Improved Methods for the Determination of Wort Color and Turbidity

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ABSTRACT

Instrumental methods for determination of wort color and turbidity either previously proposed or currently in use were reviewed. New techniques developed included a wort-color calibration based on a buffered solution of phenol red, and compensation for turbidity in determination of wort color by use of modified absorbance values obtained at 400 and 500 nm. To overcome color influence in nephelometric wort turbidity determinations, known addition methods were applied using formazin for achromatic turbidity addition to wort. Particle size distributions of turbidity in wort, formazin solutions, wort-formazin mixtures, and barium sulfate solutions were determined.

Key words: *Addition method, Color, Formazin, Phenol red, Turbidity, Wort*

Spectrophotometric methods for determination of wort and beer color were developed by comparison with visual methods (14,15,16). The ASBC definition of wort color relies on instrumental response at 430 nm and lacks external calibration standards (2). As spectrophotometers of various manufacture became available, instrumental response to wort color escaped universal definition. Analytical problems associated with spectrophotometric sensitivity to sample turbidity or changes in wavelength are enhanced in determination of wort color (16). Individual laboratories normally experience difficulty in establishing standard curves for wort and often must seek collaborative assistance. A number of color standards for wort and beer have been proposed (7). With the exception of a bromocresol purple standard proposed by Beetch and Oetzel (7), none are well suited to spectrophotometric standardization.

Determination of wort or beer color in the presence of turbidity has been particularly troublesome and the subject of considerable effort, particularly by ASBC subcommittees (5). The celite filtration method for color in turbid wort has been adopted by the ASBC (2), but is not considered exceptionally convenient or reproducible and shows substantial between-laboratory error. Several alternatives have been collaboratively examined and found unacceptable. These include 700-nm absorbance corrections (3,15), Selas filtration (3,4), membrane filtration (4), and turbidity correction factor methods (4). Two proposed methods—cuvette-shifting and color extinction (6)—have not been collaboratively tested. Cuvette-shifting requires specialized instrumentation, and

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color extinction in wort involves lengthy calibration and time-consuming analytical techniques. A dual wavelength turbidity correction for the estimation of sulfhydryl levels in milk has been reported (13). Multiple wavelength determination of beer and wort color has been examined in detail by Brandon (8), who reported enhanced color determination but did not attempt turbidity correction.

Measurement of turbidity in wort and beer has not received the attention given to color, and no recent efforts have been made to standardize analysis of turbidity within the brewing industry. Formazin suspensions have become universally accepted turbidity standards (1,2). They are reproducible, relatively stable, and contain small, uniform particle size. Nephelometry is the method of choice for measurement of turbidity. Compared to absorption or visual methods, nephelometric turbidity measurement exhibits greater sensitivity and improved color rejection (20). Instruments have been developed to eliminate color influence in determination of turbidity in colored solutions (10,18,20). Unfortunately, these modified instruments show varying degrees of success in color compensation and further confuse efforts to standardize analysis of turbidity. The nephelometric measurement of turbidity in wort can be significantly affected by wort color. This depends on the scalar magnitude of formazin standardization used by the analyst. At least two very different scales are in use within the brewing industry, and others are available (Fig. 1).

Several theoretical and philosophical explanations have been applied to the instrumental problems associated with separate quantitation of color and turbidity in wort or beer. A series of articles debating this issue were published by Thorne (17,18,19) and Chevalier (9,10). Wort color and turbidity do not conform to the ideal qualities required in theoretical explanation. The visible spectra of wort and beer do not have maxima, and some level of absorbance is retained throughout the entire spectrum (14). Particles of wort turbidity are extremely irregular in shape and are normally distributed by size within a limited range (12). The development of reliable methods for discrete measurement of wort color and turbidity must temper theory to accommodate a situation that is less than ideal.

The investigations reported in this article address what we consider to be serious deficiencies in present methods for determination of wort color and turbidity. A phenol red color standard is proposed for calibration of spectrophotometric wort color determinations. The use of a single scale for formazin standardization of nephelometers is suggested. Methods were developed to eliminate turbidity interference in spectrophotometric wort color determinations and wort color interference in nephelometric wort turbidity measurement.

EXPERIMENTAL

Reagents

Exactly 0.100 g of ACS reagent phenol red was dissolved in 100 ml of 0.01 *N* NaOH. The solution was magnetically stirred for 15 min at 200 rpm to ensure complete solubilization. Distilled water (300 ml) was added, the solution adjusted to pH 5.6 with 0.1 *N* HCl, and then brought to 500 ml final volume with distilled water. This stock solution was stored at 4° C in amber glass.

A phthalate buffer (pH 5.6) was prepared by diluting 500 ml of 0.1 M potassium biphthalate and 400 ml of 0.1 *N* NaOH to 1 L with distilled water.

Exactly 1.000 g of reagent grade hydrazine sulfate was dissolved in distilled water and brought to 100 ml volume. Reagent grade hexamethylenetetramine (10.0 g) was dissolved in distilled water and brought to 100 ml volume. Exactly 5.0 ml of each solution was pipetted into a 100-ml volumetric flask and left undisturbed for 24 hr at 25° C. The resulting suspension was brought to volume with distilled water and had a defined turbidity of 400 Nephelometric Turbidity Units (NTU). Distilled water dilutions of this suspension were prepared as required.

All worts in this investigation were from fine-grind malt prepared in accordance with ASBC Methods of Analysis (MALT-4) (2).

Instrumentation

A Bausch and Lomb Spectronic® 100 with 20-mm cuvets was used for all colorimetric analyses. Immediately prior to this study, the instrument was thoroughly checked for proper operation and calibrated according to APPENDIX-IA of ASBC Methods of Analysis.

A Hach 2100® turbidimeter calibrated with formazin turbidity standards was used for all nephelometric determinations performed in our laboratory.

Wort Color Calibration

A 2.5-ml aliquot of the phenol red stock solution described above was diluted to 100 ml with phthalate buffer (pH 5.6). An absorption spectrum in the visible region was determined for this solution (Fig. 2). Absorbance at 430 nm was equivalent to a wort color of 4.00 SRM, according to ASBC Methods of Analysis (WORT-9). Absorbance at 430 nm was determined for solutions prepared from aliquots (0.3–3.0 ml) of phenol red stock solution diluted to volume

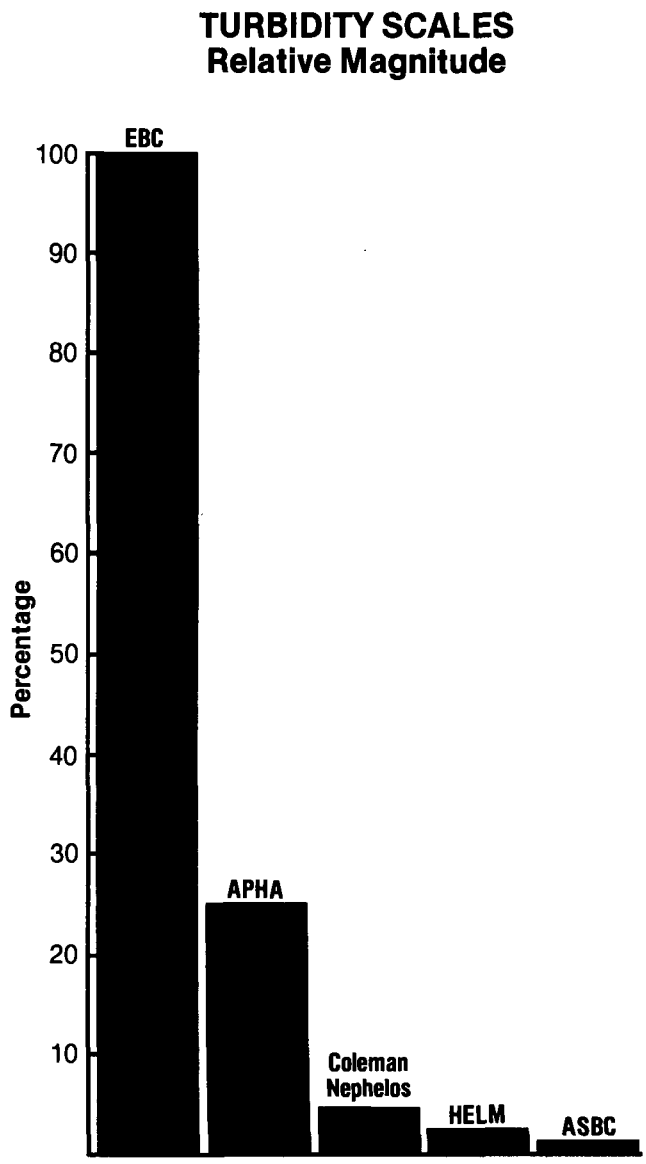

Fig. 1. Relative magnitude of common turbidity units.

with phthalate buffer (pH 5.6) in individual 100-ml volumetric flasks. From the absorbance data obtained and application of the law of Lambert-Beer, a standard curve was plotted for SRM color, as shown in Fig. 3. The phenol red curve has been offset from the ASBC definition curve for clarity. Calibration curves 1 and 2 were generated before this investigation from comparisons with ASBC collaborative data. Wort colors reported in this investigation were from the phenol red curve unless otherwise noted.

Phenol Red Investigations

The phenol red stock solution was generated five times from the same lot of ACS reagent phenol red. A 1.0-ml aliquot from each preparation was diluted to 100 ml with phthalate buffer (pH 5.6). The five resulting solutions gave absorbance values of 0.251 ± 0.002 at 430 nm.

ACS reagent phenol red was obtained from four suppliers. A stock solution was prepared for each lot, and dilutions were made as described for the initial lot. Absorbance values for three of the diluted stock solutions were 0.250 ± 0.007 at 430 nm. The remaining stock solution was slightly turbid and gave an absorbance of 0.295.

Phenol red stock solutions and phthalate-buffered dilutions were unstable at room temperature for prolonged periods, resulting in a gradual loss in color intensity. Stock solutions were stable for 30 days at 4° C in amber glass, as evidenced by consistent absorbance values for phthalate-buffered dilutions.

Sensitivity of phenol red to changes in pH was tested by preparing six phthalate buffers from pH 4.0 to 6.2 and diluting 1.0-ml aliquots of stock phenol red to 100 ml with each buffer. Absorbance values at 430 nm for these solutions were all within 0.003 units of 0.250.

Known additions of phenol red to wort were made to demonstrate compatability (Fig. 4). Additions to wort of the bromocresol purple standard proposed by Beetch and Oetzel (7) were attempted, but turbidity was immediately generated in the wort sample.

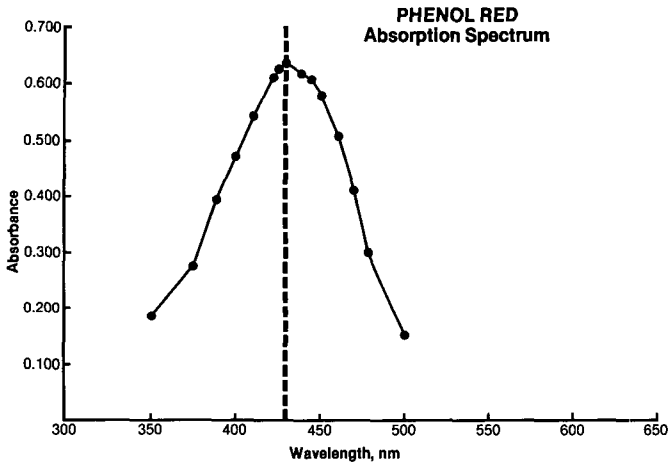

Fig. 2. Absorption spectrum for a phthalate (pH 5.6)-buffered solution of phenol red.

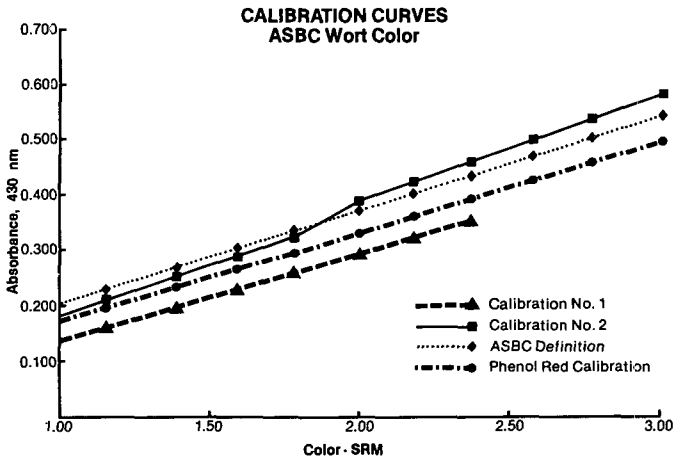

Fig. 3. Calibration curves for SRM wort color. For the phenol red curve: $X = 6.517y - 0.01$, $r = 0.999$. The ASBC definition of SRM color is (absorbance at 430 nm × 0.635 × 10) for 20-mm cuvettes.

Particle Size Distributions

A standard membrane filtration apparatus with a series of 47-mm-diameter Nucleopore® membrane filters (0.1–5.0-μm pore sizes) was used to determine particle size distributions for turbidity in worts, formazin suspensions, wort-formazin mixtures, and a barium sulfate suspension (Table I). For comparison, the values for each filtrate are reported as a percentage of the 430-nm absorbance for the sample attributed to turbidity. The size distributions for particles in wort turbidity and formazin suspensions were nearly identical, and no increase in particle size was observed in wort-formazin mixtures. Worts in this study represent several varieties of malt produced under a wide range of processing conditions. Samples were freshly prepared, and a single 100-ml aliquot taken for stepwise filtration and absorbance determination. Samples were analyzed in pairs, with approximately 30 min required for filtration analysis and an additional 15 min for centrifugation. The solution containing barium sulfate turbidity was the result of reacting a 15 mg/L SO_4 solution with $BaCl_2$ as described in Standard Methods for the Examination of Water and Wastewater (1).

Wort Color Method

A visible absorption spectrum was determined for a 30-NTU formazin suspension at 5-nm increments from 360 to 700 nm (Fig. 5). Turbidity response coefficients were approximated by dividing the absorbance at 430 nm by the absorbance at each other wavelength determined.

A low-turbidity wort was filtered through a 0.1-μm membrane filter with a 0.45-μm membrane prefilter. The color of the filtered wort was 1.64 SRM. Absorbance was determined for the filtered wort at 5-nm increments from 360 to 700 nm. The absorbance found at each wavelength was multiplied by the turbidity response coefficient for that wavelength. A pair of wavelengths bracketing 430 nm was chosen such that the difference of the multiplied

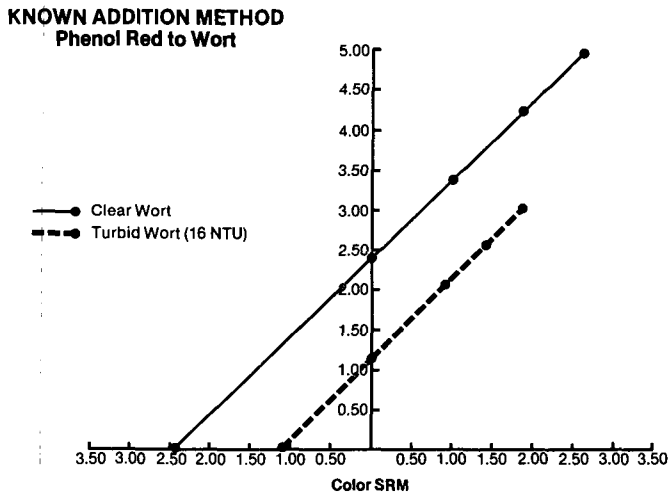

Fig. 4. Known additions of phenol red to clear and turbid worts.

absorbance values for the two wavelengths was equivalent to the absorbance required at 430 nm for 1.64 SRM color. A series of four low-turbidity worts with colors from 1.20 to 3.35 SRM were processed as described above. An average absorption spectrum for these worts is shown in Fig. 5. The wavelength pair of 400 and 495 nm was best suited for approximating 430 nm color with the spectrophotometer used in this investigation. The 495-nm wavelength was changed to 500 nm for convenience, because the error introduced by this change was insignificant.

Turbidity response curves were determined from absorbance of 5–30 NTU formazin suspensions at 400, 430, and 500-nm wavelengths, as shown in Fig. 6. A plot of absorbance for these same suspensions at 430 nm vs absorbance at 400 and 500 nm is shown in Fig. 7. The slope of each line accurately describes the turbidity response coefficient for the wavelength related to 430 nm. The coefficients were 0.874 at 400 nm and 1.345 at 500 nm. These values were within 0.004 of the approximations made earlier.

The 430-nm absorbance of clear or turbid wort was found by (absorbance 400 nm × 0.874) − (absorbance 500 nm × 1.345). Wort color was then determined from the phenol red calibration curve. Results from this method were compared with celite filtration and 430-nm color determination of the same worts (Table II). Celite-filtered worts retained turbidities from 2 to 4 NTU. The new method was applied to 0.1-μm membrane-filtered wort with 20- and 40-NTU formazin additions (Table III). When applied to phenol red solutions with or without formazin turbidity, the new method produced color values much lower than expected. Unlike wort absorption spectra (Fig. 5), the spectrum of pH 5.6 buffered phenol

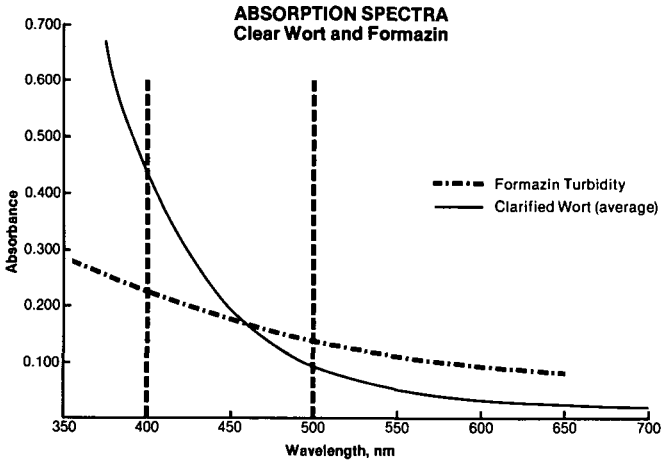

Fig. 5. Average absorption spectra of clear worts and a 30 nephelometric turbidity unit (NTU) formazin suspension.

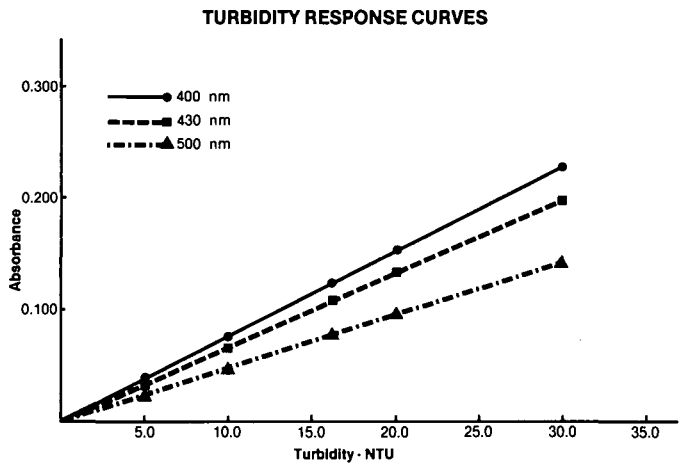

Fig. 6. Spectrophotometric turbidity response curves for increasing formazin turbidity. 400 nm: $y = 0.007X + 0.001$, $r = 0.999$; 430 nm: $y = 0.006X + 0.001$, $r = 0.999$; 500 nm: $y = 0.005 \times 0.001$, $r = 0.999$.

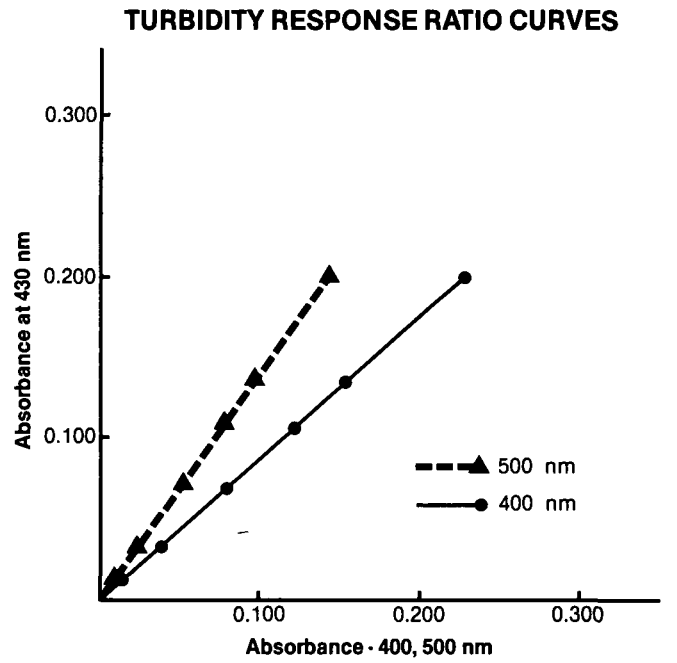

Fig. 7. Curves of spectrophotometric turbidity response at 400, 500 nm vs 430 nm. At 400 nm, $y = 0.874X + 0.001$, $r = 0.999$ and at 500 nm, $y = 1.345X + 0.001$, $r = 0.999$.

TABLE I
Particle Size Distributions

Sample	Original Absorbance[a]	Original Turbidity[b]	Absorbance of Filtered Wort-Membrane Pore Size (μm)[c] 5	3	1	0.6	0.45	0.2	0.1	Centrifuged Wort[d] Absorbance
Wort 1	.447	17	1.06	1.02	0.99	0.88	0.10	0.02	0.04	.342
Wort 2	.333	6	1.02	0.97	0.94	0.71	0.08	−0.04	0.06	.288
Wort Average (n = 10)	.431	12	1.03	0.99	0.94	0.80	0.05	0.03	0.04	.319
Formazin (20 NTU)	.133	20	1.07	0.96	0.98	0.89	0.11	0.07	0.03	···
Formazin-wort 1[e]	.301	19	1.04	0.98	0.96	0.84	0.06	0.04	−0.02	.180
Formazin-clear wort 1[f]	.225	11	0.98	1.03	0.99	0.90	0.14	0.03	0.01	.177
Barium sulfate	.171	20	1.02	0.97	0.94	0.95	0.58	0.16	0.04	···

[a] Absorbance determined at 430 nm.
[b] Corrected for color, Nephelometric Turbidity Units (NTU).
[c] Expressed as percentage of original absorbance minus absorbance of centrifuged wort.
[d] Filtrate through 0.1-μm membrane filter centrifuged at 15,000 rpm for 15 min.
[e] A 1:1 mixture of Formazin (20 NTU) and wort 1.
[f] A 1:1 mixture of Formazin (20 NTU) and 0.1 μm filtered wort 1.

TABLE II
Color of Turbid Worts
Celite Filtration and New Method

Wort Sample	Uncorrected Turbidity	SRM Color Celite Filtered	Color New Method
1	18	1.84	1.89
2	12	1.32	1.36
3	3	1.68	1.72
4	18	1.62	1.65
5	10	1.61	1.65
6	8	1.91	1.84
7	2	2.70	2.83
8	23	1.32	1.34
50	6	1.74	1.56
Mean	14	1.58	1.61
Average difference		0.07	

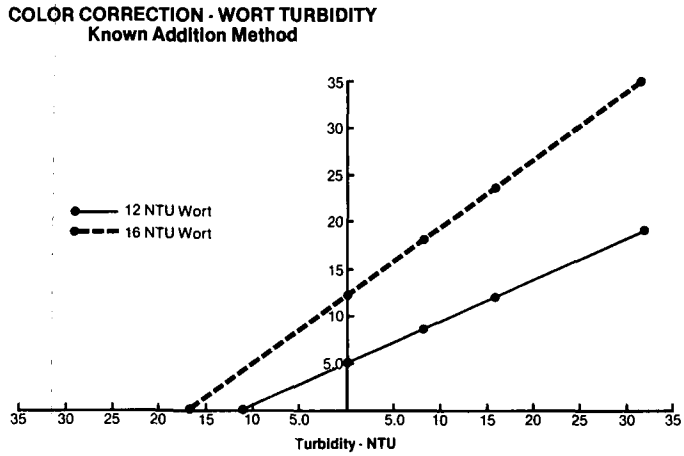

Fig. 8. Known additions of formazin to two worts.

TABLE III
Interaction of Turbidity and Wort Color

Sample	Turbidity Addition[a]	SRM Color 430 nm	Uncorrected Turbidity	Corrected Turbidity	Corrected Color
Clarified wort	...	1.32	...	...	1.34
Clarified wort	20	1.97	14	21	1.34
Clarified wort	40	2.51	22	42	1.37
Phenol red solution	...	1.33	...	...	0.61
Phenol red solution	20	1.82	16	19	0.58
Phenol red solution	40	2.30	30	40	0.61

[a] Turbidity reported in Nephelometric Turbidity Units (NTU).

TABLE IV
Wort Turbidity-Color Correction
Known Addition Method

Wort Sample	Uncorrected Turbidity	Turbidity After 16 NTU Addition	Corrected Turbidity[a]
1	3.3	12	6.3
2	3.3	12	6.3
3	3.5	11	7.5
4	3.4	11	7.2
5	5.2	12	13.0
6	4.5	13	8.8
7	23.0	30	55.0
8	27.0	34	64.0
9	7.0	16	13.0

[a] Calculated from $Tu = \frac{Ts}{(Ta/To)-1} \div 0.96$, where Tu = corrected turbidity; Ts = turbidity value of addition (16 NTU); Ta = turbidity after known addition; To = uncorrected turbidity; and 0.96 = dilution factor.

red has a well-defined maximum at 430 nm and shows little absorbance at 400 or 500 nm (Fig. 2).

Wort Turbidity Method

Two low-turbidity worts were filtered through 0.1-μm membrane filters. The colors of the filtered worts were 1.25 and 2.40 SRM. Formazin turbidity was added to each wort equivalent to 16 NTU in the 1.25 SRM wort and 12 NTU in the 2.40 SRM wort. Two-milliliter additions of formazin turbidity sufficient for 8, 16, and 32 NTU final turbidities were added to 23-ml aliquots of each wort, as shown in Fig. 8. Values for the original worts were adjusted for dilution. Extrapolation to the x-axis reveals the true original turbidity.

Turbidity was determined for 24 ml aliquots of nine worts. A single addition of 1.0 ml of 400-NTU formazin suspension was made to each aliquot and the turbidity determined again. The data were manipulated as shown in the footnotes to Table IV, providing color-corrected turbidity values for each wort.

RESULTS AND DISCUSSION

Phenol red wort color standards as described in this investigation would simplify spectrophotometric wort color calibration and minimize the effects of instrumental variation. Adjustment of phenol red curves by spectrophotometric comparison of wort colors would still be necessary for wide bandpass filter photometers as described in ASBC Methods of Analysis (BEER-10). The phenol red calibration curve closely approximated the curve defined by ASBC Methods (WORT-9) for the spectrophotometer used in this study. It should be noted, however, that a 12% adjustment of these curves was necessary to meet the median wort color for 1981 collaborative samples from the Malt Analysis Check Service of the ASBC. Calibration curves 1 and 2 (Fig. 3) show adjustments required to align our wort color values with the mean for ASBC collaborative analyses for years previous to 1981. In the absence of external calibration standards, it appears that considerable drift from ASBC definition has occurred within the industry.

Phthalate-buffered phenol red solutions are compatible with wort and can be utilized for checking spectrophotometric error with known addition methods (Fig. 4). The reproducibility and pH tolerance of phenol red wort color standards are excellent, and the phenol red stock solution is stable when stored in amber glass at 4° C. Spectral differences prevent the use of phenol red solutions for multiple wavelength modeling of wort color.

Particle size distributions for wort turbidity and formazin suspensions, (Table I), indicate that formazin is an acceptable model for wort turbidity, as suggested in previous investigations (10,11). Thorne (19) observed an increase in particle size for formazin suspended in beer. Particle size estimates were made by

TABLE V
Collaborative Analysis of Wort Turbidity
Known Addition Method

Wort Sample	Nephelometer 1[a]		Nephelometer 2[a]		Nephelometer 3[b]		Nephelometer 4[b]	
	A[c]	B[d]	A	B	A	B	A	B
1	3.0[e]	6.0	4.0	6.3	5.2	5.7	5.1	6.2
2	9.0	14.4	11.0	13.6	11.4	14.6	10.8	13.4
3	7.5	16.3	9.0	15.6	12.5	16.2	15.3	17.4
4	12.0	23.0	15.0	24.0	17.6	22.0	18.7	26.0
5	6.0	12.9	8.0	13.3	10.5	14.1	11.0	13.9
6	24.0	57.0	29.0	54.0	42.0	52.0	48.0	56.0
Mean	10.3[c]	21.6[d]	12.7[c]	21.1[d]	16.5[c]	20.8[d]	18.2[c]	22.2[d]

[a] Nephelometers without color-compensating features.
[b] Nephelometers with color-compensating features.
[c] Uncorrected turbidity values.
[d] Turbidity values corrected for color by known addition of formazin.

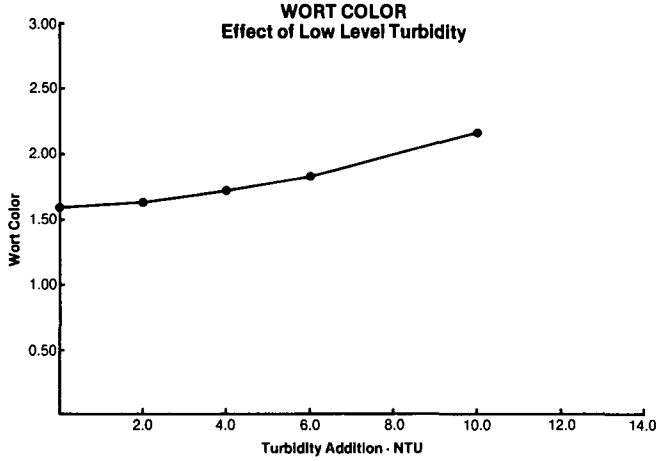

Fig. 9. The effect of addition of low levels of formazin turbidity to wort.

application of light-scattering theory to photometric determinations. Beer color may have been a contributing factor in this observation, as we found no increase in particle size in wort-formazin mixtures.

Wort color by dual wavelength absorbance determination corrected for turbidity response as we have described greatly simplifies analysis of turbid worts. The method also corrects for low levels of turbidity in wort that may not appear sufficient to warrant celite filtration but that can contribute to absorbance for the sample as shown on Fig. 9.

In theory, the slope of the relationship of spectrophotometric turbidity response at one wavelength to that at another wavelength can be predicted by $(\lambda_1/\lambda_2)^4$ (13). The requirements for this prediction include uniform particle shape and size. Wort turbidity does not fulfill either requirement. The predicted slopes of 0.749 for 400–430 nm and 1.828 for 500 to 430 nm do not agree with those actually obtained for formazin suspensions (Fig. 7). Barium sulfate turbidity contained smaller particles, resulting in slope values of 0.831 for 400–430 nm and 1.475 for 500–430 nm.

The known addition method for wort turbidity we have proposed would allow uniform nephelometric quantitation despite instrumental difference or color interference. Collaborative analysis of wort turbidity with four nephelometers utilizing the single addition method (Table IV) has produced consistent values for a limited number of samples as shown in Table V. Two of the nephelometers were of the design used in this study. The other two instruments had color-compensating design features.

The NTU scale of formazin turbidity is particularly well suited to nephelometric measurement of wort turbidity. The units are distinguishable by most nephelometric instrumentation and numerically appropriate for measurement of wort turbidity.

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