IODOMETRIC ASSAYS FOR COMPARATIVE EVALUATION OF OXIDANTS AND ANTIOXIDANTS

Shabir Ahmad Mir[1]
Division of Veterinary Pharmacology & Toxicology,
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir,
F.V.Sc. & A.H., Shuhama, Srinagar-190001, J & K, India

Zari Mohalla, Lal Bazar, Srinagar, Kashmir (India)
Formerly Professor & Head, SKUAST-K; superannuated April 1st 2014

ABSTRACT: Iodometric assays offer opportunity to test both oxidants and antioxidants. Six iodometric assays have been optimized to compare six different oxidant systems to affect quantitative oxidation of potassium iodide to generate iodine. The assays provide quantitative linear response to test oxidants, and enable assessment of their relative oxidant potential in the test system. Test oxidants over the linear range in µmoles (indicated within parenthesis) have included nitrous acid (0.02 – 0.12), alkaline pyrogallol (0.10 – 0.5), potassium dichromate (0.1 – 2.0), hydrogen peroxide (0.5 – 5.0), iron (III) chloride (0.5 – 8.0) and copper (II) acetate (1 – 10). The relative oxidant potentials have been compared on the basis of regression coefficients obtained with each test oxidant over their linear ranges on absorbance basis as well as in terms of mass of iodine released, monitored colorimetrically at 430 nm, by each oxidant as per following order: nitrite (67 X) >> pyrogallol (3.2 X)> dichromate (2.3 X) > hydrogen peroxide (1.7 X)> ferric chloride (1.1 X) > copper acetate (1 X). Published data from the Laboratory with three iodometric assays including viz., nitrous acid, alkaline pyrogallol and copper, for their relative responses to chemically diverse antioxidants including flavonoids (5), phenolics (6), thio compounds (2), reducing salts(2) and ascorbic acid has been reviewed herein to provide relative suitability of each system for differential responses to the test antioxidant. The work offers wider opportunity to screen both oxidants well as antioxidants by colorimetric iodometry.

Keywords: **Iodometric assay, Antioxidants, Nitrous acid, Pyrogallol, Dichromate, Peroxide, Iron (III), Copper (II).**

INTRODUCTION

Simple and cost-effective methods for screening antioxidants are handy for laboratories experiencing financial constraints. Iodimetric and iodometric assays are quite simpler and inexpensive to meet such eventualities. Iodimetry involves measuring of standard iodine directly. The method is particularly useful in assaying antioxidants. The method has been employed to quantify reducing agents, in terms of their iodine decreasing activity, including thiosulfates, hydrogen sulfide, sulfurous acid, stannous chloride, arsenic (III), antimony (III) and hydrogen peroxide (Jeffery et al., 1989). The method has also been used to measure ascorbic acid in fruits, vegetables (Yan et al., 2006; Choo & Yong, 2011;Yuris, 2014) and pharmaceuticals (Singh & Kapoor, 1984; Anastos et al., 2004), thiols in pharmaceuticals (Ciesielski & Zakrzewski, 2006) and to measure total antioxidant capacity of human serum (Zhang et al., 2014). In these assays, iodine has been measured titrimetrically. Of late, iodimetric assay by colorimetry has been optimized to measure antioxidant potential of a large number of chemically diverse antioxidants (Mir, 2016a).

Iodometry measures iodine released from potassium iodide solution in presence of some oxidizing agent. The assay is therefore useful in both assessment of oxidants and to antioxidants; the former generate iodine from potassium iodide and the latter reduce the iodine generated by oxidants. Iodometry has been primarily employed to standardize oxidizing agents, by their capacity to oxidize iodide to iodine, including dichromate (Jeffery et al.,1989; Mayaanjali, 2013), copper (II) (Jeffery et al.,1989; Singh & Kapoor, 1984; Mayaanjali, 2013; Agterdenbos & Tellingen, 1961; Barakat et al., 1972; Mir & Bhat, 2003; Deyanda, 2013) , hydrogen peroxide (Jeffery et al., 1989; US Peroxide, 2013), iron (III) (Mayaanjali, 2013; Agterdenbos & Tellingen, 1961; Barakat et al., 1972), nitrous acid (Mayaanjali, 2013; WHO, 1988), dissolved oxygen in water (Jeffery et al., 1989; Oulman & Baumann, 1956; APHA, 1999) and lipid peroxidation (Eric, 1987).

Lately, alkaline pyrogallol has been found to provide quite excellent oxidizing environment for an iodometric assay for antioxidants (Mir, 2015a). Oxidants capable of oxidizing KI to release iodine provide an opportunity to screen antioxidants with ability to reduce iodine content by two means: (i) to counter oxidant that releases iodine from potassium iodide, and (ii) to reduce released iodine directly by reduction. Thus iodometric assay provides a more powerful test for screening antioxidants. Since a number of oxidants can be employed to oxidize potassium iodide to iodine, therefore, a number of oxidant systems would be available to test wide variety of antioxidants. Previous work from this laboratory has demonstrated ability of iodometric assays to test chemically diverse antioxidants using oxidant systems involving alkaline pyrogallol (Mir, 2015a), copper (Mir, 2015b) and nitrous acid (Mir, 2016b). In view of these studies, the present work has been mooted to optimize three more iodometric assays including hydrogen peroxide, potassium dichromate and ferric chloride. The six iodometric assays have been compared for linear ranges of test oxidants and for their comparative abilities to release iodine from potassium iodide. Formerly published data (Mir, 2015a; Mir, 2015b; Mir, 2016b) has been employed to compare response of the iodometric assays to select antioxidants including flavonoids (5), phenolics (6), thio compounds (2), ascorbic acid, citrate and oxalate anions

MATERIALS AND METHODS

The experiments were carried out at an ambient temperature of 18.0 ± 0.8 ^{0}C. The drugs and chemicals used were of standard purity and quality obtained from reputed sources in India. Spectrophtometric measurements were made with UV-Visible Spectrophotometer, Model UVmini-1240 (Shimadzu Corporation, Japan).

Reagents

Standard iodine solution: The solution was approximately made 0.1 M by dissolving 1.4 g iodine crystals resublimed and 3.6 g potassium iodide with a drop of 10% HCl in water to make 100 mL solution. The solution was standardized against standard 0.1N sodium thiosulfate by taking 25 mL of aliquot until the color made palish-yellow; then added 0.5 mL starch solution(0.5 % w/v in boiled and cooled water) and titration continued till blue color is just discharged . The solution was appropriately diluted with distilled water as per need.

Potassium iodide solution: 5% (w/v) KI in water containing 0.001M NaOH.

Dilute HCl solution: 10 %(v/v) HCl in water.

Nitrite solution: Working solution made as 50 and 200 nmoles per mL water from stock 0.1M sodium nitrite in water.

Ferric chloride solution: Working solutions made by dilution in water from stock 25 µmole ferric chloride mL^{-1} water containing 0.001 M HCl.

Potassium dichromate solution: Working solutions were made by dilution in water from stock solution of 0.2 N potassium dichromate in water.

Pyrogallol stock solution: Stock solution was prepared by dissolving 100 mg pyrogallol (purity 98 %) in 15.8 mL water containing 0.2 mL 1 % HCl to provide 50 µmole mL^{-1}. Working solutions were made by dilution in water as per need.

Copper acetate solution: The required dilutions were made in water at the time of use from stock solution of 0.1 M copper acetate (0.2 % w/v in water).

Sodium bicarbonate solution: 2.1 % (w/v) sodium bicarbonate in water, 0.25 M.

Alkaline mixture: Each 10 mL 0.25 M sodium bicarbonate was added 10 mL water and 5 mL of 0.1 M NaOH. Each 0.5 mL aliquot provided 50 µmoles of $NaHCO_3$ and 10 µmoles of Na OH.

Hydrogen peroxide solution: Working solution of hydrogen peroxide contained 10 µmole hydrogen peroxide mL^{-1} water prepared from stock solution of 42 µmole mL^{-1} that has been standardized against standard potassium permanganate solution.

Analytical techniques

Six different oxidizing systems were evaluated for their relative ability to release iodine from potassium iodide. These included sodium nitrite, ferric chloride, copper acetate, potassium dichromate, pyrogallol and hydrogen peroxide. The standardized data for three of the six oxidant systems has been adopted from previously published works involving alkaline pyrogallol (Mir, 2015a), copper acetate (Mir, 2015b) and nitrous acid (Mir, 2016b). The present work has generated additional data using optimized iodometric

assays using other oxidants namely potassium dichromate, ferric chloride and hydrogen peroxide to enable comparative evaluation of their oxidizing potential.

Standard linearity protocol for standard iodine

A linearity testing with standard iodine solution has been made over the range 0, 1 through 5 µmoles iodine under the test conditions. The regression parameters from the linear curve have been utilized to compute iodine release for each assay system at given mass of test oxidant. This provided relative oxidizing potential of six test oxidants.

Assay 1: Iodometric assay using nitrous acid

For linearity setting, 2 mL water containing nitrite as 0, 20 through 120 nmoles was added 0.5 mL sodium bicarbonate solution, 1 mL of KI solution, and 1 mL water. The samples were mixed and added 0.5 mL dilute HCl solution to initiate action. The samples were allowed standing at room temperature for release of iodine and read at 430 nm about 1 hour following acidification. The assay has been optimized for acid requirements using 0.5 through 2 mL dilute acid and time for incubation period over 0.5 through 4 hour using appropriate mass of nitrite.

For assaying test agent, appropriate mass of test agent, added before acidification, was incubated with standard mass of nitrite (100 nmoles) and matched for solvent. The calibration for nitrite was run simultaneously used nitrite as 30, 50 and 100 nmoles nitrite with matching volume of solvent (Mir, 2016b).

Assay 2: Iodometric assay using alkaline pyrogallol

Aqueous samples containing pyrogallol as 0, 0.1 through 1.0 µmole in 3 mL water were added each 1 mL KI solution followed by addition of 0.5 mL of alkaline mixture. The samples were allowed standing at room temperature for 10 minutes, and the reaction was stopped by addition of 0.5 mL dilute HCl. The samples were read at 430 nm ca. 1 hour following addition of alkali. Iodine release by pyrogallol was computed in terms of standard iodine regression parameters.

Test antioxidants were assayed by incubating known mass in absence and presence of standard mass of pyrogallol (0.5 µmole). Activity (potentiation or antagonism) per antioxidant measured in terms of simultaneously run standard (Mir, 2015a).

Assay 3: Iodometric assay using potassium dichromate

Potassium dichromate was taken as 0, 0.1 through 2.0 µmole in 3.3 mL water, added 0.2 mL sodium bicarbonate solution, and 0.5 mL 10 % HCl in that order and finally added 1 mL potassium iodide solution. The samples were allowed standing for about 1 hour at room temperature and read at 430 nm. Each concentration required its own control containing identical mass of dichromate with all reagents except KI solution in 5 mL water. The treated samples were accordingly read with respect to corresponding control set at zero absorbance. Iodine release was estimated in terms of standard iodine regression parameters.

Assay 4: Iodometric assay using hydrogen peroxide

Hydrogen peroxide 0, 0.5 through 5.0 µmole was taken in 2 mL water and added each 0.2 mL sodium bicarbonate solution, 1 mL KI solution; 1.3 mL water and 0.5 mL dilute HCl. The samples were allowed standing at room temperature and monitored at 430 nm at about 1 hour following acidification. Iodine release was estimated in terms of standard iodine regression parameters.

Assay 5: Iodometric assay using ferric chloride

Ferric chloride was used over 0, 0.5 through 8 µmole in 3.3 mL water; added 0.2 mL sodium bicarbonate solution followed by 0.5 mL 10 % HCl. The samples were finally added 1 mL potassium iodide solution and allowed standing for about 1 hour at room temperature following acidification, and read at 430 nm for iodine release. Iodine release was estimated in terms of standard iodine regression parameters.

Assay 6: Iodometric assay using copper acetate

Copper acetate was used over 0, 1.0 though 30 µmole in 3.3 mL of water and added 0.2 mL sodium bicarbonate solution followed by 0.5 mL 10 % HCl and finally 1 mL potassium iodide solution. The samples were allowed standing at room temperature and monitored at 430 nm at about 1 hour following addition of KI solution. Iodine release was estimated in terms of standard iodine regression parameters. For assaying test agents, appropriate mass of antioxidant was taken with and without standard mass of copper (II) 5 µmole in 2 mL and treated as per outlined method, and the calibration standards were run using 1, 3 and 5 µmole copper, each matched for solvent (Mir, 2015b).

Calculations

The data were subjected to routine statistical analysis. Regression parameters notably regression coefficient

(b) and y-intercept (c) obtained from standard iodine assay were employed to estimate iodine release with each test oxidant:

Estimated iodine generated (µmoles) = (A- c)/b

Where A is absorbance with test mass of test oxidant, b and c are respectively as regression coefficient and y-intercept of standard iodine assay (over 1 through 5 µmoles). The results obtained at each mass of test oxidant were subjected to regression analysis to compute mass of iodine released per unit mass of test oxidant. Calculations for estimating antioxidant potential of test oxidants have been already given (Mir, 2015a, 2015b, 2016b).

RESULTS AND DISCUSSION

Iodine over 1 through 5 µmoles showed regression estimate of 0.2300 ± 0.0042 with perfect linearity (r= 0.999 ± 0.001, y-intercept = -0.1445). These parameters were employed to measure mass of iodine released by varying masses of test oxidants.

Nitrite based iodometric assay

As evident (Table 1), nitrous acid produced quantitative release of iodine from KI over 20 through 120 nmoles with peak regression over 40 through 100 nmoles (r ±S.E. = 0.999 ±0.001; b ±S.E. = 7.0 ±0.1). Computation revealed nitrous acid caused quantitative oxidation of iodide to iodine over the test range and each µmole of nitrous acid released 30.3 ± 0.7 µmoles of iodine.

Table 1: Nitrous acid dependent iodometric assay

Nitrite mass, µmole	Absorbance values
0.02	0.065±0.003
0.04	0.163 ±0.001
0.06	0.299 ±0.002
0.08	0.453±0.003
0.10	0.576 ±0.009
0.12	0.720 ±0.004
Statistical analysis	
r ±S.E.	0.999 ±0.001
b ±S.E.	6.5 ±0.1

The values are mean ± S.E. of five observations each; the linearity is perfect with peak regression over 0.04 through 0.10 µmoles nitrite (r ±S.E. = 0.999 ±0.001; b ±S.E. = 7.0 ± 0.1)

Pyrogallol based iodometric assay

As evident (Table 2), pyrogallol over the test range 0.1 through 1 µmole produced quantitative linear release of iodine from potassium iodide with better response over 0.1 through 0.5 µmole (0.334±0.013; 0.996 ±0.004). Estimated iodine release was found to be 1.44 ±0.07 µmoles per µmole pyrogallol over the peak range.

Table 2: Pyrogallol superoxide anion dependent iodometric assay

Pyrogallol mass, µmole	Absorbance
0.1	0.051±0.001
0.2	0.089±0.001
0.3	0.128±0.001
0.4	0.159±0.002
0.5	0.183±0.002
0.6	0.199±0.006
1.0	0.314±0.005
Statistical analysis	
r±S.E.	0.995±0.004
b±S.E.	0.284±0.011

The values are mean ± S.E. of 5 observations each. The curve is better over 0.1 through 0.5 µmole (b±S.E.0.334±0.013; r±S.E.0.996 ±0.004)

Dichromate based iodometric assay

As evident (Table 3), dichromate over the test range 0.1 through 2 µmole produced quantitative linear release of iodine from potassium iodide with better response over 0.2 through 2.0 µmole (0.238±0.004; 0.999 ±0.001). Estimated iodine release was found to be 1.03 ±0.02 µmoles per µmole dichromate over the test range.

Table 3: Potassium dichromate dependent iodometric assay

Potassium dichromate, µmole	Absorbance
0.1	0.033±0.001
0.2	0.054±0.001
0.4	0.100±0.001
0.8	0.183±0.002
1.0	0.232±0.003
2.0	0.482±0.003
Statistical analysis	
r±S.E.	0.999±0.001
b±S.E.	0.236±0.004

The values are mean ± S.E. of 5 observations each.

Peroxide based iodometric assay

As evident (Table 4), hydrogen peroxide over the test range 0.5 through 5 µmole produced quantitative linear release of iodine from potassium iodide with better response over 0.5 through 3.0 µmole (0.184±0.007; 0.997 ±0.003). Estimated iodine release was found to be 0.78 ±0.03 µmoles per µmole peroxide over the test range.

Table 4: H_2O_2 dependent iodometric assay

H_2O_2, µmole	Absorbance
0.5	0.073±0.001
1.0	0.149±0.001
2.0	0.315±0.002
3.0	0.534±0.008
4.0	0.642±0.013
5.0	0.894±0.004
Statistical analysis	
r±S.E.	0.996±0.003
b±S.E.	0.179±0.007

The values are mean ± S.E. of 5 observations each. The slope is steeper over 0.5 through 3 µmole H_2O_2, (r±S.E, 0.997±0.003; b±S.E, 0.184±0.007)

Ferric based iodometric assay

As evident (Table 5), ferric chloride over the test range 0.5 through 8 µmole produced quantitative linear release of iodine from potassium iodide with better response over 0.5 through 4.0 µmole (0.138±0.001; 0.999 ±0.001). Estimated iodine release was found to be 0.49 ±0.01 µmoles per µmole iron (III) over the test range.

Table 5: Iron (III) dependent iodometric assay

Ferric chloride, µmole	Absorbance
0.5	0.095±0.001
1.0	0.150±0.002
2.0	0.270±0.001
4.0	0.492±0.002
8.0	0.943±0.006
Statistical analysis	
r±S.E.	0.999±0.001
b±S.E.	0.113±0.001

The values are mean ± S.E. of 5 observations each.

Copper (II) based iodometric assay

As evident (Table 6), copper acetate over the test range 1 through 30 µmole produced quantitative linear release of iodine from potassium iodide with better response over 1 through 10 µmole (0.104±0.004; 0.999 ±0.001). Estimated iodine release was found to be 0.45 ±0.02 µmoles per µmole copper (II) over the test peak range.

Table 6 .Linear relation: absorbance vs copper acetate mass

Copper acetate, µmole	Absorbance
1	0.132±0.003
3	0.281±0.007
5	0.485±0.006
10	1.055±0.004
30	2.602±0.005
Statistical analysis	
r ±S.E	0.997±0.003
b ±S.E[a].	0.085 ±0.003

The values are mean ± S.E. of 5 observations each. [a] Regression is steeper over 1 through 10 µmoles (0.104 ± 0.004) and steepest over 3 through 10 µmoles (0.111 ±0.003; 0.999±0.001)

Comparative evaluation of test oxidant systems for iodometric assays

The results of six test oxidant systems have been summarized (Table 7). The assay systems have been compared with respect to test linear range of oxidants used and, regression coefficients of each test assay. Approximate relative potency has been computed using the two regression coefficients involving absorbance as well as iodine mass released with weakest test oxidant viz., copper (II) assigned unit potency. As evident, the relative sensitivity order of test oxidant systems has been found to be:
nitrite (67 X) > alkaline pyrogallol (3.2 X) > dichromate (2.3 X) > H_2O_2 (1.7 X) > $FeCl_3$ (1.1 X) > copper (II) (X).

Thus, nitrous acid provides over 20-times more sensitive iodometric assay than alkaline pyrogallol which is approximately twice as sensitive as peroxide and 1.4 times as sensitive as dichromate and nearly 3-fold as sensitive as ferric and copper assays. Ferric and cupric assays are nearly equi-sensitive in detecting antioxidants.

Iodometric assay based on nitrite makes it a strong candidate to screen for nitrous acid scavenging antioxidants. Alkaline pyrogallol system would be useful to detect superoxide anion scavenging antioxidants. Peroxide assay is good for screening peroxide scavenging antioxidants. Ferric reduction assay is widely employed in antioxidant screening but reportedly shows inability to detect thio compounds compared to cupric assay (Mir, 2015b). Dichromate reduction has been shown to screen many antioxidants (Mir, 2015b). Thus each oxidant system would be put to use, based on iodometric assay systems, to screen antioxidants. This consideration is beneficial since a single basic technique would enable detecting a given antioxidant against six different oxidants.

Table 7: Relative iodometric potential of test oxidizing agents

Oxidant	Mass range, µmole	Regression coefficient based on absorbance	Iodine released, µmole[a]	Approximate relative potency[b]
Nitrite	0.04 – 0.10	7.0± 0.1	30.3± 0.7	67 X
Pyrogallol	0.1 – 0.5	0.334± 0.013	1.44 ±0.06	3.2 X
Potassium dichromate	0.1 – 2.0	0.236 ±0.004	1.03 ± 0.02	2.3 X
H_2O_2	0.50 – 5.0	0.179±0.007	0.78 ±0.03	1.7 X
$FeCl_3$	0.5 -8.0	0.113±0.001	0.49 ±0.01	1.1 X
Copper acetate	1.0 – 10.0	0.104±0.004	0.45±0.02	X

[a] The values are regression estimate, b±S.E, relating mass of iodine released in µmole over range of oxidant mass in µmole used; [b] Relative potency based on iodine release runs parallel to those based on regression coefficients

Comparative response of selected iodometric systems against test antioxidants

A comparative evaluation of test antioxidants using three different oxidant systems such as alkaline pyrogallol (Mir, 2015a), copper (Mir, 2015b) and nitrous acid (Mir, 2016) have shown varying responses. As evident (Table 8), test antioxidants do not respond uniformly in different oxidant based iodometric assays. Copper assay responds to all test flavonoids (5/5) while nitrous acid and pyrogallol assays respond poorly (3/5) and differently such that nitrous acid assay failed to detect morin and rutin while pyrogallol assay responded by showing potentiation to quercetin and rutin. Three assay systems have responded to resorcinol (copper > pyrogallol>> nitrous acid) while 5 other phenolics worked differently: nitrous acid failed to detect others (5/5), copper detected poorly (3/5) failing to detect phenol and guaiacol; and pyrogallol showed either potentiation (gallic acid>HQ >>phenol) or no detectable response (catechol and guaiacol). Ascorbic acid and thio compounds worked well in all three test systems with greater response in copper system followed by pyrogallol and nitrous acid in that order. Pyrogallol responded to both citrate and oxalate, nitrous acid failed to detect either and copper detected only oxalate.

These observations indicate the necessity of testing antioxidants in more than one assay system for proper appreciation of any antioxidant activities. Iodometric assay offers simpler protocol to evaluate different antioxidants using different oxidants to generate iodine from potassium iodide.

Table 8. Comparative evaluation of antioxidants with selected iodometric assays mediated by pyrogallol superoxide anion, copper (II) and nitrous acid

S.No	Test agent	Alkaline pyrogallol	Copper(II)	Nitrous acid
1	Diosmin	-49±6 (1)	1208±38[a] (1)	41.3±1.3(1)
2	Daflon	-71± 3 (1)	633±57[a] (1)	45.3±2.0(1)
3	Quercetin	+ 20 ± 2 (1)	1837 ± 72[b] (1)	19.8±1.1(1)
4	Rutin	+22 ± 3 (1)	1292 ± 17[b] (1)	NAD (1)
5	Morin	-28 ± 4 (1)	3682±33[b] (1)	NAD (1)
6	Resorcinol	-83 ± 3 (1)	210±15 (5)	1.5±0.1(5)
7	Guaiacol	NAD (1)	NAD (5)	NAD (5)
8	Catechol	NAD (1)	31±9 (5)	NAD (5)
9	Phenol	+9± 0.5 (10)	NAD (10)	NAD (10)
10	HQ	+156± 4 (1)	938±12 (5)	NAD (5)
11	GA	+296 ±15 (0.2)	904±8 (2)	NAD (1)
12	Ascorbic acid	-287 ± 20 (0.5)	1402 ±19 (1)	62.3±0.2(1)
13	TGA	-231 ±2 (2)	650±36 (1)	37.9±0.7(1)
14	TU	-79 ± 2 (1)	465±66 (1)	15.1±0.7(1)
15	Citrate anion	-0.41 ±0.07 (50)[c]	NAD (50)[d]	NAD (50)[d]
16	Oxalate anion	-0.25 ±0.07 (50)[c]	60 ±1 (20) [d]	NAD (50)[d]

The values are mean ± S.E. of 5 observations each with units as nmole oxidant reduced or scavenged per µmole antioxidant. NAD, no activity detected ($p>0.1$). Test masses of antioxidants are given within parenthesis as µmole.

+ prefix stands for potentiation or pyrogallol-equivalent activity (PEA) while

- prefix stands for antagonism or pyrogallol scavenging activity (PSA).

[a] The listed values observed in absence of bicarbonate while in presence of the bicarbonate, the values for diosmin and daflon were 797±88 and 1200 ± 91, respectively; [b] The listed values observed in absence of bicarbonate while the effect was masked in presence of bicarbonate; [c] citrate and oxalate as salts; [d] citric acid and oxalic acid as test agents.

CONCLUSIONS

The work has focused on providing comparative oxidative potential of test oxidants viz., nitrous acid, alkaline pyrogallol, potassium dichromate, ferric chloride, hydrogen peroxide and copper acetate using potassium iodide based iodometric assay systems with colorimetric monitoring of iodine at 430 nm. The sensitivity order has varied with type of oxidant used with nitrous acid as most potent and copper acetate as least potent of the test oxidants. The optimized assay systems would provide opportunity for researchers to employ the convenient choice for assaying antioxidants. Test oxidants can be assayed in terms of their iodometric response with respect to test standard oxidant under identical condition. Potential of iodometric assays based on nitrous acid, copper acetate and pyrogallol have been already documented by the author. Further works are needed to exploit the other iodometric assay systems for screening antioxidants. The

assay systems have been employed routinely for testing antioxidant potential of extracts obtained from some locally available indigenous medicinal plants/herbal drugs in Kashmir (to be published separately).

REFERENCES

1. Jeffery G.H, .Bassett J, Mendham J, Denney R.C. Vogel's Textbook of Quantitative Chemical Analysis.5th Edn, Longman Scientific & Technical, UK, 1989.
2. Yan LY, Teng L.T, Jhi TJ. Sunway Academic Journal 2006; 3:9-20.
3. Choo WS, Yong WK. Advances in Applied Science Research, 2011; 2 (3): 418-425
4. Yuris A. Jr. Food Studies. 2014; 3(1):40-56.
5. Singh H, Kapoor V. K. Basic and Pharmaceutical Practical Chemistry, V. Prakashan, New Delhi, 1984
6. Anastos N, Barnett NW, Hindson BJ, Lenehan CE, Lewis SW. Talanta. 2004; 64(1):130-134.
7. Ciesielski W, Zakrzewski R. Chem. Anal. (Warsaw), 2006; 51: 653.
8. Zhang M, Liu N, Liu H. Bioinorganic Chemistry and Applications, 2014, Article ID 928595, Hindawi Publishing Corporation; http://dx.doi.org/10.1155/2014/928595
9. Mir SA.International Journal Pharm Tech Research 2016a ; 9(1): 70-78.
10. Mayaanjali. Iodometry and iodimetry, 2013, HubPages, Inc. http://mayaanjali.hubpages.com/hub/Iodometry-and-Iodimetry
11. Agterdenbos J, Tellingen EJv.Talanta 1961; 8:532-534.
12. Barakat M.Z., Bassiouni M, El-Wakil M. Jr Sci Food Agric. 1972; 23(6):685-686
13. Mir S A, Bhat AS. Asian Jr Chemistry 2003; 13(1):255-258
14. Deyanda F. http://www.ehow.com/about_5794702_iodometric-titration-method.html eHow Contributor , last updated April 16, 2013
15. US Peroxide. Iodometric titration. Copyright © 2013 U.S. Peroxide, LLC; 900 Circle 75 Parkway, Suite 1330, Atlanta, GA 30339 http://www.h2o2.com/technical-library/analytical-methods/default.aspx...
16. WHO. The International Pharmacopoeia, 3rd Edn, volume 3 (Quality Specifications), Geneva, Pp.197-199, 1988.
17. Oulman CS, Baumann ER. Sewage Ind. Wastes 1956; 28:1461.
18. American Public Health Association. In: Standard Methods for the Examination of Water and Wastewater, USA, 1999.
19. Eric DW. In: Biochemical Toxicology: A practical approach, Snell K, Mullock B (Eds.), IRL Press, Washington DC, pp.127-152, 1987.
20. Mir SA.International Journal Pharm Tech Research 2015a; 7(2): 266-274
21. Mir SA International Journal Pharm Tech Research 2015b; 7(2): 276-286.
22. Mir SA. International Journal Pharm Tech Research 2016b; 9(1): 60-69.