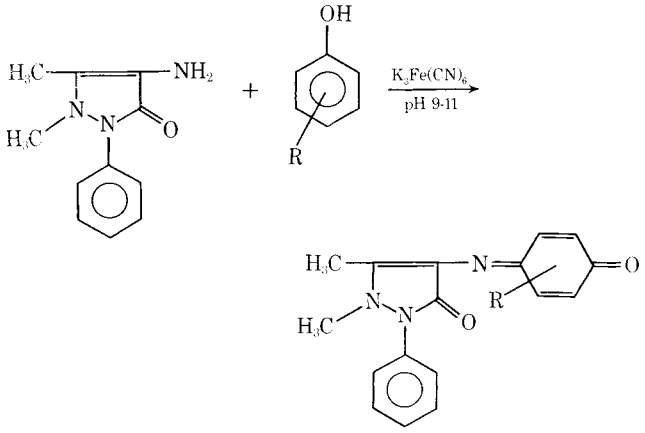
Interference by Surface-Active Agents with the 4-Aminoantipyrine Determination of Hexachlorophene and Other Phenols

K. F. BROWN*, D. E. GUTTMAN†, and A. S. ANDERSON

Abstract □ The colorimetric method for the estimation of phenols which is based on oxidative condensation with 4-aminoantipyrine (4-AAP) yielded erroneous results when performed in the presence of nonionic surfactants and related materials. For example, solutions of hexachlorophene gave greatly diminished responses when the color-forming reaction was conducted in the presence of Brij 30, Brij 35, Pluronic F108, PEG 6000, and bovine serum albumin. This effect appeared to result from the binding of hexachlorophene. Apparently only free hexachlorophene was capable of reaction. In contrast, solutions of *o*-chlorophenol yielded significantly higher absorbance values in the presence of Brij 35 than in its absence. This was shown to result from an effect on the spectrum of the condensation product caused by the presence of surfactant. The spectral effect was observed with a number of phenolic compounds. Only *p*-substituted phenols, however, were found to exhibit significantly reduced reactivities with 4-AAP in the presence of surfactant. The specificity of reaction inhibition by surfactants permitted a determination of phenol in the presence of hexachlorophene.

Keyphrases □ Hexachlorophene, phenols analysis—4-aminoantipyrine method □ Surfactants, interference—hexachlorophene, phenols analysis □ Structure effect—phenol-dye interaction □ Colorimetric analysis—spectrophotometer

The colorimetric reaction between phenols and 4-aminoantipyrine (4-APP) was first described by Emerson *et al.* (1–4). The reaction involves the oxidative condensation of phenols with 4-AAP, in basic solution, to produce intensely colored pyrazolone dyes. The general reaction can be written (Scheme I):



Scheme I

Emerson (2) recommended that potassium ferricyanide be used as the oxidant. However, Ochynski (5) found that potassium persulfate was also suitable. Reaction conditions must be sufficiently basic to prevent the formation of antipyrine red, a dye formed from the oxidation of AAP by potassium ferricyanide at pH values below 8.5 (6). Absorption maxima for reaction products formed with most phenols are reported to be between 460–600 mμ (6–8, 19, 20).

Emerson and Beegle (3) outlined the structural requirements which are prerequisite for a positive 4-AAP test. In general, phenols which are unsubstituted in the *para* position or those which are substituted in the *para* position with halogen, hydroxyl, sulfonic acid, or carboxylic acid groups give a positive reaction. It was theorized that the *para* substituents are expelled in the reaction. *Para* substitution by alkyl, aryl, nitro, or aldehyde groups blocks the reaction, although Gottlieb and Marsh (8) pointed out some exceptions to these empirical rules.

A number of reports have appeared in the literature describing the application of the reaction to the quantitative estimation of phenols (5–21). These include a number of extensive reviews of methodology (6, 7, 9, 21). Assay methods based on the reaction are claimed to possess specificity, sensitivity and convenience.

The 4-AAP method has been applied to the estimation of hexachlorophene in a variety of products (7, 22–24). However, there have been reports that the method gave unreliable results for the analysis of hexachlorophene in the presence of soaps (26–28). The present investigation was prompted by an observation, by one of the authors (A.S.A.), that the 4-AAP reaction gave strikingly low estimates for hexachlorophene when applied to preparations which contained nonionic surfactants. The rather interesting nature of the interference prompted a detailed investigation and, as will be seen, the results demonstrate that surfactant binding was responsible for the observed effect.

EXPERIMENTAL

Materials—Bovine serum albumin, fraction V, B grade (BSA) was obtained from Calbiochem Laboratories (lot 801274). Polyethylene glycol (PEG) 6000 was purchased from Ruger Chemical Co., Inc. (lot B777). The nonionic surfactants used were: Brij 30SP [polyoxyethylene (4) monolauryl ether], Brij 35SP [polyoxyethylene (23) monolauryl ether], obtained from Atlas Chemical Industries (lots 8406BB and 1340B, respectively), and Pluronic F108 (a polymer containing 20% polyoxypropylene and 80% polyoxyethylene of molecular weight 16000), obtained from Wyandotte Chemicals Corp. (lot D-50056-R). Hexachlorophene was recrystallized from chloroform, m.p. 160.5–162.5. Anhydrous sodium carbonate, potassium ferricyanide, and phenol were purchased from Fisher Scientific Co. 4-Aminoantipyrine, 4-chloro-3-methyl phenol, 4-chloro-3,5-dimethyl phenol, *o*-chlorophenol, *p*-chlorophenol, 2,4,6-trichlorophenol, *m*-hydroxybenzoic acid, and *p*-hydroxybenzoic acid were obtained from Eastman Organic Chemicals. *o*-Cresol, *m*-cresol, *m*-xylenol, 2,4-dichlorophenol, 2,2′-methylene bis-(4-chlorophenol) (dichlorophene), 4,4′-isopropylidene diphenol, and salicylanilide were purchased from Aldrich Chemical Co. With the exception of hexachlorophene, all materials were used as received.

Procedure—Reaction solutions were prepared in glass-stoppered test tubes. Suitable concentrations of phenol and (where appropriate) surfactant or related materials were included in the form of concentrated stock solutions. All aqueous media were buffered

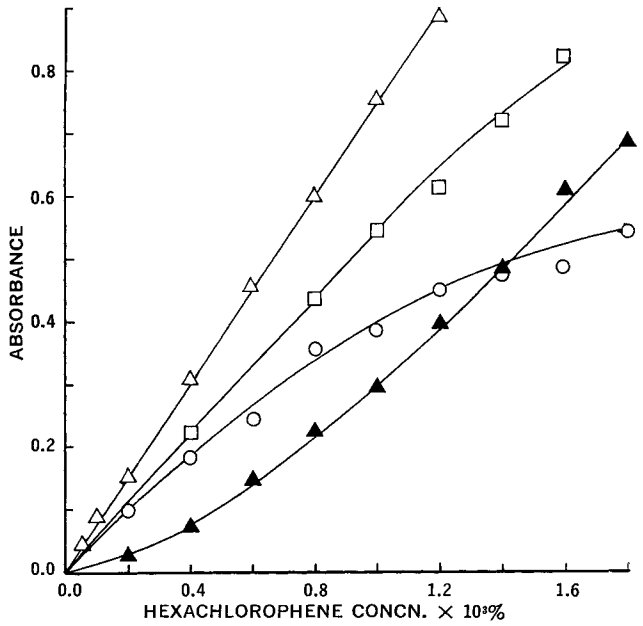


Figure 1—*A plot showing the inhibitory effects of various additives on the coupling reaction between hexachlorophene and 4-aminoantipyrine. Key:* △, *aqueous buffer;* □ *6% PEG 6000;* ▲, *0.02% Brij 35;* ○ *0.1% Pluronic F108.*

with 0.03% anhydrous sodium carbonate and the solution volume was adjusted to 25 ml. with distilled water. The final pH of the solution was 10.4. Stock solutions of potassium ferricyanide (16%) and 4-aminoantipyrine (2%) were prepared in distilled water and stored protected from light. After thorough mixing of the reaction solution 0.25 ml. of 4-AAP solution was added and after further mixing, 0.25 ml. of potassium ferricyanide solution. These reagent volumes were constant throughout the study and calculations for the concentrations of the solution components were based on a final volume of 25 ml. The reagent concentrations used were based on the recommendations of previous authors (7, 8, 21).

Equilibrium Dialysis—Hexachlorophene was dialyzed between aqueous buffer solutions and buffered solutions of Pluronic F108

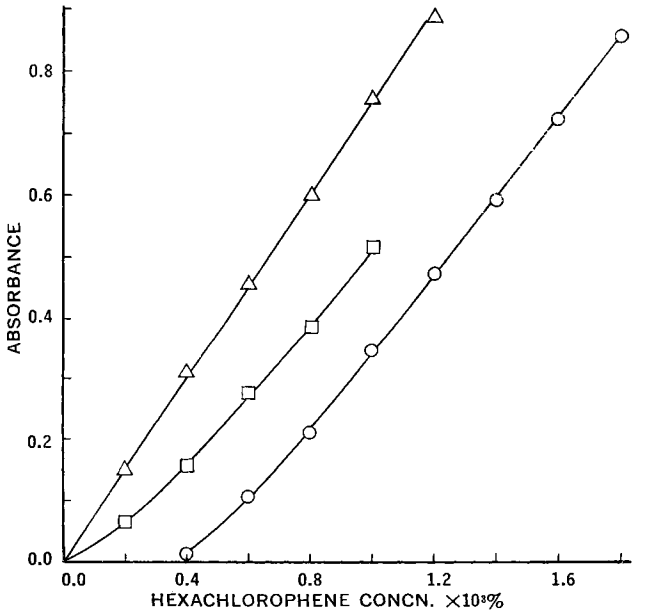


Figure 2—*A plot showing the inhibitory effects of various additives on the coupling reaction between hexachlorophene and 4-aminoantipyrine. Key:* △, *aqueous buffer;* □, *0.003% Brij 30;* ○, *0.02% bovine serum albumin.*

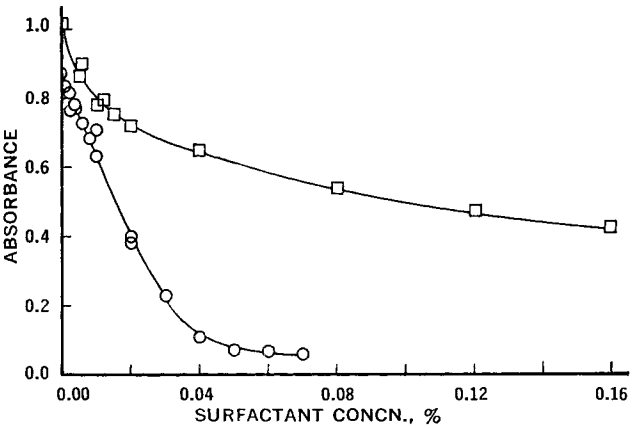


Figure 3—*A plot showing the influence of surfactant concentration on the coupling reaction between hexachlorophene and 4-aminoantipyrine. Key:* □, *Pluronic F108 with 1.6* × 10^{-3}% *hexachlorophene;* ○, *Brij 35 with 1.2* × 10^{-3}% *hexachlorophene.*

at pH 10.4. Dialysis bags were prepared from seamless Visking cellulose tubing (Union Carbide Corp.). The tubing was cut into 22.86-cm. (9-in.) lengths and pretreated by heating at approximately 70° for 4 hr. in several changes of distilled water. After thorough rinsing with distilled water, each length was double-knotted at one end and dried with paper towelling. Twenty-five milliliters of buffer solution containing Pluronic F108 and hexachlorophene was placed in the bags which were then sealed by knotting and partially immersed in 50-ml. volumes of aqueous buffer, contained in 250-ml., covered conical flasks. The flasks were agitated for 12–14 hr. on a reciprocal shaker. Preliminary experiments demonstrated that equilibration occurred within this time period. No apparent leakage of Pluronic F108, from the bags was observed. The bags were removed and the contents collected. Ten-milliliter samples of both surfactant and aqueous solutions were taken and subjected to the dye-forming reaction by treatment with 0.1 ml. of 4-AAP stock solution and 0.1 ml. of potassium ferricyanide stock solution.

The absorbance of each solution was measured on a Beckman DU spectrophotometer equipped with a Gilford photometer and power supply. Spectra of the dyes were measured with a Beckman DB recording spectrophotometer equipped with a Sargent model SR recorder. All spectral measurements were made using an appropriate reference solution.

RESULTS AND DISCUSSION

Figures 1 and 2 summarize the results obtained when various hexachlorophene-containing systems were treated with 4-AAP and potassium ferricyanide. Here absorbance, determined at a wavelength specific for the pyrazolone dye, is plotted as a function of the hexachlorophene concentration for an aqueous sodium carbonate solution and corresponding solutions containing various nonionic surfactants, PEG 6000, and bovine serum albumin. It is apparent from these data that inclusion of colloidal materials in the reaction solution caused a considerable reduction in dye production. Figure 3 illustrates the effect of varying concentrations of Pluronic F108 and Brij 35 on the absorbance produced by fixed concentrations of hexachlorophene. The data of Figs. 1–3 demonstrate marked interference with the dye-forming reaction and indicate that the relative magnitude of the interference was dependent on the nature and concentration of the additive and on hexachlorophene concentration.

It is important, in order to gain an insight into the mechanism of the interference, to note some significant characteristics of the reaction between phenols and 4-aminoantipyrine. The generation of dye in both the presence and absence of additive was extremely rapid with maximum and stable absorbance values being attained in a matter of seconds following the addition of oxidant. The order in which reagents are mixed is an important determinant of the amount of dye which is formed (6, 7, 11). Thus, addition of potassium ferricyanide to solutions of 4-AAP in the absence of phenol, rapidly and irreversibly oxidizes the 4-AAP. No dye is produced with subsequent addition of phenol. Similarly after 4-AAP con-

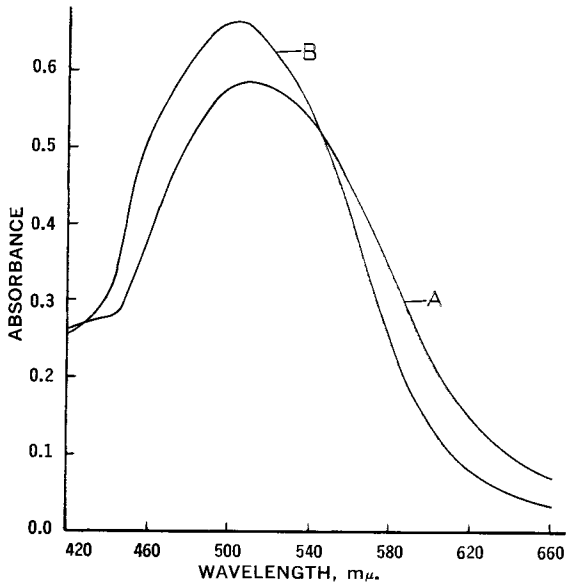


Figure 4—*Spectra of dye formed by the reaction of hexachlorophene with 4-aminoantipyrine. Key:* A, *dye generated in aqueous buffer;* B, *dye generated in aqueous buffer and Pluronic F108 added, to a concentration of 0.8%, after completion of reaction.*

denses with phenol, the excess 4-AAP reagent is rapidly destroyed and no further color formation occurs with further additions of phenol. From a consideration of these facts and the data of Figs. 1–3 it is proposed that binding of hexachlorophene by the interfering materials was responsible for the interference. It is suggested that the bound hexachlorophene possessed negligible or greatly diminished reactivity and that during the dye-forming reactions, the overall rate of release and condensation of bound hexachlorophene was slower than the rate of consumption of 4-AAP by the oxidant. Based on this hypothesis, the concentration of hexachlorophene indicated by the 4-AAP method should approximate that free in the system. It was thought that equilibrium dialysis experiments could conveniently test this possibility and Pluronic F108 was chosen as the surfactant for this purpose primarily because of its molecular size which can be retained by a cellophane membrane.

Prior to conducting dialysis experiments, the influence of surfactant on the spectral characteristics of preformed hexachlorophene-4-AAP dye was investigated. Figure 4 illustrates a typical result by comparing the spectra of dye in the absence and presence of Pluronic F108. It is apparent that the presence of surfactant, incorporated after dye formation, resulted in a hypsochromic shift with concomitant hyperchromism in the spectrum of the dye. These spectral effects are similar to those reported by Becher (29) and Martin and Standing (30) which were encountered in a study of the association of an azo dye, benzopupurin-4-B, with nonionic surfactants. Thus two modes of interference, acting in different directions, are operant in hexachlorophene-surfactant systems exposed to the 4-AAP assay: (*a*) a decrease in the amount of dye generated and (*b*) an increase in the absorptivity, at the $\lambda_{max.}$, of the dye which is formed. Relative to the latter effect, it was found that

Table I—Comparison of the Concentration of Hexachlorophene Involved in Dye Formation, with the Concentrations of Free and Bound Hexachlorophene in Solutions Containing Pluronic F108

Pluronic F108 Concentration, %	Hexachlorophene Concentration, % × 10^3			Fraction of Bound Hexachlorophene Resulting in Dye-Formation
	Bound	Free	Reactive[a]	
0.1	1.68	0.290	0.432	0.085
	2.66	0.390	0.572	0.069
	3.67	0.480	0.720	0.096
	4.18	0.592	0.800	0.049
	4.82	0.662	0.845	0.038
	5.25	0.800	1.000	0.038
	5.97	0.845	1.027	0.035
	6.60	0.920	1.172	0.038
0.2	1.96	0.140	0.410	0.139
	3.06	0.180	0.515	0.109
	4.07	0.230	0.600	0.091
	4.64	0.300	0.640	0.073
	6.92	0.340	0.775	0.063
	7.51	0.401	0.835	0.058

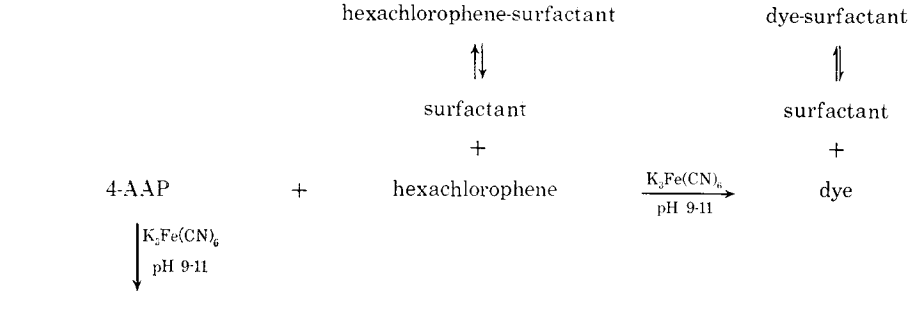
[a] Concentration of hexachlorophene responsible for dye formation in surfactant solution.

Beer's law was obeyed for systems containing a constant concentration of surfactant which was added after dye-formation had occurred.

The results of equilibrium dialysis studies are summarized in Table I. The concentration of free hexachlorophene was determined by assaying the outside, surfactant-free, solution of the dialysis system by the 4-AAP method and interpolating the concentration of hexachlorophene from a standard curve. The concentration of bound hexachlorophene was calculated from a knowledge of the total and free concentrations. The concentration of "reactive" hexachlorophene was determined by assaying the surfactant-containing solution inside the dialysis bag by the 4-AAP method and interpolating the concentration from the appropriate standard curve. It is evident that the concentration of "reactive" hexachlorophene is somewhat greater than the concentration of free species. It is, however, significantly and markedly lower than the total concentration of hexachlorophene on the surfactant side of the membrane. In fact, as shown by the table, the fraction of bound hexachlorophene which resulted in generation of dye was usually less than 0.1. It should be noted that another surfactant, Brij 35, is much more effective in inhibiting dye formation and can, at a sufficiently high concentration, completely inhibit the reaction. It would appear that in the Pluronic F108 system, some bound hexachlorophene was released during the course of the reaction and had the opportunity to condense with 4-AAP in competition with the reaction which resulted in oxidative destruction of 4-AAP.

Based on these observations, Scheme II is suggested to explain the basis of surfactant interference in the determination of hexachlorophene by the 4-AAP method.

A number of other phenols were examined to determine whether or not the extent and nature of the interference by surfactants depended on the structure of the phenolic compound. For each compound, spectra of three differently prepared systems were deter-



Scheme II

Table II—Effect of Brij 35 on Dye Formation and on the Spectral Properties of Dyes Produced by Various Phenols

Phenol	Concentration, g./l. × 10^3	Molecular Weight	Apparent Molar Absorptivity × 10^{-3}			$\lambda_{max.}$, mμ		
			Aqueous Solution[a]	Brij 35 (2%)[b]	Brij 35 (2%)[c]	Aqueous Solution[a]	Brij 35 (2%)[b]	Brij 35 (2%)[c]
Hexachlorophene	8.0	407	29.1	0.00	39.8	507	—	500
Dichlorophene	8.0	269	13.4	0.84	20.7	534	—	506
4,4′-Isopropylidene diphenol	8.0	228	19.7	2.57	21.2	532	—	516
o-Chlorophenol	4.0	129	16.8	23.6	23.6	538	516	516
p-Chlorophenol	4.0	129	11.6	9.19	12.9	531	514	520
2,4-Dichlorophenol	4.0	163	9.38	7.95	14.3	539	515	515
2,4,6-Trichlorophenol	40.0	197	Unstable	0.86	2.66	—	530	530
o-Cresol	4.0	108	9.72	11.5	11.7	528	506	506
m-Cresol	4.0	108	10.8	14.2	14.2	524	498	498
m-Xylenol	12.0	122	5.82	5.59	6.20	590	566	566
p-Chlorometacresol	8.0	143	10.4	11.1	14.1	528	487	486
p-Chlormetaxylenol	16.0	157	6.31	4.61	6.79	587	586	584
m-Hydroxybenzoic acid	4.0	138	8.56	10.7	10.9	553	553	553
p-Hydroxybenzoic acid	4.0	138	15.9	17.6	17.6	532	515	515
Salicylamide	4.0	213	18.7	30.5	32.5	544	536	536
Phenol	3.2	94	13.5	14.8	14.8	530	518	514

[a] No surfactant added. [b] Brij 35 included in the reaction mixture. [c] Brij 35 added to a solution of the preformed dye.

mined and compared; *A*, the dye was generated in aqueous carbonate buffer; *B*, the dye was generated in aqueous carbonate buffer containing 2% Brij 35; and *C*, the dye was generated in aqueous carbonate buffer to which was added, after dye formation, sufficient Brij 35 to yield a 2% solution. Thus, comparison of Spectra *A* and *C* provides a measure of the influence of surfactant on the spectral characteristics of the dye, while a comparison of Spectra *B* and *C* provides an indication as to whether surfactant influenced the reactivity of the phenolic compound in the condensation reaction.

Typical results are represented by spectra for *o*-chlorophenol and *p*-chlorophenol which are shown in Fig. 5. It is seen that the *B* and *C* systems for the *ortho* compound yielded coincident spectra demonstrating that inclusion of surfactant in the reaction solution caused no inhibition of dye formation. A comparison of these spectra with those of System *A*, however, shows that the presence of surfactant caused a hypsochromic shift with concomitant marked hyperchromism. Spectra for the *para* compound show a somewhat different pattern. Neither the shift nor the hyperchromism was as marked. There were, however, large differences in the relative absorbances produced by Systems *B* and *C* indicating that some inhibition of dye formation occurred. Table II summarizes spectral data obtained, in the previously described manner, for a variety of phenols. It is interesting to note that while the presence of surfactant affected, to a greater or lesser degree, the spectral characteristics of dyes formed from all of the phenols, reactivity in the condensation reaction was reduced in only a few instances. This may be interpreted to mean that some phenols are less strongly bound to surfactant than others and that the rate of release from surfactant association is faster. Alternatively, the differences can be explained on the basis of intrinsic reactivity in the dye-forming reaction, *i.e.*, some phenols react so rapidly with 4-AAP that a slowing of reaction rate due to a reduction in reactant concentration does not result in a detectable reduction in the yield of dye. However, with others the rate of reaction is somewhat slower so that a decrease in readily available reactant slows the reaction to such an extent that dye-formation no longer competes as effectively with the oxidative side reaction for 4-AAP. The data of Table II offer some support to the latter possibility in that those phenols which produced decreased yields of dye in the presence of surfactant are substituted in the position *para* to the hydroxyl group. Emerson and Beegle (3) proposed that *para* substituents must be expelled in order for condensation to occur and it seems likely, therefore, that the intrinsic reactivity of phenolic compounds in the dye-forming reaction depends on the ease of expulsion of the *p*-substituent.[1] Thus, Brij 35 inhibited dye formation with all of the *p*-chloro compounds but not with phenols which were unsubstituted in the *para* position. It is notable that surfactant inhibition was not detected with *p*-hydroxybenzoic acid and suggests, perhaps, that in this reaction mechanism the carboxyl group is a more effective leaving group than chlorine.

Previous authors (31) have studied the effect of the nonionic surfactant Triton X100 [polyoxyethylene(9-5)diisobutylphenyl ehter] on the kinetics of dye formation from the oxidative coupling of 4-chloro-1-naphthol with 3-methyl-4-amino-*N*,*N*-diethylaniline. In this system the coupling reaction also involved elimination of the *p*-chloro group from the coupler (32). It was found that 2.5% of the surfactant caused a two-fold reduction in the rate constant for the reaction. In this system, however, dye formation was not antagonized significantly by a competitive side reaction.

The marked increase in molar absorptivity of dye caused by the presence of surfactant is of interest and suggests that, for some compounds, incorporation of surfactant in the reaction mixture

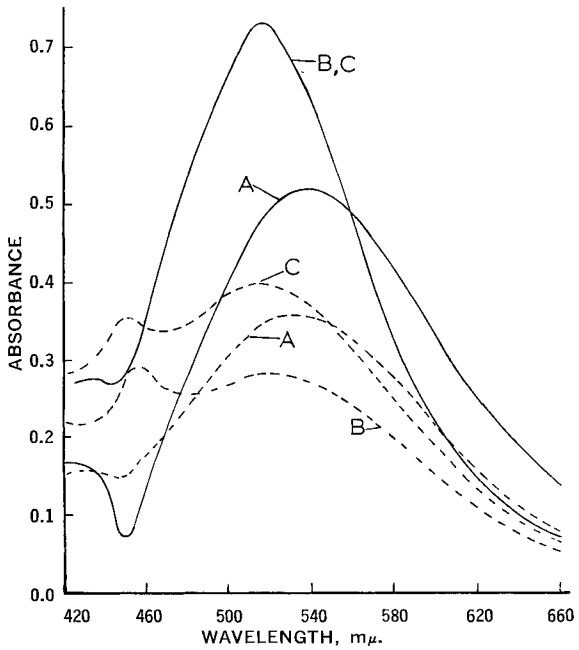


Figure 5—*Spectra of dyes formed by the reaction of 4-aminoantipyrine with* o-*chlorophenol* ——, *and* p-*chlorophenol* - - -. *Key:* A, *dye formed in aqueous buffer;* B, *dye formed in aqueous buffer containing 2% Brij 35;* C, *dye formed in aqueous buffer and Brij 35 added, to a concentration of 2%, after completion of reaction.*

[1] The reviewer kindly brought to the authors' attention the publication of K. H. Muller, B. Christ, and M. Schneider, *Arch. Pharm.*, **293**, 567(1960). These authors presented evidence to indicate that with *p*-substituted phenols, dye formation can occur by *o*-condensation. This suggests an alternative explanation of the observed differences: that dye-formation involving *o*-condensation normally proceeds at a slower rate than that involving *p*-condensation.

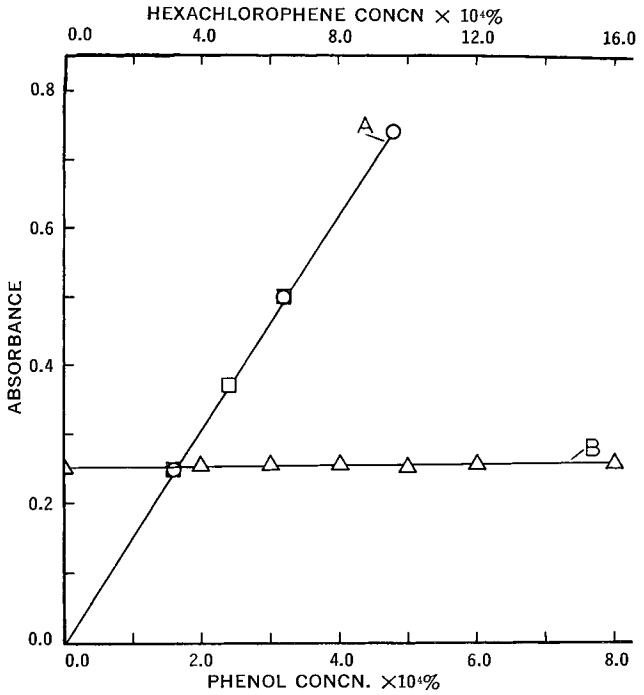


Figure 6—*A plot illustrating the basis for a differential assay for phenol and hexachlorophene. Key:* A, *a Beer's law plot for phenol in the presence of 2% Brij 35 and 4 × 10⁻⁴% hexachlorophene* (○) *and 8 × 10⁻⁴% hexachlorophene* (□); B, *the phenol concentration was maintained constant at 0.16 × 10⁻⁴% and the hexachlorophene concentration was varied as indicated by the upper abscissa values.*

might be usefully employed to increase the sensitivity of assays using the 4-AAP method. For example the addition of 2% Brij 35 to dye produced by salicylamide (Table II) causes an apparent increase of 74% in molar absorptivity. This almost doubles the assay sensitivity.

It may be seen from Table II that while the reaction of hexachlorophene was completely quenched by the presence of Brij 30, the reactivity of phenol was not affected by the surfactant. This suggests the possibility of using this system to estimate phenol in the presence of hexachlorophene. Preliminary studies to this end are summarized in Fig. 6 where absorbance data for phenol in phenol-hexachlorophene systems are plotted. Curve A represents a Beer's law plot for phenol in the presence of 2% Brij 35 and two concentrations of hexachlorophene. The linearity of this plot suggests that the absorbance due to phenol was not influenced by the hexachlorophene. Curve B represents the absorbances of solutions containing a single small concentration of phenol and a wide range of hexachlorophene concentration. These data indicate that dye formation was due only to the concentration of phenol and that hexachlorophene made a negligible contribution to the absorbance. If the absorbance produced by reacting a mixture of the two phenols is determined first in the presence of surfactant, added after dye generation, and then in solutions in which the surfactant was included in the reaction mixture it may be possible to estimate both substances simultaneously. The absorbance value of the first system represents the sum of contributions by each compound. The second value represents phenol only, while the difference between the two measurements is directly related to the hexachlorophene concentration. The concentration of each component may be obtained by reference to the appropriate Beer's law plot.

Table III compares results for such determinations. In general good recoveries were obtained for phenol. However, the recoveries for hexachlorophene tended to be low. The low recoveries of hexachlorophene may have resulted from non-adherence of the systems to Beer's law because of the relatively high total phenol concentrations encountered in the study.

In spite of the fact that the extent to which surfactants interfere with the 4-AAP reaction is not necessarily a quantitative measure of binding behavior, some qualitative insights concerning the binding of hexachlorophene can be interpolated from Figs. 1 and 2.

Table III—Estimation of Mixtures of Phenol and Hexachlorophene by the 4-AAP Reaction Using 2% Brij 35

Phenol Concentration, % × 10³		Hexachlorophene Concentration, % × 10³	
Added	Recovered	Added	Recovered
0.160	0.158	0.200	0.200
0.160	0.160	0.800	0.740
0.160	0.160	0.200	0.198
0.160	0.160	0.400	0.385
0.160	0.160	0.600	0.565
0.080	0.085	0.100	0.100
0.320	0.325	0.200	0.190
0.080	0.087	0.400	0.387
0.320	0.325	0.400	0.350
0.800	0.087	0.600	0.525

It is apparent, for example, that bovine serum albumin (BSA) strongly binds hexachlorophene since no dye-formation occurred until a critical concentration of hexachlorophene was present in the system. The shape of the BSA curve strongly suggests that this concentration was almost sufficient to saturate binding sites on the protein. PEG 6000 was found to be a relatively weak inhibitor and indicates that interaction between the phenol and polyoxyethylene chains was not primarily responsible for the binding indicated by this experimental method. Micellar solubilization is probably the mechanisms giving rise to the marked effects observed with Brij 30, Brij 35, and Pluronic F108. The former compounds were significantly more potent in their inhibitory action than the latter and might reflect a lower CMC and/or the generation of micelles better able to incorporate the hexachlorophene. It is interesting to note that the curves characterizing the behavior of the Brij compounds indicate saturable binding whereas those for PEG 6000 and Pluronic F108 indicate a cooperative effect where initial binding appears to promote subsequent binding.

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New 1,2,4(H)-Triazole Derivatives as Diuretic Agents

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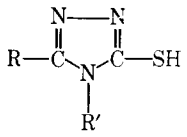
Abstract □ Sixty-three new 1,2,4(H)-triazole derivatives have been prepared and their diuretic activity studied in rats. Sequential screening showed 14 compounds possessing significant diuretic activity. 3-Phenyl-4-allyl-5-mercapto-1,2,4(H)-triazole and 3-*o*-chlorophenyl-4-allyl-5-mercapto-1,2,4(H)-triazole were the most potent compounds in the present series.

Keyphrases □ Diuretic activity—1,2,4(H)-triazole derivatives □ Mercapto-triazoles—synthesis □ Structure-activity relationship—triazole rings

In a previous communication (1), the authors reported the diuretic activity of some 1,2,4(H)-triazoles (I). Recently, Yale and Piala (2) have also reported the diuretic properties of some *s*-triazole derivatives amongst which 3-(*p*-aminophenyl)-*s*-triazole-5-thiol (I, R = *p*-NH_2-C_6H_4 and R′ = H) has been claimed to possess good diuretic activity. In view of these interesting results the work has now been extended to some more 3,4-disubstituted-5-mercapto-1,2,4(H)-triazoles.



I

The mercapto-triazoles were synthesized from the corresponding thiosemicarbazides by cyclization with sodium hydroxide or sodium carbonate. Some triazole derivatives were obtained directly in one step from acid hydrazides and the isothiocyanates by heating in excess alkali. When this reaction was carried out at room temperature, it proceeded only as far as the formation of the 1,4-disubstituted thiosemicarbazides.

The list of triazoles prepared, their melting points, yields, analytical data, and diuretic activity are given in Table I.

The requisite thiosemicarbazides were obtained by the reaction of acid hydrazides and isothiocyanates by literature methods. The new thiosemicarbazides are listed in Table II along with their melting points and analytical data.

Since many sulfamoyl compounds are being used clinically as potent diuretics, the conversion of some of the 5-mercapto-1,2,4(H)-triazoles into the corresponding 5-sulfamoyl derivatives was attempted by the usual oxidative chlorination followed by the action of ammonia (3, 4). The 5-sulfamoyl derivatives were obtained in two cases while in some other instances the desired compounds could not be isolated due to extensive decomposition. Moreover, the two sulfamoyl derivatives thus obtained showed activity of lower order than the parent mercapto compounds, *cf.* Yale and Piala (2), hence the preparation of other sulfamoyl derivatives was not pursued.

PHARMACOLOGY

All the 3,4-disubstituted-5-mercapto-1,2,4-triazoles were screened for the diuretic properties in rats at their optimal responsive dose levels by the sequential method of Modi *et al.* (5).

Method—Albino rats (male) weighing about 180–200 g. were taken in groups of four in each cage per test dose. Prior to the experiment the rats were allowed food and water *ad libitum*. During the experiment each group of four animals was housed in an improved metabolism cage described by Modi *et al* (6). One group was used as untreated control and received orally the vehicle only, consisting of 0.5 ml. of 2% starch solution. Another group received hydrochlorothiazide (2.5 mg./kg.) as reference compound, suspended in the vehicle. The other groups received the various test compounds in the same vehicle. The urine was collected for 24 hr. If the total volume of urine in Cage I exceeded 19.3 ml., the compound was considered active and if below 3.7 ml., inactive. However, if the volume was in between these two values, a further evaluation with another cage of four rats was made. If the total volume of urine in Cages I plus II exceeded 30.8 ml. the compound was considered active but if less than 15.2 ml. it was considered inactive. In case the volume was again between the two limits a third cage was taken and similarly a fourth one if necessary as per criteria in Table III.

The compounds that did not meet activity criteria in the fourth cage experiment were given up as not sufficiently active.

Among the compounds with acceptable activity, those which produced urinary volumes more than 125% of controls were selected