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Refractometry for Quality Control of Anesthetic Drug Mixtures

Jennifer M Stabenow, Mindy L Maske, and George A Vogler

Injectable anesthetic drugs used in rodents are often mixed and further diluted to increase the convenience and accuracy of dosing. We evaluated clinical refractometry as a simple and rapid method of quality control and mixing error detection of rodent anesthetic or analgesic mixtures. Dilutions of ketamine, xylazine, acepromazine, and buprenorphine were prepared with reagent-grade water to produce at least 4 concentration levels. The refraction of each concentration then was measured with a clinical refractometer and plotted against the percentage of stock concentration. The resulting graphs were linear and could be used to determine the concentration of single-drug dilutions or to predict the refraction of drug mixtures. We conclude that refractometry can be used to assess the concentration of dilutions of single drugs and can verify the mixing accuracy of drug combinations when the components of the mixture are known and fall within the detection range of the instrument.

Anesthetic mixtures incorporating ketamine, xylazine, and acepromazine are used often for rodent and rabbit anesthesia.[5,10] These combinations are not commercially available and are formulated and mixed locally according to published and anecdotal information, experience, and personal preference. Potent drugs such as buprenorphine are often diluted to increase the accuracy and convenience of administration.[6] Unanticipated or unusual responses to a 'standard' dose of anesthetic or analgesic do occasionally occur, and it is useful in the course of investigating these incidents to be able to quickly ascertain whether the drug mixture was compounded correctly.

Most analytical methods for assessing dilution and compounding accuracy are time-consuming and require technical expertise and instrumentation not readily available in many laboratory animal facilities. By contrast, refractometers are comparatively simple and rapid to use. Small handheld instruments are used frequently in veterinary medicine to determine urine specific gravity and serum total protein. Refractometers also are used in industrial and laboratory applications as a sensitive and rapid means of assessing solute concentration.[8] With few exceptions, the refraction of a solution varies directly with the solute concentration. These instruments take advantage of the fact that the speed of light varies with the density of the medium through which it passes. The velocity of light is greater in air than in water and, as light passes from air into water, it slows, and its path is deflected (refracted). Using a series of prisms, refractometers detect and measure the refraction of light, comparing the velocity of light in one medium to that in another.[7] Laboratory and research instruments display the ratio of the 2 velocities as the index of refraction, a dimensionless number. Because the index of refraction can vary with temperature and with the wavelength of light, both of these variables are controlled carefully in research and analytical instruments, making them capable of very high precision and accuracy.

Small, handheld refractometers are made for a variety of specialized uses. Although they are less sophisticated than research or analytical instruments, these small refractometers are considerably less expensive, easier to use, and sufficiently accurate and precise for a wide range of purposes. Knowledge of the refractive properties of a material allows the construction of specialized scales for direct determination of concentration.[9] Hence, many clinical refractometers have scales that indicate the concentration of serum protein or urine specific gravity. Some instruments also include a scale for refraction, defined as the difference between the index of refraction of water and that of the solution being examined. Automatic temperature compensation contributes significantly to accuracy and reproducibility and is a feature of many, but not all, clinical instruments. More recently, small handheld digital refractometers have been marketed that offer improved accuracy, precision, and measurement range compared with those of traditional handheld instruments.

Clinical refractometers have been used by hospital pharmacies to detect illicit diversion of some controlled drugs, such as midazolam, morphine sulfate, and meperidine hydrochloride, as determined by the refractive properties of the solution.[1-4] Building on these reports, we investigated the use of refractometric methods for quality control of drug mixtures prepared in-house that contain anesthetic and analgesic agents.

Materials and Methods

Instrument. A temperature-compensated clinical refractometer with an illuminated table stand was used for analysis (Figure 1). The instrument has scales for urine specific gravity, protein concentration, and refraction. Readings were taken with the refraction scale to allow for an extended range of measurement. Before each series of analyses, the instrument zero point was verified with reagent-grade water, according to the manufacturer's instructions.[9]

Drugs. The drugs selected for evaluation in this study are used often in our facility in either combination or diluted form. The following drugs were used: ketamine HCl (100 mg/ml; Ketaset, Fort Dodge Animal Health, Overland Park, KS); xylazine HCl (20 and 100 mg/ml; AnaSed, Lloyd Labs, Shenandoah, IA); acepromazine maleate (10 mg/ml; Boehringer Ingelheim Vetmedica, St Joseph, MO); and buprenorphine HCl (0.3 mg/ml; Reckitt and Colman, Hull, England).

Single-drug dilutions. Single-drug dilutions of the listed drugs were prepared to assess the refractive properties of the

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Saint Louis University School of Medicine, Department of Comparative Medicine, St Louis, Missouri.
**Corresponding author. Email: stabenow@slu.edu*

Figure 1. Refractometer used in this study.

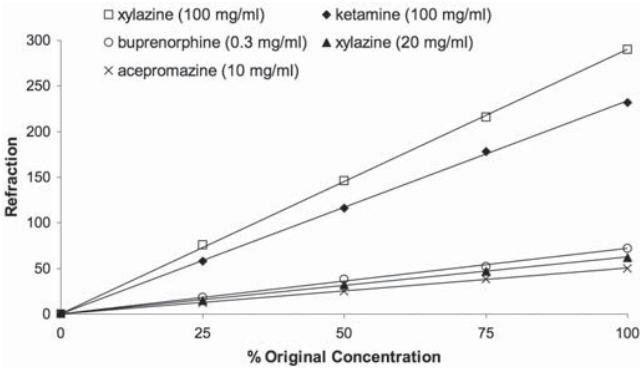

Figure 2. Effect of dilution with reagent-grade water on the refraction of various anesthetic and analgesic agents.

individual drugs. All of the drugs examined in this study were manufactured in aqueous solutions, which allowed the use of reagent-grade water as the diluent without alteration of the matrix of the compound. The refractometric properties of each drug were examined by use of the undiluted (stock) drug to establish maximal refraction (100% point) and reagent-grade water for minimal refraction (zero point). Adjustable-volume, positive-displacement precision pipettes (Scientific Manufacturing Industries, Emeryville, CA) were used to make primary dilutions of the drug. Dilutions were made using reagent-grade water as the diluent, and a minimum of 3 dilutions (75%, 50%, and 25%) according to the labeled concentration of the undiluted stock drug were made. One to two drops (approximately 100 µl) of each dilution was placed in the sample area of the refractometer, and the refraction of each dilution was obtained. The result of each reading was added to a graph generated by using a commercial spreadsheet program (Excel, Microsoft, Redmond, WA) in which the percentage of the stock drug in the dilution was plotted along the X axis and the refraction was plotted along the Y axis. A trend line then was analyzed for linearity (Figure 2).

Drug mixtures. Drug mixtures commonly used in our facility were examined to determine whether the refractive properties of each drug were additive. From data generated in the previous section, the refractive contribution of each drug at its final concentration was calculated. These values then were added, yielding the predicted refraction of the final mixture. Table 1 illustrates the method used for calculating refraction of a drug mixture.

The calculations in Table 1 were performed as follows.

Stock drug concentration is the undiluted labeled concentration of each drug component.

Volume added is the volume, in ml, of each component added to the mixture.

Drug weight is the weight of each component added to the mixture and is calculated as: (volume of drug added) × (stock drug concentration). For example, 2.25 ml ketamine (concentration, 100 mg/ml) was added to the mixture (Table 1). The drug weight of this component is: (2.25 ml) × (100 mg/ml) = 225 mg.

Final concentration is the concentration of each component in the final mixture and is calculated as: (drug weight) / (final volume of drug mixture). In Table 1, 225 mg ketamine was added to the mixture, with a final mixture volume of 6.0 ml. The final concentration of ketamine in this example is: (225 mg) / (6 ml) = 37.5 mg/ml.

The *calculated refraction of each component* was calculated from the data obtained from the single-drug dilutions (Figure 2). The graphs generated in Figure 2 were used to calculate the slope of the linear regression line, and the slope then was used to calculate the refraction of each component at various concentrations. In the equation used to calculate slope, $y = mx + b$, y is refraction, m is slope, x is drug concentration, and b is the y-intercept. Reagent-grade water was used as the diluent in these trials, resulting in a y-intercept (b value) of 0. The slope from Figure 2 for ketamine was 2.3382 (data not shown). Using this value, the refraction of ketamine in the mixture in Table 1, where the concentration of ketamine in the final mixture is 37.5 mg/ml, is calculated as: $y = (2.3382 \times 37.5) + 0 = 88$. Therefore the calculated refractive contribution from the ketamine component in this drug mixture is 88. Note that if a diluent other than water is used, its refractive contributions must be factored into the equation.

The *calculated refraction of final mixture* is the sum of the refraction of each component at its concentration in the final mixture. The mixture in Table 1 shows that ketamine is calculated to add 88 to the refraction of the mixture, xylazine (20 mg/ml) will add 16, and water will add 0. The sum of the refractive contributions of these components is 104, which is the calculated refraction for this mixture. Using the method described above, the calculated refraction was compared against actual refraction for various drug mixtures.

Error detection. Drug mixtures were evaluated with the refractometric method to determine whether mixing errors could be detected. Test compounds representing 4 types of mixing errors were prepared and refraction values obtained. Incorrectly formulated mixtures prepared by departmental personnel normally responsible for drug mixture preparation were interspersed with correctly prepared mixtures, and presented for refractometric analysis as the 'correct formulation' described in Table 2. Actual measured refraction of the mixtures was compared with the calculated refraction of the correct mixture, as described in the previous section.

Table 1. Method for calculating refraction of a drug mixture

Drug	Stock concentration (mg/ml)	Volume added (ml)	Drug weight (mg)	Final concentration (mg/ml)	Refraction
Ketamine	100	2.25	225	37.5	88
Xylazine	20	1.50	30	5.0	16
Water	0	2.25	0	0	0
Total volume		6.00	Calculated refraction		104

Table 2. Refractometric detection of mixing errors

Component	Correct formulation		Error 1 Ratio inversion		Error 2 Drug omission		Error 3 Diluent omission		Error 4 Wrong concentration	
	ml	R	ml	R	ml	R	ml	R	ml	R
Ketamine (100 mg/ml)	2.25	88	1.5	58	0	0	2.25	140	2.25	88
Xylazine (20 mg/ml)	1.5	16	2.25	24	1.5	25	1.5	25	0	0
Xylazine (100 mg/ml)	0	0	0	0	0	0	0	0	1.5	73
Water	2.25	0	2.25	0	2.25	0	0	0	2.25	0
Final volume	6.00	—	6.00	—	3.75	—	3.75	—	6.00	—
Total refraction	—	104	—	82	—	25	—	165	—	161

The correct formulation is shown in the 1st row, with the correct volume of each component added and the corresponding refraction (R) of each component contribution. The sum of the R of each formulation is shown at the bottom of the columns.

Results

Single-drug dilutions. For all drugs examined, the refraction varied linearly with concentration in the ranges tested (Figure 2). The coefficient correlation (r^2) value of each drug is as follows: xylazine (100 mg/ml), $r^2 = 0.9997$; ketamine (100 mg/ml), $r^2 = 0.9997$; buprenorphine (0.3 mg/ml), $r^2 = 0.9975$; xylazine (20 mg/ml), $r^2 = 0.9995$; and acepromazine (10 mg/ml), $r^2 = 0.9997$. According to the r^2 values, refraction is an accurate measure of concentration in single-drug dilutions.

Drug mixtures. Figure 3 illustrates the measured refraction compared with the calculated refraction, determined with the formula described in Table 1, for 10 independent drug preparations. The actual refraction measured for each drug mixture was within 2 units of the calculated refraction, which is within the error limit claimed by the manufacturer.[9] The refraction of a drug mixture is the sum of the refraction of each component at the concentration present in the final mixture. This additive nature of the refractive properties allows for accurate prediction of refraction of the final mixture.

Error detection. The actual measured refraction of each mixing error shown in Table 2 was within 2 units of the calculated refraction (data not shown). Each of the tested erroneous mixtures was clearly detectable as differing from the expected value for a reference mixture.

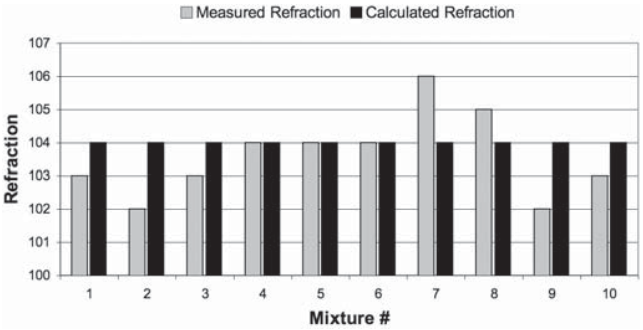

Figure 3. Measured refraction compared with calculated refraction for 10 preparations of formula mixture described in Table 1.

Discussion

Refractometry is an old and well-established technique to determine solute concentration.[4,9] The application of refractometry to detect tampering with controlled substances is also well documented.[1-4] In this report, we demonstrated the use of the technique as a means of quality control and error detection for injectable anesthetic and analgesic mixtures used in laboratory animals.

For the drugs we examined, refraction varied linearly with concentration, as expected, and provided a reliable means of establishing concentration in single-drug dilutions. Further, we confirmed that the refraction of each component of a drug mixture was cumulative at the concentration present in the final mixture. The additive nature of the refractive properties allows for accurate prediction of the refraction of a drug formulation. In light of these findings, the ability of refractometry to identify incorrectly compounded mixtures was substantiated: refractions for the incorrectly formulated mixtures described were clearly different from that of the correct formulation. However, it was also apparent from the relative similarity of the refractions resulting from diluent omission (error 3) and the use of a wrong concentration of xylazine (error 4) that refractometry cannot be depended upon to identify the nature of the mixing error (Table 2).

In using refractometry as a means of quality control, it is important to consider that the refractive properties of injectable anesthetics or analgesics cannot be attributed to the drug alone; rather they are the cumulative result of all of the solutes in the manufactured drug, including buffers, preservatives, and so on.[2] To this extent, the accuracy of the method depends upon the accuracy of the drug label. It is also worth remembering that, because of differences in compounding, drugs with the same concentration of active agent but from different manufacturers may well have different refractive properties. Although we have found little lot-to-lot variation in the refractive values of drugs from the same manufacturer, it is prudent to periodically confirm the refraction of the stock drug. All the scales that appear on refractometers are based on the index of refraction. In measuring solute concentration it is not important which scale is used, as long as it encompasses the full range of dilutions needed to establish a standard curve. The increment in concentration for each scale unit can then be determined and an acceptable degree of variation for drug dilutions can be assigned. According to

the refraction scale of our instrument, values within 2 refraction units of the calculated value for single-drug dilutions will fall within 4% of the target concentration for most drugs.

In preparing the drugs for laboratory analysis, we used reagent-grade water. However, when preparing drug mixtures for clinical use, we use either preservative-free sterile water for injection (USP) or 0.9% sodium chloride injection (USP). The refraction of reagent-grade water and sterile water for injection USP is the same. When 0.9% sodium chloride injection is used, its refractive contribution is included.

There are some limitations to this method. The components used in the mixture, whether drugs or diluents, must be known and consistent, and reference values must be established for the mixture. For very potent drugs, including opioids such as fentanyl, which are compounded in very low concentrations, a clinical refractometer may not be sufficiently sensitive to distinguish the sample from pure water. Some other materials, such as ethanol, do not behave in a linear fashion, so that it may not be feasible to establish a standard curve. Finally, the method does not definitively identify an unknown drug or drug mixture. Within these limitations, however, the method provides an inexpensive and rapid method of quality control and mixing error detection.

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