

# Sensitive, Simple and Rapid Spectrophotometric Method for Monitoring Treatment Concentration of Chloramine-T on Fish Culture Facilities and Environmental Water Samples using Green Analytical Reagent

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When studying the effectiveness of the drug for controlling bacterial gill disease. N-sodium-N-chloro-rho-toluenesulfonamide (chloramine-T) concentration must be monitored during treatments of fish. A simple, sensitive and rapid spectrophotometric method, using phenoxazine Cisapride (CSP) and Metaclopramide as green analytical reagents for determination of chloramine-T (CAT) was proposed. The methods are based on the oxidation of Cisapride (CSP) and Metaclopramide (MCP) by chloramine-T in acidic media and coupling with phenoxazine to produce red colour product that has maximum absorption at 520 nm. The calibration curves are linear over 0.50 – 4.50  $\mu\text{g mL}^{-1}$  and 0.45 - 4.00  $\mu\text{g mL}^{-1}$  respectively. The concentration measurements are reproducible within a relative standard deviation of 1% and recoveries are 98.0 -102.0 %. The performance of proposed methods were evaluated in terms of Student's t-test and Variance ratio F-test that indicate the significance of proposed methods over the standard spectrophotometric method. The proposed method was successfully applied in the determination of chloramine-T in fish culture facilities and environmental water samples.

**Keywords:** Chloramine-T, Spectrophotometric method, Fish Culture Facilities, Phenoxazine cisapride, green analytical reagents.

## 1. INTRODUCTION

Amoebic gill disease (AGD) is a significant disease affecting the culture of Atlantic salmon [1]. Over recent years AGD outbreaks have been reported in a number of countries [2]. The chosen treatments are fresh water bathing; however, the effectiveness of this treatment has declined over time, that is because the process is time-consuming and expensive. The use of additives to prevent and control the diseases would therefore represent a potential reduction in treatment costs, provided it satisfy several criteria. It might be toxic to the undesired organism, while non-toxic to the fish. The action of the treatment needs to be rapid, along with not causing an additional stress to the fish beyond that of handling. Chloramine-T, sodium salt of N-chloro-p-toluene sulfonamide, (CAT) is extensively used as an additive as it satisfies most of the criteria. Besides, being a cost-effective, also the environmental impact is low, accordingly the treatment can be repeated on a daily basis without the need for major water changes [3,4]. One of the major reasons for its wide popularity over other reported additives such as chlorine dioxide, hydrogen peroxide, organic phosphate, per acetic acid/hydrogen peroxide/

acetic acid mixture is that it can be used both as a disinfectant and as a therapeutant [5]. Currently, there is an interest in obtaining an approval from the Food and Drug Administration for the use of CAT in aquaculture. Thus one of the requirements for approval is documentation of its efficiency during scientifically valid or pivotal treatments of infected fish. This calls for periodic monitoring of CAT [6].

HPLC unquestionably is the most extensive analytical method is used to determine the concentrations of CAT during the experimental treatments of fish in the course of studying the effectiveness of the additive for controlling gill disease [7]. However, this technique is not feasible at the hatchers as it requires a trained personnel for the maintenance of such a chromatographic equipment. Furthermore, elaborating a precautionary measures have to be taken to transport the sample to the laboratory for analysis. Attempts were made to develop a spectrophotometric method as surrogate method to replace HPLC which shows characteristic features, such as, on-site analysis, analysis time, cost, safety, sensitive and reliability. The reported surrogate spectrophotometric method [6] utilizes (N, N-diethyl-p-phenylenediamine) as a chromogen which develops a red color in presence of chlorine released form CAT in water samples. N,N diethyl-p-phenylene diamine (DPD) based chlorine kit available in the market measures free and total chlorine and there is no mention in the literature that DPD can be used for the determination of CAT. There are conflicting reports in the literature of the degradation mechanism of CAT to  $H^+$ ,  $OCl^-$  which ultimately release a chlorine molecule. This is contradicted with a recent report that indicates CAT ion is highly stable and remains over an extended period of time [8]. One of the recent reports mentioned chloramine-T ion as the major active species in aqueous media. Hence, there is an urgent need to develop an alternative spectrophotometric method for an accurate determination of CAT as chloramine-T ion rather than molecular chlorine, hypochlorous acid (HOCl) or hypochlorite ion ( $OCl^-$ ); the stoichiometry of which is not very well established.

The majority of compendial and approved by Food and Drug Administration (US) analytical methods utilize organic solvents, corrosive and toxic chemicals such as tetrahydrofuran, benzene, chlorinated reagents, etc. which are hazardous pollutants and/or carcinogens, with no other options currently available [9]. Development of analytical methods that meet both requirements of efficient applications and environmental considerations is an ambitious and quite new approach not only occurring in academia but also in industry especially, if the Commitment for development of eco-friendlier analytical techniques was mandatory by regulatory authority and/or has economical and financial merits.

With the objective of utilizing the non-toxic chemicals like phenoxazine, Cisapride and Metaclopramide as green analytical agents which is a 'control exercise' for the formation of hazardous substances, we report a simple, sensitive, rapid and novel spectrophotometric method based on the electrophilic coupling of CSP or MCP with phenoxazine (PNZ) as chromogenic reagent for the determination of chloramine-T. The results show that the methods are simple, sensitive, rapid and reliable. Besides, the reagents offer clear advantages over chromogenic reagent currently reported for the purpose and the procedure shows positive features over for reference, DPD colorimetric method [10].

## 2. EXPERIMENTAL

### 2.1. Solutions and Methods

Phenoxazine (PNZ) (Aldrich, India), MCP from Ipca Laboratories Ltd., India, CSP from USV Ltd., India, were obtained as gift samples. Stock solutions of CAT was prepared by dissolving (0.1%) w/v of CAT in 1 liter of distilled water. The solution was standardized by iodometric method [9]. Solutions of the required strength were prepared by diluting this stock solution with distilled water just before the experiment. Appropriate dilutions were prepared from the standard solutions. Stock solution of PNZ 0.05% (w/v) was prepared by dissolving 50 mg in distilled ethyl alcohol and diluting quantitatively to 100 ml with distilled ethyl alcohol. 0.5 % (w/v) solutions of CSP and MCP were prepared by dissolving 500 mg each and diluting to 100 ml with water. Solutions of diverse ions were prepared by dissolving their corresponding salts.

### 2.2. Apparatus

All spectral and absorbance measurements were carried out on a Specord 50 UV-vis spectrophotometer with 1.0-cm silica quartz matched cell.

### 2.3. Preparation of different Water Quality

The proposed method was initially evaluated with chloramine-T solutions. Subsequently, the water quality was evaluated at temperatures of  $7 \pm 1$ ,  $12 \pm 1$  and  $17 \pm 1^\circ\text{C}$ ; at pH's of  $6.5 \pm 0.2$ ,  $7.5 \pm 0.2$  and  $8.5 \pm 0.2$ ; at hardnesses of  $44 \pm 5$ ,  $170 \pm 10$ , and  $300 \pm 20$  mg/l as  $\text{CaCO}_3$  [9]. The selected of water conditions as standard (temperature =  $12 \pm 1^\circ\text{C}$ , pH =  $7.5 \pm 0.2$ , and hardness =  $170 \pm 10$  mg/l as  $\text{CaCO}_3$ ) were held constant as the range of one quality parameter was tested.

### 2.4. Spectrophotometric Procedure

Appropriate volumes of standard CAT solution containing known concentration as mentioned in Table 1 were transferred to a series of 25-ml calibrated flasks, 1ml of PNZ, 1 ml of 2M HCl and 1ml of CSP or MCP were added, The red color was formed almost instantaneously and made up to the mark with distilled ethyl alcohol and the absorbance were measured at 520 nm in 1.0-cm quartz cell against reagent blank which was prepared in the same manner but in the absence of CAT.

## 3. RESULTS AND DISCUSSION

The optical characteristics for the determination of chloramine-T with PNZ using CSP and MCP are detailed in the Table 1.

The effect of PNZ reagent was studied in the volume of 0.50 – 3.00 mL of the solution gave good result. Hence, 1 mL of (0.025 % w/v) PNZ solution in 25 mL standard flask was selected for further studies. Similarly, the same procedure was adopted to ascertain the amount of CSP or MCP required for getting constant and maximum color intensity. It

was found that 0.50 – 3.00 mL of the solution were needed to get good result. Hence, 1 mL of (0.15% w/v) CSP or MCP solutions is sufficient to get reproducible results.

Maximum intensity of the red color was achieved in the range of 1-6 mL of 2M HCl. Therefore, for getting the best results, 1 mL of 2M HCl in 25 mL was used.

Experiments were carried out to optimize temperature and time of the reaction. It was found that the maximum color developed 1 min at room temperature and remained almost stable for about 2h.

### 3.1. Order of Addition of Reactants

During the course of the investigation it was observed that the sequence of addition of reactants was also important as it influence the intensity and the stability of the color of the product to great extent as the reaction (i) and (ii) produced radical cation, while, electrophilic reaction was evident in the order (iii).

The sequence (i) CAT–CSP or MCP–PNZ–HCl and (ii) CSP or MCP–CAT–HCl–PNZ gave less intense and unstable color, while (iii) CAT–PNZ–CSP or MCP–HCl gave more intense and stable red color.

### 3.2. Effects of water quality

Concentrations of chloramine-T were measured by the reference DPD for the entire water quality variations. The proposed spectrophotometric method, solutions of chloramine-T (1, 3, and 5 mg/l) were prepared for comparative analyses in a variety of water quality conditions. The average accuracy of the proposed spectrophotometric method relative to the DPD colorimetric method was 99.1% when the effect of temperature was assessed as tabulated in Table 2, 99.7% when the effect of pH was assessed as tabulated in Table 3 and 98.0% when the effect of hardness was assessed as shown in Table 4.

### 3.3. Analytical Figures of Merit

The proposed spectrophotometric method was evaluated under the optimum conditions with respect to linearity, accuracy, precision and interference.

### 3.4. Linearity (Beer's Law Application), Accuracy, Precision

The linearity of the spectrophotometric method for the determination of CAT was evaluated under optimum conditions. The calibration curve was linear over the range 0.50–4.50  $\mu\text{g mL}^{-1}$ . The regression calibration equation obtained under optimum conditions for CSP with PNZ as example was:  $Y = 0.011 + 0.144 X$ ;  $r = 0.993$  and  $n = 7$ , where Y is the absorbance and X is the CAT concentration as  $\mu\text{g mL}^{-1}$ , r is the correlation coefficient. The detection limit was calculated as  $(DL = 3.3\delta/m)$ , where " $\delta$ " the standard deviation of the blank, "m" is the slope of the calibration curve. Keeping CSP as an example, the calculated detection limit was 0.098  $\mu\text{g mL}^{-1}$  of CAT. The accuracy of the proposed system was evaluated by comparing the results obtained with using the

proposed spectrophotometric method as well as the DPD spectrophotometric method [9]. The % R.S.D. was found to be  $< 1.4$  ( $n=7$ ). The proposed method was found as accurate and precise as that of DPD method.

Recovery tests by standard addition technique were performed known amounts of standard solutions at two different levels were added to a fixed amount of real sample and the mixtures were analyzed by the proposed procedure; each test was repeated 5 times to validate the accuracy of the proposed methods, the results presented in Table 5 show good recoveries and non-interference from common ions presented in the environmental sample such as  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ .

**Table 1:** Spectral data for the determination of CAT using CSP or MCP as electrophilic coupling agent and PNZ as chromogenic agent.

Parameters	CSP	MCP
Color	Red	Red
$\lambda_{\text{max}}$ (nm)	520	520
Stability (h)	2	2
Beer's law ( $\mu\text{g mL}^{-1}$ )	0.50-4.50	0.45-4.00
Recommended ion concentration ( $\mu\text{g mL}^{-1}$ )	2	2
Molar absorptivity ( $\text{L mol}^{-1}\text{cm}^{-1}$ ) $\times 10^4$	1.152	1.111
Lower Detection Limit (LOD) ( $\mu\text{g mL}^{-1}$ )	0.098	0.084
Regression equation*:		
Slope (a)	0.144	0.143
Intercept (b)	0.011	0.005
Correlation coefficient	0.9991	0.9972
R.S.D** % ( $n=7$ )	0.98	1.10

\* $y = ax + b$  where  $x$  is the concentration of CAT in  $\mu\text{g mL}^{-1}$ .

\*\* Relative Standard Deviation,  $n$ —number of the replicates.

**Table 2:** Accuracy and precision for measuring CAT concentrations at selected temperatures and with hardness of 170 mg/l as CaCO<sub>3</sub> and pH of 7.5.

Temperature (°C)	Chloramines-T added (mg/l)	DPD method (mg/l)	Proposed method (mg/l)	Average accuracy	R.S.D
15	1	1.10	0.98	98	1.20
	3	2.98	2.96	99	0.90
	5	5.10	4.91	98	1.80
20	1	0.96	0.95	95	2.10
	3	2.89	3.10	103	1.70
	5	5.09	5.06	101	0.45
24	1	1.09	1.10	101	0.67
	3	2.91	2.97	99	1.20
	5	4.83	4.92	98	1.80

Measurements are means of n=7.

R.S.D= relative standard deviation; Grant main = 99.1%.

**Table 3:** Accuracy and precision for measuring CAT concentrations of various PHs at hardness of 170 mg/l as CaCO<sub>3</sub> and temperature of 12°C.

pH	Chloramines-T added (mg/l)	DPD method (mg/l)	Proposed method (mg/l)	Average accuracy	R.S.D
6.5	1	1.20	1.10	92	0.43
	3	2.98	3.10	104	0.98
	5	4.93	4.96	99.50	0.63
7.5	1	0.98	1.02	104	0.31
	3	2.97	2.91	98	1.20
	5	5.09	5.10	102	1.80
8.5	1	0.96	0.93	97	0.28
	3	2.89	2.95	102	0.63
	5	5.01	4.97	99	0.47

Measurements are means of n=7.

R.S.D= relative standard deviation; Grant main = 99.7%.

**Table 4:** Accuracy and precision for measuring CAT concentrations of various hardness (as CaCO<sub>3</sub>) and pH of 7.5 and temperature 12°C.

Hardness (as CaCO <sub>3</sub> )	Chloramines-t added (mg/l)	DPD method (mg/l)	Proposed method (mg/l)	Average accuracy	R.S.D
44	1	1.08	1.06	98	1.23
	3	2.95	2.98	101	0.81
	5	4.91	4.93	100.4	0.31
170	1	6.98	0.93	95	0.18
	3	2.97	2.89	97	1.03
	5	4.93	5.08	103	1.40
300	1	1.04	0.99	95	0.93
	3	3.03	2.97	98	0.71
	5	5.03	4.93	98	0.38

Measurements are means of n=7.

R.S.D= relative standard deviation; Grant main = 98.00%.

**Table 5:** Determination of CAT in different environmental samples using CSP and PNZ.

Sample	CAT Added (g/ml)	Proposed Method		Reported Method		t - Value**	F- Value***
		Found (µg)	Recovery % ± RSD *	Found (µg)	Recovery % ± RSD *		
1	-	0.52	-	0.51	-	1.1	1.6
	0.5	1.03	101.9±0.31	1.02	101.0±0.85	1.3	2.5
	1.5	2.02	100.5±0.81	1.995	99.2±0.31	1.8	4.2
2	-	0.35	-	0.36	-	1.3	2.9
	0.5	0.865	100.6±1.01	0.997	98.7±0.51	0.79	4.23
	1.5	0.52	-	2.035	101.2±0.63	2.1	2.57
3	0.5	0.498	99.6±0.67	0.489	98.0±0.25	1.1	2.57
	1.5	1.498	99.86±1.03	1.502	100.1±1.40	1.98	3.98
4	0.5	0.492	98.4±0.52	0.491	98.2±0.92	0.38	5.31
	1.5	1.492	99.5±0.37	1.496	99.7±0.60	0.61	4.98

\*Average of five determinations  $\pm$  relative standard deviation.

\*\*Tabulated t-value at 95% confidence level is 2.78.

\*\*\*Tabulated F-value at 95% confidence level is 6.39.

### 3.4. Interferences

The tolerable limit of a foreign species was taken as a relative error which is not greater than 3%. It was found that 800-fold excess of Chloride  $\text{Cl}^-$ , Bromide  $\text{Br}^-$ , Nitrate  $\text{NO}_3^-$ , Sulfate  $\text{SO}_4^{2-}$ , 500-fold of Oxalate  $\text{C}_2\text{O}_4^{2-}$ , Hydrogen phosphate  $\text{HPO}_4^{2-}$ , Dihydrogen Phosphate  $\text{H}_2\text{PO}_4^-$ , EDTA and 1600-fold of potassium ion  $\text{K}^+$ , Sodium ion  $\text{Na}^+$ , Magnesium ion  $\text{Mg}^{2+}$ , Barium ion  $\text{Ba}^{2+}$ , Calcium ion  $\text{Ca}^{2+}$  and tartarate did not interfere with the proposed method. Foreign species interference were tested by analyzing a standard solution of  $3.0 \mu\text{g mL}^{-1}$  chlorine to which increasing amounts of interfering species were added.

### 3.5. Method Validation

To validate the proposed spectrophotometric method, Student's t-test was performed on the results of four real samples as shown in Table 5. Comparison was made between the proposed spectrophotometric method and the DPD method to find out whether the two methods give the same results at the 95% confidence level. The t-test with multiple samples was applied to examine whether the two methods for CAT determination differ significantly at the 95% confidence level. The calculated Student's t-value and F-value did not exceed the tabulated value indicating that the proposed methods are as accurate and precise as the DPD method.

## 4. CONCLUSION

Environmental analytical methods showing high performance but which are not environmentally friendly tend to be unacceptable and this will stimulate the development of cleaner methods. A first-ever use of PNZ, CSP and MCP as green analytical reagents for Monitoring Treatment Concentration of CAT on Fish Culture Facilities and Environmental Water Samples involving electrophilic coupling reaction with CSP or MCP are proposed. Although a variety of methods are available for the determination of chloramine-T, the proposed methods, besides, being simple, sensitive reliable and cost-effective also have the advantage of determination without the need for extraction or heating and not pH dependent. Simplicity of the procedures and less cost of the instrument are the hallmark of spectrophotometry. The methods do not involve troublesome reaction conditions and can be compared in sensitivity and reliability with standard method.

The replacement of old procedures by attractive methods exploiting available myriad molecules in the field of pharmaceutical chemistry will result in the phenomenal increase in the utilization of less or non-toxic reagents, which consequently will result in the reduction of toxic waste. This will develop an essential environmental conscience for the future.



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