Spectrophotometric determination of niclosamide Via Schiff's base formulations in pharmaceutical and veterinary preparations

Determinación por espectrofotometría de la niclosamida a través de formulaciones base de Schiff en preparaciones farmacéuticas y veterinarias

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ABSTRACT

Introduction: Niclosamide(NICS) its chemical name 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenz-amide]is the only commercially existing molluscicide optional by the WHO for large extent use in schistosomiasis be in charge of programs . NICS and its two new synthesized derivatives constructed to float on the water surface were able to kill cercariae, also obsessed promising activity in vitro nearby to an apicomplexan parasite Toxoplasma (4). Few spectrophotometric methods have been reported for the estimation of NICS as pure and in formulations, approximately these methods depend on reduction of nitro group (almost with zinc powder in acidic medium) followed by reaction with different reagents. The method based on reduction of nitro group of NICS then reaction of reduced-NICS with para- N,N-dimethylaminobenzaldehyde in non-aqueous medium (methanol) to form a colored product that has been proved successfully for the estimation of NICS in pharmaceutical and veterinary formulations **Material and method** :All reagents used are of analytical grade and are obtained from Fluka or Aldrich , NICS was supplied from SIGMA companies. Methanolic solution of para- N,N- dimethylanimobenzaldehyde (Fluka)3%, weighing 3 g and dissolved in 100 ml methanol in a volumetric flask. All other reagents were prepared by dissolving the propriety weight in perfect solvent. A volume in the range of 0.1 to 1.7 ml of 100 µg.ml-1RNICS solution was transferred to 10 ml calibrated flasks.2ml of PNNDMABA (3.0 %) was added, and the volume was made up to 10 ml by adding methanol. The yellow Schiff's base was measured at 454 nm versus a blank solution. **Results and Discussion**:The optimum pH for reaction of NICS with para-N, N- dimethylanimobenzaldehyde equal to 3 which resulted by mixing the components of the reaction. The absorbance increase with increasing reagent concentration (para-N,N- dimethylanimobenzaldehyde) and reached maximum on adding volume of 2.0 ml of (3%), which also gives the highest value of determination coefficient (R2).The experimental data indicated that methanol was the optimum solvent used in dilution according to high intensity of Schiff's base and the good stability. The formation of the yellow Schiff's base being complete after mixing the components of reaction and the absorbance remained constant for at least 2 hours. Conclusion: Accurate and sensitive spectrophotometric method was described for the estimation of NICS. The present method has been successfully applied for the estimation of NICS in pharmaceutical and veterinary preparations.

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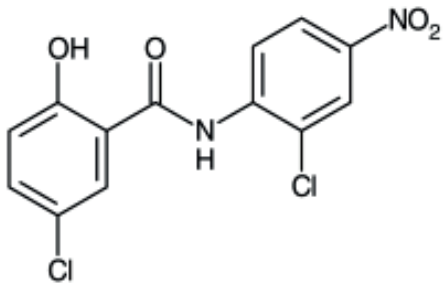
INTRODUCTION

Niclosamide, its chemical name 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenz-amide(Scheme I)] is the only commercially existing molluscicide optional by the WHO for large extent use in schistosomiasis be in charge of programs [1] .



Scheme I: Chemical structure of niclosamide

NICS and its two new synthesized derivatives were constructed to float on the water surface were able to kill cercariae [2,3], also obsessed promising activity in vitro nearby to an apicomplexan parasite Toxoplasma [4]. Few spectrophotometric methods have been reported for the estimation of NICS as pure and in formulations, approximately these methods depend on reduction of nitro group(almost with zinc powder in acidic medium) followed by reaction with different reagents such as metol and K2Cr2O7 in acidic medium (pH 3.0) to produce a red color,it measured at530 nm [5], 2,6 –dihydroxybenzoic acid after diazotization to form yellow azo dye having maximum absorbance at 456 nm [6] ,1,10 –phenanthroline to give red complex, its maximum absorbance at 510 nm [7] and p– benzoquinone to form a pink product which absorbs at 506 nm [8]. The derivative spectrophotometric methods have also been used in simultaneous determination of NICS in presence of author drug such as: drotaverine hydrochloride, [9] thiabenazole [10] in the same formulations.NICS in tablets has been estimated by dissolving the tablets in 0.1M NaOH solution ,then measuring the absorbance at 375 nm [11]. Various chromatographic methods include high performance liquid chromatography [12-15], LC-MS-MS [16,17] gas chromatography. [18]. The electrochemical methods for the determination of NICS via square – wave voltammetry [19], cyclic voltammetry at a glassy carbon electrode [20]. The purpose of the current work was the description of a simple and sensitive spectrophotometric estimation of NICS . The method based on reduction of nitro group of NICS then reaction of RNICS with NNDMABA in non-aqueous medium (methanol) to form a colored product that has been proved successfully for the estimation of NICS in pharmaceutical and veterinary formulations.

MATERIALS AND METHOD

Apparatus

Spectrophotometric measurements were carried out on Shimadzu UV-Visible Spectrophotometer UV-160, using 1-cm quart cells.

Reagents

All reagents used are of analytical grade and are obtained from Fluka or Aldrich, NICS was supplied from SIGMA companies.

Reagents preparation

Methanolic solution of PNNDMABA (Fluka)3%, weighing 3 g and dissolved in 100 ml methanol in a volumetric flask. All other reagents were prepared by dissolving the propriety weight in perfect solvent.

Reduction of nitro group in NICS

A 0.25 g of pure NICS was dissolved in a 15 ml of a mixture containing ethanol: acetone (1:1) (1), and the volume was completed to 25 ml in volumetric flask with the same mixture. A 5 ml of solution was treated with 0.1g of zinc powder and 5 ml of hydrochloric acid (1M). After standing for 15 minutes in water-bath at 90 C°, the solution was cooled in ice-bath, then; the solution was filtered out the insoluble material. The remains were washed with distilled water, and the volume of the filtrate completed up to 100 ml with distilled water in a volumetric flask. The final solution of RNICS in work containing 100 µg.ml-1 was prepared by further dilution.

Recommended procedure

A volume in the range of 0.1 to 1.7 ml of 100 µg.ml-1RNICS solution was transferred to 10 ml calibrated flasks.2ml of PNNDMABA (3.0 %) was added, and the volume was made up to 10 ml by adding methanol. The yellow Schiff's base was measured at 454 nm versus a blank solution.

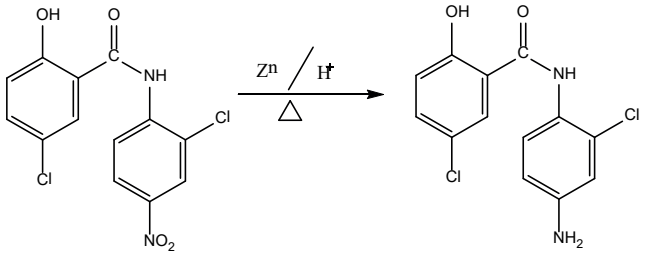
Analysis of NICS in pharmaceutical and veterinary preparations.

Ten tablets (for each formulation individually) were weighed and finely powdered. The powder amount equivalent to 0.25 g of NICS was taken and the procedure mentioned before is followed (the procedure for reduction of the nitro group). Solutions (100 $\mu g.ml^{-1}$) of pharmaceutical and veterinary preparations were prepared, and then the suggested procedure was proceeded.

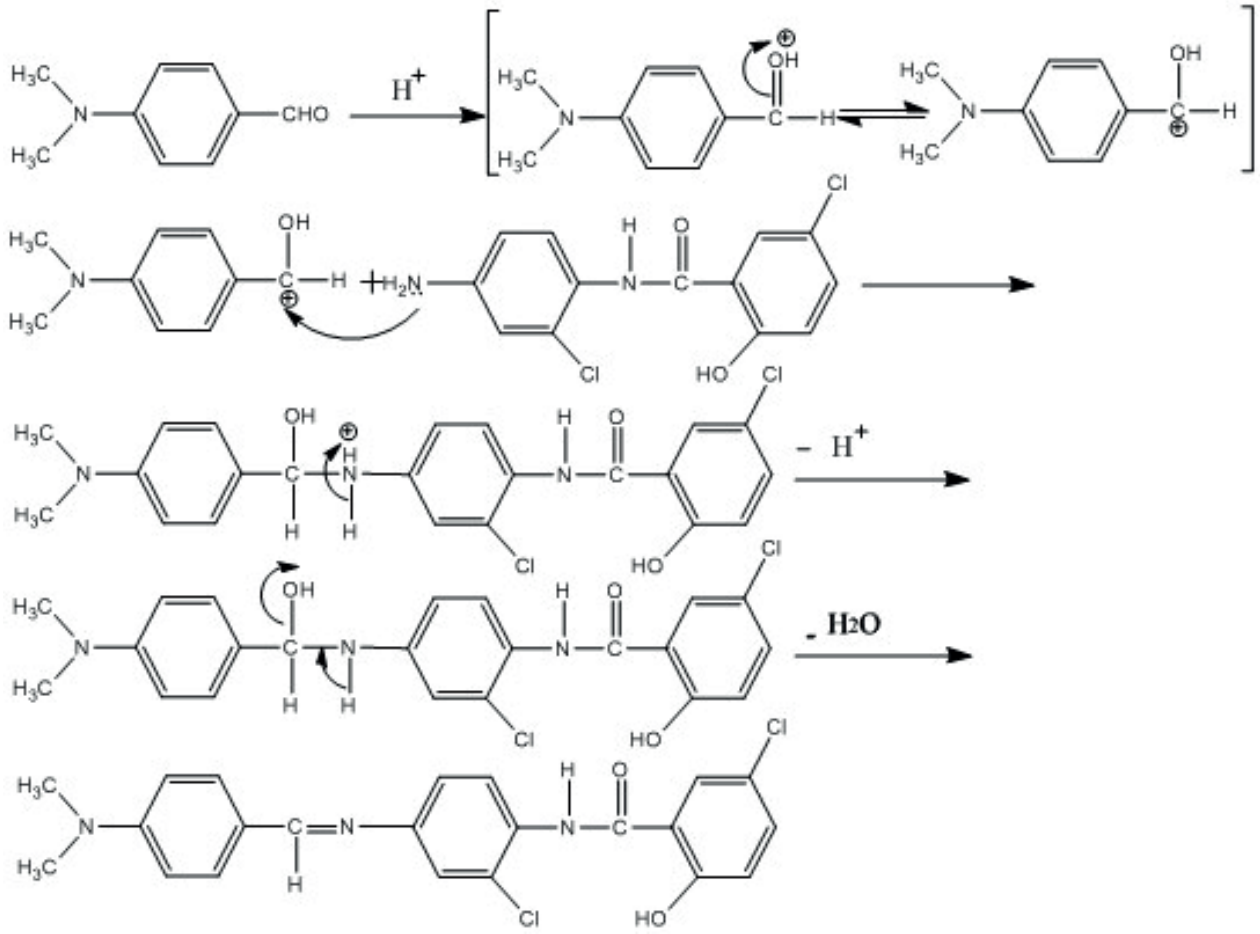
RESULTS AND DISCUSSION

The principle of reactions

Reduction of the nitro group in NICS by adding zinc powder and hydrochloric acid (as mention above) to produce RNICS.



The aromatic amine (RNICS) in presence of acid undergoes a condensation reaction in to form a yellow Schiff's base with a maximum absorbance at 454 nm (scheme 2).



Scheme 2. The formation of yellow Schiff's base.

All parameters affected the A of the yellow Schiff's base were investigated in order to construct the optimum conditions.

Selection of aldehyde.

Table 1 contains the results of using different aldehydes. The results illustrated in Table 1 indicated that PNNDMABA gives the highest intensity of colored product and good color contrast, so that it selected in the next experiments.

Table 1. The selection of aldehyde

Reagents (3%)	A	λmax (nm)	*Δλ (nm)	ε $l.mol^{-1}.cm^{-1}$
Benzaldehyde	**0.285**	**346**	**45**	0.932×10^4
Vaniline	**0.383**	**349**	**38**	1.252×10^4
***p*-Dimethylamino - benzaldehyde**	**0.441**	**454**	**70**	1.442×10^4
***p*-Diethylaminobenzaldehyde**	**0.398**	**454**	**66**	1.301×10^4
***p*-Nitrobenzaldehyde**	**0.369**	**368**	**38**	1.207×10^4
Cinnamaldehyde	**0.274**	**460**	**56**	0.896×10^4

* Color contrast (Δλ) = lmax S - lmax B S=Schiff's base and B=Blank

The effect of pH

Different amounts of 0.1M acetic acid or 0.1M sodium bicarbonate(individual) were added to the medium of the reaction ,the results indicated that the optimum pH =3 which resulted by mixing the components of the reaction(Table2).

Table 2. The effect of pH on absorbance.

0.1 M CH_3COOH (ml)	A*	0.1M $NaHCO_3$ (ml)	A
0.25	0.424	0.25	0.010
0.5	0.381	0.5	0.009
1.0	0.356	1.0	-0.003
1.5	0.338	1.5	-0.005
2.0	0.288	2.0	-0.007
pH =2.9-2.1		pH =3.2-6.0	

*Absorbance without acid or base = 0.449 at pH=3.00

Table (3) included the results of using four types of buffer solution with pH =3, there was a decrease in absorption intensity compared to the absence of these solutions; therefore the addition was eliminated in subsequent experiments. The addition of the reaction components, which gives the optimal pH, has been maintained.

Table 3. The effect of buffer solutions.

Buffer solution pH 3.0	Absorbance /min				
	0	10	15	20	30
Formic acid/KOH	**0.187**	**0.198**	**0.207**	**0.210**	**0.215**
Tartaric acid/ NaOH	**0.145**	**0.205**	**0.217**	**0.238**	**0.297**
KH phathalate/HCl	**0.145**	**0.250**	**0.247**	**0.281**	**0.291**
Glycine/HCl	**0.247**	**0.374**	**0.398**	**0.396**	**0.404**
Without buffer	**0.430**	**0.445**	**0.446**	**0.445**	**0.446**

The effect of PNNDMBA concentration

To a series of solutions containing 50-150 µg. of RNICS, various volumes of PNNDMBA (3.0%), then addition of methanol in a 100ml volumetric flasks. The obtained results indicated that the absorbance increase with increasing reagent concentration and reached maximum on adding volume of 2.0 ml of (3%) PNNDMABA, which also gives the highest value of determination coefficient (R2). Therefore, the addition of 2ml reagent was recommended for the subsequent experiments (Table 4).

Table 4. The effect of PNNDMBA concentration.

Volume of 3% PNNDMBA (ml)	Absorbance/ µg RNICS				R^2
	50	75	100	150	
0.5	0.226	0.395	0.446	0.790	0.98746
1.0	0.434	0.527	0.787	1.054	0.98966
1.5	0.473	0.782	0.930	1.289	0.99781
2.0	0.506	0.739	1.021	1.442	0.99829
2.5	0.502	0.720	1.013	1.426	0.99771
3.0	0.493	0.703	1.009	1.406	0.99664

The medium of dilution

Water, organic solvents and mixtures of solvents in different ratio have been tested for optimum condition. The results illustrated in Table 5.

Table 5 indicated that methanol was the optimum solvent used in dilution according to high intensity of Schiff's base and the good stability.

Table 5. The medium of dilution.

Solvent	λ_{max}(nm)	A	ε , l.mol^{-1}. cm^{-1}
Ethanol	454	0.833	$\times 10^4$ 2.724
Methanol	454	1.030	$\times 10^4$ 3.369
Formic acid	454	0.955	$\times 10^4$ 3.123
Acetone	Turbid	_____	_____
n-Propanol	453	0.775	$\times 10^4$ 2.535
Water	Turbid	_____	_____
Methanol: Water (1:1)	455	0.709	$\times 10^4$ 2.319
Ethanol: Water (1:1)	456	0.639	$\times 10^4$ 2.090

The stability of the yellow Schiff's base

The effect of time on the development and stability period of the Schiff's base was investigated under optimum conditions described before. The formation of the yellow Schiff's base being complete after mixing the components of reaction and the abs. of yellow Schiff's base remained constant for at least 2 hours.

The optimum conditions

The optimum conditions which constructed from the previous experiences were illustrated in table 6, which were maintained.in subsequent experiments

Table 6. The optimum condition.

Variable	Optimum
Wavelength	455 nm
Volume of p-NNDMABA	2ml (3.0 %)
pH	3
Time of measurement	After dilution
Medium of reaction	Non-aqueous solvent (methanol)

Estimation of absorption maximum

RNICS was coupling with PNNDMABA in methanolic medium to produce a yellow Schiff's base. The absorption spectra were taken against blank in the range 200-800 nm. Maximum absorption wavelength for RNICS was established to be 454 nm. Under the optimum conditions, the blank showed a negligible abs. at the related lmax (Fig. 1)

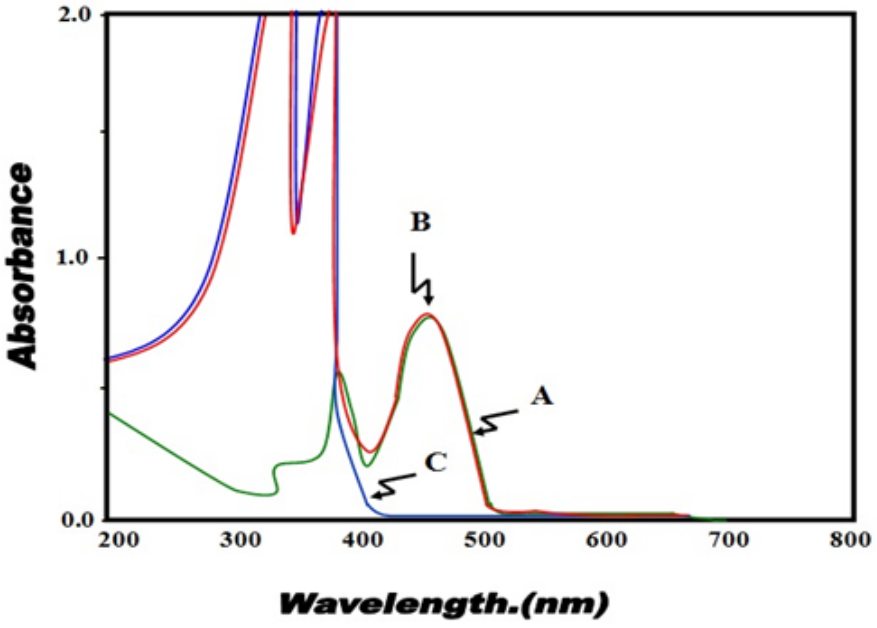


Fig.1. Absorption spectrum of: A-The Schiff's base against blank B- Schiff's base against distilled water. C- Blank against distilled water.

The Linearity

Fig 2, showed a good strength line from 10 to170 µg / 10 ml (1 to 17 µg/ml).

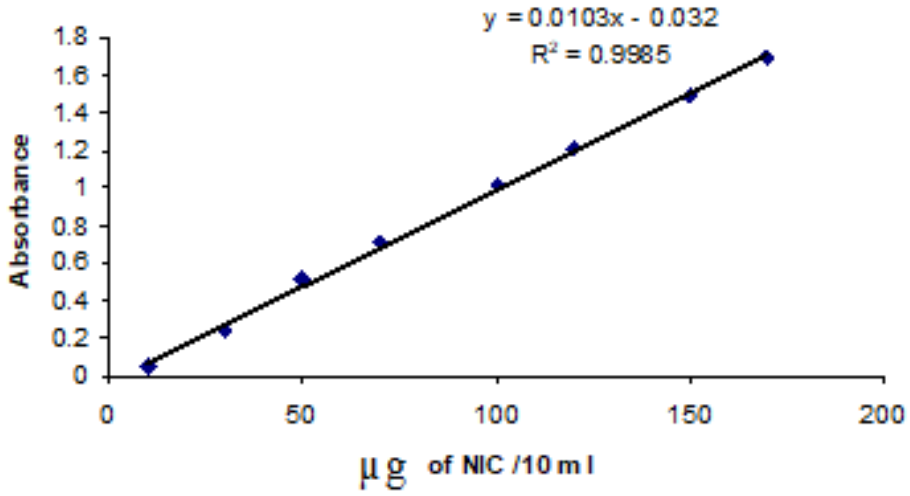


Fig. 2. Calibration graph for NICS determination using the proposed method.

Optical characteristics

Analytical variation such as the range of Beer's law, molar absorptivity factor and Sandell's sensitivity index are given in Table 7.

Table 7. Some optical characteristics.

Parameters	Value
λ_{max} (nm)	454
Limits of Beer's law (µg. ml^{-1})	1.0-17.0
Molar absorptivity factor(l. mol.$^{-1}$ cm^{-}1)	3.369×10^4
Sandell's sensitivity y index (µg.cm^{-2})	0.0097
Regression equation	Y = bX - C
Slope (b)	0.0103
Intercept (c)	-0.032
Determination coefficient (R^2)	0.9984
Relative standard deviation (R.S.D%)	0.616± 0.205 to ±
LOD (µg. ml^{-1})	0.00813
LOQ (µg. ml^{-1})	0.02711
Stability (hrs.)	2
Color	Yellow

Application part

The method was successfully applied to assay NICS in its pharmaceutical (Tablet) and veterinary drugs form formulations (Table 8 and 9 respectively)

Table 8. The results of determination NICS in tablet.

Drug	µg RNICS present	µg RNICS measured	Recovery (%)*
Yomesan 500mg /Tablet	50	49.8	99.6
	100	100.3	100.3
	150	149.2	99.2

*Average of five determinations.

Table 9. The results of determination NICS in veterinary preparations.

Veterinary preparations	µg RNICS present	µg RNICS measured	Recovery (%)*
Niclosamide 1250mg/Tablet	50	49.0	98.0
	100	98.8	98.8
	150	148.8	99.2
Niclosan 250mg/Tablet	50	47.4	95.6
	100	97.8	97.8
	150	142.9	95.3

*Average of five determinations.

The performance of the suggested method was assessed by calculation of t-test compared with the typical method (1) for 95% confidence rank with eight degrees of freedom. The calculated value was less than the critical value, indicated that there were no significant differences between the present and standard methods for NICS estimation.

CONCLUSION

Accurate and sensitive spectrophotometric method was described for the estimation of NICS.. The present method has been successfully applied for the estimation of NICS in pharmaceutical and veterinary preparations.

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