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Determination of Iodide, Iodate, Dichromate, Bismuth(III) and Hydrogen Peroxide Through Spot Tests Quantification by Computational Image Scanning Densitometry

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Analytical methods based on image digitization followed by computational quantification have been devised for the micro determination of various ions and compounds which can otherwise be determined by iodometry directly or indirectly. All methods were based on iodometric reactions to produce intense coloured spots of starch-iodine complex which were then determined by image scanning densitometry. A sample aliquot was transferred to a reagent impregnated TLC plate and the colour intensity of the resulting spot was quantified using a flat-bed scanner connected to a computer equipped with especially developed software. The technique has been applied for the determination of various ions in commercial and synthetic samples at micro level through image scanning densitometry. Iodide, iodate, dichromate, bismuth(III) and hydrogen peroxide were successfully determined up to ppm level by the this technique. Effects of the concerned factors have been studied and the precision of method was verified statistically. Standard deviation was calculated to check the precision of the method.

Keywords: Iodometry, Spot tests, Image Scanning, Densitometry, Computational Quantification.

INTRODUCTION

In last few decades, a number of researchers have employed optical densitometers for the quantitative measurement while working on TLC and paper chromatography. Radecka and colleagues performed assay of tetracycline by TLC[1]. After developing the chromatogram the intensity of spots was quantified by densitometry. Malinowska *et al.*[2] developed a quick densitometric method for quantitative total carbohydrate determination as well as for preparation of elution profiles of polysaccharides. Peter with his co-workers proposed spot test method for the determination of the total cholesterol concentration in human serum[3]. An aliquot of the octane extract was spotted on a thin-layer plate and the cholesterol in the spot was rendered visible using an aqueous phosphomolybdic acid staining solution followed by heat treatment for the colour development and the resulting coloured spot was scanned with a densitometer for quantification[3]. Another group of researchers separated glucosamines from plant extracts on a silica gel TLC plate[4]. The brownish-red chromatographic zones on a colourless background were quantified by reflectance scanning densitometry. Nzai and Proctor[5] developed a rapid reliable method to measure vegetable oil phospholipid content by thin-layer chromatography-imaging densitometry. Densitometry was employed by another group of workers for the determination of moxifloxacin by TLC using aluminium plates coated with silica gel[6]. Computational densitometry was first time employed as an alternative of spectrophotometry by our group in 2010 for the determination of various cations at micro-level[7].

Recently a novel micro-level technique has been developed by our group which comprises computational quantification of coloured spots resulting from the interaction of analyte and the chromophoric reagent on a paper or a TLC plate. In actual practice equivolume micro-liter aliquots of standards and the sample are applied on a plate which has been already impregnated with the reagent. The resulted coloured spots are quantified by measuring the colour intensity of each spot by using a flat-bed scanner or digital camera to digitize the result and quantitative analyses on PC running a specially developed software.

In computational densitometry a measured volume (usually 1 µL) of standards and sample are transferred to a suitable medium (TLC plate or Chromatographic paper) which may be reagent impregnated or the reagent (s) subsequently transferred thereto. After sufficient drying, a digital image of the medium containing the developed spots is digitized by a flatbed scanner at 300 dpi, 24 bit RGB mode. The RGB colour components of each area of selected spot are analyzed and quantified subsequently by simply adding up the RGB values of each

pixel. The higher the colour depth (or darker the spot) the higher will be the resulting figure. The explanation of RGB colour model will be helpful in understanding the underlying principle of image colour to density conversion.

Out of many devised colour models the RGB colour space is a subtractive colour model. Each of the respective RGB component can have a value from 0-255. The bright white colour would have RGB components are at maximum value (*i.e.*, 255, 255, 255), a grey colour would have R = G = B = 128 and a pure black colour would have R = B = G = 0. For calculating the optical density value, a subtraction from the maximum value (255) would result in RGB = 0 for White, 128 for Grey and 255 for Black. The same is practically feasible for all other colours or combinations thereof. The darker the colour or a higher concentration of analyte produces a higher colour depth. As there are few thousands of pixels inside the digitized image of a spot (a 1µL spot on silica coated aluminium TLC encloses 0.2 cm in diameter circle which when digitized at 300 dpi consists of approximately 2800 pixels), the collective colour density value of the typical spot comes out as 6 digit figures.

As the polychromatic light is used during the digitization process, the method does not offer a spectral selectivity as in absorption spectrophotometry, it must be kept in mind while selecting the developing reagents as they must be analyte specific or the interfering species may be removed or masked in the analyte prior to proceeding for the proposed methodology. The technique was employed for arsenic, mercury and lead at ppb level. By incorporating the technique with Gutziet method the sensitivity and accuracy of the method for arsenic determination was improved[8]. The technique was also employed for the determination of mercury in fish sample[9]. Sulphide was also determined by computational densitometry of dark-brown spots of lead sulphide[10].

Besides this minor limitation the present technique has some advantage of gigantic proportion which no other method of analyses may offer; a very small volume of sample is required as compared to wet chemistry or spectrophotometric analysis which require few mLs of sample. Secondly and most importantly it is the only applicable method when the analytical-reagent reaction results in precipitation-the spectrophotometry fails but the proposed method performs well even for black coloured spots. It is not only applicable to small volume and for precipitation analytical systems, it can be made portable for field work by using a digital camera and laptop.

Iodometry is a pure chemical classical analytical technique which is being used in routine and research laboratories since 19th century. A large number of anions, metal ions and neutral compounds which can easily undergo redox reactions with iodine, iodide or iodate were the species which normally being determined directly or indirectly by this technique. The field of titrations where iodine solution is directly employed as a titrant is normally termed as iodimetry. The famous iodine-starch test which yields a deep blue colouration when iodine reacts with starch established iodometry as a sensitive and selective analytical technique for a number of ions and compounds. One of the drawback of volumetric analytical methods is that they need at least a few mL of the sample. This drawback enlists the volumetric analysis as macro-level technique. Use of a special flask for iodometric titrations, volatility of iodine at elevated temperatures and uncertainty of end point are other weak points of the classical iodometry. In present work the image scanning densitometry is employed for various direct and indirect determinations by iodometry.

EXPERIMENTAL

Micropipette (2 µL, pipettemen), TLC plates (aluminium strips (kieselgur F254), Reflective flat bed colour scanner with computer. Standard solutions and synthetic samples were prepared by dissolving the AnalaR grade reagents in doubly distilled water. Subsequent dilution gave the desired concentration.

Determination of iodide: The iodide standards (50-250 ppm) were prepared by diluting the stock solution followed by acidification by 2 mL of dilute nitric acid solution. TLC plate was soaked with 10 % starch solution and 10 % acidified potassium iodate solution. After drying the plate, 2 µL aliquots of each of the iodide standards and synthetic sample were applied on the plate with the help of micropipette. Blue spots of varying intensity were immediately produced. The plate was scanned on the scanner and the resulting image was imported to the software to compute and digitalize the colour intensity of each spot. Calibration line was appeared on the computer screen which also gave the concentration of the sample. Same procedure was repeated for determination by dichromate and 35 % hydrogen peroxide.

Determination of iodate, dichromate, nitrite and peroxide: The redox reactions of iodide with iodate, dichromate and peroxide were also used to determine all these species. In one set of experiments plates were soaked with iodate, dichromate, nitrite and peroxide solutions along with starch and aliquots of iodide were applied with the help of micropipette as sample. In second set of experiments, plates were soaked with iodide and starch whereas iodate, dichromate and hydrogen peroxide were applied as samples. All the reactions were performed in acidic conditions.

Determination of bismuth(III) by using iodide: TLC plate was soaked with acidified potassium iodide and starch (both 10 %) and dried in oven at 100 °C. 2 µL aliquots of bismuth(III) standards (500-2000 ppm) were applied with the help of micro pipette. Bright yellow spots were produced immediately. The plate was scanned and intensity of each spot was scanned and digitalized with the help of the scanner attached to computer.

Effect of time: To check the effect of time on the colour intensity of spots obtained in various experiments 2 µL aliquots of iodide standards (100 and 200 ppm) were applied on oxidizing reagent impregnated TLC plate with micropipette and colour density of blue spots was determined after every five minutes interval of time. To observe the variation, the intensity values were plotted against time intervals.

Determination of standard deviation: To check the validity and precision of the method, standard deviation was calculated by transferring 10 equi-volume (2 µL) aliquots of iodide standard (100 ppm) on a TLC plate impregnated with acidified iodate solution and starch. The intensity of the spots obtained was digitalized and standard deviation was calculated.

RESULTS AND DISCUSSION

In present work the chemistry of iodometry is combined with image scanning densitometry to enhance its sensitivity many fold and to bring it among the techniques which need micro-liter quantities of the sample. By combining it with newly developed ISD (image scanning densitometry) not only it has become capable for analyzing quantitatively the micro-liter samples with compatible precision and accuracy but also one can overcome the afore mentioned practical problems. The new technique requires less sophisticated instruments and can be handled with modern computers, digital cameras and laptop devices. The analysis results are quick, accurate and precise and provide high sensitivity with micro samples then conventional titration methods.

Calibrations: In this work the TLC plate was impregnated with the suitable reagent (iodide, iodate, dichromate or peroxide) with reference to sample along with 10 % starch and after drying the plate in oven at 80-90 °C, micro-liter aliquots of the sample were applied. The spots of blue to violet colouration (Figs. 1-5) were appeared immediately which were scanned on a scanner and digitalized through a special software. The calibrations for various ions and hydrogen peroxide (Figs. 6-10) were drawn. The synthetic and real samples were quantified by inserting the intensity values in

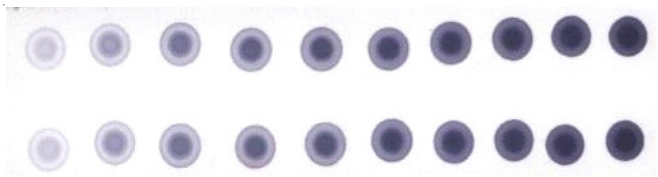

Fig. 1. Iodide determination by iodate

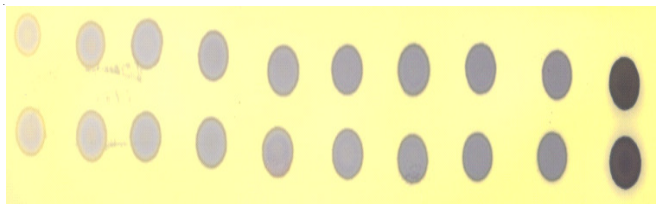

Fig. 2. Iodide determination by dichromate

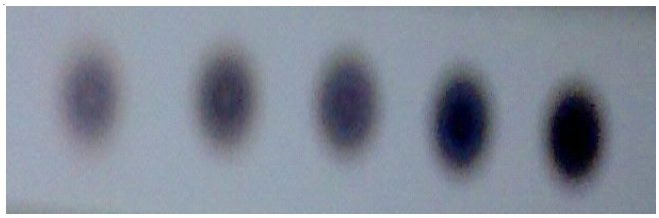

Fig. 3. Iodate determination by Iodide

Fig. 4. Hydrogen per oxide determination by Iodide

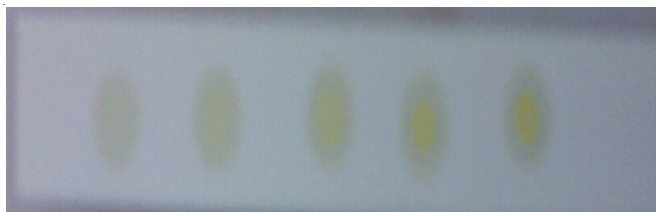

Fig. 5. Bismuth determination by iodide

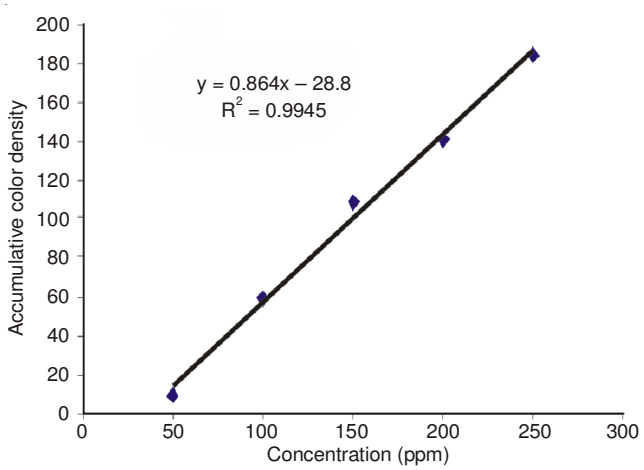

Fig. 6. Calibration for determination of iodide

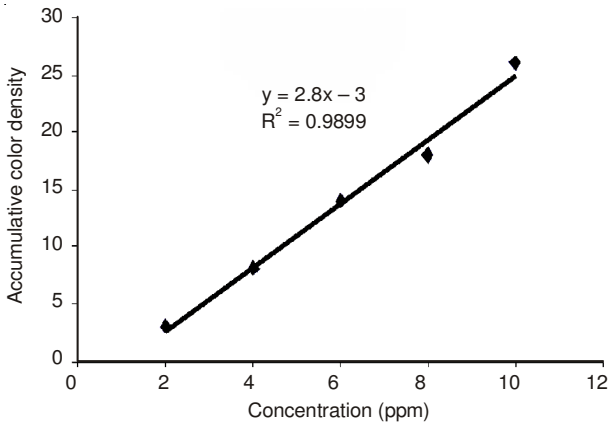

Fig. 7. Calibration for determination of iodate by iodide

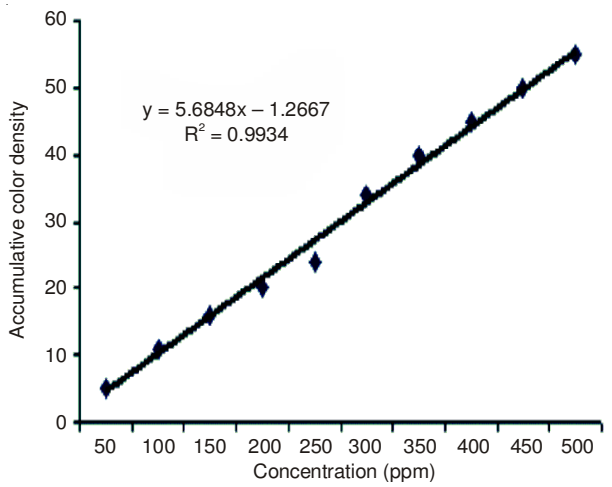

Fig. 8. Calibration for determination of dichromate ion by iodide

calibrations. As a principle all the species which are estimated iodometrically otherwise, can be determined by employing the image scanning densitometry. However in this work only iodide, iodate, dichromate, hydrogen peroxide and bismuth(III) were determined.

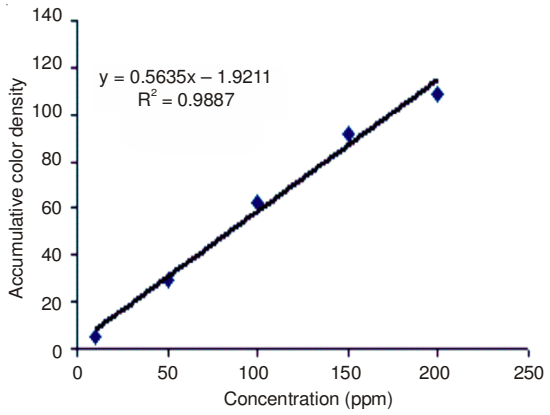

Fig. 9. Calibration for determination of H_2O_2 by iodide

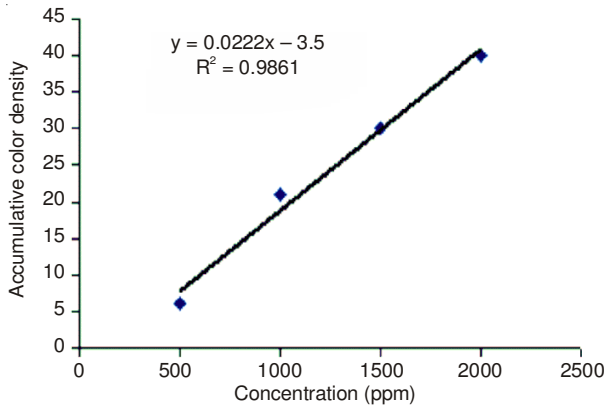

Fig. 10. Calibration for determination of Bi(III) ion by iodide

Effect of time on colour intensity of spots: To check the effect of time on the colour intensity of spots obtained in various cases, same spots were repeatedly scanned on the scanner and intensity was digitalized again and again. As shown in the Figs. 11 and 12 the colour intensity slightly decreased with time. This is due to slow vaporization of iodine from the starch-iodine complex when exposed to air.

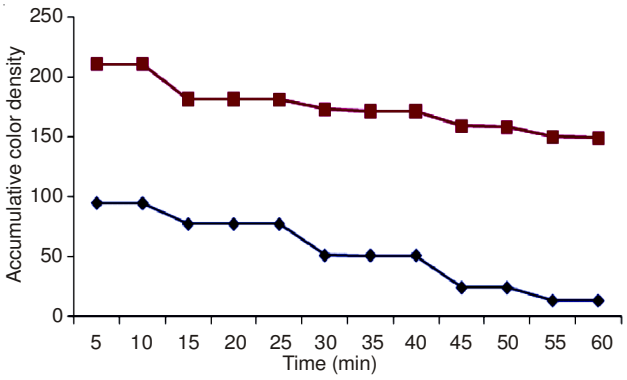

Fig. 11. Effect of time on colour intensity on determination of iodide ion by iodate (Lower line is for 150 ppm and upper line is for 300 ppm standard solution)

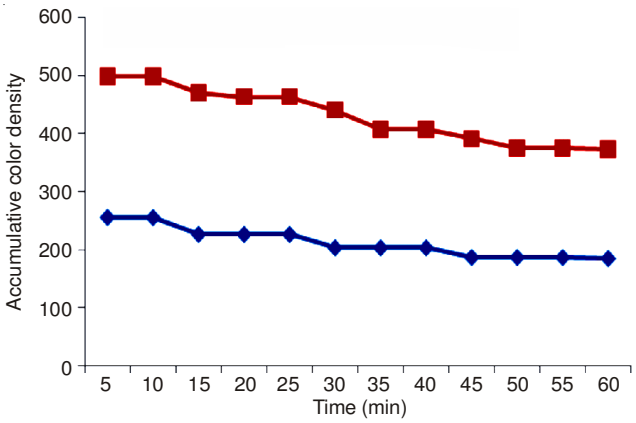

Fig. 12. Effect of time on colour intensity on determination of iodide ion by dichromate (Lower line is for 150 ppm and upper line is for 300 ppm standard solution)

Sample analysis: A number of real and synthetic samples containing iodide, iodate, dichromate, hydrogen peroxide and bismuth(III) were analyzed by the combined technique (iodometry through ISD) as well as conventional iodometry. Iodide has been determined in pharmaceutical preparations *i.e*. Lugol's Iodine (Lugol's Solution B.P) and Tincture Iodine U.S.P. Similarly iodized table salt samples collected from local market (National iodized salt and Shan iodized salt) were analyzed for iodate. Three samples of each the commercial product was analyzed. Assay was found in the range of 95-102 %. Samples of metal salts were prepared synthetically by dissolving appropriate quantities of the metal salts in 100 mL of water. These were also analyzed by the described technique as well as by reference methods. In all the cases the recovery was not less than 98.5 %.

Conclusion

This work employs the image scanning densitometry for the determination of iodide, iodate, hydrogen peroxide and Bi(III) in micro-liter samples. The spots produced were scanned on a flat bed scanner and digitalized through software loaded on a computer. The technique used is simple, low-cost, rapid, precise and accurate.

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