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Colorimetric strip tests: a comparison of visual and reflectometric measurements for quantitative applications

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Abstract

The use of indicator-based colorimetric strip tests for measuring chromate and nitrate in spiked and waste water samples was validated. Test strip readings made by reflectometry were significantly more accurate and reproducible than those made by a visual comparisons to a reference color chart. Each of the analytes was consistently detected at 1 to 2 µg/ml, using the reflectometer. There was an excellent correlation between the analytical results obtained with the strip tests and those obtained using the more traditional laboratory methods of inductively coupled plasma/mass spectrometry and ion chromatography. This study shows that the use of strip tests for field screening, and as a prescreen in the laboratory, can be a time- and cost-effective alternative or adjunct to currently used laboratory methods.

1. Introduction

The magnitude of the problems associated with environmental monitoring and waste management has signaled a need for the use of analytical methods that are more rapid and cost effective than those currently being used [1–3]. As part of a program to validate such methods for the Department of Energy (DOE), a number of indicator strip-based colorimetric tests have been evaluated [4–8]. Colorimetric strip tests are undoubtedly the fastest and least expensive analytical methods available. They can be used to monitor a wide variety of analytes. Despite the apparent advantages, there appears to be only limited usage of strip tests for environmental monitoring and waste management.

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Evaluations of strip tests for nitrate and chromate in water are presented in this paper. Both spiked and waste water test samples were used. Test strips were read by asking individuals to make visual comparisons to a reference color chart (visual readings) and by the use of a reflectometer (reflectometric readings). Comparisons of the reproducibility and accuracy of visual readings versus reflectometric readings were made. The accuracy of the strip test methods was assessed relative to more traditional laboratory methods for analyzing these analytes, i.e., inductively coupled plasma/mass spectrometry (ICP/MS) [9] and ion chromatography (IC) [10].

2. Materials and methods

The general procedure used to evaluate the strip test methods was to prepare, or obtain, spiked and waste water samples containing the analytes over a range of concentrations. Waste water samples that were found to be outside the strip test range were appropriately diluted prior to analysis. Replicate samples at each analyte concentration were tested and the strips read either by visual comparison to a reference color chart or with a reflectometer using a preprogrammed concentration curve. Results obtained for chromate and nitrate analyses were compared with those determined by ICP/MS and IC, respectively.

2.1. Test strips

All the test strips used in this study were manufactured by E. Merck, Darmstadt, Germany, and were supplied by E.M. Science, Gibbstown, NJ, USA.

Chromate analysis

The Merckoquant® 10012 chromate test strips were used when visual readings were made and the Reflectoquant® System 16988 was used when the reflectometer was used to read the strips. The two types of test strips differ only in that the System 16988 test strips have two reaction zones, instead of one, to adapt them for being read in the reflectometer. The programmed concentration curve for the System 16988 is 0 to 45 µg/ml chromate. The Merckoquant® 10012 test color chart consists of different hues of red–violet, corresponding to 0 to 60 µg/ml chromate.

The chromate color test is based on a redox reaction between chromate ions and diphenylcarbazide. Chromate ions oxidize diphenylcarbazide in acidic solution to produce diphenylcarbazone. The Cr^{3+} ions formed in this reduction react with the enolate form of diphenylcarbazone in a 1:1 molar ratio to produce a colored complex. In practice, the pH of the test sample is adjusted to <1 with sulfuric acid, then the reaction zone of the test strip is wetted in the test sample, removed, and 'blotted' to remove excess liquid. The reaction zone remains white in the absence of chromate and changes to varying shades of red–violet in the presence of chromate.

Nitrate analysis

The Merckoquant® 10020-1 nitrate test strips were used when visual readings were made. The Reflectoquant® Systems 16995 and 16971 were used when reflectometric

readings were made. The reaction zones of the test strips from each of the three test systems are identical. The Systems 16995 and 16971 test strips are different from the 10020-1 test strips in that they have two identical reaction zones to facilitate their reading in the reflectometer. Systems 16995 and 16971 differ from each other only in the barcode strips provided for programming the reflectometer. The two barcodes permit the instrument to be programmed with either a low range (0–90 μg/ml nitrate – System 16995) or a high range (0–225 μg/ml nitrate – System 16971) concentration curve. The color chart of the Merckoquant® 10020-1 test provides incremental red–violet reference hues corresponding to a range of 0 to 500 μg/ml nitrate.

The nitrate color test is based on a series of reactions starting with the reduction of nitrate to nitrite. In the presence of an acid buffer the nitrite is then converted to nitrous acid which diazotizes an aromatic amine (sulfanilic acid). The diazotized sulfanilic acid couples with *N* [naphthyl(1)]-ethylenediamine to produce a red–violet azo dye. In practice, the reaction zone of the test strip is wetted in the test sample, removed and 'blotted' to remove excess liquid. The pale yellow-colored reaction zone changes to white for negative samples and to varying shades of red–violet in the presence of nitrate.

Potential interferants are discussed in the instructions included with each strip test kit. Information on how to identify and/or remove interfering substances is also included in the instructions and in the Merck Rapid Test Handbook [11].

2.2. Reflectometer

The reflectometer used in these studies was the Merck RQflex, obtained from EM Science. The RQflex is a small ($19 \times 8 \times 2$ cm; 275 g), push-button controlled reflectometer. The light source is 4 LEDs producing wavelengths of 570 and 657 ± 10 nm and uses double optics. The instrument is capable of making over 1000 measurements using 4 AAA batteries as the power source. All necessary information to run the instrument is communicated via a barcode strip. This includes analyte-specific reaction times, wave length corrections and reference curves for concentration. Periodically, the instrument is blanked by the use of a barcode for calibration and a white standard calibration strip. The instrument can be preprogrammed, using separate barcodes, for up to 5 different methods (analytes). Up to 50 measurements for each method, with time and date, are stored in memory. Data in memory can be transferred to a printer or PC via an available software program. The RQflex is designed to operate in humidity below 90% and at temperatures between 5 and 40 °C.

The RQflex was used according to instructions in the Merck RQflex manual. Important features in the setup of the instrument are as follows: (1) *Setting date and time* – This is important so that measurement data stored in memory can be identified. (2) *Blank recalibration* – The instrument should be periodically recalibrated with the blank, white standard test strip. (3) *Coding* – The instrument should be coded, or recoded, when loading a new method for a different analyte or concentration range. Coding involves (a) choosing the barcode that is specific for the lot of Reflectoquant®

test strips being used, (b) choosing a test number to correspond to the new code being stored, and (c) inserting the barcode, repeating if necessary, until the coding process is successfully completed.

In practice, a test strip is read by inserting it into the strip adapter of the RQflex. The average of the percent reflectance of both reaction zones is determined, relative to a white standard blank strip. The results are calculated using the programmed reference concentration curve, stored in memory and displayed on the screen in μg/ml (ppm).

2.3. Water samples for test

Both spiked and waste water samples were used in this study. Spiked samples were made by diluting stock solutions of either sodium chromate or sodium nitrate, at nominal concentrations of 1000 μg/ml CrO_4^{2-} or NO_3^-, into simulated ground water (SGW). SGW is deionized water to which 0.165 g/l of sodium chloride and 0.148 g/l of sodium sulfate has been added (contains 100 μg/ml each of SO_4^{2-} and Cl^-) [12]. Chromate-containing waste water samples were taken from a metal-plating operation and had been treated by reverse osmosis as a method of extracting and concentrating the chromate from the waste stream. Fractions for each sample set included FEED – the untreated waste stream, PERM – the permeate from the treatment and CON – the concentrate from the treatment. Three sample sets, a total of nine samples, were analyzed by ICP/MS and by the strip test for comparison of the methods. Nitrate-containing waste water samples were collected from either seepage or sampling wells around a solid waste storage area on the Oak Ridge DOE reservation. Six samples were analyzed by IC and by the strip test for methods comparison. The reference concentrations of chromate and nitrate in the stock solutions and in the waste water samples were determined by ICP/MS and IC, respectively.

2.4. Procedures for chromate testing

Visual readings

Spiked test samples were made by diluting the stock solution of sodium chromate to concentrations between 0.5 and 50 μg/ml. One-ml volumes of the spiked samples were tested in duplicate. Four blank, or negative, samples were tested. The samples were acidified to a pH of <1 with 5 drops (0.143 ml) of the reagent, sulfuric acid. Samples were randomized and assayed. The test strips were briefly immersed in the test solutions, and excess liquid was removed by wiping the test strip against the test tube rim. After 15 s, the color of the reaction zone was compared to the reference color chart. Five analysts independently read the test strips and recorded the concentrations with reference to the color chart. The chromate concentrations defined by the color chart are 0, 3, 10, 30 and 100 μg/ml. Assignments between those values were also made. To evaluate the sensitivity and accuracy of the method, numerical values were assigned to the results obtained from each strip as follows: a reading of 10 μg/ml was given the value 10, a reading of between 10 and 30 was given the value 20, and so on. Because of the volume of acid used in the sample preparation, the concentration

values were corrected for dilution by a factor of 1.14 (see comments below on the differences in the preparation of chromate test samples for the Merckoquant® and the Reflectoquant® tests). Test results were compared with those obtained from the ICP/MS analysis of the stock chromate solution and with those obtained by reflectometric readings of the test strips.

Reflectometric readings

Spiked test samples were made by diluting the stock solution of sodium chromate to concentrations between 0.25 and 40 µg/ml chromate. Two-ml volumes of the spiked samples were tested in duplicate. Four blank, or negative, samples were tested. Samples were acidified with two drops of the reagent, sulfuric acid. Samples were assayed by immersing the test strips briefly in the test solutions. Then excess liquid was removed by shaking, or blotting, the test strip; and the reaction timer on the RQflex was started. After 30 s, the strip was inserted into the strip adapter of the RQflex for reading. After five additional seconds, the measurement result was displayed on the screen and automatically stored in memory.

Waste water samples were diluted with SGW, if necessary, so that the concentration would fall within the concentration range of the RQflex. This was readily done by visually comparing the intensity of the samples' yellow color with a standard solution. Duplicate two-ml volumes of each of the waste water samples, and four blank samples, were tested as described above for the spiked samples.

The test results obtained for the spiked samples were compared with those obtained by visual readings and with the results based on ICP/MS analysis. The results obtained for the waste water samples were corrected for sample dilution, if necessary, and compared with those determined by ICP/MS.

It should be noted that the manufacturer's recommendations for test sample treatment were different for the Merckoquant® (visual readings) and the Reflectoquant® (reflectometric readings) chromate strip tests. Instructions for the Merckoquant® kit call for the test sample to be acidified to a pH below unity with the sulfuric acid reagent (25%). For samples in SGW this required five drops of acid per ml of sample. The Reflectoquant® instructions require the addition of only one drop of acid per ml of sample. Although the final pH values for the samples were significantly different, it was empirically determined that the test results were the same regardless of which acidification protocol was used, provided the sample dilution that occurred in the Merckoquant® test was taken into account.

2.5. Procedures for nitrate testing

Visual readings

Spiked test samples were prepared by diluting the stock solution of sodium nitrate to concentrations between 5 and 250 µg/ml nitrate. One-ml volumes of the spiked samples were analyzed in triplicate. Six blank, or negative, samples were tested. No preparation is required for nitrate-containing test samples that are in the pH range 1–12. The samples were randomized and tested by immersing the test strips briefly in the test solution and removing excess liquid by wiping the test strip against the test

tube rim. After the color formed, four analysts independently read the strips and recorded the concentrations with reference to the color chart. The nitrate concentrations defined by the color chart are 0, 10, 25, 50, 100, 250 and 500 ppm. Increments between these values were also used. In order to evaluate the accuracy of the method, numerical values were assigned to the results obtained from each strip as follows: a reading of 50 ppm was given the value 50; a reading of between 50 and 100 was given the value 75 and so on. Results were compared with those obtained by IC analysis of the stock nitrate solution and with those obtained by reflectometric readings.

Reflectometric readings

Spiked test samples were made by diluting the stock solution of sodium nitrate to concentrations between 2 and 250 µg/ml nitrate. Duplicate 2-ml volumes of the spiked samples were tested. Eight blank, or negative, samples were tested. Samples were assayed by immersing the test strips briefly in the test solutions. Excess liquid was shaken, or 'blotted', from the test strip and the reaction timer on the RQflex started. After 60 s, the strip was inserted into the strip adapter of the RQflex to be read. After five additional seconds, the measurement result was displayed on the screen and automatically stored in memory. Test samples up to concentrations of 90 µg/ml nitrate were tested using the low concentration range Reflectoquant® Systems 16995 test strips and barcode. Test samples in the entire range of 0 and 250 µg/ml nitrate were tested using the high concentration range Reflectoquant® Systems 16971 test strips and barcode.

The six waste water samples that were analyzed had nitrate concentrations in the range 0–66 µg/ml and were tested with the Reflectoquant® Systems 16995 test strips and barcode without dilution. At least four replicates of each waste water sample were tested as described above for the spiked samples.

The test results obtained for the spiked samples were compared with those obtained by visual readings and with the results based on IC analysis. The results obtained for the waste water samples were compared with those determined by the IC method.

3. Results and discussion

3.1. Chromate analyses

When the chromate test strips were visually read by several individuals, the average of these readings produced a linear response (Fig. 1). (Note that in this experiment all 20 readings for the blank samples were zero, thus all data points are superimposed as one; while each of the 10 readings for the positive samples were either one of two concentrations, thus all the data points are superimposed as two points.) However, there was substantial variation in the readings made by different individuals (Fig. 1). Statistical analysis of a similar set of data showed that over a range of about 2.5 to 50 µg/ml the coefficient of variation (CV) among six readers, at six different concentrations, varied between 20% and 50% (data not shown). Furthermore, a similar analysis showed that the CVs for readings of several different strips, used on the same
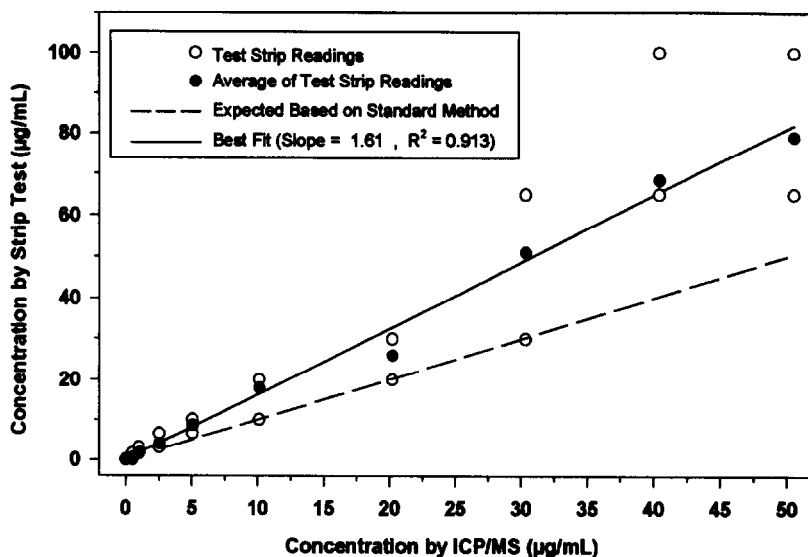
Fig. 1. Chromate strip test versus ICP/MS using spiked water samples and visual readings. Four blank samples and duplicate samples for each of nine different concentrations of spiked samples were tested. A single test strip was used for each test sample. Each test strip was read independently by five different analysts ($n = 20$ for blanks; $n = 10$ for each spiked sample).

sample, by a single reader, were similar to those obtained for multiple readers reading the same strip, i.e., in the 20–50% range.

The results shown in Fig. 1 also indicate that, on the average, the actual chromate concentration is significantly overestimated, the slope of the best fit line being 1.6. Several factors indicate that this was an artifact involving the color chart on the Merckoquant® 10012 test kit. First, analysis of chromate-spiked water samples in the same concentration range as shown in Fig. 1, using the Reflectoquant® System 16988 test strips and the RQflex reader, produced a best fit line with a slope of 1.2 (Fig. 2). Second, the Reflectoquant® System 16988 test strips and the Merckoquant® 10012 test strips gave identical colors when compared using the same chromate solutions. And finally, readings made on the same test strips in the RQflex and with reference to the color chart were consistently indicated to correspond to a higher concentration when using the color chart.

The data in Fig. 2 show that the accuracy and reproducibility of reading colorimetric test strips was dramatically increased by using the reflectometer. The R^2 value of greater than 0.99 indicates a strong linear association between the RQflex measurements and the ICP/MS measurements. The slope of 1.2 indicates that the strip test is still slightly overestimating the chromate concentration in the samples. However, it should be pointed out that the reference methods of ICP/MS and IC used in this study are not infallible, as will be discussed below in the case of nitrate contaminated waste water. It is obvious from a comparison of Figs. 1 and 2 that reflectometric readings are much less variable than human readings, as could be expected. CVs for
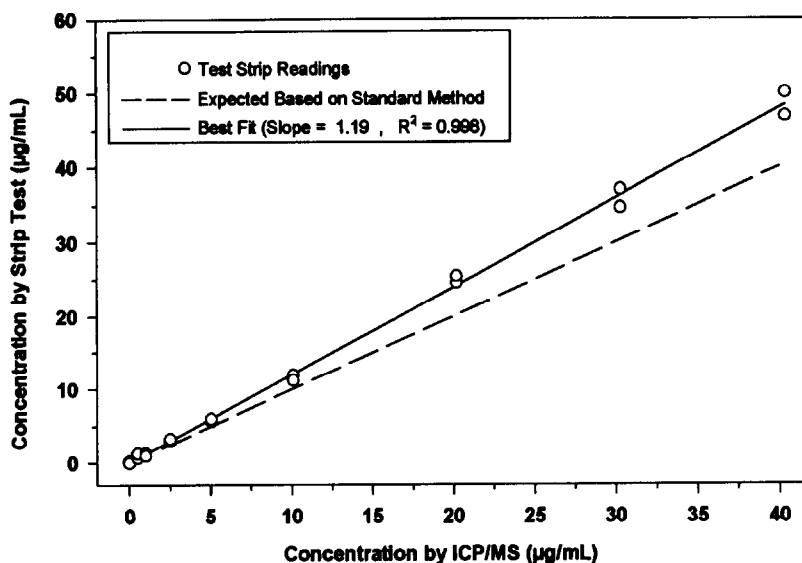
Fig. 2. Chromate strip test versus ICP/MS using spiked water samples and reflectometric readings. Four blank samples and duplicate samples for each of nine different concentrations of spiked samples were tested. A single test strip was used for each test sample. Each test strip was read once in the RQflex ($n = 4$ for blanks; $n = 2$ for each spiked sample).

the measurements shown in Fig. 2 over the range 2.5–40 µg/ml varied between 2.5 % and 6.7%.

The utility of chromate test strips and the RQflex reader for measuring chromate in waste water is illustrated in Fig. 3. As indicated in the section Materials and Methods, the test samples were diluted to within the range of the Reflectoquant® test and the results then corrected for the dilution factor. An excellent association between the chromate strip test, using the RQflex reader, and the results of ICP/MS analysis is indicated by the R^2 of greater than 0.99. The slope of 0.88 indicates that the strip test may slightly underestimate the actual chromate content of these waste water samples.

Overall, these data (Figs. 1–3) indicate that visual readings of the chromate test strips may limit the use of the test to qualitative or semiquantitative applications. By using reflectometric readings, however, a role in more quantitative applications is indicated.

3.2. Nitrate analyses

For the most part, the results obtained using the strip test for nitrate in spiked water mimic those obtained for chromate. As for chromate, there was considerable variability in the visual readings made by different individuals (Fig. 4). The R^2 value of 0.85 indicates an even poorer association of the visual readings for nitrate with expected concentrations than was observed with the chromate test (compare Figs. 1 and 4). Average values of all individual visual readings yielded a best fit line with a slope of
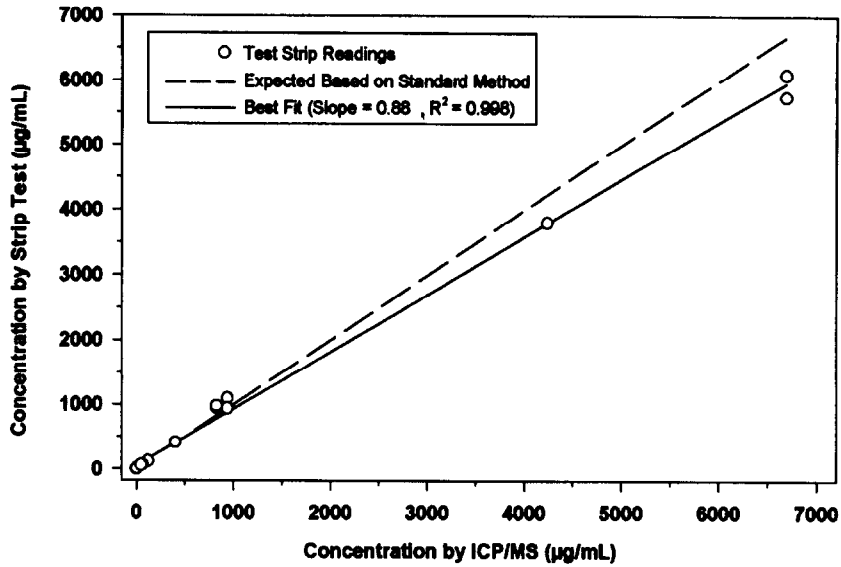
Fig. 3. Chromate strip test versus ICP/MS using waste water samples and reflectometric readings. Four blank samples and duplicate samples for each of nine different waste water samples were tested. A single test strip was used for each test sample. Each test strip was read once in the RQflex ($n = 4$ for blanks; $n = 2$ for each waste water sample).
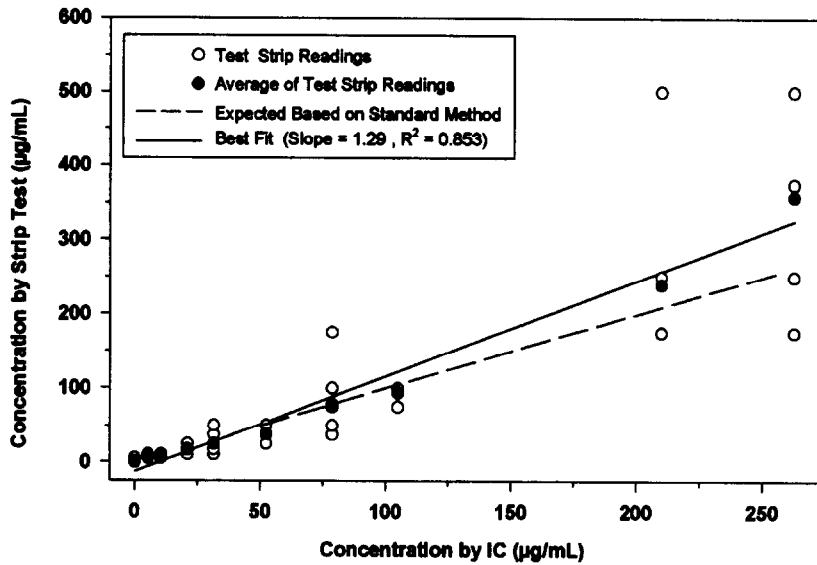

Fig. 4. Nitrate strip test versus IC using spiked water samples and visual readings. Six blank samples and duplicates for each of nine different concentrations of spiked samples were tested. A single test strip was used for each test sample. Each test strip was read independently by four different analysts ($n = 24$ for blanks; $n = 8$ for each spiked sample).

1.3, indicating that the test is somewhat overestimating the nitrate concentration in the samples.

Reflectometric readings of the strip test for nitrate in spiked water were a remarkable improvement over visual readings (Fig. 5). There was good agreement between the four overlapping samples that were read using both the low and high range concentration curves. The R^2 of greater than 0.99 and the slope of 0.94 indicate a very good correlation between the strip test and IC methods for measuring nitrate in water.

The results obtained for the nitrate-containing waste water samples using the RQflex reader (Fig. 6) were very similar to those obtained for the spiked samples (Fig. 5). Again, there was good agreement between the strip test and IC methods of analysis with a slope of 0.98 and an R^2 of greater than 0.99.

Mention was made above to concerns about using traditional laboratory methods as references for less familiar technologies. A case in point are the data shown in Fig. 6. RQflex measurements of the most concentrated sample tested gave a mean value of 63.6 µg/ml ($n = 6$) with a sample standard deviation of 3.2. However, the value obtained by IC was 49.3 µg/ml; clearly a poor agreement between methods. A subsequent analysis of the sample by IC gave a value of 82 µg/ml. The average of the two IC measurements was used in Fig. 6. This value of 65.6 µg/ml is in very good agreement with the strip test measurement. In this instance, it is obvious that a reference value obtained by standard laboratory methods may not always be
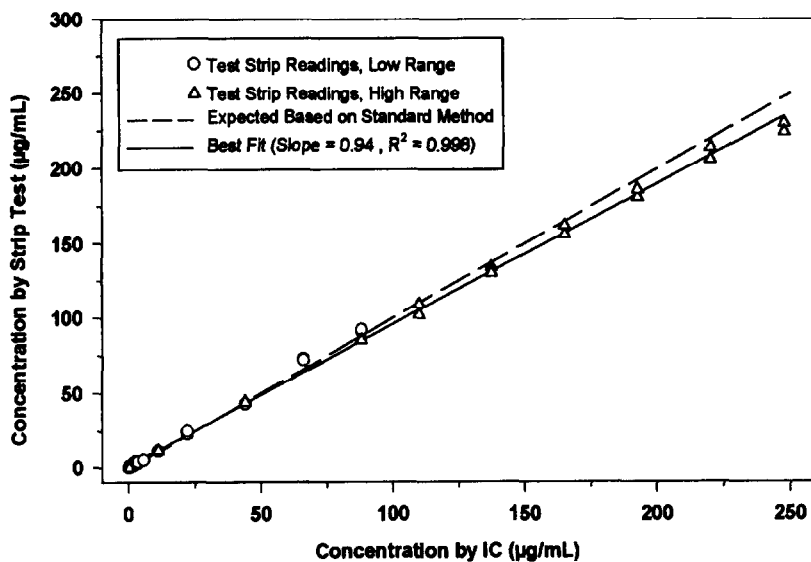

Fig. 5. Nitrate strip test versus IC using spiked water samples and reflectometric readings. Four blank samples and duplicate samples for each of 14 different concentrations of spiked samples (three of these concentrations were read in both the low and high range tests) were tested. A single test strip was used for each test sample. Each test strip was read once in the RQflex ($n = 8$ for blanks; $n = 2$, or 4, for each spiked sample).
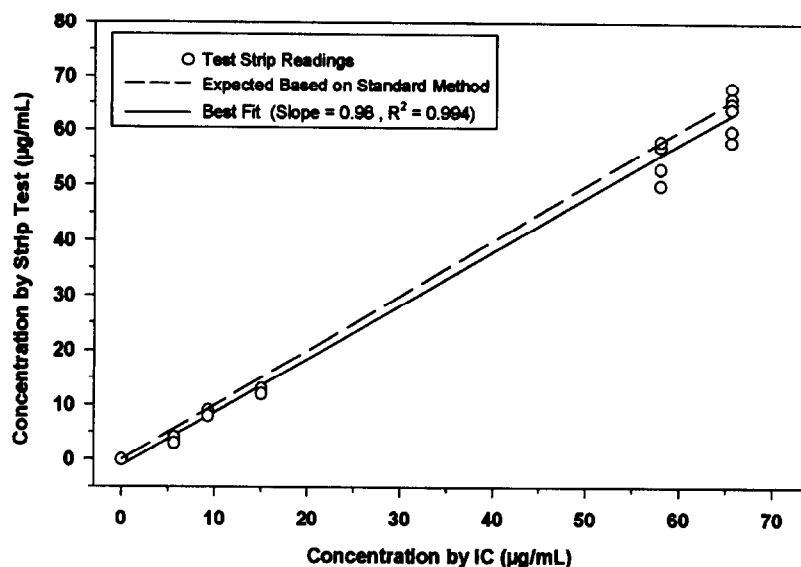
Fig. 6. Nitrate strip test versus IC using waste water samples and reflectometric readings. At least four replicates of six different waste water samples were tested. A single test strip was used for each test sample. Each test strip was read once in the RQflex (n = at least 4 for each waste water sample).

correct. This possibility should be taken into account when the performances of innovative, or alternative, technologies are being evaluated against the more traditional laboratory methods.

4. Conclusions

The use of colorimetric strip tests to measure chromate and nitrate in spiked and waste water samples was evaluated with reference to the more traditional laboratory methods of ICP/MS and IC. It was determined that although visual readings of test strips against a color chart may be useful for qualitative, or semiquantitative assessment of the concentration of these analytes in water, it would not be practical or cost effective to perform the multiple tests required to generate the average value needed to make the test quantitative. Such a requirement defeats the purpose of a rapid field screening method.

The use of a reflectometer to read the test strips dramatically increased the reproducibility and accuracy of this test method. This is undoubtedly due to the elimination of bias and random error inherent in making visual readings of the test strips. By using a reflectometer to read the test strips, quantitative results comparable to those obtained by standard laboratory methods were produced. Therefore, it was concluded that the use of indicator-based colorimetric strip tests, in conjunction with a reflectometer to read them, is a practical and cost effective alternative, or adjunct, to

traditional laboratory methods for field screening and as a prescreen for samples to be analyzed in the laboratory.

Acknowledgements

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