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Human visual perception of haze and relationships with instrumental measurements of turbidity. Thresholds, magnitude estimation and sensory descriptive analysis of haze in model systems

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Abstract

Spherical polymer beads (0.769, 2.600 and 10.300 μm diameter) were suspended in clear, yellow and red liquids. The samples were measured by turbidimetry and assessed by panelists. Thresholds were determined by the Ascending Method of Limits and ranged from 0.384 to 0.815 NTU. The results were influenced by both particle size and solution color. Visual intensity (assessed by Magnitude Estimation) rose linearly with particle concentration until it reached a plateau. A regression model was developed that expressed visual haze intensity as a function of particle concentration and size, and liquid color ($R^2 = 0.949$). A relationship between visual and instrumental responses was also developed ($R^2 = 0.870$); when particle size was included, this improved to $R^2 = 0.978$. Turbidimeter response could be predicted from particle concentration and size ($R^2 = 0.986$). Principal Components Analysis was applied to Descriptive Analysis results and showed that two factors accounted for 99% of the observed variation. Suspensions of large particles at intermediate concentrations appeared non-homogeneous.

Keywords: Particle size; Particle concentration; Color; Turbidimeter; Beverage; Haze; Visual perception; Instrumental; Turbidity

1. Introduction

Product appearance is an important quality parameter for both clear and cloudy beverages. Clear beverages are intended to remain clear until the product is consumed. Development of visually discernible haze is considered a significant product defect. Cloudy beverages, on the other hand, are intended to have a pleasant appearance that is stable throughout their shelf life. Perception of haze mainly results from light scattering by colloidal size particles, although direct observation of larger particles may occur in some cases. Important questions in this area are how little haze can be seen in clear beverages and how does the quantity of particulate material influence the intensity of perceived scattering and product appearance. The relationships between human and instrumental perceptions of haze are also of considerable interest.

Cloudy beverages typically contain pieces of fruit pulp or other particles derived during processing that are not removed from the product. Haze particles in clear beverages can originate from a number of possible sources. These include fragments of processing aids (filter media or adsorbents) as well as inorganic or organic particles that form in the package. Examples of the latter are oxalates in beer, tartrates in grape juice and wine, polysaccharides, and protein–polyphenol complexes. Some of these substances form regular crystals while others are amorphous.

Haze or turbidity in a transparent medium is the optical phenomenon due to small suspended particles that divert light from its regular course (Rayleigh, 1871). Turbidity, as a precise physical concept, is defined as the extinction coefficient due to light scattering (Thorne, 1963). Thus turbidity is the total light scattered in all directions from the incident beam as it traverses a suspension. In order to obtain an exact measurement of turbidity it would be necessary to measure the total amount of light scattered in all directions. For clear solutions, the light transmission difference from a blank provides an estimate of turbidity.

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With colored samples, some of the reduction in transmission is caused by absorbance. On average, the scattered light travels through the same optical path length in the sample as transmitted light (halfway in and halfway out again). This means that essentially the same opportunity for absorbance occurs with scattered as with transmitted light, so less scattered light reaches a detector or a human observer with a colored solution compared with a clear one.

In any practical light scattering instrument it is necessary to measure the intensity of scattering at one or, at most, a few angles to the incident light beam. It has been demonstrated that the intensity of scattering observed is dependent on the wavelength of light, the size of the particle and the angle at which scattering is observed (Thorne, 1963). It is known, for example that smaller colloidal size particles produce a greater response in a 90° light scattering instrument while larger particles cause more scattering at narrow angles. The relationship of the wavelength of the light used to measure scattering to the particle radius also exerts a strong influence on results. Since the effective wavelength may be changed in a sample that absorbs light strongly, this is an additional way that color could influence instrumental and possibly also visual perception of turbidity.

Turbidimetry has been used to study the intensity of light scattered from non-homogeneities in a wide variety of systems (MacRae, Robinson & Sadler, 1993b); including beverages such as apple juice (Malcolmson, Jeffrey, Sharma & Ng, 1989), beer (Morris, 1987), and wine (Dubourdieu, Serrano, Vannier & Ribereau, 1988). Nephelometry (90° scattering) provides an estimate of turbidity that has been widely used in the brewing industry (Thorne & Nannestad, 1959; Thorne & Svendsen, 1962). Chapon (1993) reported that nephelometry can detect as little as a few mg/liter of haze material. Morris measured beers and synthetic samples with light scattered at angles of 90° and 13° to the beam of transmitted light. He concluded that 90° detection is oversensitive to particles < 0.5 μm in diameter (giving rise to 'invisible haze'), while measurements at 13° are oversensitive to particles > 1 μm in diameter.

Flavor threshold determinations for various compounds added to beverages have frequently been made using the Ascending Method of Limits (ASTM, 1979; Meilgaard, 1991). No references describing determinations of turbidity perception thresholds were found.

In 1953, Stevens related the perceived sensations of brightness and loudness using ratio scales and established Magnitude Estimation (ME) as the method of choice for these perceptions. Indow and Stevens (1966) used ME to establish the relationship between sensory and physical estimates of saturation and hue. Lawless and Malone (1986) compared the sensitivity of ME to that of other scaling methods for evaluation of hue and the amount of shine on a surface. ME has been widely applied to the sensory analysis of foods, particularly to flavor and texture perceptions (Moskowitz, 1975, 1977, 1977b). It has also been applied to assess the turbidity of apple juice (Malcolmson et al., 1989).

Descriptive Analysis, a technique for detection and description of both qualitative and quantitative sensory aspects, is suitable for all of the appearance, flavor and texture properties of a product (Meilgaard, Civille, & Carr, 1987; Stone & Sidel, 1985). However, no references reporting the application of Descriptive Analysis to turbidity were discovered.

A few studies of the relationship of turbidity to human sensory perception have been carried out. Pieczonka and Cwiekala (1974) compared the clarity of 23 samples of commercial apple juice measured with a Pulfrich nephelometer to visual assessments on a 5-point scale (10-member panel); this resulted in a correlation coefficient of -0.81. The authors suggested that since the nephelometric method is simple and much more accurate than visual assessment, it may successfully be used instead. Venkatasubramanian, Saini, and Vieth (1975) related turbidity values from a nephelometer to six descriptive categories of clarity ranging from 'brilliant' to 'veil'. Leedham and Carpenter (1977) related the results of beer samples measured with the Radiometer Haze Meter (90° scattering) and electronic particle counting to visual assessments and found that particle sizes exceeding 5 μm diameter and particle concentrations exceeding 1000 per ml were commercially significant. Hough, Briggs, Stevens and Young (1982) related different instrumental haze measurements to five descriptive categories ranging from 'very hazy' to 'brilliant'. Pangborn (1982) applied quantified visual assessments of turbidity by highly trained judges to coffee preparations and showed that preparation technique and contact time between water and ground coffee influenced haze intensity. Malcolmson, Jeffrey, Sharma, and Ng (1989) related sensory estimates of clarity produced by magnitude estimation to instrumental assessments of turbidity on a series of apple juices. This study found a linear relationship:

$$\log(\text{perceived clarity}) = -0.54 \times \log(\text{instrumental turbidity}) + 1.83$$

This equation was used to predict sensory clarity from instrumental turbidity values for commercial apple juice samples. Several other studies attempted to relate particle size to haze in beer (Morris, 1987; Siebert, Stenroos & Reid, 1981; Thorne & Svendsen, 1962) or apple juice (McKenzie & Beveridge, 1988).

No published studies have reported how turbidity, particle size, particle concentration and human sensory perception relate. The work reported here was designed to compare human visual perception of haze with instrumental results for clear or colored (yellow or red) samples containing particles of several sizes in the colloidal range (small, medium, large). The questions to be answered were:

1. Is human visual perception of haze influenced by color or particle size?
2. Are instrumental measurements of haze influenced by color or particle size?
3. How do instrumental measurements relate to visual perceptions of haze?

The objectives of this study were to determine visual perception thresholds for uniform synthetic beads of several known sizes in artificial colloidal suspensions, to determine the haze intensities of selected suspensions and correlate these with instrumental turbidity, and to develop descriptors that could be used to profile haze appearance in model systems and beverages by sensory descriptive analysis.

2. Materials and methods

2.1. Reagents

Regular styrene/vinyl copolymer microspheres with carboxylate functions of three different sizes: 0.769 μm (small), 2.600 μm (medium), and 10.300 μm (large) mean diameters were obtained from Bangs Laboratories, Inc. (Carmel, IN). The 0.769 μm diameter microspheres had a standard deviation of 0.012 (μm) and a density of 1.065 g/cm^3. The 2.6 μm diameter microspheres had an unspecified standard deviation and a density of 1.060 g/cm^3. The 10.3 μm diameter microspheres had an unspecified standard deviation and a density of 1.060 g/cm^3. Red and yellow food coloring (McCormick and Co., Inc., Hunt Valley, MD) were purchased locally. Sucrose (Certified A.C.S.), and the 95×25-mm sample cells were obtained from Fisher Scientific (Pittsburgh, PA). Polyethylene glycol (PEG, avg. mol. wt.: 8,000) was purchased from Sigma Chemical (St. Louis, MO).

2.2. Preparation of hazy model suspensions

HPLC-grade deionized water was mixed with sucrose and PEG, with or without the addition of red (116 mL/L water) or yellow (1.1 mL/L water) food coloring. The mixtures were degassed, vacuum filtered through 0.2 μm porosity nylon membrane filters, and autoclaved (15 min, 121°C). The liquids and particles were added to vials in a laminar flow hood to avoid introducing microbial contamination; the vials were then closed with screw caps.

2.3. Samples

Suspensions of each of the three sizes of synthetic particles were prepared in a range of concentrations in each of three colored solutions. Suspensions were prepared in 2-fold dilution steps from almost no haze to very high hazes. These were measured with the turbidimeter. Particle number concentrations were calculated from particle weight concentrations using the particle density and the formula for the volume of a sphere.

2.4. Apparatus

Haze was measured with a Hach 2100AN laboratory turbidimeter (Hach Co., Loveland, CO) using 95 mm tall, 25 mm diameter, 30 mL capacity sample cells. This instrument was used to measure haze in Nephelometric Turbidity Units (NTU). It was operated in the ratio mode (results <40 NTU are from simple 90° scattering; higher NTU values are ratios of 90° scattering to an unspecified function of the transmitted and forward scattering results). Formazin suspensions were prepared every three months and used as primary standards. Gelex® preparations (Hach) were used as secondary standards for daily calibration.

2.5. Selection of stimuli

Subsets of the samples were selected from each of the three particle sizes and three suspension colors to fulfill the requirements of the various sensory methods. For threshold determinations, the stimuli were of low concentration (1–3620 μg/L and 0.29–3 NTU). For magnitude estimation (see Table 1), stimuli were noticeably above threshold, i.e. they had a distinct and perceptible visual haze. For sensory descriptive analysis (see Table 2), stimuli consisted of a small population of samples chosen to represent a range of descriptors.

2.6. Testing conditions

The test presentation conditions were established before definitive testing was carried out. Preliminary studies were conducted with the help of one person and considered illumination (spectral distribution and intensity) and viewing conditions (geometric considerations, viewing time, sample area and juxtaposition, background and glare). Based on the preliminary study, conditions were fixed throughout the testing sessions by construction of a viewing box. All testing sessions were carried out using the viewing box.

2.7. Viewing box

To control as many variables as possible, a viewing box was designed for the visual sensory perception experiments. The viewing box was a rectangular box 98×66×47 cm (38.5×26×18.5 inches), constructed of plywood and painted black. There was a centered, open window (20 cm wide×20 cm high), (8×8 inches) in one of the long sides through which panelists viewed the

Table 1
Characteristics of samples used for magnitude estimation

	Small (0.769 μm diameter)		Medium (2.60 μm diameter)		Large (10.3 μm diameter)	
Color	(particles/mL) ($\times 10^6$)	Haze (NTU)	(particles/mL) ($\times 10^6$)	Haze (NTU)	(particles/mL) ($\times 10^6$)	Haze (NTU)
Clear	2–919	1–1560	0.13–134	1–5100	0.002–0.92	1–185
Yellow	2–919	1–1540	0.13–73	1–3210	0.002–1	1–204
Red	2–919	1–1280	0.13–73	1–2890	0.002–1	1–194

Table 2
Characteristics of samples used for descriptive analysis

	Small (0.769 μm diameter)		Medium (2.60 μm diameter)		Large (10.3 μm diameter)	
Color	Concentration (mg/L)	Haze (NTU)	Concentration (mg/L)	Haze (NTU)	Concentration (mg/L)	Haze (NTU)
Clear	0.227	0.721	2.560	1.82	1.088	1.15
Clear	1.818	4.2	10.240	6.35	8.704	2.46
Clear	14.541	34.1	40.960	29.5	34.816	6.39
Clear	58.163	194	163.840	152	139.264	25.7
Clear	232.653	1581	655.360	1157	557.056	189
Yellow	0.454	1.32	1.280	1.72	5.120	1.01
Yellow	3.635	8.49	5.120	6.2	40.960	7.33
Yellow	14.541	33.2	20.480	25.4	163.840	33.5
Yellow	58.163	188	81.920	120	327.680	80.7
Yellow	232.653	1541	655.360	2850	655.360	197
Red	0.454	1.47	1.280	1.37	2.560	0.646
Red	3.635	7.85	10.240	10.1	10.240	13.3
Red	14.541	28.6	40.960	48.6	163.840	29.6
Red	58.163	161	163.840	254	327.680	72.1
Red	232.653	1276	655.360	2720	655.360	182

samples. In one of the short sides there was another window (25.4 cm wide×25.4 cm high), (10×10 inches) through which the samples were introduced and removed. Inside the box there were four tungsten-filament lamps in a rectangular arrangement (in the directions of the box corners) with all the lights directed toward the center of the box (high levels of illumination are preferred for maximum sensitivity). Samples were placed on a platform (22 cm wide×12 cm high) in the center of the box. The inside of the box, including the platform, was covered with black velvet to minimize reflections.

2.8. Subjects

A pool of 19 visually healthy, unpaid, volunteer panelists including students, faculty, and staff members were recruited from the Food Science and Technology Department (see Table 3). Each panelist reported lack of colorblindness and vision that was normal or corrected to normal. Panelists for each sensory test were drawn from this pool according to their availability. Subjects were rewarded with cookies at the end of each panel session.

2.9. Threshold determinations

Threshold determinations were performed using the Ascending Method of Limits, ASTM E-679.

2.10. Threshold panel training

Training sessions were carried out over three days during which 19 panelists were presented with each sample (small, medium and large particles, each suspended in clear, yellow and red liquids) at least once to assure familiarity with their nature and the test format. Each set of samples consisted of 10 three-alternative forced choices, each containing two controls and one sample with added particles. The 10 concentrations presented increased by factors of 2.0. Panelists evaluated three sets a day (each set consisted of a single size particle at various concentrations in one of the three liquid colors). The panel leader spent approximately 30 min each day with each panelist familiarizing him or her with the samples and test format.

2.11. Threshold determination

The testing sessions were conducted in the same manner as the panel training sessions.

2.12. Threshold statistics

The particle concentration equivalent to each individual's Best Estimate Threshold (BET) was calculated as the geometric mean of the highest particle concentration missed, and the next higher particle concentration. The thresholds were expressed as particle weight and particle number concentrations, and as instrumentally measured

Table 3
Characteristics of sensory panelists (panelists were drawn from a pool of 19 unpaid volunteers, most of whom participated in all the sensory tests)

Category	Number in category	Males	Females
Faculty	1	1	
Graduate students	6	1	5
Visiting scientists	1	1	
Staff	11	8	3
Totals	19	11	8

hazes (NTU). The group thresholds were calculated as the geometric mean of the BETs of the individual panelists.

Complete block ANOVA designs using panelists as blocks were carried out with the SAS® general linear models (GLM) procedure (SAS® Institute Inc., Cary, NC), expressing the thresholds as weight concentration, number concentration or haze. In each case, log transformed individual geometric means were used. The treatment structure was a 2-way factorial design (size and color). The GLM procedure was used to test for significant differences (using Fisher's Least Significant Difference at the 95% confidence interval) between the thresholds attributable to size and to color for each of the three ways of expressing thresholds.

2.13. Magnitude estimation (ME) sensory assessment of haze

The relative intensities of selected suspensions were evaluated using the principles of magnitude estimation (Moskowitz, 1975, 1977a, 1977b; Stevens, 1956, 1961).

2.14. ME selection of stimuli

Stimuli for Magnitude Estimation (Table 1) were noticeably above the haze visual threshold. Suspensions of three particle sizes in clear, red and yellow liquids in the range 1–5000 NTU were used for non-modulus ME. The stimuli were equally spaced in terms of ratios (increasing in concentration by factors of 2).

2.15. ME panel training

Eighteen panelists were trained with each particle size and color suspension before the formal testing sessions. They were also trained using geometric figures of varying sizes to familiarize each panelist with the basis of the method. Each subject was informed that he or she would be presented with a reference sample with an unspecified intensity (non-modulus), followed by a random series of samples with intensities both less than and greater than the reference intensity. The panelist's task was to estimate the haze intensity of the unknowns relative to the reference sample. The panelist was instructed to use whatever integers (excepting zero) seemed appropriate and to judge each sample separately.

2.16. ME testing sessions

The testing sessions were carried out over 3 days and included each particle size in all three colors. One presentation of a sample set consisted of 10 to 12 samples of different concentrations of one particle size in one liquid color.

2.17. Non-modulus magnitude estimation

A standard in the middle of the range of stimulus intensities in a set was chosen and shown as the first sample to the panelists; it remained visible for comparison during the entire test. Panelists were told to assign an arbitrary integer value representing the intensity of the haze in this reference sample. Observers were instructed to rate the second sample and each successive sample relative to the first sample. The presentation orders of the samples after the standard were independently randomized for each observer to avoid bias due to the presentation sequence. The stimuli were rated in a complete block design (each panelist rated each sample once).

2.18. ME statistics

Examination of the data revealed that the variance of results increased in proportion to the mean. Logarithmic transformations were applied to the perceived haze intensity results expressed as particle concentration, number concentration and turbidity. The modeling procedures used for analysis of the data included nonlinear regression, and forward and backward stepwise multiple linear regression; these were applied with STATISTICA® 4.1 (StatSoft, Inc., Tulsa, OK).

2.19. Descriptive analysis

Descriptive Analysis of turbid samples was carried out using the methodologies that have typically been applied for sensory descriptive analysis of flavor perceptions (Stone & Sidel, 1985).

2.20. Descriptive analysis stimuli

Clear and colored (yellow and red) samples containing particles of a single mean size at concentrations ranging from 0.227 to 655 mg/L (see Table 2) were used. Standards or anchors for each attribute were provided.

2.21. Descriptive analysis selection of standards

Standards chosen were as follows: clear (0.233 NTU), yellow (0.237 NTU), and red samples (0.263 NTU) without any suspended particles were chosen as the 'clear', 'transparent', 'dark', and 'not glowing' anchors. Medium size (2.600 μm) particles in clear (1311 mg/L particle concentration, 5101 NTU), yellow (715 μg/L particle concentration, 3213 NTU), and red (700 mg/L particle concentration, 2895 NTU) suspensions were presented as anchors for "turbid", "opaque", "glowing" and "light". Two clear 0.769 μm samples (0.45 mg/L, 1.220 NTU and 29 mg/L, 76.8 NTU), one yellow (7.27 mg/L, 15 NTU), and one red suspension (7.27 mg/L, 13.9 NTU) were presented as anchors for "homogeneous". Samples of 10.300 μm particles in clear (139 mg/L, 26.2 NTU), yellow (163.8 mg/L, 31.7 NTU), and red (163.8 mg/L, 29.9 NTU) suspensions were presented as anchors for "particulate".

2.22. Descriptive analysis training sessions

The 14 panelists who participated in this study were trained before the actual test. A representative subset of the samples was chosen for training purposes. The first draft ballot had 25 descriptive terms (bright, brilliant, clean, clear, cloudy, dark, dense, dispersed, dull, glaring, hazy, lucid, luminous, lustrous, milky, muddy, murky, opaque, particulate, scattered, shining, thick, translucent, transparent, turbid). Further training and discussion sessions with panelists reduced these to six final descriptors (actually six descriptors with their antonyms): turbid/clear, homogeneous/non-homogeneous, particulate/fine, glowing/not-glowing, opaque/ transparent, and dark/light. A line scale (10 cm) with increasing intensity from left to right was used. Panelists were asked to rate the relative intensity of a particular attribute by marking a vertical slash across the horizontal line at the point that best reflected the intensity of the particular term compared with standards or anchors presented for each attribute.

2.23. Descriptive analysis testing sessions

All samples were tested in duplicate by the 14 panelists over 6 days. Sessions were held throughout the day and panelists were scheduled to come individually to a session that lasted approximately 30 min. Fifteen samples were tested in each session. Standards were available within the sensory box for comparison throughout the test. Each day a sample set of one color was tested until all suspensions from each color were rated. Samples were presented to the panelists in random sequence and were identified with three-digit random numbers.

2.24. Descriptive analysis statistics

The descriptive analysis data were analyzed by Principal Components Analysis (PCA) using STATISTICA® 4.1. PCA treatments were performed on both the raw data from all samples (including both duplicate results), and averaged raw data from the duplicate samples. PCA loading plots were used to assess the relationships among the attributes. PCA score plots were used to display relationships between the samples.

3. Results and discussion

The synthetic polymer beads were used to prepare stable suspensions in liquids. The amount of sucrose needed to make a particular size microsphere neutral in buoyancy, and thus a suspension of it indefinitely stable, was determined empirically. This was more critical for the larger particles, which had a greater tendency to settle. PEG was used as a dispersing agent to reduce the tendency for the microspheres to aggregate. The amounts of food coloring used were selected to roughly mimic the appearance of a beer (pale yellow) and a red wine (dark red). The clear or colored suspending liquids were autoclaved to prevent microbial growth and the liquids and particles were added to vials in a laminar flow hood to avoid introducing microbial contamination. The clear, yellow and red colored solutions with no added particles (controls) had low, but non-zero turbidity.

3.1. Thresholds

3.1.1. Comparison of thresholds

Table 4 contains the group visual thresholds for small, medium and large particles for each color (clear, yellow and red) expressed as the particle weight concentration, particle number concentration and the turbidimeter measurement (a simple 90° scattering result). The GLM procedure was used to calculate Fisher's Least Significant Difference at the 95% confidence level for each of the three ways of expressing thresholds; this was used to compare the means of the nine treatments (3 colors$\times$3 sizes). For the weight concentration data, the lowest group BET found was for large particles in clear samples (25 μg/L) and the highest was for large particles in red samples (1127 μg/L). There was a 45-fold concentration difference between these two group BET values. Higher particle concentrations were uniformly needed for detection of haze in the red suspensions than for the clear and yellow suspensions. The log standard deviations for all the samples were similar, ranging from 0.202 to 0.711; the largest and smallest values both occurred with medium sized particles. The variation between individual panelists appeared smaller for the large particles. The complete block ANOVA design using panelists as blocks showed highly significant differences ($p = 0.0001$) in thresholds expressed as weight concentration, number concentration or turbidimeter observation (in each case log transformed individual geometric means were used). There were significant effects of color and the size$\times$color interaction for all three expressions of the thresholds (Table 5). The size treatment was not significant for the threshold data expressed as turbidity, but it was for thresholds expressed as weight or number concentration.

Haze perception thresholds expressed as particle concentrations were quite different for small, medium and

Table 4
Best estimate visual thresholds expressed as particle weight concentration, particle number concentration, and turbidimeter observation as a function of particle size and suspension color

Size and color	No. of panelists	Expressed as weight concentration (µg/L)			Expressed as number concentration (particles/mL)			Expressed as turbidimeter measurement (NTU)		
		Group threshold	Log s.d.[a]	Individual BET[b] range	Group threshold	Log s.d.	Individual BET range	Group threshold	Log s.d.	Individual BET range
0.769 µm Clear	16	35c[c]	0.474	1–161	136,000b	0.474	4,960–635,000	0.384d	0.077	0.353–0.595
0.769 µm Yellow	16	37c	0.630	5–643	145,000b	0.630	19,800–2,540,000	0.441cd	0.248	0.289–1.732
0.769 µm Red	19	112b	0.253	80–643	441,000a	0.253	317,000–2,540,000	0.709ab	0.155	0.577–2.058
2.600 µm Clear	16	123b	0.711	28–1,810	12,600c	0.711	2,900–186,000	0.483cd	0.217	0.322–1.140
2,600 µm Yellow	16	113b	0.504	28–1,810	11,600c	0.504	2,900–186,000	0.451cd	0.225	0.291–2.275
2.600 µm Red	19	727a	0.202	453–1,810	74,500b	0.202	46,400–186,000	0.815a	0.181	0.533–1.840
10.300 µm Clear	16	25c	0.403	12–95	41e	0.403	20–157	0.493cd	0.100	0.412–0.696
10.300 µm Yellow	16	46c	0.376	28–453	75e	0.376	47–745	0.529c	0.102	0.454–0.798
10.300 µm Red	19	1,127a	0.334	453–3,620	1,860d	0.334	745–5,960	0.574bc	0.155	0.422–1.080

[a] s.d. = standard deviation.
[b] BET = best estimate threshold.
[c] Thresholds in a column with the same letter are not significantly different at the $p = 0.05$ level.

large particles. However, they were remarkably similar in measured turbidity; including all colors and particle sizes examined, the group thresholds ranged from 0.384 to 0.815 NTU. This demonstrates that 90° light scattering measurements are very suitable for detecting low level hazes of commercial significance across the entire colloidal size range (particle diameters from <1 to 10 µm), even in products in which the size of the particles varies or is unknown. For each particle size, the distribution patterns of the perception thresholds in dark red colored samples were shifted to higher haze compared with the clear and pale yellow samples; this reached statistical significance for the small and medium sized particles.

While there were no literature reports relating visual haze perception thresholds to concentrations of uniformly sized particles, there was anecdotal comment that people can just see hazes of about 5 NTU. That the results in this study are so much lower (0.384–0.815 NTU), yet so consistent between individuals, is presumably due to the use of high intensity lighting, viewing under optimized conditions, and the use of a sensory procedure designed for threshold determination.

3.1.2. Threshold frequency distributions

The frequency distributions of the individual panelists' responses to the nine color/particle size combinations were evaluated. For the small size particles (Fig. 1), the frequency distribution of individual thresholds was unimodal for the red suspensions, and apparently bimodal for clear suspensions, but the situation with the yellow suspensions was less apparent. One panelist was able to perceive haze in clear suspensions at 1.26 µg/L (0.353 NTU), which was much lower than the group threshold. Results for the medium size particles are shown in Fig. 2. For red suspensions, there was a shift to higher thresholds compared to the clear and yellow suspensions. A bimodal distribution was evident for clear suspensions as there were two widely separated groups of results, one at very low concentration (28 µg/L) and the other closer to the thresholds found for the red suspensions (727 µg/L). In the case of the yellow suspensions, one panelist had a threshold for haze perception (1810 µg/L) that was much higher than the rest of the responses. For the large particles (Fig. 3), the frequency distributions were unimodal for all sample colors. There was a shift to higher thresholds for the red suspensions.

Individual flavor thresholds for a given substance have been reported to vary as much as 10,000-fold (Stevens, Cain & Burke, 1988). In a comprehensive study of individual differences in sensory thresholds for aroma chemicals added to beer, (Meilgaard, 1993) found that individual flavor thresholds for panelists ranged between 1.6 and 2011-fold (average 119 and median 36); for highly trained panelists, results were

Table 5
ANOVA assessments of significance of size and color effects on threshold determination results expressed as particle weight concentration, particle number concentration and turbidimeter measurement (NTU)

Source	DF[a]	Mean square	F value	Pr > F[b]
	Particle weight concentration			
Size	2	5.23344876	25.36	0.0001
Color	2	12.06088486	58.45	0.0001
Size×color	4	1.37636083	6.67	0.0001
	Particle number concentration			
Size	2	116.4102242	565.55	0.0001
Color	2	12.0323985	58.46	0.0001
Size×color	4	1.3656071	6.63	0.0001
	Turbidimeter measurement (NTU)			
Size	2	0.04123211	1.51	0.2246
Color	2	0.50239422	18.42	0.0001
Size×color	4	0.07882352	2.89	0.0250

[a] DF = degrees of freedom.
[b] Pr > F is the probability that the calculated F value is statistically significant.

slightly more consistent, ranging between 1.6 and 1135-fold (average 87 and median 32). In the study reported here, individual visual thresholds ranged from 4- to 128-fold expressed as concentration and 1.7- to 7.8-fold expressed as instrumentally measured turbidity, with corresponding averages of 49- and 3.5-fold. The medians were 16- and 3.4-fold, respectively (Table 6). Visual perception, at least as performed under carefully controlled conditions, does not present the individual variations experienced with flavor perception. People responded more uniformly in this study, and this would also be expected for visual perception of wine, beer, and fruit juice samples viewed under similar conditions.

3.2. Magnitude estimation

Maintaining a standard stimulus reference during testing is not customary in ME, although it has been done in some previous studies. Reference samples have previously been kept present throughout evaluation (Meilgaard & Reid, 1979) or presented periodically during sessions (Lawless & Malone, 1986). It appears that one of the main disadvantages of having a standard present in flavor ME work, fatigue from repetitive sampling, is not a problem with visual sensory perception, where there is also no carryover between samples.

In ME studies reported in the literature, score values of zero have usually been permitted. In some prior studies scores of zero have been omitted (Giovanni & Pangborn, 1983; MacRae, Robinson & Sadler, 1993a; Pangborn, Guinard & Meiselman, 1989; Stone & Oliver, 1969). In the present case all the ME samples had measured hazes >1 NTU, which was shown to be above threshold, and scores of zero were accordingly not permitted.

3.2.1. Panelists results vs. turbidimeter measurements

The panelists' perceptions of the haze intensities of samples were assessed using non-modulus magnitude estimation. The perceived haze intensities were calculated as the geometric means of the 18 panelists' responses for each sample. Figs. 4–6 show the results for non-modulus ME for each particle size and color compared with turbidimeter measurements. While

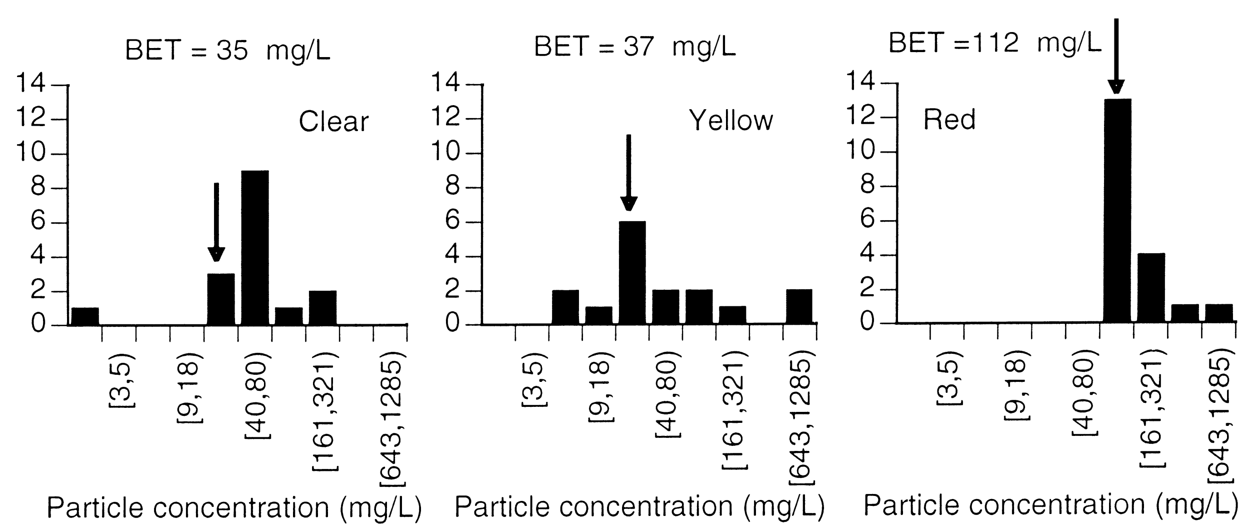

Fig. 1. Frequency distributions of individual thresholds (BETs expressed as weight concentration) for small (0.769 μm diameter) particles suspended in clear, yellow and red solutions. The positions of the arrows indicate the group thresholds.

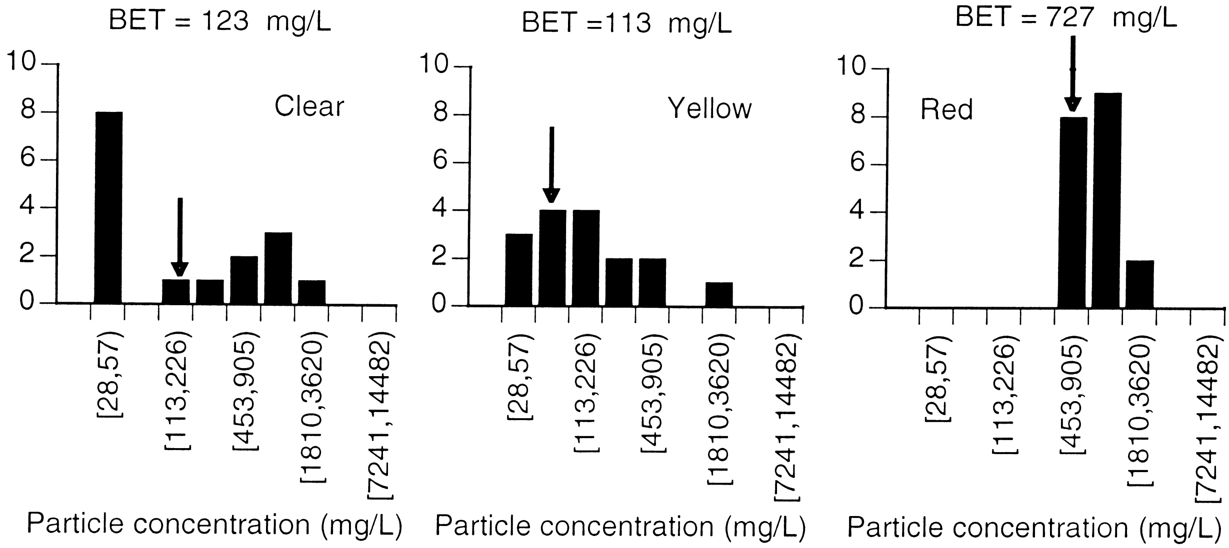

Fig. 2. Frequency distributions of individual thresholds (BETs expressed as weight concentration) for medium sized (2.600 μm diameter) particles suspended in clear, yellow and red solutions. The positions of the arrows indicate the group thresholds.

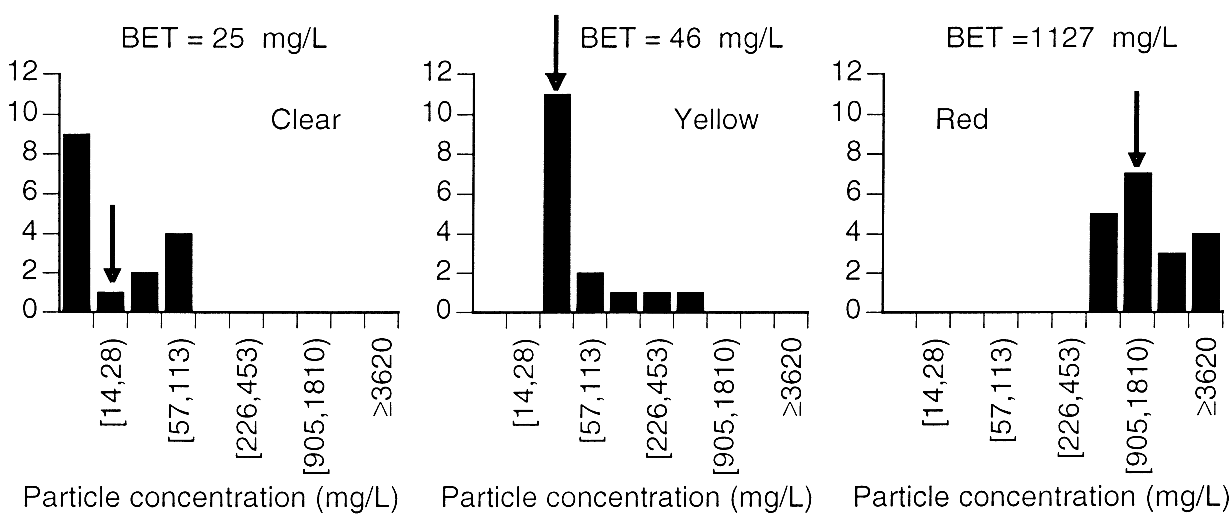

Fig. 3. Frequency distributions of individual thresholds (BETs expressed as weight concentrations) for large (10.300 μm diameter) particles suspended in clear, yellow and red solutions. The positions of the arrows indicate the group thresholds.

Table 6
Comparison of individual panelist responses for the same color/particle size conditions expressed as the ratio of the highest individual BET/lowest individual BET

	Threshold range	Average	Median
Weight concentration (μg/L)	4–128	49	16
Number concentration (particles/mL)	4–128	49	16
Turbidimeter result (NTU)	1.7–7.8	3.6	3.4

panel-determined haze intensities appeared to follow a negative exponential function versus concentration, the instrumental results followed a positive exponential function. This is a major and important difference between human perception and instrumental measurements. Panelist responses became saturated at high turbidity levels, but this did not occur with turbidimeter results. The panelist saturation level varied with particle size. For small particles (see Fig. 4) saturation occurred with particle concentrations between 0.1 and 0.15 mg/mL, corresponding to an instrumental turbidity of about 430 NTU. Saturation appeared at slightly higher particle weight concentrations for yellow and red suspensions compared to clear suspensions. For medium size particles (Fig. 5), panelist turbidity perception reached saturation at particle concentrations of about 0.3 mg/mL; this corresponded to a turbidity of about 400 NTU for clear samples and about 900 NTU for the yellow and red suspensions. For large particles (see Fig. 6), panelist saturation was not observed until particle

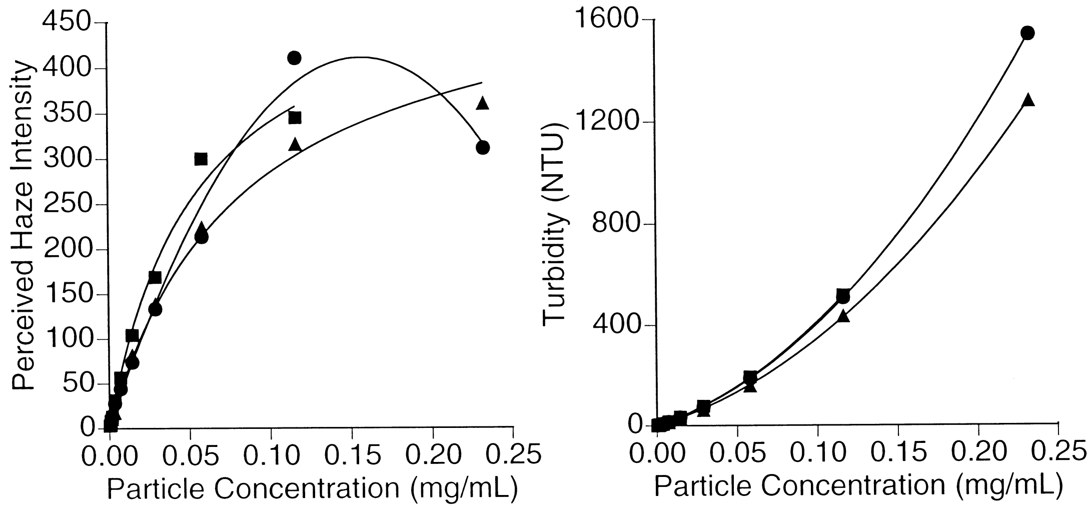

Fig. 4. Haze intensities (geometric means) perceived by sensory panelists using non-modulus magnitude estimation (left) and instrumentally measured turbidity (right) vs. particle concentration for small (0.769 μm diameter) particles in clear (■), yellow (●) and red (▲) liquids.

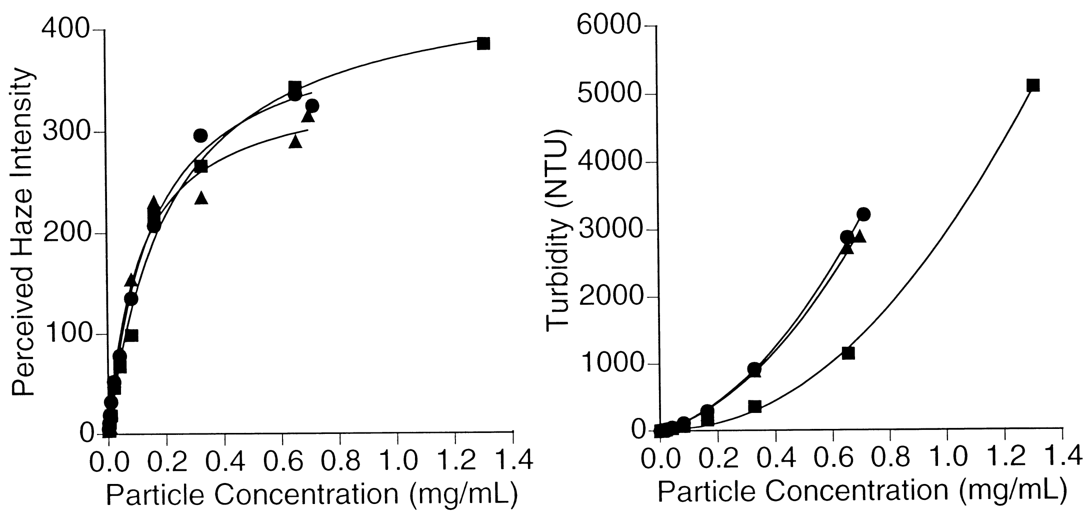

Fig. 5. Haze intensities (geometric means) perceived by sensory panelists using non-modulus magnitude estimation (left) and instrumentally measured turbidity (right) vs. particle concentration for medium (2.600 μm diameter) particles in clear (■), yellow (●) and red (▲) liquids.

concentration levels reached 0.6 to 0.7 mg/mL, corresponding to about 180 NTU.

3.2.2. *Modeling of perceived haze intensity results*

It was of interest to see if the perceived haze intensities could be predicted as a function of turbidimeter results or sample properties. Panelists used different scales to rate haze intensity, resulting in substantial inter-panelist variability. Normalization of the data applies an allowable multiplicative transformation (Moskowitz & Jacobs, 1988) that reduces unwanted variability by making the size of numbers similar across panelists. These authors wrote that statisticians like data to behave perfectly and that for magnitude estimation, statisticians find that often the scale values are neither perfectly normally nor log normally distributed. The magnitude estimates may not distribute log normally, even after normalization. While the Kolmogorov–Smirnov One-Sample Test showed that the turbidimeter and panel results obtained in this study were not normally distributed, the log transformed variables did not differ significantly from a normal distribution. As a result, further normalization was not applied. Multiple linear regression analysis was applied in an attempt to model the sensory perception and instrumental turbidity results as a function of particle concentration and size and solution color.

Haze intensity scores for each sample from 18 panelists were mathematically averaged and this result was modeled as a function of the averaged instrumental measurements or known sample properties (concentration or color or particle size). Color was coded as a

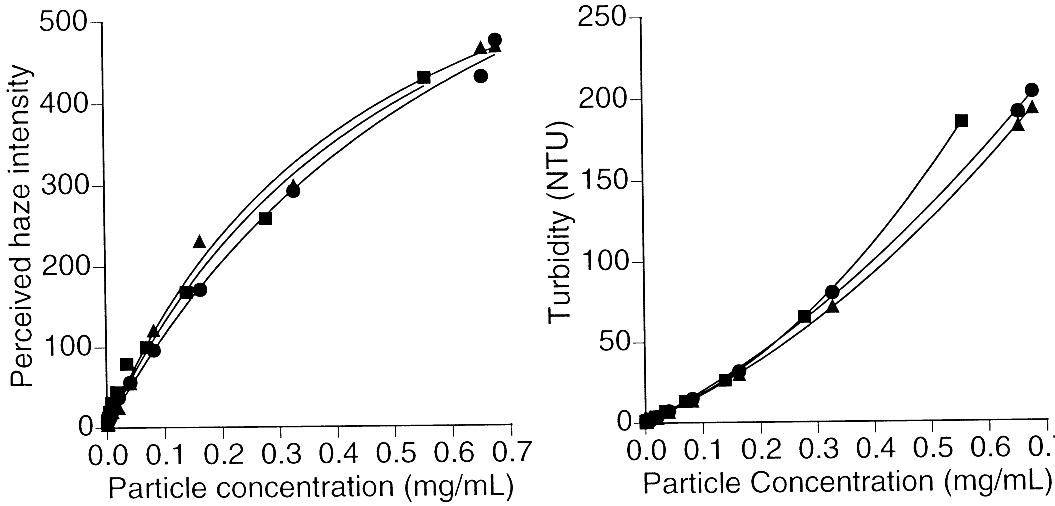

Fig. 6. Haze intensities (geometric means) perceived by sensory panelists using non-modulus magnitude estimation (left) and instrumentally measured turbidity (right) vs. particle concentration for large (10.300 μm diameter) particles in clear (■), yellow (●) and red (▲) liquids.

category variable (clear = 1, yellow = 2, red = 3). Multiple regression analysis was used to fit to linear, exponential, power, sigmoidal and polynomial forms. The R^2 values obtained from a number of the regressions were high but in some cases the plots of the predicted results versus residuals, normal probability, predicted versus observed results, and observed results versus residuals showed poor fits.

Stepwise multiple linear regression was used to select polynomial terms involving log (instrumental turbidity) to predict log (human sensory perception of intensity) for the entire data set including samples of all colors, particle concentrations and particle sizes. The equations were chosen to balance complexity (number of terms) against explanatory power (judged by R^2). The models that best explained the perceived intensity scores are shown in Table 7. Two terms, log(NTU) and $(\log(\mathrm{NTU}))^4$, were sufficient to produce a model between instrumentally measured turbidity and human estimated haze intensity with an R^2 of 0.870 [Eq. (a) in Table 7]. Fig. 7 displays the predicted vs. observed plot for this relationship. It is readily apparent that the data fall along two or possibly three different diagonal lines. The data for the large particles (▲) are all along the lower line while the small (■) and medium sized particle (●) results fall along two overlapping populations above this line. This pattern indicates that an additional factor influences the results. Addition of two size terms (size and size^4) [see Eq. (b)], improved the fit to $R^2 =$ 0.978 (see Fig. 8). This fit very well to a linear relationship between the predicted and observed values without any indication of separation by particle size. It is clear that particle size influences the relationship between instrumental and human perceived turbidity. Solution color either has no influence on the relationship between instrumental results and human perception or it influences both human and instrumental perceptions similarly.

When particle concentration, particle size, and solution color were used as independent variables, an equation (c) predicting log (human perceived haze intensity) was obtained ($R^2 = 0.949$) (Fig. 9). This equation has terms for both particle weight and number concentration; if the particle size and density are known, one of these can be estimated from the other. The data points for the small particle samples (squares) appeared to curve upward slightly at low haze intensity values while those for large particles (triangles) curved downward. This divergence from the fitted line appears mainly

Table 7
Best fit equations relating the log (perceived intensity) assessed by modulus (= 50) and non-modulus magnitude estimation to instrumentally measured haze and known sample properties for the whole data set (including small, medium and large particles suspended in clear, yellow, and red liquids)[a]

Formula	Non-Modulus	R^2
a	Log Int = 0.9192 + 0.8778 (Log NTU)–0.008636 (Log NTU)^4	0.870
b	Log Int = 0.7716 + 0.9341 (Log NTU)–0.007926 (Log NTU)^4–0.06788 (Size) + 0.000096 (Size)^4	0.978
c	Log Int = –0.7167 + 0.1189 (Size)–0.0197 (Color) + 0.3293 (Log Conc) + 0.4284 (Log NPart)	0.949

[a] Log Int = log (perceived haze intensity); Size = particle diameter in μm; Color = sample color where clear = 1, yellow = 2 and red = 3; Log Conc = log (μg/mL); Log NPart = log (number of particles/mL); Log NTU = log (turbidity measurement).

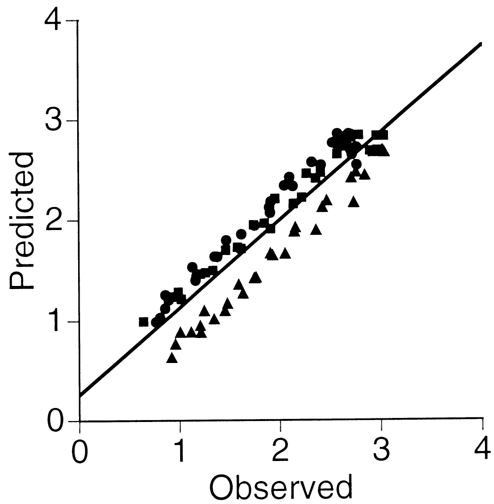

Fig. 7. The relationship of the observed log (perceived haze intensity) vs. that predicted from the turbidimeter result by Eq. (a) [Log Int = 0.9192 + 0.8778 (Log NTU)−0.008636 (Log NTU)^4]. $R^2 = 0.870$. Results from small (■), medium (●) and large (▲) particles.

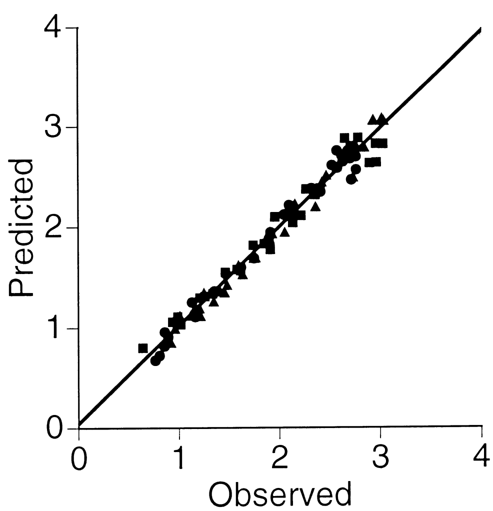

Fig. 8. The relationship of the observed log (perceived haze intensity) vs. that predicted from Eq. (b) [log Int = 0.7716 + 0.9341 (log NTU)−0.007926 (log NTU)^4−0.06788 (Size) + 0.000096 (Size)^4]. $R^2 = 0.978$. Results from small (■), medium (●) and large (▲) particles.

below an observed log (haze intensity) of 1.6. It should be noted that this point corresponds to 40 NTU [log (40) = 1.6], below which the turbidimeter employs direct 90° scattering and above which it uses a ratio of 90° scattering to the transmitted and narrow angle scattering. Ratio results should, and apparently do, correct for an effect of color. The samples that deviated from the line at lower hazes contained either large particles in clear solutions (△) or large (▽) or medium sized (⊙) particles in yellow solutions.

Taken together, the results indicate that human perceived haze can be predicted reasonably well from a turbidimeter result alone ($R^2 = 0.870$). If the size of the

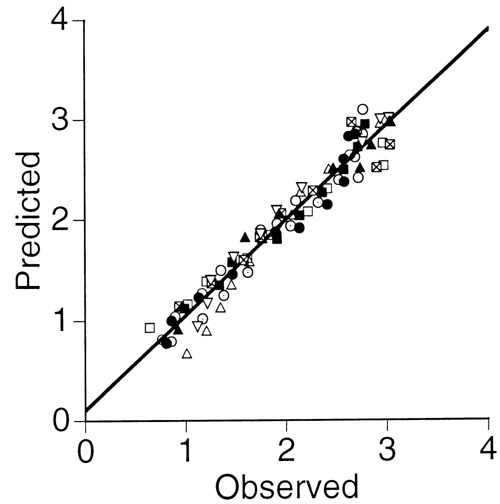

Fig. 9. The relationship of the observed log (perceived haze intensity) vs. that predicted from Eq. (c) [log Int = −0.7167 + 0.1189 (Size)−0.0197 (Color) + 0.3293 (log Conc) + 0.4284 (log NPart)]. $R^2 = 0.949$. Results from small clear (□), small yellow (⊠), small red (■), medium clear (○), medium yellow (⊙), medium red (●), large clear (△), large yellow (▽), and large red (▲) samples.

particles is known, however, the prediction can be substantially improved ($R^2 = 0.978$). The results also indicate that human perceived haze can be predicted from the particle weight and number concentrations, together with particle size and solution color ($R^2 = 0.949$).

Table 8 displays the formula (d) obtained with the turbidimeter result expressed as a function of particle size and concentration, see Fig. 10. This result indicates that the turbidimeter measurements were independent of the color of the samples, but were affected by the particle size and concentration ($R^2 = 0.986$). The turbidimeter was again used in its ratio mode (which gives a ratio of 90° scattering to some unspecified function of transmitted and narrow angle scattering above 40 NTU), which should theoretically correct for color differences. Since there was no need for a color term in the equation, it appears that this operates as designed. Note in Fig. 10 that at very low haze values, the predicted values tended to be lower than the observed results. All of the samples that deviated from the regression line contained large particles in clear (△) or yellow (▽) solutions, but the large particles in red samples did not exhibit this behavior. Presumably the deviation occurred because only a few of the samples without added particles had measured haze values less than 0.8 NTU [and log (0.8) = −0.1], while there were no limitations on the predicted values.

3.3. Sensory descriptive analysis

During the preliminary trials and the discussion phase of the Descriptive Analysis work the panelists agreed to use bipolar scales with antonyms (e.g. turbid/clear)

Table 8
Regression equation relating the log (instrumentally measured haze) and known sample properties for the whole data set (including small, medium and large particles suspended in clear, yellow, and red liquids)[a]

Formula	NTU with ratio ON	R^2
d	Log NTU = 2.5396 + 1.242 (Log Conc) + 0.1231 (Log Conc)^2–0.000303 (Size)^3 + 0.001855 (Log NPart)^3	0.986

[a] Log NTU = log (turbidity measurement); Size = particle diameter in μm; Log Conc = log (μg/mL); Log NPart = log (number of particles/mL).

anchoring each end. This is not the normal procedure for DA. Perhaps this particular situation (and maybe even visual perception in general) differs from classical DA in that there are not so many attributes (as in flavor, for example) and at least some are conceptually opposite (hazy vs. clear, particulate vs. uniform).

3.3.1. PCA on visual attributes

Principal Components Analysis (PCA) was performed on duplicate results obtained from the 14 panelists and on the same data after averaging. The two PCA treatments gave virtually identical results. In both analyses, the first factor explained approximately 68% of the variance in the data set and the second factor added about 30.6%, for a total variance explained of 98.3–99.1%. The fact that two PCs were so effective in explaining the variance in the data set indicates that the panel, in using the six descriptor terms, responded to two fundamental properties of the samples.

The PCA factor loading plot is shown in Fig. 11. Descriptors loaded heavily on the factor 1 axis were 'turbid', 'opaque' and 'glowing' in one direction, and 'dark' in the opposite direction. The locations of the terms 'opaque', 'turbid', and 'glowing' were superimposed, indicating a very high correlation (in this case all 0.98), and thus redundancy, between the terms. From the relationships between 'dark' and the rest of the descriptors located along factor 1, it can be seen that dark is highly negatively correlated with 'turbid', 'opaque' and 'glowing' (all had $r = -0.98$). This indicates that very turbid samples were perceived as 'light' rather than 'dark'; presumably this could be different with particles of darker colors. The particles here appeared white or slightly off-white and samples were viewed against a black background. Factor 2 was loaded almost entirely by two attributes, 'homogeneous' and 'particulate'. The 'homogeneous' descriptor had a very high negative correlation with 'particulate' ($r = -0.97$), as expected from the opposite directions for the two attributes along the factor 2 axis.

The PCA factor scores from the standardized and then averaged data were plotted for the three particle sizes in Figs. 12–14. Small and medium particle size suspensions were mostly defined by the first PC (i.e. they remained close to the horizontal axis in Figs. 12 and 13). The more turbid the samples were (particle

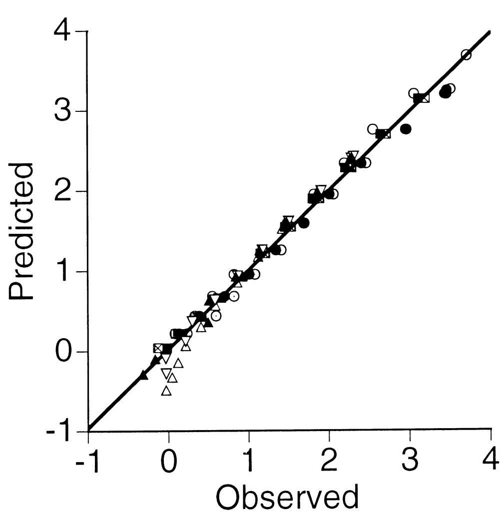

Fig. 10. The relationship of instrumentally measured haze [log (turbidity)] vs. that predicted from Eq. (d) [log NTU = 2.5396 + 1.242 (Log Conc) + 0.1231 (Log Conc)^2–0.000303 (Size)^3 + 0.001855 (Log NPart)^3]. $R^2 = 0.986$. Results from small clear (□), small yellow (⊠), small red (■), medium clear (○), medium yellow (⊙), medium red (●), large clear (△), large yellow (▽), and large red (▲) samples.

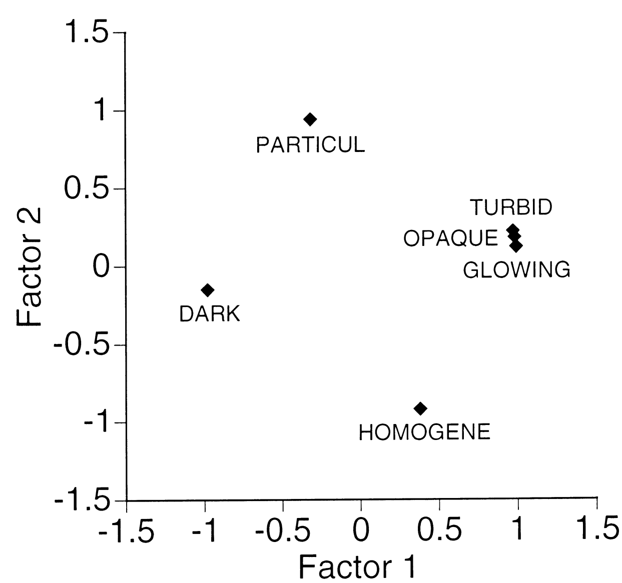

Fig. 11. Factor loadings from Principal Components Analysis of averaged descriptive analysis sensory data [rated on scales for Glowing, Turbid, Opaque, Particulate (Particul), Homogeneous (Homogene), and Dark].

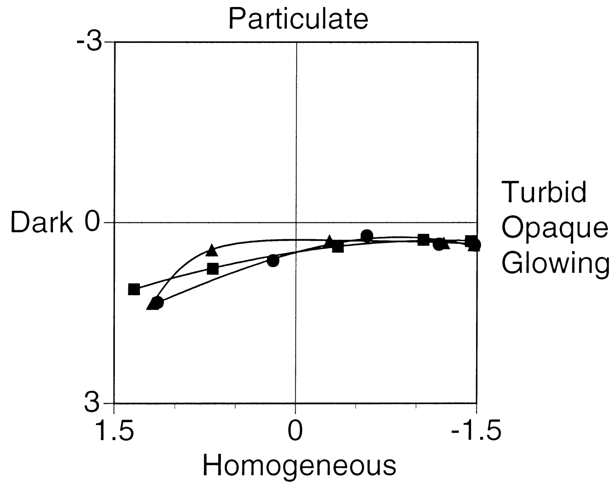

Fig. 12. PCA Factor scores for standardized, averaged sensory results for small (0.769 μm diameter) particles suspended in clear (■), yellow (●) and red (▲) solutions. Concentrations increase from left to right.

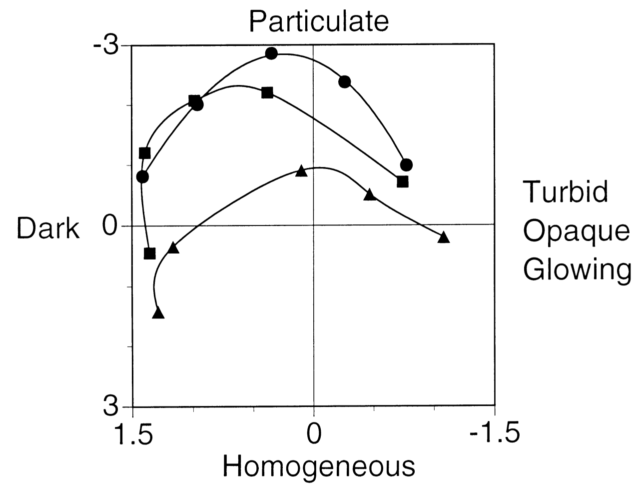

Fig. 14. PCA Factor scores for standardized, averaged sensory results for large (10.300 μm diameter) particles suspended in clear (■), yellow (●) and red (▲) solutions. Concentrations increase from left to right.

concentrations increase from left to right), the more ‘opaque’, ‘glowing’ and ‘light’ the samples were perceived to be. Large particle size suspensions also moved along the PC1 axis from left to right as they increased in concentration (Fig. 14); however, they showed movement on the PC2 axis as well. As turbidity increased from essentially none to modest, the samples were described as increasingly particulate (and non-homogeneous), but when the particle concentration increased further yet, the samples were described as less particulate and more homogeneous. All of the panelists responded in the same manner. This phenomenon was described by some panelists as the sense of seeing individual large particles or of seeing disturbances in the suspensions as a result of local conglomerations of particles. When samples were very turbid these conglomerates seemed to disappear and the samples again appeared homogeneous. Theoretically the unaided human eye cannot resolve 10 μm and so should not be able to directly perceive particles of this diameter. If it could, it should have been possible to see them also in the more dilute suspensions. Perhaps what happens is that the mass is randomly distributed, resulting in parts of the sample with momentarily higher localized particle concentrations and more intense scattering relative to other parts of the sample. With large particles at medium concentrations, the difference between the localized heavy and light concentrations would be accentuated compared with the same weight concentration of smaller particles. This situation may appear to the human observer as ‘particulate’ or ‘lumpy’. In general, the ‘particulate’ attribute was more difficult to perceive in the red colored suspensions compared to the yellow and clear suspensions.

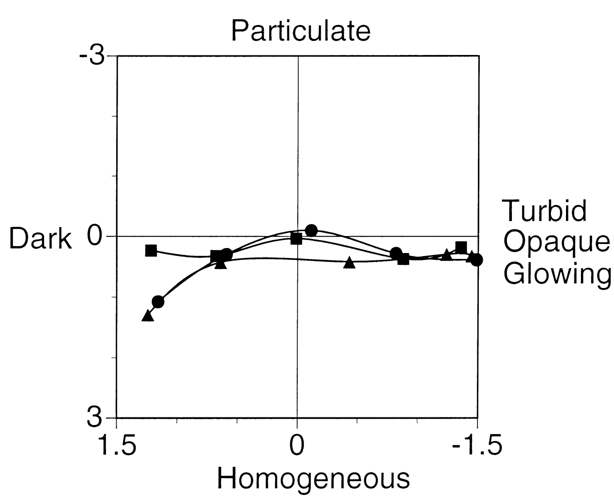

Fig. 13. PCA Factor scores for standardized, averaged sensory results for medium (2.600 μm diameter) particles suspended in clear (■), yellow (●) and red (▲) solutions. Concentrations increase from left to right.

The major conclusions from the Descriptive Analysis were that two components accounted for almost all visual attributes (98–99% of the total variance). ‘Homogeneous’ was opposite to ‘particulate’ in one dimension, while ‘dark’ was opposite to ‘glowing’, ‘opaque’ and ‘turbid’ in the other. Large particle size suspensions at medium concentrations were characterized as particulate, while small and medium particle size suspensions were uniformly characterized as homogeneous. Samples were judged more turbid with increasing particle concentration for all sample colors and particle sizes.

4. Practical implications

Producers of clear products are primarily interested in the threshold of haze perception, where the unaided eye of a consumer can just perceive turbidity. The Ascending Method of Limits gave visual haze perception threshold results that were consistent within and between panelists and appeared to be quite suitable for application to visual perception. The threshold results were influenced somewhat by particle size and solution color, but it can be seen that a simple (non-ratio) scattering measurement made with white light at an angle of 90° to the incident light beam gives a good indication of what can be seen with the naked eye regardless of the sample color or haze particle size. The observed thresholds were much lower than the few anecdotal estimates in the literature, and, under good viewing conditions, were in the range of 0.38–0.82 NTU (this corresponds to 0.09–0.19 EBC units or 2.4–5.3 nephelos units).

Producers of cloudy products are mainly concerned with the suprathreshold turbidity region. Human perceptions assessed by non-modulus Magnitude Estimation and 90° instrumental measurements (in the non-ratio mode below 40 NTU and in the ratio mode at or above 40 NTU) agree at lower particle concentrations, but diverge at higher levels (>200 NTU) due to the saturation of the human response. Equations were developed that successfully modeled the relationships between the nature of the sample and human and instrumental responses. Particle size influenced these relationships. There was essentially no effect of solution color when the turbidimeter was operating in the ratio mode, but small deviations were associated with sample color in the direct 90° mode.

The overall visual appearance of cloudy samples (also in the suprathreshold region) includes both the haze intensity and the degree of granularity (non-uniformity). As with Magnitude Estimation, panelist response on the clear-cloudy axis increased as a smooth function of particle concentration. Non-homogeneity was observed only with larger particles at intermediate concentrations. With both lower and higher particle numbers or with the small and medium sized particles, the appearance was homogeneous.

5. Future work

A number of interesting aspects remain to be investigated. These are the effects of additional solution or particle colors, perceptions of samples with particles of two or more sizes rather than monodisperse systems, and the effects of viewing with different backgrounds (e.g. white or gray in comparison to black) and types of illumination (sidelighting, backlighting, etc.)

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