due to the high solubility of PEG.

A rather unique powdered emulsion base containing PEG 4000 was prepared by Ward and Sperandio (39). When six parts of the powder were mixed with four parts water, the result was a hydrophilic ointment base compatible with many medicinals. A Veegum**-PEG mixture as a major component of ointment bases has been described in the literature (40).

Incorporation of the Spans into oleaginous bases increases their hydrophilic properties (41). A 10% concentration of one of the Span surfactants gave an increase of 1200% in the water uptake of petrolatum.

Dental ointments of ethyl aminobenzoate have been prepared employing nonionics (42). These may contain as high as 35% of the anesthetic and have an excellent rating.

The hydrophile-lipophile balance (HLB) value of

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*Trademark Atlas Powder Co., Wilmington, Delaware for sorbitan esters of the common fatty acids.

**Trademark of R. T. Vanderbilt Co., New York City for colloidal magnesium aluminum silicate.

the base appears to exert an effect upon the release of incorporated medicinals (32). Diffusion of erythromycin, chlortetracycline hydrochloride, neomycin sulfate, bacitracin, and hexachlorophene from ointment bases consisting of a nonionic surface-active agent and petrolatum varied considerably with the change in the HLB of the base.

The utilization of the nonionics for preparing lotions and emulsions represents another important pharmaceutical application. Addition of Tween 80 and bentonite to Benzyl Benzoate Lotion is reported to give a more stable preparation with lower pH values than that which was official in the USP XIV (43). If Span 80 is added during the extemporaneous compounding of kaolin-pectin suspension, the resultant product has less tendency to settle and cake and can be resuspended with only a little agitation (44).

Emulsions of sulfadiazine prepared with a mixture of Tween 80 and Span 80 gave greater absorption and higher blood levels of the antibacterial than either suspensions or tablets of equivalent dosage (45). The release of antiinfectives from dermatological emulsions is apparently not adversely affected by the variation in the nonionic content (46). Vithal, Nadkarni, and Zopf (47) recommended deletion of bentonite magma and glycerine in the USP XIII Calamine Lotion and replacing them with 8% PEG 400 and 3% PEG 400 monostearate.

There have been many investigations into the use

of nonionics in preparing fat emulsions for intraveneous administration. Most of these have been concerned with the application of the Tweens, particularly Tween 60, for this purpose (48). After studying tissue reactions at the site of injection, Carpenter and Shaffer (49) recommended that the PEG compounds be given a clinical trial for use as vehicles for certain drugs administered parenterally. The more stable and desirable emulsions result from the concurrent use of two emulsifiers, one of high HLB mixed with one of low HLB (50). The principle of using two emulsifiers of differing hydrophilic properties is often used and recommended (45,51). The emulsifier blend promotes greater stability and often reduces the work of emulsification.

In the presence of anti-infectives nonionics give varied and sometimes unpredictable results. Bacitracin is slowly inactivated in the presence of Tweens, Spans, and PEG compounds and rapidly destroyed by PEG 400 and its stearate (52). However, the potency of bacitracin is reportedly enhanced in the presence of nonionics (53). These detergents favor mold growth (54,55) and exhibit an inhibitory effect upon fungistatics and preservatives against molds (54,56). Hexachlorophene can be solubilized by using Tween 20 or Tween 80 and its potency in ointment bases is greatly increased in the presence of these agents (55). In contradiction is the report that Tween 80 is more effective than serum, serum albumin, and globin in

inactivating the antibacterial properties of as much as 0.1% hexachlorophene in broth (57).

Polyethylene glycols have been recommended for the treatment of Trichomonas vaginalis in concentrations of 25% to 50% (58). The addition of gentian violet or diamond green to aqueous solutions of PEG 4000 exhibits a synergistic effect upon the killing time of the solutions for both Trichomonas vaginalis and Paramecium caudatum.

Many other problems involving the incompatibility or instability of prescription formulations can be solved with the aid of the nonionics. The Spans and Tweens are particularly useful for this purpose and are highly recommended (59).

The addition of a nonionic to the hydro-alcoholic menstrum increased the efficiency of extraction in preparing fluidextracts of several crude drugs (60). The total yield of the combined anthracene derivatives of Cape Aloe is also increased when a nonionic is employed in the aqueous extraction (61).

With the recent ruling of the FDA (62) concerning labeling requirements of inert ingredients, the components of many preparations are no longer secret. Examination of the labels of pharmaceuticals now reveals their entire contents and the extent to which the nonionics have been employed can be fully appreciated.

PREPARATION OF THE POLYOXYETHYLENE NONIONICS

General Review

In the general preparation of the polyoxyethylene nonionics ethylene oxide is added to the appropriate hydrophobe, generally an alcohol, phenol, or carboxylic acid, usually in the presence of an alkaline catalyst (63). These reactions proceed by a stepwise addition of ethylene oxide resulting in the build-up of a hydrophilic polyether chain onto the hydrophobe. The first mole of ethylene oxide adds to form a beta-hydroxy compound. The second mole condenses on the beta-hydroxy group and lengthens the chain by two carbon units. Successive units are serially added and the chain of ethenoxy groups grows progressively longer.

$$R\text{-}OH + CH_2\overset{O}{\diagup\diagdown}CH_2 \longrightarrow R\text{-}O\text{-}CH_2\text{-}CH_2OH$$

$$R\text{-}O\text{-}CH_2\text{-}CH_2\text{-}OH + CH_2\overset{O}{\diagup\diagdown}CH_2 \longrightarrow R\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}OH, \text{ etc.}$$

By this process it is possible to build chains containing fifty or more of these units. The properties of the finished surfactant are highly dependent upon the number of units present. In general, increasing the length of the ethenoxy chain increases water solubility. Other properties,

such as wetting power, detergency, and solubilizing power are also dependent upon the number of these groups in the molecule (64).

It is significant that these types of surfactants are almost never homogeneous with respect to the length of the hydrophilic chain, but are a heterogeneous mixture of compounds having a wide range of chain lengths (65). Since the commercial surfactants are not pure compounds, their nomenclature is based upon the molar ratio of the reactants rather than the products of such reactions (65). Thus, the reaction of one mole of lauric acid with ten moles of ethylene oxide might be referred to as "lauric acid ten mole ethoxylate." When the polyoxyethylene esters are prepared by direct esterification of an acid with preformed polyethylene glycol, they are generally referred to as polyethylene glycol "x" monoester. The "x" denotes the molecular weight of the polyethylene glycol used. The above example prepared in this manner, would be termed polyethylene glycol 440 monolaurate.

The solubilizing power of the polyoxyethylene chain is considerably less than ionogenic groups, such as-SO_3H, -COOH, or quaternary nitrogen (66). For most of the reasonably water soluble polyether nonionics the weight of the hydrophilic chain exceeds that of the hydrophobe by a substantial margin. Solubilization is effected by the ability of the ether linkages to hydrate (67,68). Both heat and

monovalent ions render these compounds less soluble by dissociating these hydrates (69). Residual PEG can be removed from commercial polyoxyethylene nonionics by extracting them with hot brine solutions, followed by ion exchange procedures to remove the salt (70).

The question has been raised concerning ionization of the nonionics in solution. It is highly improbable that they form ions in the sense of electrically charged dissociation products of single molecules. Their micelles may bear a charge since they have been shown to migrate in an electric field (71). Waibel (72) believes these surfactants exist in solution as polyoxonium ions and are not nonionic, but weakly cationic.

Preparation of the Polyethylene Glycols

The polyethylene glycols can be prepared by adding ethylene oxide to glycol or to water and are a mixture of polyglycols of varying molecular weights (65). These compounds are produced commercially under the trade name of "Carbowax" (Carbide and Carbon Chemicals Corp., New York, New York) and are available in various molecular weights ranging from PEG 200 to 6000. The lower members of the series are liquids; the higher members above 1200 are waxy solids. They are all water soluble and have found extensive use in the preparation of pharmaceuticals.

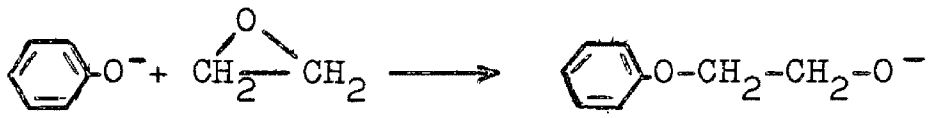
Flory (73) published a theoretical treatise on the

synthesis of these compounds and showed that the distribution of chain lengths should follow the Poisson curve. His derivation is based upon two assumptions: (1) that the total number of molecules capable of reacting is constant, i.e., that each reacting molecule possesses a propagating functional group and the addition of each unit of monomer regenerates such a group, and (2) that the rate constant for each step in the process is identical. This conclusion was used to predict the composition of the commercial PEG 400, although there was no experimental evidence to confirm it in the literature (74).

Preparation of the Polyoxyethylene Ethers

These surface-active agents are prepared by the addition of ethylene oxide to alcohols or phenols. The products of these reactions are again a heterogeneous mixture of compounds of varying chain lengths (65). Miller, Bann, and Thrower (74) applied the considerations of Flory to the reaction of ethylene oxide with phenols and alkylphenols in the presence of sodium and sodium hydroxide. Their results confirmed his predictions for chain lengths of up to five ethenoxy units. Kinetic studies by these and other investigators (75) indicate the following mechanism of reaction: First, phenol is ionized to phenoxide ion

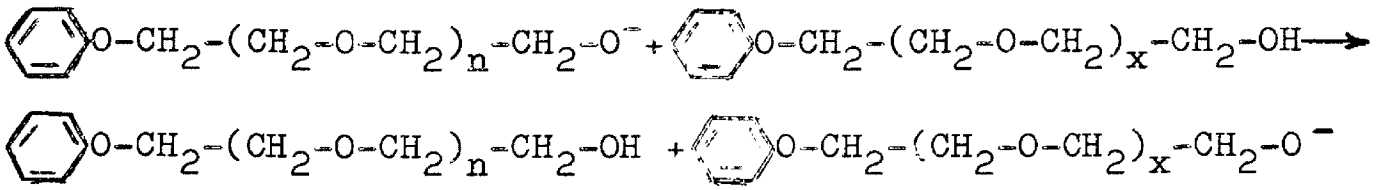
which attacks epoxide to form the phenoxyethanolate ion.



This ion then reacts with unionized phenol effecting proton transfer resulting in the formation of another phenolate ion and an aliphatic hydroxyl group.

This is the first step in the process and it proceeds to complete consumption of phenol to the exclusion of side reactions. When all the phenol is ethoxylated, the second step of adding a unit of monomer to the phenoxyethanolate ion ensues yielding the ion of the next member of the series.

In this step-wise manner additional units of ethylene oxide are added. Generation of new ions can come about by the interaction of ion with hydroxyl.



Since "x" may have any value, the formation of this alkoxyl ion can occur with any polymer hydroxyl independent of chain length. This leads to the conclusion that all hydroxyl groups are equivalent and any reaction of this nature is

entirely dependent upon probability. With the exception of the first step leading to complete consumption of phenol, it follows that subsequent addition of ethylene oxide occurs via a series of kinetically similar steps. The rate constant for the first step differs from those of the succeeding, but those of the latter are essentially the same.

Preparation of the Polyoxyethylene Esters

There are two methods of preparing the polyethenoxy esters (65). The first is to condense ethylene oxide on a hydrophobic acid in the presence of an alkaline catalyst. The second involves direct esterification of the hydrophobe with preformed PEG. The products of either reaction are a mixture of at least three components: moncesters, diesters, and free PEG. As in the ether surfactants these esters are not pure compounds, but are mixtures of varying chain lengths, the distribution of which closely resembles that of the ethers (65). Reaction of ten moles of ethylene oxide with one mole of lauric acid resulted in a mixture, at equilibrium, of monoester; diester; free PEG in the ratio of 2:1:1 respectively.

Kinetic studies of this type of reaction (75) reveal a similar mechanism to the etherification process already described. In the presence of a base the addition of an epoxide to caprylic acid becomes highly selective, resembling very closely the sequence of events obtained with the

reactions of phenol, leading to complete consumption of acid before further addition of epoxide occurs.

$$R\text{-}COOH + Base \longrightarrow R\text{-}COO^-$$

$$R\text{-}COO^- + \underset{\diagdown\,O\,\diagup}{CH_2\text{-}CH_2} \longrightarrow R\text{-}COO\text{-}CH_2\text{-}CH_2\text{-}O^-$$

$$R\text{-}COO\text{-}CH_2\text{-}CH_2\text{-}O^- + R\text{-}COOH \longrightarrow R\text{-}C(=O)\text{-}O\text{-}CH_2\text{-}CH_2\text{-}OH + R\text{-}COO^-$$

The results of their studies lead Sechter and Wynestra (75) to propose almost identical mechanisms for free base catalyzed alcohol-glycidyl ether, phenol-glycidyl ether, and acid-glycidyl ether reactions.

NONIONICS EMPLOYED IN THIS STUDY

The Tweens

The Tween series of surfactants are prepared by treating esters of anhydrosorbitols with various amounts of ethylene oxide (51). The resulting products are generally soluble or dispersible in water and have excellent emulsifying properties. The hydrophilic portion of the molecule consists of the anhydrosorbitol-ethylene oxide condensate. The hydrophobic portion is the esterifying long chain fatty acid residue.

Dehydration of sorbitol leads to a mixture of hexitans and hexides, which are then esterified with one or more moles of the common fatty acids. Subsequent addition of ethylene oxide yields the Tween.

$$\text{Sorbitol} \xrightarrow{-H_2O} \text{Hexitans and Hexides} \xrightarrow{HOOCR}$$

$$\text{Anhydrosorbitol esters (Span or Arlacel)} \xrightarrow{\text{Ethylene Oxide}} \text{Tween}$$

Since the esterified anhydrosorbitols make up the Span and Arlacel series, the Tweens can be considered as essentially polyoxyethylene derivatives of the Span type products.

There are nine Tween products available of which all but two (Tween 60 and 61) are yellow or amber oily liquids. Of these, only four (Tween 20, 40, 60, and 80)

are water soluble. The others are insoluble (Tween 61, 65, and 81), or form translucent to milky dispersions (Tween 21 and 85). The water soluble members are oil-in-water emulsifiers and solubilizers. When used in conjunction with a corresponding Span or Arlacel, the Tweens tend to alleviate some of the work of emulsification required in preparing water-in-oil emulsions.

The Myrj Series

The Myrj products, representative of the polyoxyethylene fatty acid esters, are all ethylene oxide adducts of stearic acid differing from each other in the length of their hydrophilic polyether chain (51). They are all ivory solids, water soluble, and thus tend to form water-in-oil emulsions. Their chief advantage lies in their effectiveness to function in the presence of astringent salts.

The following reaction characterizes their synthesis:

$$R\text{-}COOH + n\ \underset{\diagdown O \diagup}{CH_2\text{-}CH_2} \rightarrow R\text{-}COO\text{-}CH_2\text{-}CH_2\text{-}O(CH_2\text{-}CH_2\text{-}O)_{n-2}\text{-}CH_2\text{-}CH_2\text{-}OH$$

The Brij* Series

These compounds are characteristic of the polyethenoxy fatty ethers (51). They are prepared in an

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*Trademark Atlas Powder Co., Wilmington, Delaware for polyoxyethylene stearate.

analogous manner to the Myrj products, but utilize a long chain fatty alcohol as the hydrophobe, rather than an acid. They are all ethylene oxide condensates of lauryl alcohol varying in the ether content of their hydrophilic chain. They are generally soluble or dispersible in water. Since they are ethers, they are stable under the more extreme conditions of pH beyond the ranges employed for ordinary emulsifiers, particularly in mediums expected to be highly alkaline. Brij 30 is a colorless oily liquid, fairly lipophilic in nature. Brij 35 is a white solid, water soluble and an excellent oil-in-water emulsifier.

Their preparation is illustrated by this equation.

$$R\text{-}OH + n\ CH_2\text{-}\underset{\diagdown\ \diagup}{O}\text{-}CH_2 \longrightarrow R\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}(CH_2\text{-}CH_2\text{-}O)_{n-2}\text{-}CH_2\text{-}CH_2\text{-}OH$$

INTRODUCTION TO THE PROBLEM

The incorporation of dyes into various formulations is a common practice, but this wide-spread use has not occurred without attending problems. Quite often the finished product is the result of considerable effort on the part of the formulator to create a color stable preparation. Many dyes are, in themselves, photosensitive and cannot be utilized unless specific measures are taken to alleviate this condition. Also, incompatibilities arising from various components may lead to color loss. Addition of antioxidants, chelating agents, and/or light absorbers may be of value only in specific formulations and cannot be relied upon to promote stability in all cases.

Although this problem is generally recognized in the industry, there is little published information specifically concerned with this aspect of pharmaceutical manufacturing. The absence of experimental evidence can be attributed to several factors among which are (2): (1) lack of a suitable exaggerated light stability cabinet in which to study photosensitivity reactions, (2) absence of proper analytical methods for the measurement of color changes, (3) discarding a dye which is unsuitable in a given formulation without determining the reasons for its instability, and (4) confinement of such data to the confidential files within a particular company.

Previously, the formulating chemist had a greater array of dyes from which to choose and by simple screening techniques could select one having the desired stability characteristics for a specific preparation. With the list of certified dyes shrinking annually, it is becoming more difficult to find a suitable coloring agent. Therefore, it is necessary to investigate these problems more thoroughly.

There have been several studies involving color stability in tablet formulations (76-80). All of these were conducted under exaggerated light conditions and were designed to determine color fastness, but not the mechanism of color fading. Swartz, et al., (2) studied the influence of temperature and pH on the surface color and total dye content of tablets colored with certified dye.

Common pharmaceutical materials may react with a dye to promote fading as was shown by Kuramoto, et al., (81). They demonstrated the deleterious effects of reducing sugars, such as dextrose, lactose, and mannitol on F D & C Blue #2. Lachman (82) showed that three commonly used quaternary ammonium compounds can interact with F D & C Red #1, F D & C Blue #1, and D & C Yellow #10 yielding insoluble complexes. This finding was extended to include several other dyes (2). Garrett and Carper (83) studied kinetically the color loss of a liquid multisulfa preparation. Since their object was to predict the shelf life of the product, they made no attempt to elucidate the

mechanism of reaction.

Although there have been several studies and reports concerning the interaction of the polyoxyethylene nonionics with phenols and related compounds (38,84,85), there has been little experimental evidence pertaining to the effects of these agents on the stability of certified dyes. The first such study was conducted by Scott, Goudie, and Huetteman (86) who described the accelerated fading of several certified colorants in the presence of six ethoxylated surfactants. In all but 4 of the 30 systems examined, fading occurred. Also, Hayashi (87) reported on the interaction of Congo Red with Tween 80.

While the problem of color stability in pharmaceutical preparations is primarily one for the manufacturer, the possibility of its occurrence in extemporaneous compounding cannot be ignored. Quite often the pharmacist is required to mix proprietaries or add ingredients which could lead to an incompatibility resulting in color loss. Since he has neither sufficient time to observe fading nor pertinent information enabling him to predict such an event, it may go completely unnoticed. The present investigation was prompted by the observation that Phenobarbital Elixir exhibited a gradual loss of color when Tween 80 was added to the formula. Amaranth is the coloring agent in this elixir and for this reason it was chosen as the dye for study. Although there is published data on the fading

of other azo dyes in the presence of Tween 80 and other polyethenoxy nonionics (86), there has been, to date, no specific evidence in the literature relating to the stability of amaranth in solution with these agents. This study was undertaken to determine if these ethoxylated compounds were responsible for the observed color loss.

EXPERIMENTAL PROCEDURE

The materials utilized in this study consisted of a certified dye, normally stable to light, and four nonionic surfactants of the polyoxyethylene type. The dye was amaranth (F D & C Red #2) and the surfactants were Tween 80, Myrj 52, Brij 35, and Carbowax 400. In addition, three antioxidants were used to ascertain their effects upon the dye-surfactant systems. These were sodium bisulfite, ascorbic acid, and hydroquinone.

For convenience, stock solutions were prepared of all reagents on a weight to volume basis. The stock solution of amaranth was 70 mg.% and of each surfactant 25%. The solutions of sodium bisulfite and ascorbic acid were 4% and the stock solution of hydroquinone was 2%.

The surfactants were dissolved in hot distilled water to facilitate solution, and the resulting solutions were allowed to stand overnight to attain room temperature. Sufficient distilled water then was added to bring them to correct volume.

Preliminary test runs with these surfactant solutions showed a high degree of mold contamination. To preclude a recurrence of this contamination, the test sample bottles and the stock solutions of surfactants and dye were autoclaved.

Following preparation, the solutions to be

sterilized were transferred to pint amber bottles, plugged with gauze stoppers, and autoclaved at 15 pounds for twenty minutes. After removal from the autoclave, the gauze stoppers were replaced with sterile screw caps and the solutions were cooled to room temperature with intermittent agitation to prevent permanent phase separation.

Previous experience indicated that the sodium bisulfite solutions deteriorate when they are autoclaved. It was feared that the solutions of ascorbic acid and hydroquinone might do likewise. To overcome this deterioration these stock solutions were prepared just prior to use by employing well-cleaned glassware and sterile distilled water. (Sterile water was distilled water previously boiled for at least ten minutes and cooled to room temperature.) If these solutions were not immediately used, they were transferred to sterile amber bottles for storage. However, all solutions were used within four days of preparation.

To determine if the concentration of amaranth had changed during the process of sterilization, two duplicate samples were prepared by diluting 5.0 ml. of the stock solution to 50.0 ml. with distilled water and read on the Fisher electrophotometer, A-C model, using a 425B filter. A further check was conducted by reading a test sample control solution for concentration. In no case was evaporation of solvent or destruction of dye, if it occurred, great enough to effect a change in the prepared concentration.

It was not desirable to autoclave the flasks, burettes and pipettes used for measuring. In order to render them as aseptic as possible, they were first washed well with a laboratory detergent and rinsed with hot tap water, followed by three rinses with sterile distilled water.

To prepare the test solutions 50.0 ml. of sterile amaranth solution was transferred to a 500 ml. volumetric flask by pipette. To this was added approximately 100 ml. of sterile distilled water, followed by the desired amount of surfactant solution. Finally, sufficient sterile water was added to make 500 ml. In preparing the test solutions containing the antioxidants, the same procedure was followed, except that just prior to the addition of the final amount of water, 25.0 ml. of the desired antioxidant solution was introduced. The test solutions then were 7 mg.% amaranth and 0%, 1%, 3%, or 5% surfactant for each nonionic employed. Those solutions containing the antioxidants were prepared in a like manner, but contained in addition, a final concentration of 0.1% sodium bisulfite, 0.1% ascorbic acid, or 0.05% hydroquinone.

Immediately following preparation these solutions were transferred to their respective containers in 60 ml. portions. This amount was instilled into each of two 2-ounce clear, two 2-ounce amber, two 4-ounce clear, and two 4-ounce amber prescription bottles. The entire series of a surfactant-dye combination consisted of 32 sample

bottles, each containing 60 ml. of solution. The pH of each test solution was then determined with a Beckman Zeromatic pH meter.

To overcome any pH changes which might result from the use of soft glass bottles, the following procedure was adopted. Sixteen sample bottles were washed repeatedly with a laboratory detergent and rinsed well until they showed no change of greater than 0.1 unit, when stored overnight, in the pH of distilled water. The remainder of the bottles used in this study were cleansed similarly and rinsed several times with distilled water before being autoclaved. A further check on a few of these cleansed bottles for pH changes showed negative results and it was assumed that the rest of the containers would conform likewise. In subsequent studies which employed the antioxidants, these bottles were reused after cleaning in the above manner, but since no pH studies were undertaken in these series, no check was made for pH change.

The test samples were placed in a window on the shady side of the building to permit exposure to sufficient light, but not to direct sunlight. These were allowed to stand for a period of several weeks, after which their dye concentrations were determined by readings on the electrophotometer. Although the time lapse between preparations and reading of the samples varied for each surfactant series, the solutions containing the antioxidant were read

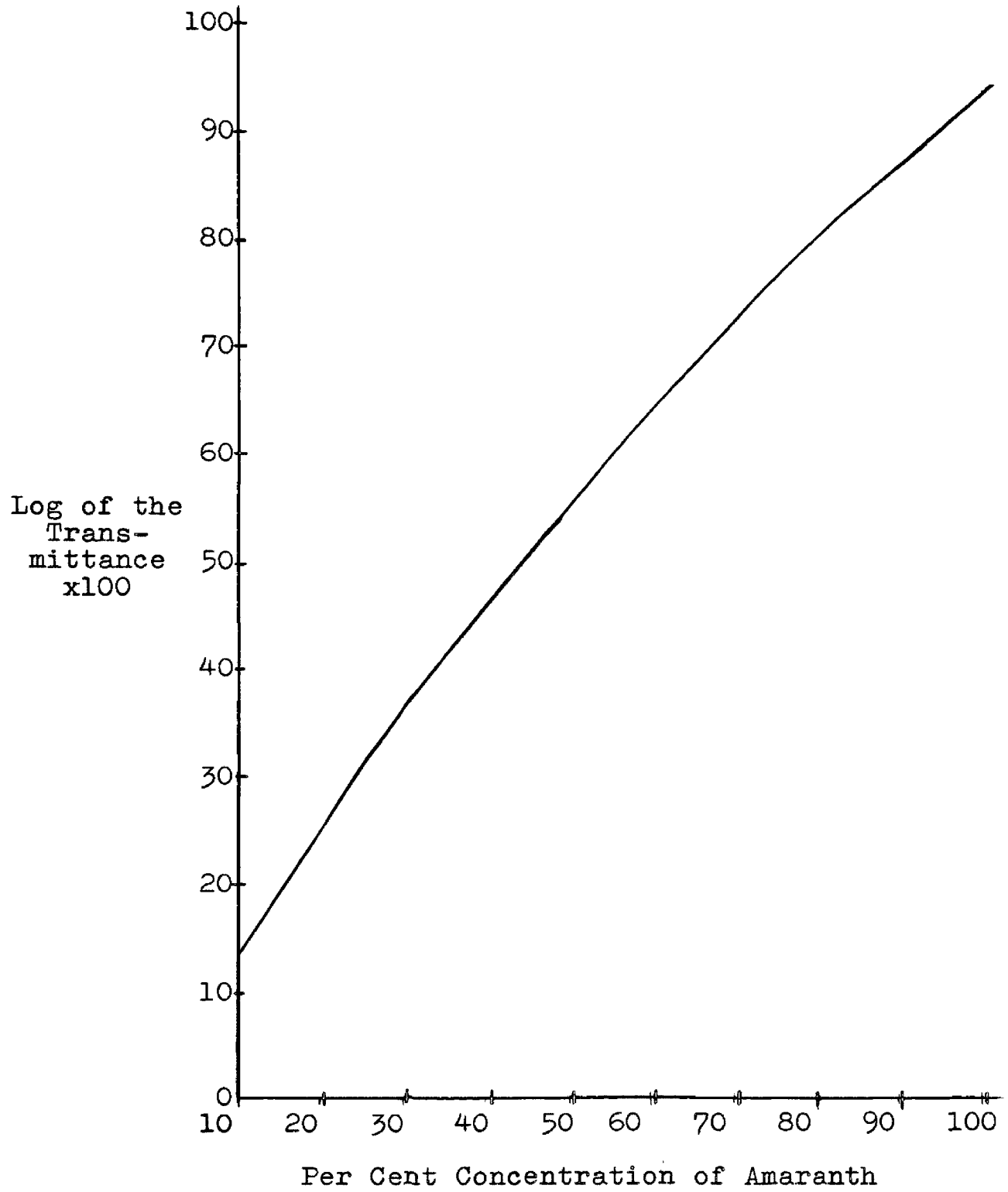
at the close of an identical time period as the corresponding solutions containing no antioxidant.

Determination of a Standard Curve

Since Beer's Law was not valid over the range of concentration studied, a standard curve was determined by plotting the log of the transmittance as a function of the concentration of dye. In establishing this curve a 7 mg.% solution of amaranth was chosen as 100% concentration and its transmittance was determined on the electrophotometer. Nine different dilutions were prepared. Each dilution corresponded to 10% increments in dye concentration from 10% through 90%. Transmittance was determined using distilled water as a blank. The curve was constructed from these data. Since the higher concentrations of surfactant caused a slight shift in this curve when read against distilled water, a corrective blank containing the appropriate amount of surfactant was used for reading the test solutions. The low concentrations of antioxidants employed were disregarded and no corrective measures concerning the antioxidants were undertaken in reading these sets of solutions. Figure I is the standard curve.

FIGURE I

STANDARD ABSORPTION CURVE OF AMARANTH



RESULTS AND DISCUSSION

Tables III and IV represent the data obtained from the Tween 80-amaranth and Myrj 52-amaranth studies, and Tables V and VI for that of the Brij 35- and Carbowax-amaranth studies. With the exception of the original pH values, which were determined before the solutions were instilled in their respective containers, all data represent an average of the two sample figures. The Tween 80-amaranth system was read at the end of thirty-five days; the Myrj 52-amaranth system at the end of thirty-one days; the Brij 35-amaranth system at the end of forty days; and the Carbowax 400-amaranth system at the end of thirty-five days.

Examination of the tables reveals that in all cases where the surfactant-dye combination was stored in clear bottles, complete loss of color ensued. For the most part, the samples contained in amber bottles exhibited only partial fading. The exceptions to this were the 2-ounce amber bottles containing the Brij and Myrj systems. No controls faded during the course of the experiment. Generally, increasing concentrations of surfactant resulted in a greater degree of fading, but there were exceptions.

Since the 4-ounce amber bottle samples faded less than the 2-ounce amber bottle samples, the presence of air may have exerted some protective action.

TABLE III

PER CENT CHANGE IN DYE CONCENTRATION AND pH CHANGES OF AMARANTH-TWEEN 80 COMBINATION IN 35 DAYS

Sample	Conc. of Surfactant, % w/v	Original pH	Final pH	Change in pH	Final Reading log of Trans-mittance	Change in Dye Conc., %
2 oz. clear	0	6.78	6.87	0.09	95	0
	1	4.56	3.84	-0.72	c[a]	100
	3	4.46	3.94	-0.52	c	100
	5	4.30	3.98	-0.32	c	100
4 oz. clear	0	6.78	6.74	-0.04	95	0
	1	4.56	3.66	-0.90	c	100
	3	4.46	3.68	-0.78	c	100
	5	4.30	3.72	-0.58	c	100
2 oz. amber	0	6.78	6.91	0.13	95	0
	1	4.56	4.62	0.06	89	9
	3	4.46	4.15	-0.31	86	14
	5	4.30	4.22	-0.08	89	9
4 oz. amber	0	6.78	6.97	0.19	95	0
	1	4.56	4.96	0.40	95	0
	3	4.46	4.42	-0.04	95	0
	5	4.30	4.25	-0.05	93	3

[a]c. colorless

TABLE IV

PER CENT CHANGE IN DYE CONCENTRATION AND pH CHANGES OF AMARANTH-MYRJ 52 COMBINATION IN 31 DAYS

Sample	Conc. of Surfactant, % w/v	Original pH	Final pH	Change in pH	Final Reading log of Trans-mittance	Change in Dye Conc., %
2 oz. clear	0	6.48	6.91	0.43	95	0
	1	4.30	3.42	-0.88	c[a]*	100
	3	3.96	3.36	-0.60	c	100
	5	3.84	3.38	-0.46	c	100
4 oz. clear	0	6.48	6.91	0.43	95	0
	1	4.30	3.26	-1.04	c*	100
	3	3.96	3.16	-0.80	c*	100
	5	3.84	3.06	-0.78	c*	100
2 oz. amber	0	6.48	7.12	0.64	95	0
	1	4.30	4.23	-0.07	87	13
	3	3.96	3.69	-0.27	c	100
	5	3.84	3.67	-0.17	c	100
4 oz. amber	0	6.48	7.12	0.64	95	0
	1	4.30	4.51	0.21	93	3
	3	3.96	3.96	0.00	88	11
	5	3.84	3.81	-0.03	84	15

[a] c. colorless
* turbidity present

TABLE V

PER CENT CHANGE IN DYE CONCENTRATION AND pH CHANGES IN AMARANTH-BRIJ 35 COMBINATION IN 40 DAYS

Sample	Conc. of Surfactant, % w/v	Original pH	Final pH	Change in pH	Final Reading log of Transmittance	Change in Dye Conc., %
2 oz. clear	0	6.56	6.95	0.39	95	0
	1	3.53	3.10	-0.43	c[a]*	100
	3	3.10	2.95	-0.15	c*	100
	5	3.08	2.89	-0.19	c*	100
4 oz. clear	0	6.56	6.97	0.41	95	0
	1	3.53	3.03	-0.50	c*	100
	3	3.10	2.89	-0.21	c*	100
	5	3.08	2.80	-0.28	c*	100
2 oz. amber	0	6.56	7.16	0.60	95	0
	1	3.53	3.26	-0.27	c*	100
	3	3.10	3.05	-0.05	c*	100
	5	3.08	2.98	-0.10	c*	100
4 oz. amber	0	6.56	7.21	0.65	95	0
	1	3.53	3.42	-0.11	84	15
	3	3.10	3.13	0.03	88	10
	5	3.08	3.01	-0.07	88	10

[a] c. colorless
* turbidity present

TABLE VI

PER CENT CHANGE IN DYE CONCENTRATION AND pH CHANGES IN AMARANTH-CARBOWAX 400 COMBINATION IN 35 DAYS

Sample	Conc. of Surfactant, % w/v	Original pH	Final pH	Change in pH	Final Reading log of Trans-mittance	Change in Dye Conc., %
2 oz. clear	0	6.56	6.95	0.39	95	0
	1	5.92	3.42	-2.50	c[a]	100
	3	5.45	3.32	-2.13	c	100
	5	5.02	3.27	-1.75	c	100
4 oz. clear	0	6.56	6.97	0.41	95	0
	1	5.92	3.28	-2.64	c	100
	3	5.45	3.08	-2.37	c	100
	5	5.02	3.06	-1.96	c	100
2 oz. amber	0	6.56	7.16	0.60	95	0
	1	5.92	6.86	0.94	94	1
	3	5.45	6.31	0.86	93	3
	5	5.02	4.30	-0.72	86	14
4 oz. amber	0	6.56	7.21	0.65	95	0
	1	5.92	7.20	1.28	95	0
	3	5.45	6.69	1.24	94	1
	5	5.02	6.98	1.96	93	3

[a] c. colorless

Again from the tables, III, IV, V, and VI, the pH of the samples generally dropped with aging, but the control sample pH increased consistently. This would seem to indicate that, even though precautions were taken to prevent a pH change, in sufficient time the soft glass still imparts alkalinity to the solutions. This decrease in pH was less for increasing concentrations of surfactant, but this may have been due to the fact that the original solutions had initially lower pH values with higher percentages of surfactant. The decrease in pH was greatest where total loss of color occurred. In the amber bottles, there were several exceptions to this generally downward trend of pH. This variable behavior is attributed to the alkalizing effects of the glass containers. Although there was a difference between members of a pair as high as 0.24 pH unit (5% Carbowax-amaranth in 4-ounce amber bottles), in all cases where increase or decrease in pH was noted, both members of the pair exhibited the same response, but some differed in degree. Nine sample pairs showed difference in pH between members of 0.1 unit or greater. This decrease in pH is in accord with the results of Scott, et al. (86), in their experiments on the fading of certified dyes in the presence of these surface-active agents.

In the Myrj- and Brij-dye combinations there was turbidity in almost all solutions which exhibited total color loss. The precipitate in the Myrj system may be the

result of hydrolysis, since even the stock solution of this nonionic showed a heavy precipitate after several weeks storage. Because Brij 35 is an ether, rather than an ester, the presence of a precipitate in this system is more difficult to explain. There was no precipitate in any samples packaged in amber bottles, except for the decolorized Brij solutions.

That the pH drop, per se, does not cause accelerated fading was again shown by Scott and coworkers (86), who obtained accelerated fading of their surfactant-dye solutions prepared in Sorensen's phosphate buffer (pH 6.98) in which the hydrogen ion concentration was constant throughout the experiment.

In order to gain some insight into the reaction occurring in these dye-surfactant systems and in an attempt to overcome this incompatibility, several antioxidants were added to these systems to determine their effect. Tween 80 solutions were used as pilot runs and when an antioxidant was found which showed promise, the entire series of four surfactants was tested in the presence of this agent. For this purpose only those samples which experienced a total loss of color were studied. Thus, all samples contained in clear bottles and the 3% and 5% 2-ounce amber bottle Myrj samples and all 2-ounce amber bottle Brij samples were involved.

The first two attempts were conducted with sodium

bisulfite and ascorbic acid respectively, at a concentration of 0.1%. In both cases deterioration of the solutions occurred, especially in the presence of a surfactant. Although the controls also faded in these studies, their rate of fading was much less than that of the solutions of nonionics. Ascorbic acid was particularly active, effecting total color loss of the Tween-dye solutions within two days.

In the third experiment hydroquinone at a concentration of 0.05% was added to the Tween system. Since this system showed no apparent fading at the end of one week, it was decided to expand the experiment to include all four surfactants. No pH studies were undertaken. The results are tabulated in Tables VII--X.

From the tables it is noticed that there was an increase in density of color, leading to an apparent increase in concentration of dye. Since there was no evaporation of solvent, this is at present unexplainable, unless it stems from changes in the hydroquinone. After several days the stock solution of this agent darkened upon standing and if this occurred in the test solutions, it would give a greater concentration reading. The readings over 100 are estimated, since the electrophotometer scale extends only to this value. In order to tabulate these results the values of the final readings (second column in Tables VII--XI) were reduced proportionately to give a corresponding reading based upon original dye concentration.

TABLE VII

PER CENT CHANGE IN DYE CONCENTRATION OF TWEEN 80-HYDROQUINONE-AMARANTH COMBINATION IN 35 DAYS

Sample	Conc. of Surfactant, % W/V	Final Reading Log of Transmittance	Change in Dye Conc., %
2 oz. clear	0	99	0
	1	98	2
	3	91	12
	5	86	20
4 oz. clear	0	99	0
	1	99	0
	3	90	13
	5	88	16
2 oz. amber	5	99	0

TABLE VIII

PER CENT CHANGE IN DYE CONCENTRATION OF MYRJ 52-HYDROQUINONE-AMARANTH COMBINATION IN 31 DAYS

Sample	Conc. of Surfactant, % W/V	Final Reading Log of Transmittance	Change in Dye Conc., %
2 oz. clear	0	101	0
	1	97	6
	3	78	32
	5	68	43
4 oz. clear	0	101	0
	1	98	5
	3	80	29
	5	75	34
2 oz. amber	0	101	0
	3	95	9
	5	94	10

TABLE IX

PER CENT CHANGE IN DYE CONCENTRATION OF BRIJ 35-HYDROQUINONE-AMARANTH COMBINATION IN 40 DAYS

Sample	Conc. of Surfactant, % W/V	Final Reading Log of Transmittance	Change in Dye Conc., %
2 oz. clear	0	101	0
	1	94	10
	3	77	33
	5	75*	34
4 oz. clear	0	101	0
	1	97	6
	3	78	32
	5	77	33
2 oz. amber	0	101	0
	1	97	6
	3	96	7
	5	96	7

*one sample of pair was colorless.

TABLE X

PER CENT CHANGE IN DYE CONCENTRATION OF CARBOWAX 400-HYDROQUINONE-AMARANTH COMBINATION IN 35 DAYS

Sample	Conc. of Surfactant, % W/V	Final Reading Log of Transmittance	Change in Dye Conc., %
2 oz. clear	0	101	0
	1	93	12
	3	78	32
	5	78	32
4 oz. clear	0	101	0
	1	94	10
	3	83	25
	5	81	28

TABLE XI

PER CENT CHANGE IN DYE CONCENTRATION OF TWEEN 80-HYDROQUINONE-AMARANTH COMBINATION IN 35 DAYS

Sample	Conc. of Surfactant, % W/V	Final Reading Log of Transmittance	Change in Dye Conc., %
2 oz. clear	0	102	0
	1	101	1
	3	91	14
	5*	c**	100
4 oz. clear	0	101	0
	1	102	0
	3	88	19
	5*	c**	100

*no hydroquinone present in these samples.
**colorless.

From these calculated values, the percentages of column 3 were obtained by interpolation on the standard curve. It is assumed that all solutions exhibited a similar increment of color density.

With the exception of one sample, the loss of color was less in this system than the original which contained no hydroquinone. Since the two series were not run simultaneously, but at different seasons, the first in early summer and the last in late summer, it might be inferred that any comparison of results would not be valid. That the results are indicative, from a qualitative standpoint, can be seen from Table XI in which this Tween-dye-hydroquinone system showed almost identical results for the

control and 1% and 3% surfactant concentrations as in Table VII. The 5% surfactant samples contained no hydroquinone and faded completely. This experiment was conducted at the same time as the Myrj-, Brij-, and Carbowax-amaranth-hydroquinone solutions during late summer. The corresponding Tween-dye-hydroquinone set in Table VII was run during early fall.

It is quite difficult to distinguish differences in dye concentrations of less than 5% with the naked eye and it would appear from the results that in many cases no change could be detected without the aid of an instrument, since color loss was less than 5%. However, the electrophotometer used in this study was not sensitive to slight changes in absorption maximum. Several samples which gave near identical readings on the instrument were easily detectable by visual examination as having different colors. The general trend in fading of these solutions was red through orange to yellow and with increased fading the solutions acquired more orange character. As an example, in the Carbowax-dye-hydroquinone system the 1% 4-ounce clear samples had a noticeable orange cast while the 1% 2-ounce amber samples did not and were red, yet both solutions had identical readings. Thus, the instrument was not able to distinguish this shift in absorption maximum. Therefore, it was necessary to examine all samples visually to determine any color change. There was no precipitate in any

sample of this series.

Scott (86) stated that the presence of nonionics did not cause a shift in absorption maximum of their dye solutions, and that such a shift did not occur during the fading process. Whether or not this happened to the original dye-surfactant solutions of this study was not recorded, since this event was not considered. The shift was evident when the antioxidant was present. The characteristic transition toward the yellow side of the spectrum did not occur in any samples contained in amber bottles. There was no visual difference in these samples when compared to their controls either in color or, with the exception of the Myrj series, degree of fading.

As before, the presence of air seemed to exert some protective action. This fact, along with accelerated fading in the presence of sodium bisulfite and ascorbic acid, seem to indicate that the reaction is one of reduction. Inskeep and Kretlow (88) state that azo dyes commonly take up hydrogen to form amine compounds.

That hydroquinone exerts a protective action on the fading probably does not reside in its ability to act as an antioxidant, but rather in other properties of the molecule. A more plausible answer may be that it absorbs light in the ultraviolet wavelengths known to be in sunlight (89,90). Out of curiosity a duplicate sample of hydroquinone in 5% Tween-amaranth in a 2-ounce amber bottle was run

concurrently with the other Tween-dye-hydroquinone solutions. No fading occurred and the solutions could not be distinguished either visually or on the electrophotometer from the control. (See Table VII.) It would appear that the supplemental action of this agent and amber glass affords a fairly high degree of protection to the photosensitivity of amaranth in the presence of Tween 80.

Although the use of amber bottles and a light absorber do offer a substantial degree of protection to the surfactant-dye system, they do not preclude the possible occurrence of a thermally catalyzed reaction. Fading might well occur in the absence of light, but at a much slower rate at room temperature. In the short term the activating energy of light proves more detrimental, but over an extended time the same deleterious results might be obtained even if light were excluded.

The significance of the pH drop upon aging eludes definition. This occurs both in the presence and absence of dyes (86). It could be that this is the result of some interaction of surfactant with aqueous solvent leading to release of hydrogen ion. Buffering the solutions would not prevent such a reaction, but would only remove the hydrogen ion as it formed. Involvement of the solvent might explain the pseudo-first order reaction rates obtained in kinetic studies reported by Scott. He stated that preheating a surfactant before addition of dye caused no change in

reaction rates and concluded that the mechanism does not involve (stable) decomposition products of the surfactant.

A comparison between the data of the two series, with and without hydroquinone, was deliberately avoided, since the results are not truly quantitative. They are indicative because of the readings obtained with the two different sets of Tween-hydroquinone solutions (Compare Tables VII and XI). The unsuitability of sunlight or daylight as a source of radiant energy for photocatalyzed reactions is fairly well recognized (2). Several light stability cabinets have been designed and are produced for quantitative studies of photosensitivity (2,77). However, the use of daylight seems justified for this present study, since the results are in fairly close agreement.

It is interesting that no hypsochromic shift occurred in the samples contained in amber bottles. The beneficial effects of this glass in protecting various pharmaceuticals and dyes from photocatalyzed reactions is well utilized. In clear bottles the presence of light may stimulate other reactions which lead to a shift in absorption maximum. Ordinary glass absorbs the shorter ultra-violet as well as the longer infra-red radiation found in sunlight (80,91). Photodecomposition would then be due to visible light and/or the ultra-violet and infra-red radiation nearest in wavelength to visible light.

In view of the scant information available on the

interaction of these nonionics with dyes, it is not possible to propose any mechanism of reaction. Since the ethenoxy chain is common to all agents used, it is likely that this portion of the molecule is responsible for the observed phenomenom. However, as Scott (86) has pointed out, trace contaminants present in the surfactants as a result of manufacturing processes could also be suspected.

The results of this study and that of Scott and coworkers (86) indicate that these nonionics are not as inert as they were once thought to be (38,92). Further, their activity may not be restricted purely to dyes. If such effects were to occur with potent medicinals, such as alkaloids, which are in many cases of the same order of concentration as these colorants, the results could be total loss of activity.

SUMMARY

A qualitative investigation into the effects on amaranth of several polyoxyethylene nonionics and a polyethylene glycol has been conducted. The results of this study indicate that these ethoxylated surfactants exert a deleterious effect upon the stability of the dye. Addition of sodium bisulfite and ascorbic acid, as well as the presence of air, imply that a reduction reaction occurs leading to loss of color. This reduction presumably takes place at the azo linkage destroying the chromophore. Amaranth has been shown to be normally stable to light, but in the presence of these surface-active substances it is quite photosensitive. Hydroquinone offers some protection and it is thought that its activity resides in its ability to absorb light, rather than function as an antioxidant. The results suggest that the greatest protection to the detrimental effects of surfactants and light is afforded by the concurrent utilization of a light absorber and packaging in amber bottles. That the reaction can occur in the absence of light is also discussed. No mechanism of reaction is proposed, although it seems likely that the polyethenoxy chain could be implicated. The possibility that trace contaminants in the surfactants can cause the loss of color cannot be overlooked.

Although this study was conducted with a dye, the

question concerning the ability of these surfactants to elicit a similar action in the presence of therapeutically active substances is raised. Not only should these non-ionic surfactants be more thoroughly investigated for their chemical activity, but also, as Swartz and Cooper (2) have pointed out, "basic studies are needed in the field of chemical reactivity of colorants with other components of medications /and/ the stability of individual or mixed colorants in various environments . . .".

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