Investigation of the Chemistry of a Diazo Micromixing Test Reaction

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The azo coupling of 1-naphthol with diazotized sulfanilic acid has been studied in detail focusing on the practical use of this reaction as a micromixing test reaction, as developed by Bourne and coworkers. The reaction is a fast, competitive, consecutive reaction whose final product distribution is affected greatly by mixing. Problems that occur in the isolation of the pure-dye products and quantification of the product distribution are addressed. Previously unreported information is given about the structure and properties of one of the products as well as the existence of an additional unknown product. The reaction was used to characterize the spatial heterogeneity of micromixing in a 14-L stirred-tank fermenter. Results show large differences in the product distribution dependent on the depth and radial position of the feed pipe in the tank.

Introduction

It has long been recognized (Danckwerts, 1958; Zwietering, 1959) that for nonfirst-order reaction kinetics the residence time distribution of a reactor is insufficient information to predict conversion. In such cases, it is necessary to consider the degree and intensity of segregation, defined by Danckwerts, which the molecules experience while undergoing chemical reaction. Ultimately, molecules are mixed by small-scale turbulent eddies whose size and velocity depend on the local rate of energy input. Consideration of mixing at the scale of the smallest turbulent eddies is usually termed micromixing. Micromixing becomes important particularly in the case of multiple simultaneous reactions where it can affect the selectivity of a desired product. Some general requirements for a reaction to display mixing sensitivity have been outlined by Bourne et al. (1977) and David and Villermaux (1987).

Mixing effects are also seen in bioreactors, in which microorganisms are grown (Dunlop and Ye, 1990; Toma et al., 1991). For the purpose of oxygen transfer, these reactors are often highly agitated, which may lead to the assumption of "perfect" mixing in the design of bioreactors. However, changes in micro-organism productivity as a function of substrate injection location (Hansford and Humphrey, 1966), stirrer speed (Toma et al., 1991), or grid generated turbulence (Fowler and Dunlop, 1989) suggest that the mixing is often less than perfect. Since micro-organisms are of the same length scale or smaller than the smallest turbulent eddies in a fermenter (Dunlop and Ye, 1990), micromixing concepts may be able to explain these effects. A micromixing test reaction, described below, is being used in the present study to measure micromixing in biochemical reactor systems.

Although much work has been done to model the process of micromixing (see, for example, Baldyga and Rohani, 1987; David and Villermaux, 1987; Patterson, 1981), there still exists no way to predict its effects for an arbitrary reactor design and reaction kinetics. Micromixing effects, however, can be measured through the use of reactive tracers. These tracers are well-defined chemical reactions that display micromixing sensitivity. Through the use of reactive tracers, reactor designs can be evaluated with respect to their ability to efficiently micromix chemically reacting species, rather than simply create an "ideal" residence time distribution.

The azo coupling of 1-naphthol with diazotized sulfanilic acid has been developed by Bourne and coworkers as a micromixing test reaction. This reaction is usually described by the scheme:

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$$A + B \xrightarrow{k_1} R \tag{1}$$

$$R + B \xrightarrow{k_2} S \tag{2}$$

where $k_1/k_2 \approx 2{,}500$. Reaction 1 is essentially instantaneous, so that when A and B are reacted with B as the limiting reagent, product R should predominate. Deviations from perfect mixing cause the newly formed R molecules to remain in contact with a high concentration of B molecules, so that product S is formed before more A molecules can mix with the B molecules. Imperfect mixing therefore leads to a relatively high concentration of S in the bulk solution. A convenient index of micromixing intensity has been described by:

$$X_S = \frac{2C_S}{2C_S + C_R} \tag{3}$$

which is the yield of S based on B. As a micromixing index, X_S gives numerical values that are inversely proportional to micromixing intensity. The products of reaction R and S are dyes and can be quantified spectrophotometrically.

Since its inception as a micromixing test reaction (Angst et al., 1979; Bourne et al., 1981), the reaction has been used to measure the degree of micromixing in various reactor geometries. The influence of power input, impeller design (Angst et al., 1979), and feed position (Bourne and Hilber, 1990; Rice and Baud, 1990) has been studied in stirred-tank reactors ranging from laboratory scale to production scale (Angst et al., 1979). Mixing in tubular reactors (Bourne and Tovstiga, 1988), rotor-stator mixers (Bourne and Garcia-Rosas, 1986), and centrifugal pumps (Bolzern and Bourne, 1985) have all been studied; the effects on micromixing of gas bubble dispersion have also been measured using the reaction (Ye, 1984). Dunlop and Ye (1990) have used the reaction to correlate mixing variables with microbial growth behavior in bioreactors.

The kinetics and products of the reaction have been studied extensively by Bourne and coworkers (1985, 1990). Recently, Bourne et al. (1990) have reported that a second isomer of monazo dye R, the ortho form, is produced in significant amounts. Thus, according to Bourne, the reaction scheme must be modified to the series parallel scheme (Figure 1):

$$A + B \rightarrow p-R,\quad o-R \tag{4}$$

$$p-R,\quad o-R + B \rightarrow S \tag{5}$$

According to Bourne et al. (1990), the definition of X_S must thus be extended to include this additional product:

$$X_S = \frac{2C_S}{2C_S + C_{p-R} + C_{o-R}} \tag{6}$$

This definition will be retained through the remainder of this article, although its relationship to the two-component definition will be examined.

The problems associated with this micromixing test reaction are as follows:

- Published reference spectra for bisazo dye S have shown substantial variations among researchers, both in shape and magnitude. This information is necessary for accurate quantification of S in the final product mixture.
- While the reaction scheme in Eqs. 1 and 2 is usually assumed, two other colored products of this reaction have been observed (Ye, 1984; Bourne et al., 1985). One of these was identified as the ortho coupled isomer of R by Bourne et al. (1990). The other was observed by Bourne et al. (1985) as the product of a side reaction and concluded that it is the degradation of bisazo dye S by excess diazonium ions. The importance, or lack thereof, that these products have in micromixing experiments has yet to be fully demonstrated.
- Material balances on the final product mixture have been found by several workers to average 96-97% closure (Ye, 1984; Rice and Baud, 1990). While at first sight this seems adequate, the error indicated by this "unaccounted for" material is substantial compared to the 1-10% of bisazo dye S typically found in the product mixture.

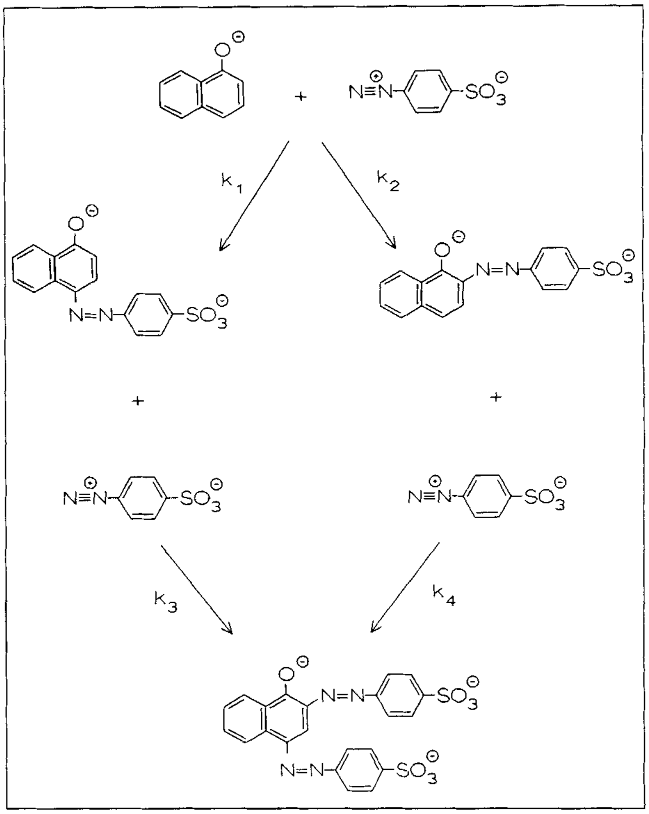

Figure 1. Azo coupling of 1-naphthol with diazotized sulfanilic acid.

Background

Extinction coefficients of bisazo dye S

The product distribution X_S is measured using a spectrophotometer. Therefore, to accurately determine the concentration of each dye in the sample, the molar extinction coefficients of the pure dyes must be known. Generally, such a determination involves isolating a pure sample of the dye, preparing a solution of known concentration and measuring its absorbance spectrum. For $o-R$ and $p-R$, this approach has presented little problem. For S, however, problems typi-

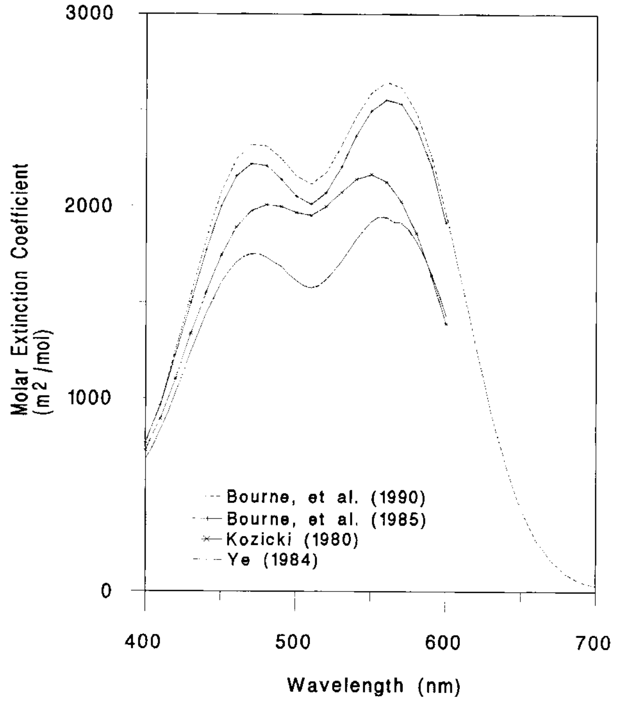

Figure 2. Previously reported visible spectra for bisazo dye *S*.

cally arise in isolating, purifying and quantifying a sample. Hence, literature values for *S* extinction coefficients are highly varied. This impedes any attempts to accurately determine the amount of *S* in a given sample.

Some published extinction coefficient spectra for *S* are given in Figure 2. A brief review of the methods used to determine these spectra is useful:

Kozicki (*1981*). This spectrum was obtained by coupling one mole of diazotized sulfanilic acid with one mole of $p-R$. A solid product was obtained by precipitation, dried and weighed with no purification steps.

Ye (*1984*). This sample was prepared as above, but the dried product was washed with 1/1 v/v ethanol/water solution 12 times until paper chromatography showed only one spot.

Bourne et al. (*1985*). This sample was also prepared by $p-R$ and diazotized sulfanilic acid coupling. The crude product mixture was separated by thin-layer chromatography, and a small amount of *S* was recovered from the plate. It was quantified by titration with titanium (III) chloride, which reduces the azo groups.

Bourne et al. (*1990*). This sample was prepared by coupling one mole of $o-R$ with less than one mole of diazotized sulfanilic acid. By assuming 100% yield and subtracting out the spectrum of the residual $o-R$, the spectrum of *S* was obtained. It should also be noted that these latest extinction coefficients are reported at an ionic strength of 444 mol/L, 11.1 times higher than the conditions of Bourne et al. (1985). The effect of ionic strength on extinction coefficients has been described as significant (Bourne et al., 1990), but not quantified.

The differences in these spectra have a significant effect on the analysis of a product mixture. Furthermore, they are disturbing since the chemistry of a micromixing test reaction should be unequivocally understood. It was therefore our approach to attempt to isolate a pure, crystalline sample of *S*, of which the structure and molecular weight could be confirmed.

Table 1. Index of Purity

Reference	*Ip*
Bourne (1990)	1.25
Bourne (1985)	1.27
Kozicki (1980)	1.08
Ye (1984)	1.23

Index of Purity. It was observed that most of the published visible spectra of *S* had essentially the same saddle shape, but differed in magnitude. It was also assumed that differences in shape corresponded to impurities in the sample. Since the other known products have absorption maxima in the region near 510 nm, where *S* has an absorption minimum, impurities tend to flatten the saddle shape. Therefore, a convenient way to compare the shapes of the spectra is to define an index of purity I_p where

$$I_p = \frac{D_{560}}{D_{510}} \qquad (7)$$

Using this definition, published spectra for *S* were compared, as shown in Table 1.

Experimental Results and Discussion

Isolation of the dyes

When this investigation was started, it was assumed that the reaction was adequately described by the scheme in Eqs. 1 and 2. Subsequently, Bourne et al. (1990) reported that substantial amounts of $o-R$ are formed and recommended the reaction scheme be modified to that described by Eqs. 4 and 5. To confirm the importance of these two isomers and include them separately in the analysis, they were synthesized and their extinction coefficients were determined along with those of *S*.

Diazotization of Sulfanilic Acid. Sulfanilic acid (Aldrich 25, 191-7) was diazotized by following the procedure of Kozicki (1980). The acid was first dissolved in an aqueous sodium carbonate solution. The solution was cooled to 15°C and sodium nitrite was added, followed by a mixture of hydrochloric acid and ice. The diazo salt, seen as a white precipitate, is stable for several hours when kept in slurry form in an ice bath. (The precipitate should not be isolated as it is an explosion hazard when dried.) Urea was added to the solution to destroy excess nitrite ions.

Preparation of $p-R$. This monazo dye was prepared by coupling 1-naphthol(Aldrich N199-2) with equimolar amounts of diazotized sulfanilic acid in aqueous ethanol solution. It was recrystallized three times from aqueous ethanol, at which point paper chromatography showed only one spot (Whatman 3MM chromatography paper; 30:47:3:20 volume basis mixture of *n*-butyl acetate, pyridine, conc. NH_4OH, H_2O). The dye was isolated as the free acid having a theoretical molecular weight of 328.1.

Preparation of $o-R$. This isomer of *R* was prepared from 1,2-naphthoquinone using the method of Bourne et al. (1990). It was recrystallized from warm water four times until paper

Table 2. Elemental Analyses ($o-R$, $p-R$)

	Theoretical	Found
$o-R$, Sodium Salt		
%C	54.84	54.89
%H	3.14	3.24
%N	8.00	7.96
$p-R$, Free Acid		
%C	58.52	58.55
%H	3.66	3.71
%N	8.53	8.49

chromatography showed only one spot. $o-R$ is isolated as the sodium salt having a theoretical molecular weight of 350.1.

Preparation of Crude S. As described earlier, isolation of this bisazo compound has proven difficult. The general approach taken was that of Kozicki (1980), reacting equimolar quantities of $p-R$ and diazotized sulfanilic acid. The reaction was carried out under a nitrogen atmosphere in an ethanol/water solution using sodium acetate as a buffer.

Previous attempts (Kozicki, 1981; Ye, 1984; Bourne et al., 1985) at purifying a similarly obtained product by recrystallization from ethanol solutions were unsuccessful. This was also the case here. After washing the product with 95% ethanol 12 times, paper chromatography still indicated impurities by showing two spots, one purple and one orange. The product, however, was thought to be reasonably pure and was submitted for elemental analysis.

A visible spectrum of this product was measured and was found to have an index of purity $I_p = 1.18$.

Elemental analysis

The three dyes prepared above were sent for elemental analysis (MHW Laboratories, PO Box 15149, Phoenix AZ 85018). Results for $o-R$ and $p-R$ are given in Table 2.

The results for S did not have such good agreement with theoretical values. Table 3 shows results obtained in two analyses of S, along with values obtained by Kozicki (1980). Theoretical values for S correspond to the free acid structure.

The most notable facet of the data is the significantly low values for % C and % N obtained both by us and by Kozicki. It is especially important to note that $o-R$ or $p-R$ impurities in the sample would have increased, rather than decreased, the apparent % C.

Also important is the presence of sodium in the sample. Previous researchers (Kozicki, 1980; Ye, 1984) assumed that S is isolated as the free acid. The data indicate that it is isolated as a sodium salt.

If it is assumed that our S molecule has 22 carbons, the data from sample 2 can be reduced to the molecular formula $C_{22}H_{18.1}N_{3.56}S_{1.83}O_{10.4}Na_{0.99}$, or nominally, $C_{22}H_{18}N_4S_2O_{10}Na$ (MW = 585.5). A monosodium salt of S would have the molecular formula $C_{22}H_{15}N_4S_2O_7Na$ (MW = 534.4); thus, this calculation implies that there are three extra OH groups per molecule of S in sample 2. Although it did not fit the above analysis exactly, we examined the simpler case and hypothesized that water in the sample may have been the cause of the extra oxygen and hydrogen. Dyes containing sulfonic groups are known to be very hygroscopic and difficult to dry (Venkataraman, 1952). This hypothesis was checked by mass spectroscopy and Karl Fischer titration.

Table 3. Elemental Analyses (S)

	Theoretical	Kozicki (1980)	Sample 1	Sample 2
%C	51.54	42.9	42.68	45.5
%H	3.12	2.81	3.43	3.12
%N	10.93	8.65	8.25	8.60
%S	9.78	—	—	10.1
%Na	0.0	—	—	3.91
%O	21.87	—	—	28.7

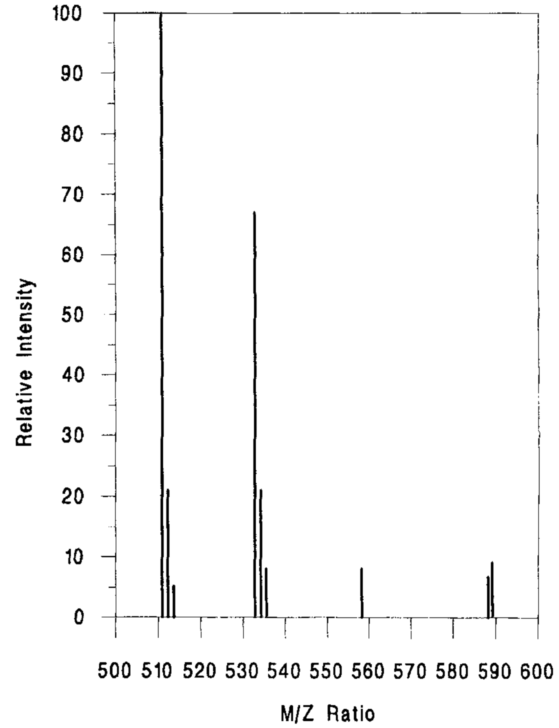

Figure 3. Fast atom bombardment mass spectrum of crude S sample.

Mass spectroscopy

A sample of crude S was submitted for fast atom bombardment mass spectrometry (Midwest Center for Mass Spectrometry, Univ. of Nebraska-Lincoln, Lincoln, NE 68588-0362). The results are shown in Figure 3. Molecular ion peaks were found at 511 and 533 m/z, confirming a monosodium salt structure for S. Peaks were also found at 558, 588 and 589 m/z, indicating the presence of higher molecular weight species. A free acid and a monosodium salt of S with three associated water molecules would have apparent molecular weights of 566 and 588, respectively. These data suggest that the sample may have contained S molecules strongly associated with three molecules of water.

Karl Fischer titration

A sample of S, obtained by the procedure described earlier, had been dried in a 50°C oven and was analyzed by Karl Fischer titration. The sample, which had been stored in equilibrium with ambient air, was found to contain 10.5% water (by weight)

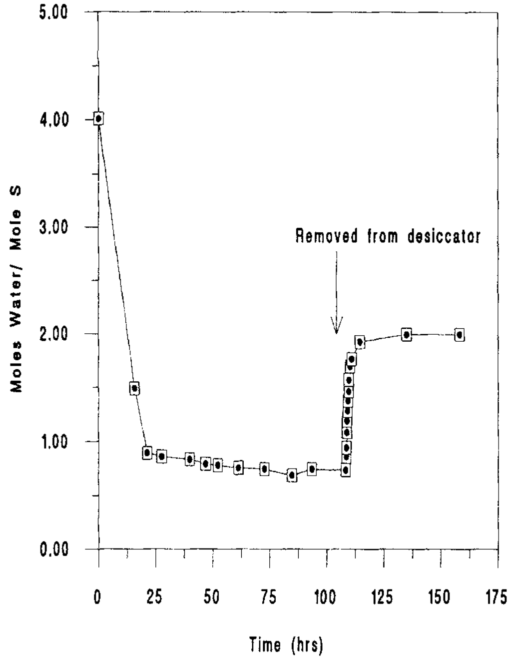

Figure 4. Water desorption/adsorption from a crystalline sample of bisazo dye *S*.

or 3.5 moles of water per mole of monosodium salt *S*. A different sample, stored in a desiccator over $CaSO_4$ (Drierite), was found to contain 1.6 moles of water per mole of *S*. Similarly, dried samples of $p-R$ and $o-R$ contained 0.038 and 0.34 moles of water per mole of dye, respectively.

The mass of another sample of oven-dried, air-equilibrated *S* was followed as a function of time, while being stored under vacuum over Drierite. The results are shown in Figure 4, where the sample, originally containing 4.1 moles of water per mole of *S*, lost 9.5% of its mass before reaching a constant mass of 100 h; 96% of this weight loss, however, occurred within the first 24 hours.

After 100 hours, the above sample was allowed to come back into equilibrium with ambient air. The results are also plotted in Figure 4. The sample regained 61.4% of the moisture lost, with most of this occurring within the first six hours of equilibration.

Another sample of *S*, dried for several days under vacuum over Drierite, was analyzed by Karl Fischer titration and was still found to contain 0.823 moles of water per mole (2.7% water). This was assumed to be the maximum dryness of *S* that could be achieved in a vacuum desiccator.

These results suggest that crystalline samples of *S* are extremely hygroscopic and rapidly come into equilibrium with substantial amounts of water. This naturally affects any attempts to determine molar extinction coefficients from such samples. It appears that the difficulties in obtaining spectra of consistent magnitude (rather than shape) have originated from this hygroscopic property. In fact, Bourne et al. (1990) speculated that differences in extinction coefficients were due to inaccuracies in isolating and weighing purified solid *S*.

Table 4. Extinction Coefficients for the Three Dyes*

Wavelength (nm)	$p-R$ (m^2/mol)	$o-R$ (m^2/mol)	S (m^2/mol)
700	0.15	0.92	20.2
695	0.20	0.85	25.9
690	0.05	1.00	37.8
685	0.20	1.18	51.9
680	0.22	1.21	67.6
675	0.35	1.13	89.7
670	0.49	0.90	122
665	0.67	0.87	161
660	0.86	0.85	211
655	1.06	0.90	272
650	1.66	1.41	348
645	2.37	2.03	434
640	3.46	2.95	533
635	5.06	4.36	648
630	7.73	6.46	776
625	12.0	9.69	918
620	19.0	14.5	1,070
615	30.3	22.1	1,230
610	48.7	33.4	1,390
605	77.8	49.9	1,540
600	122	74.1	1,690
595	215	120	1,830
590	313	173	1,950
585	443	253	2,070
580	614	357	2,160
575	819	495	2,240
570	1,050	655	2,290
565	1,300	830	2,310
560	1,570	1,020	2,320
555	1,840	1,230	2,310
550	2,120	1,430	2,280
545	2,380	1,630	2,240
540	2,610	1,810	2,180
535	2,820	1,980	2,120
530	2,980	2,120	2,050
525	3,090	2,230	1,990
520	3,160	2,320	1,940
515	3,190	2,370	1,900
510	3,170	2,380	1,880
505	3,100	2,360	1,890
500	3,000	2,310	1,920
495	2,860	2,230	1,950
490	2,710	2,130	1,990
485	2,540	2,010	2,030
480	2,360	1,880	2,050
475	2,190	1,740	2,070
470	2,010	1,610	2,060
465	1,860	1,470	2,040
460	1,710	1,350	2,010
455	1,570	1,230	1,940
450	1,440	1,120	1,860
445	1,340	1,010	1,770
440	1,230	914	1,660
435	1,120	824	1,540
430	1,020	740	1,420
425	916	664	1,290
420	807	595	1,170
415	703	553	1,040
410	608	480	933
405	523	437	830
400	441	407	749

*$T = 25°C$; $I = 0.0440$ mol/L; pH = 10.0

Purification of crude S

To obtain material for extinction coefficient determination,

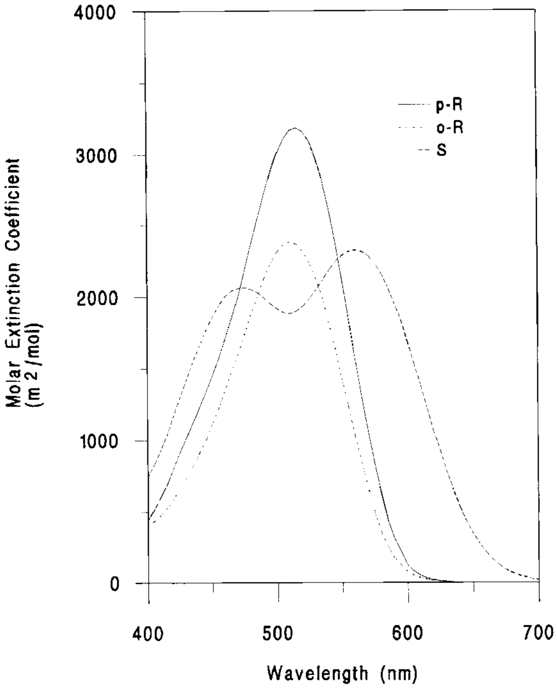

Figure 5. Molar extinction coefficients for the three dyes.

$T = 25°C$; $I = 0.0440$ mol/L; pH = 10.0.

the crude S described earlier was purified in two ways: by thin layer chromatography and by recrystallization from methanol.

Thin-Layer Chromatography. A solution of crude S in methanol was spotted on to TLC plates (Merck, silica gel 60, F_{254}) and developed in a 1.8/1 (v/v) mixture of ethyl acetate and methanol. S was seen as a purple spot ($R_f = 0.325$) together with an unknown orange spot ($R_f = 0.240$). $p-R$ ($R_f = 0.506$) and $o-R$ ($R_f = 0.41$) were not detected in the mixture.

A small quantity of the S was isolated from the plate, but could not be quantified. Its visible spectrum was measured and gave an $I_p = 1.27$.

Crystallization. A sample of crude S (0.1896 g) was dissolved in 35 mL of boiling MeOH. This solution was kept in an ice bath overnight. A small amount of crystals were formed (7.18 mg). These crystals showed only one spot on a TLC plate and had an $I_p = 1.24$. The crystals were dried for 36 hours in a vacuum desiccator over Drierite before being weighed. It was assumed that, like the crude S, the purified S came into equilibrium with the Drierite at a water content of 2.7%.

Extinction coefficients for dyes

Molar extinction coefficients for all three dyes were determined, as shown in Table 4 and Figure 5 from 400 to 700 nm. For $p-R$, the reported data from 400 to 595 nm are an average of five solutions ranging in concentration from $3.67(10^{-6})$ to $4.59(10^{-5})$ mol/L, and the data from 600 to 700 nm are from a solution concentration of $4.05(10^{-4})$ mol/L. For $o-R$, the data from 400 to 595 nm are an average of five solutions ranging in concentration from $7.10(10^{-6})$ to $8.52(10^{-5})$ mol/L and the data from 600 to 700 nm are from a solution concentration of $3.90(10^{-4})$ mol/L. The extinction coefficients are compared to those reported by Bourne et al. (1990) in Figure 6. For S, the data are the result of a single measurement, using a molecular weight of 534.4, corrected to account for 2.7% water in the sample. Figure 7 compares the extinction coefficients determined for S to those reported by Bourne et al. (1990). The reason for the discrepancy in these results is unknown. In any case, calculated X_s values are about 10% lower when the extinction coefficients of Bourne et al. (1990) are used.

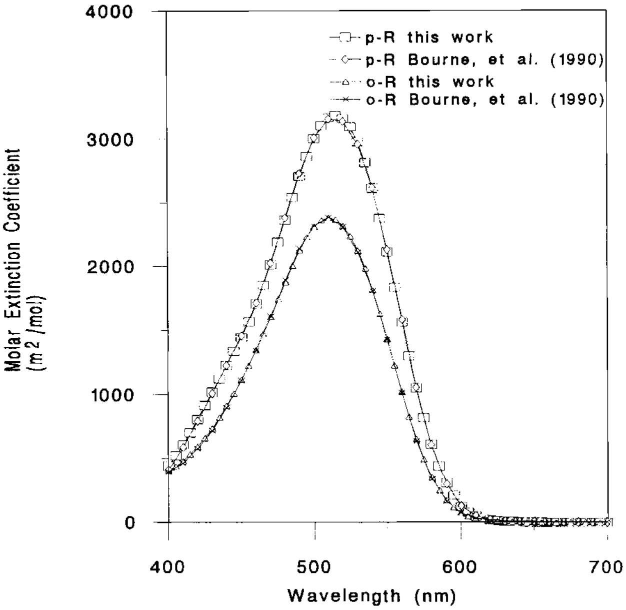

Figure 6. Comparison of $o-R$ and $p-R$ spectrum with most recent spectrum published by Bourne et al. (1990).

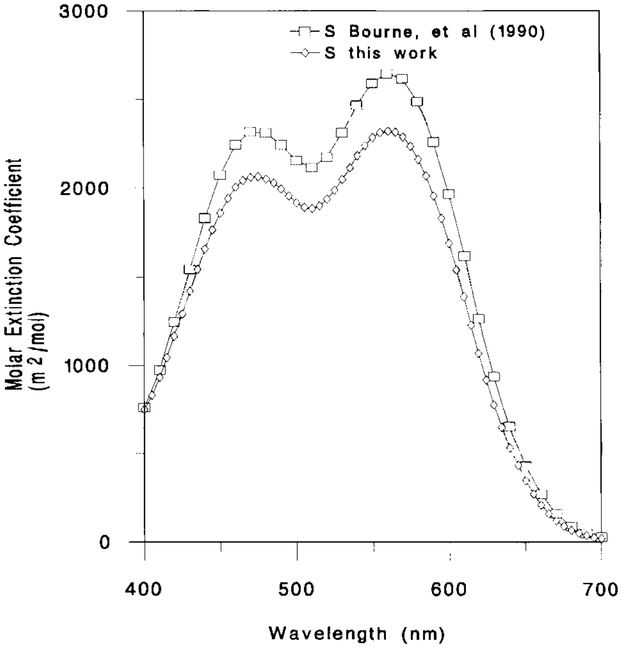

Figure 7. Comparison of bisazo dye S spectrum with most recent spectrum published by Bourne et al. (1990).

High-Pressure Liquid Chromatography

To check the spectrophotometric analysis and verify that the three products are formed in mixing experiments, samples of the individual dyes as well as product mixtures from mixing experiments were analyzed by HPLC (Beckman, Model 126). This method also has the potential to determine dye concentrations in a mixture without the problems associated with overlapping visible spectra.

The stationary phase was a Beckman Ultrasphere C18 column with 5-micron particle size. The mobile phase was a variable mixture of acetonitrile and double distilled water which increased linearly from 20% to 90% acetonitrile over 15 minutes. The flow rate was 0.75 mL/min, and the injected volume was 20 μL. Individual dye samples were prepared by dissolving the dye in an equimolar solution of $Na_2CO_3/NaHCO_3$ ($I = 0.220$ mol/L) and neutralizing the solution to pH 7.0 with 8M HCl. Similarly, product mixture samples were diluted to $I = 0.220$ mol/L and neutralized. HPLC analysis was performed within 5 minutes of the neutralization. The dyes were detected at 515 nm by a diode array detector (Beckman, Model 168).

For the reference dye samples, the largest peaks observed eluted from the column with the retention times:

S	5.3 min
$p-R$	6.1 min
$o-R$	7.0 min

For the product mixtures, the peaks were identified both by retention time and visible spectrum measured by the diode array detector. The retention times observed for the three dyes are given below:

S	5.1 min
$p-R$	6.1 min
$o-R$	7.7 min

In addition, a major peak (comparable in size to that of $o-R$) was observed at a retention time of 6.45 min. This unknown product was also observed as an impurity in reference samples of $p-R$ and S, but not $o-R$. The peak was observed in dye mixtures resulting from 1-naphthol obtained from two different suppliers (Aldrich N199-2, Fluka 70438) and was not increased by adding an impurity of 2-naphthol (Fluka 70448). The visible spectra of the three dyes and the unknown product under the chromatographic conditions are shown in Figure 8. The shapes of the $o-R$, $p-R$, and S spectra are similar to those reported by Bourne et al. (1990) at pH 1.2. In addition, the shape of the unknown product spectrum is similar to the decomposition product observed by Bourne et al. (1985). This similarity suggests that some of this decomposition product may be formed in the micromixing environment where an excess of B is present.

Experimental Results: Mixing Effects

This reaction has been used to characterize the varying degrees of micromixing in a stirred tank fermenter by Ye (1984) and Dunlop and Ye (1990). A comprehensive study of the effects of injection point location, gassing rate, and stirring speed is reported in these references. The injection point data were used to explain losses in microbial yield that occurred when the substrate injection point was changed. The experimental procedure used for the dye experiments, semibatch addition of B to A, was similar to that developed by Bourne et al. (1981).

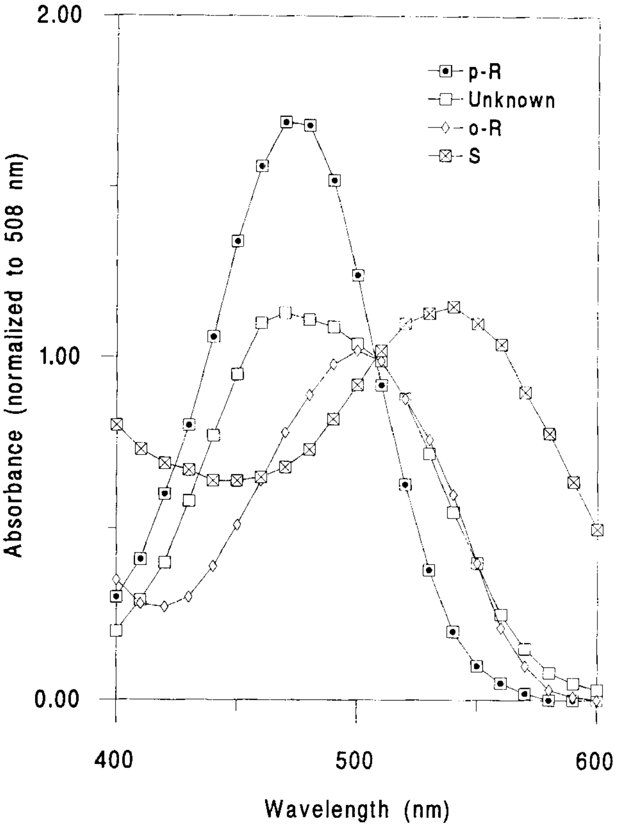

Figure 8. Visible spectra of four colored compounds detected in a product mixture by HPLC.

pH and ionic strength conditions are those of the HPLC analysis.

To illustrate the improvements gained by the present chemical investigation, the reaction was used to study the distribution of micromixing intensity in a 14-L fermenter.

Description of the experiment

A schematic of the reactor is given in Figure 9. It is a 14-L New Brunswick stirred-tank fermenter, equipped with a four-bladed Rushton turbine impeller. A single-orifice sparger is used to introduce gas into the reactor 12 cm below the impeller disk. The tank is equipped with four baffles, each 2.0 cm wide. As shown in Figure 9, the tank was fitted with ten different feed pipes. These pipes were welded into place to ensure reproducibility of location. The pipes were 5.0 mm in diameter with a 1.0-mm orifice.

The experiments took place in semibatch mode, as described by Bourne et al. (1981). The reactor was initially charged with $5.78(10^{-3})$ mol of 1-naphthol in 10 L of double distilled water. This solution was buffered with 1.1 mol each of $NaHCO_3$ and Na_2CO_3, giving an ionic strength of 0.440 mol/L. The pH of this solution remained constant throughout the experiment at 10.1.

$5.50(10^{-3})$ mol of diazotized sulfanilic acid was prepared as described above and diluted to 1.0 L with double distilled

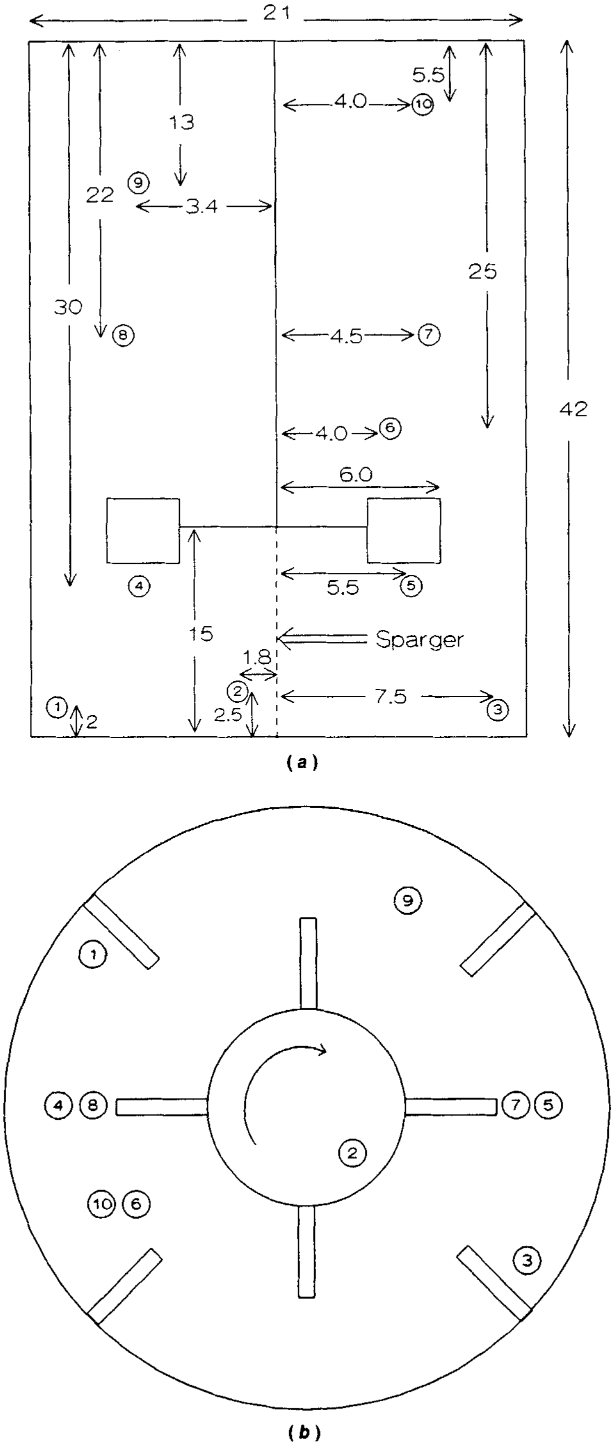

Figure 9. Experimental reactor: a) side view; b) top view.
Dimensions are in cm.

Table 5. Injection Point Effects

IP#	X_S	σ	M (%)	σ
1	0.107	0.011	100.1	0.7
2	0.130	0.015	99.4	0.7
3	0.086	0.009	100.1	1.1
4	0.020	0.005	98.8	0.3
5	0.030	0.001	99.7	0.6
6	0.071	0.005	99.3	0.4
7	0.082	0.005	99.6	0.4
8	0.079	0.002	101.0	1.1
9	0.117	0.012	100.4	0.5
10	0.106	0.006	100.1	1.1

water. Both the A and B solutions were degassed with nitrogen for 1 hour prior to each experiment and were continuously purged throughout the experiment.

The B solution was pumped through the experimental injection point at a flow rate of 42 ± 1 mL/min by a peristaltic pump (Masterflex). A flow integrator eliminated pulsations in the flow. The stirrer speed was manually set to 600 ± 1 rpm as measured by an optical tachometer (Ametek). Nitrogen flow rate into the tank was maintained at 12.3 std. L/min by a mass flow controller (Brooks). Temperature was maintained at $25 \pm 0.5\,^{\circ}$C by a water bath outside the tank. The stainless steel portions of the tank were coated with a silicone sealant to eliminate possible undesirable side reactions with the metal.

After each injection, which lasted about 24 minutes, the product solution was immediately sampled for analysis. The sample was diluted to standard conditions ($I = 0.0440$ mol/L) and was analyzed in the spectrophotometer. Although the analysis was always completed within 10 minutes of the end of the experiment, no significant changes in the spectrum were observed after one hour.

Analytical method

The absorbance of the product mixture was recorded as a function of wavelength from 400 to 700 nm by a Varian Cary 3 scanning spectrophotometer. These data were then subjected to a three variable linear regression according to the Lambert-Beer law:

$$\frac{D}{d}(\lambda) = \beta_{o-R}(\lambda)C_{o-R} + \beta_{p-R}(\lambda)C_{p-R} + \beta_S(\lambda)C_S \qquad (8)$$

X_S can then be calculated from the concentrations.

The analysis was checked by using a mass balance on B. The percent closure M is given by the equation:

$$M = (2N_S + N_{o-R} + N_{p-R})\,\frac{1}{N_B} \times 100 \qquad (9)$$

Effects of feed point location

The above experiment was carried out four times at each injection point (IP#). The results are summarized in Table 5, where the listed X_S and mass balances (M) are averages for each injection point and σ is the standard deviation.

The average of all the mass balances obtained was 99.9% with a standard deviation of 0.9%. The standard deviation of X_S as a percentage of X_S at a given injection point averaged 9.2% which agrees well with the repeatability reported by Rice and Baud (1990).

The results show a nearly sixfold change in the product distribution from the most to least intensely mixed portions of the tank. This demonstrates the effect that local turbulence intensities can have even in a highly agitated vessel. The differences between extremes of the tank are similar to those observed by Rice and Baud (1990) and Dunlop and Ye (1990).

As reported by Bourne et al. (1990), the analytical method fails to distinguish reliably between $o-R$ and $p-R$ due to the similarity of their spectra. In 17.5% of the analyses, the $o-R$ concentration was determined to be less than zero by the regression analysis. In addition, values of C_{o-R} at a given injection point showed a large degree of irreproducibility. However, neither the X_S nor the M calculated values were affected by these C_{o-R} values. Thus, it was concluded, as by Bourne et al. (1990), that the sum of C_{o-R} and C_{p-R} is correctly determined by the regression method. In the range of X_S values obtained in these experiments, the three component analysis gave X_S values that were on average higher by 0.002 than those obtained using a two-component analysis (neglecting $o-R$). Bourne et al. (1991) have reported a correlation between "old" (1985) and "new" (1990) values of X_S, which implies a more significant difference (average of 0.011) between the three-component and two-component analyses in the range of X_S found in this work. Most of this difference, however, can probably be ascribed to updated extinction coefficients of S between the two studies.

Local rates of energy dissipation

While values of X_S give a quantitative description of the degree of micromixing, it is more valuable for reactor design to have a physically meaningful parameter related to X_S. Using a micromixing model developed by Baldyga and Bourne (1989), local rates of turbulent energy dissipation can be estimated from the product distribution X_S. This model treats the rate of engulfment of fresh A solution into the growing reaction cloud as the rate-controlling step in reaction of A and B. Experimental and theoretical evidence supporting this mechanism is given by the authors. The model can be summarized by the equation:

$$\frac{dc_i}{d\alpha}=E(<c_i>-c_i)+R_i \qquad (10)$$

The engulfment rate E in the viscous-convective part of the concentration spectrum can be related to the local rate of energy dissipation by Eq. 11 (Baldyga and Bourne, 1989):

$$E=0.05776\left(\frac{\epsilon}{\nu}\right)^{1/2} \qquad (11)$$

The use of this model for the experimental situation used here (slow semibatch addition of B to A) is also detailed by the authors. Briefly, the B feed is discretized into 20 or more parts, and the set of ordinary differential equations described by Eq. 10 is solved for each portion of the feed. Each feed portion represents a reaction cloud that grows exponentially by the engulfment mechanism. When the concentration of B in the growing reaction cloud reaches zero, the bulk concentrations are updated and Eq. 10 is again solved for the next feed portion.

Using this model, a value of X_S was predicted for various values of the engulfment rate or by Eq. 11, ϵ. If the model holds true, the rate of turbulent energy dissipation present in the reaction zone can then be estimated from experimental values of X_S. The predictions are given in Figure 10. While ϵ predicted in this manner is necessarily averaged over the reaction zone and additionally depends on the assumptions made by the mixing model, it gives a quantitative description of the local turbulence that has physical meaning. Energy dissipation as predicted by the model varies from 0.21 to 19 W/kg in the areas of the tank studied in the above experiments. This agrees qualitatively with an overall rate of energy dissipation of 9.7 W/kg as predicted by standard correlations (Treybal, 1980).

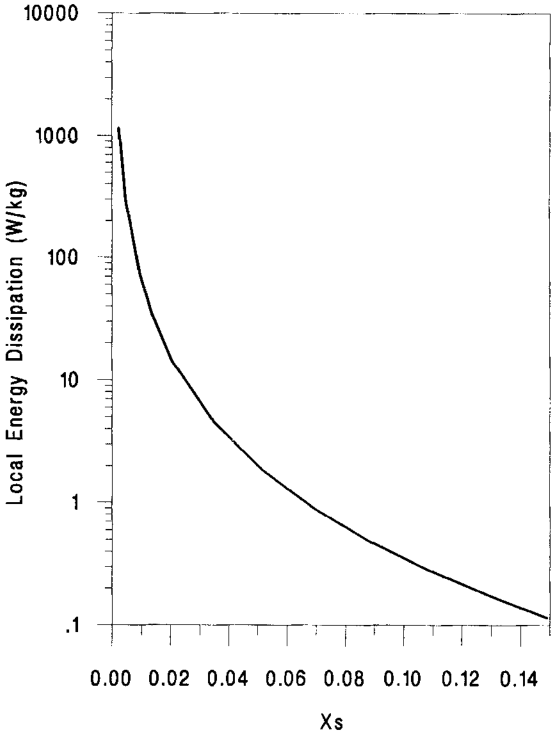

Figure 10. Predicted values of local energy dissipation as a function of the product distribution X_S.

Conclusions

An examination of the properties of bisazo dye S has yielded information important to its quantification in micromixing experiments:

- Pure S was obtained by azo coupling $p-R$ with diazotized sulfanilic acid and purifying by recrystallization from methanol. This purification method is previously unreported and yields sufficient quantities of crystalline S for gravimetric quantification.
- Crystalline S was found to form a monosodium salt with a molecular weight of 534.2. This information is necessary to determine accurate extinction coefficients.
- Crystalline S was found to be extremely hygroscopic, containing as much as four water molecules per mole. This may explain differences in published extinction coefficients determined by isolating crystalline samples of S.
- Reference extinction coefficients for $o-R$, $p-R$, and S were obtained for the experimental conditions of the micromixing test reaction. These provide accurate quantification of the three species, as demonstrated by closure of the mass balance.

In addition, analysis of products obtained by semibatch addition of B to A has yielded potentially useful information:

- Use of the micromixing test reaction in a 14-L stirred-tank fermenter showed substantial heterogeneity within the tank. The heterogeneity manifested itself in a sixfold change in the product distribution from the best to the worst injection point. This demonstrates the large effect that micromixing can have even in a highly agitated vessel.
- HPLC analysis of product mixtures from mixing experiments has shown that another product is formed in addition to $o-R$, $p-R$ and S. The product is formed in significant amounts and appears to be a decomposition product of S.
- Presence of the unknown compound did not adversely affect the mass balance in the mixing experiments, nor did the inclusion of $o-R$ in the analysis substantially affect these results. Consequently, it can be recommended that the two-product model described by Eqs. 1 and 2 is adequate for practical purposes, with the recognition that it is a great simplification of the chemistry involved.

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Notation

A = 1-naphthol
B = diazotized sulfanilic acid
c = concentration
c_i = concentration of species i in the reaction zone
$<c_i>$ = concentration of species i in the bulk
d = path length
D = light absorbance
E = rate of turbulent fluid engulfment
I_p = index of purity for S
M = percent closure of material balance
N = number of moles
$o-R$ = 2-(1,4 sulfophenylazo)-1-naphthol or its ion in aqueous alkaline solution
$p-R$ = 4-(1,4 sulfophenylazo)-1-naphthol or its ion in aqueous alkaline solution
R_i = rate of reaction of species i
S = 2,4 bis(1,4 sulphophenylazo)-1-naphthol or its ion in aqueous alkaline solution
X_S = product distribution

Greek letters

α = vortex age
β = molar extinction coefficient
ϵ = turbulent energy dissipation rate
ν = kinematic viscosity
λ = wavelength of light

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