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Developing and Validating a Spectrophotometric Method for Estimating Antifungal (Nystatin) in its Pure Form Pharmaceutical Formulation Using Tetrachloro-1,4 benzoquinone

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Abstract

The UV–VIS absorption spectroscopy technique was used to study the formation of a new complex of charge transfer (CT) between bioactive organic molecules as (Nystatin) containing both a π-electrons from a conjugated system and lone-pair of electrons (amine) with Tetrachloro-1,4 benzoquinone (TCBQ) as a π-acceptor in which the transferred electron goes into its vacant anti-bonding molecular orbitals. The Tyrian purple-colored complex formed was quantitatively measured at 544 nm. This complex shows obeying Beer's law within the concentration range of (10-90) µg.ml-1The stoichiometry of the formed complex between the (Nys.) and (TCBQ) was found 1:2 as evaluated by continuous variation (Job's method) and mole ratio method The value of molar absorptivity was calculated at 7038.2840 L.mol-1.cm-1, while Sandell's sensitivity value was estimated to be 0.01315 µg.cm-2, while LOD and LOQ were found to be 0.5661and 1.71558 µg.ml-1, respectively. The Charge-Transfer complex association constant (KCT) value was evaluated using Benesi-Hildebrand equation and was found to be 3.00E+03 L.mol-1. This procedure was successfully involved in the analysis of pharmaceutical formulations.

Keywords

UV–VIS absorption spectroscopy, Charge-Transfer Complex, Nystatin (Nys.) & tetrachloro-1,4- benzoquinone (TCBQ).

Nystatin is an antifungal polyene drug of Streptomyces noursei and it was one of the first antifungal drugs to be discovered and used clinically. It exhibits fungicidal activity against a wide spectrum of fungal pathogens (e.g. Candida albicans, Candida glabrata, Candida kruse and Candida tropicalis), including azole-resistant strains of Candida and, in some cases, amphotericin B-resistant strains of Candida albicans. It is used for the treatment of cutaneous, intestinal, oropharyngeal, and vulvovaginal candidiasis. [1,2,3]. Nystatin is a stereoisomeric (1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E,21E,25E,27E,29E,31E,33R,35S,36R,37S)-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl) oxy] 1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo [33.3.1] nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid [4]

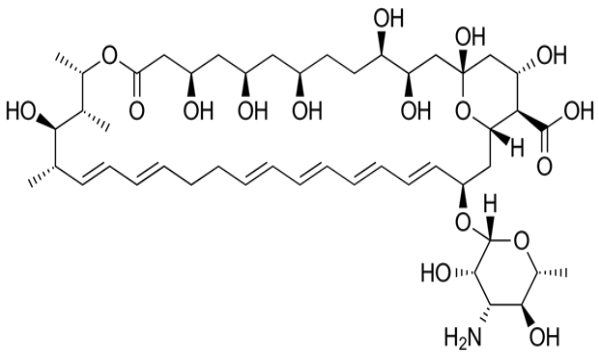
It is a yellowish or slightly brownish powder, hygroscopic. Practically insoluble in water, freely

soluble in dimethyl formamide and in dimethyl sulphoxide, slightly soluble in methanol, and practically insoluble in alcohol. [4,5]

Nystatin acts by binding its hydrophobic part to ergosterol in the cell membrane of sensitive fungi and causes extreme permeability of the plasma membrane, allowing potassium permeability to be increased, which leads to reversible fungistasis. While higher doses result in the formation of multimeric pores of 40–105 nm. These pores lead to increased membrane permeability to the cations Mg^{2+}, Ca^{2+}, and Mg^{2+}, with consequent fungal cell death.

Nystatin, in addition to its action of making the cell membrane more permeable, potentiates the entry of drugs such as 5-flucytosine and tetracycline into Candida albicans cells Nystatin and other polyene antifungal drugs may also contribute to the activation of the host's innate immune system [2,6,7]. It is safe to use during pregnancy to treat oral Candida infections. When applied topically or taken orally, it is not absorbed into the systemic circulation. This Drug may also be used to treat thrush in either the pregnant woman or the nursing infant [2].

The spectrophotometric method is one of the widest methods for the quantitative study of (Nys.) because of its sensitivity in the determination of drugs. [8-16) In addition, there are many other methods used for the quantitative determination of (Nys.) like HPLC. [17-21].



Scheme (1): The structural formula of Nystatin. [6,22]

1. Experimental Part

1.1 Instruments

- Visible Spectrophotometer with 1mL glass cuvettes (ROAYA LAB Model 721).
- UV-Vis 1800 Spectrophotometer- Shimadzu-(Kyoto-Japan) has match glass cuvettes (1 mL) was used for the determination of maximum Absorption which was used in all measurements.
- Inolab pH-7110 (Germany) pH meter ,WTW GmbH, has a combined glass electrode. It was used to test the pH value of solutions. An analytical sensitive electronic balance having a readability of ±0.0001 g ,(Kern & Sohn GmbH Germany) ACS 120-4 , was used for weighing substances.
- Water bath sonicator, LabTech Model LUC-405.
- Laboratory Oven, LabTech Diahan Model LDO-030E.
- Hot plate with magnetic stirrer, Ijlassco, India.

1.2 Materials and Reagents

All the chemicals used in this study were reagent grade with the highest purity and used without any additional purification. Tetrachloro-1,4 benzoquinone (TCBQ) (99.9%, BDH), Pharmaceutical grade of Nystatin (Nys.) notarized to be 99.89% pure was obtained from the State Corporation for Drug Industries and Medical Appliances Samara-Iraq (SDI). Nystatin (500.000 IU Nys./Coated tablet) from "BELMEDPREPARATY" RUE, Republic of Belarus,220007, Minsk,30,Fabritsius St.

1.3 Reagents solutions:

Tetrachloro-1,4-benzoquinone (TCBQ) 0.3%(w/v): The stock solution was freshly prepared by dissolving an accurate weight of 0.15 g of (TCBQ) in 1,4-Dioxane and diluted to 50mL with the same solvent. The solution is prepared freshly before the routine work.

Glucose, Sucrose and lactose 3000('g. mL^{-1}): The stock solutions of those saccharides' types were prepared by dissolving an accurate weight of 0.075 gm of all previous types of saccharides in distilled water and then diluted to the mark in 25 mL volumetric flask individually.

Starch 3000('g. mL^{-1}): 0.075g was mixed with 5mL of cold distilled water stirring until was gained a fluffy paste, and 10 ml of boiling distilled water was added to the paste. The mix was heated for a few minutes till a clear solution is gained. Then the solution was completed to the mark in a volumetric flask of 25 mL

1.4 Prepare the Standard Stock Solution for Nystatin

Nystatin standard stock solution 1000(µg. mL^{-1}) was prepared by dissolving an exacted weight of 0.05 g of pure drug in 5.5mL of Tri ethyl amine and sonicated for 5 min to ensure the complete dissolution of the drug. The volume of solution was achieved to the mark in a 50mL volumetric flask with Methanol. The working routine solutions were freshly prepared by consecutive diluting with methanol and analyzed by the proposed method

1.5 Recommended procedure

Into a series of Pyrex test tubes, (1) mL of various concentrations of a standard solution of (Nys.) ranged (10-100) µg. mL^{-1}, 1.6 mL of 0.3%(w/v) (TCBQ) as a (π-acceptor reagent) were added carefully. The sealed Pyrex test tubes were placed in a water bath at 70 °C for 15 minutes. After the Test tubes were left for 2min, reaction mixture was diluted to final volume 5 mL with isopropanol by adding 2.4 mL. The maximum absorbance of the Tyrian Purple Charge-transfer complex was discovered at 544nm against a blank solution that was prepared similarly excluding drug substance.

1.6 Sample Preparation

Ten tablets each containing 500.000 IU of (Nys.) were accurately weighed individually and was ground to finely powdered. An average weight was calculated. 0.1417gm of Belarusian Nystatin, weighed accurately, and dissolved in 5.5mL of Tri ethyl amine and 10 mL Methanol. The previous mixture was sonicated for 20 min to ensure the complete dissolution. The volume solution was achieved to the mark in a 50mL volumetric flask with the Methanol and left to stabilize. After (24) h the solutions were filtered using Whatman filter paper No.540 to avert all Pendent or undissolved substances before the use. The resulting filtrate was moved into a volumetric flask of 50 ml to get 1000 (µg. mL-1). The solutions were freshly prepared by successive dilutions with methanol and studied by the suggested Method.

1.7 Results and Discussion

a) The Analytical Procedure Optimization

Many optimum conditions were investigated in this research to identify their effect on the immediate and quantitative formation of a colored charge-transfer complex including stability and sensitivity by a number of primary experiments.

b) Absorption spectra

The reaction of (Nys.) as n, π-donor, with (TCBQ), as π-acceptors, yielded an intense Tyrian Purple colored complex in an organic medium having a new band with maximum absorption at 544 nm while the corresponding blank gave a low absorbance at this wavelength (Fig. 1). The new absorption peak is the outcome of the charge-transfer complex production by the interaction of (TCBQ) as a π-acceptor and the considered drug (Nys.) as n & π-donors followed by the formation of radical anion.

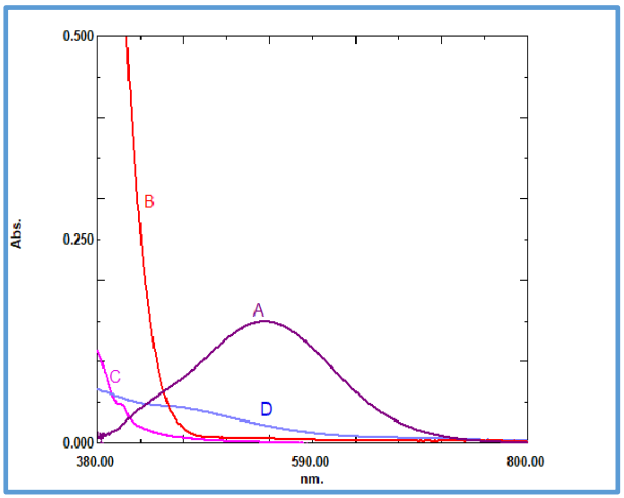


Figure 1: Absorption spectrum of (A) [(Nys.) + (TCBQ)Charge Transfer (10 µg. mL^{-1} Nys.)], (B) (TCBQ), (C) (10 µg. mL^{-1} Nys.), and (D) Blank-blank.

c) Effect of Reagent Concentration

The effect of (TCBQ) concentration was studied by adding 1 mL of 50(µgmL^{-1}) Nys. to which different con. of (TCBQ) in series test tubes. After the reaction mixture was left for 5min, diluted to 5 ml with (1,4 Dioxane). The absorbance of the Tyrian Purple Charge-transfer complex was measured at an initial 544nm against the corresponding blank which is prepared in the same manner. The results show (Fig. 2)

that the highest absorbance was obtained with a concentration of reagent equal to 0.3% which was used during this study.

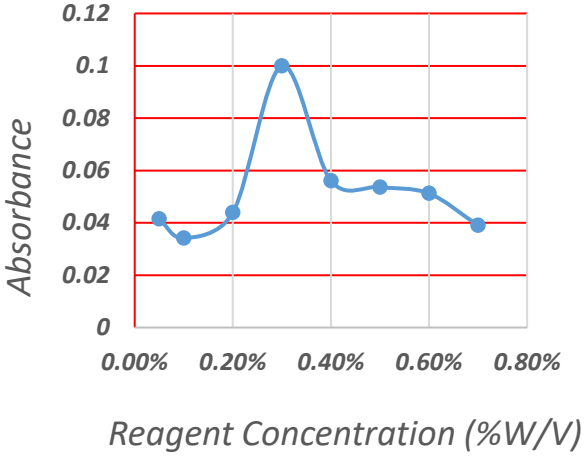


Figure 2: Effect of Reagent Concentration.

d) Effect of the Volume of Reagent

The effect of (TCBQ)volume was studied by adding 1 mL of 50(μgmL^{-1}) (Nys.) to which different volumes of 0.3% (w/v) (TCBQ) in series test tubes. The After the reaction mixture was left for 5min and diluted to 5 ml with (1,4 Dioxane). The absorbance's of the Tyrian Purple Charge-transfer complex were measured at 544nm against the corresponding blank which is prepared in the same manner. Results demonstrate that the best volume of 0.3%(w/v) (TCBQ)is 1.6 mL, as shown in (Fig. 3).

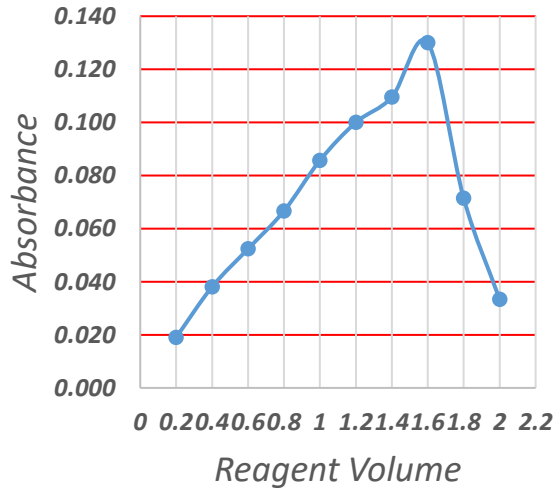


Figure 3: Effect of the Volume of Reagent

e) Effect of Diluting Solvents

Different solvents were studied as diluting solvent to ensure which one is the best for the complete formation of the complex. The results exhibited that the ideal diluting solvent to attain extreme sensitivity and stability of the colored complex was Isopropyl alcohol. (Fig. 4). [Distilled water was tested as a dilution solvent, but the resultant solution was turbid].

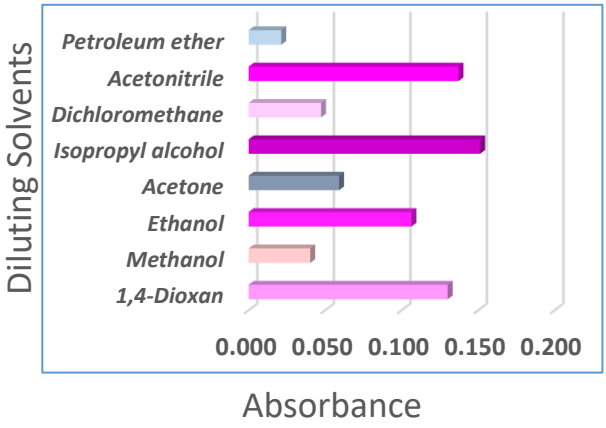


Figure 4: Effect of Diluting Solvents.

f) Effect of Heating Temperature

The effect of heating temperature on the entire formation of the charge-transfer complex was carried out by changing the water-bath temperature (10° to 80°) °C. The 70°C was chosen as an optimum temperature. (Fig 5).

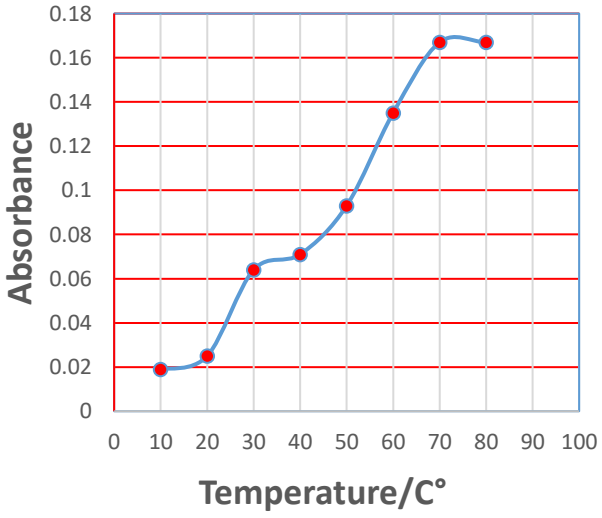


Figure 5: Influence of heating temperature on charge-transfer complex formation.

g) Effect of Heating Time

The heating time effect on the charge-transfer complex formation was investigated in this research by heating the mixture at optimum temperature70^{0}C during different time periods. The colored charge-transfer complex was attained the maximum absorbance at 15min. (Fig 6).

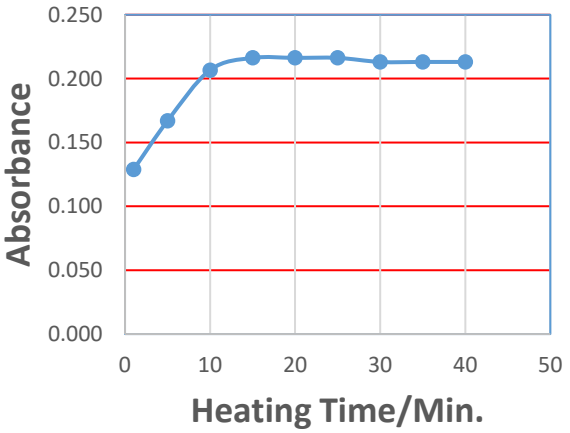


Figure 6: Influence of heating time on charge-transfer complex.

h) Stability of Complex

The complex absorption was measured every five minutes to determine its stability (Fig 7). As can be seen from the figure, the complex showed a higher stability in the absorption values within a moment measured to 75 minutes. After that, the complex recorded a slight rise in absorbance within the range of (75-90) minutes. This gives the proposed procedure an advantage in measuring the absorption of the complex within 35 minutes.

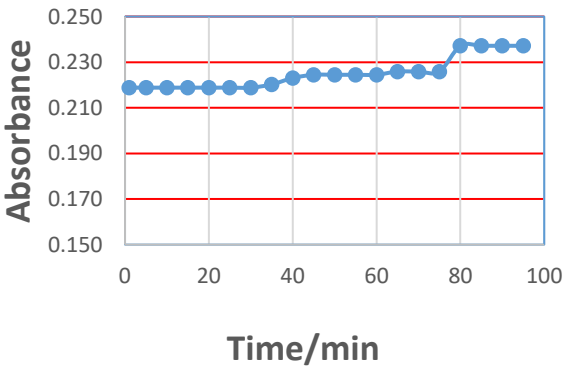


Figure 6: Showed the Stability of charge-transfer complex.

i) Effect of Interferences

Different compounds like (Glucose, Sucrose, Lactose and Starch) were studied as interferences in order to investigate their effect as commonly encountered compounds present in Nystatin dosage form (Table 1). The results showed that good recovery was obtained in tablets analysis by applying the proposed procedure

Interference	Absorbance
Glucose	0.204
Sucrose	0.219
Lactose	0.207
Starch	0.201

j) Composition of (Nys.-TCBQ) Complex

The stoichiometry of **(Nys.-**TCBQ) Charge-transfer complex was determined spectrophotometrically by job's and mole ratio methods. [23,24] equimolar concentrations of the Nys. and TCBQ (5.399×10^{-4}) M were used. Different volumes of the drug and reagent were taken with maintaining a fixed final volume of the complex at 5 ml. The resultant Charge-transfer complex absorbance was measured at the optimum wavelength according to the general procedure. The results demonstrated that1:2 (drug: reagent) Charge-transfer complex is formed as shown in (Fig 6,7).

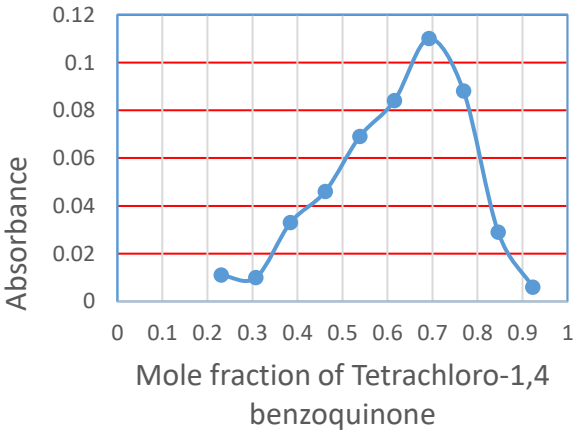


Figure 6: Job's method plot for Charge-transfer complex of

Nys. $=5.3990\times10^{-4}$ at 544 nm.

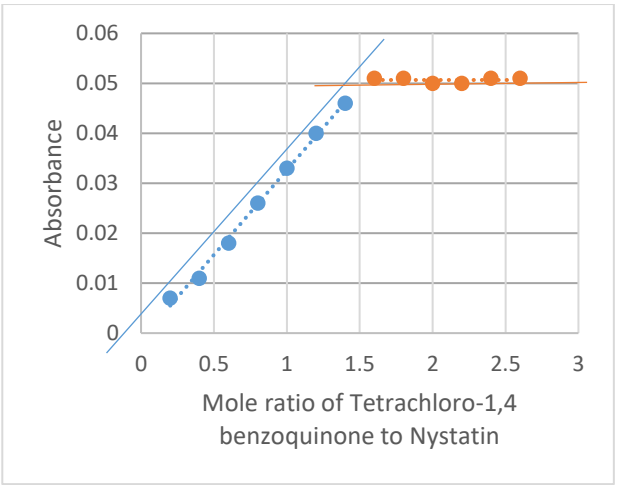
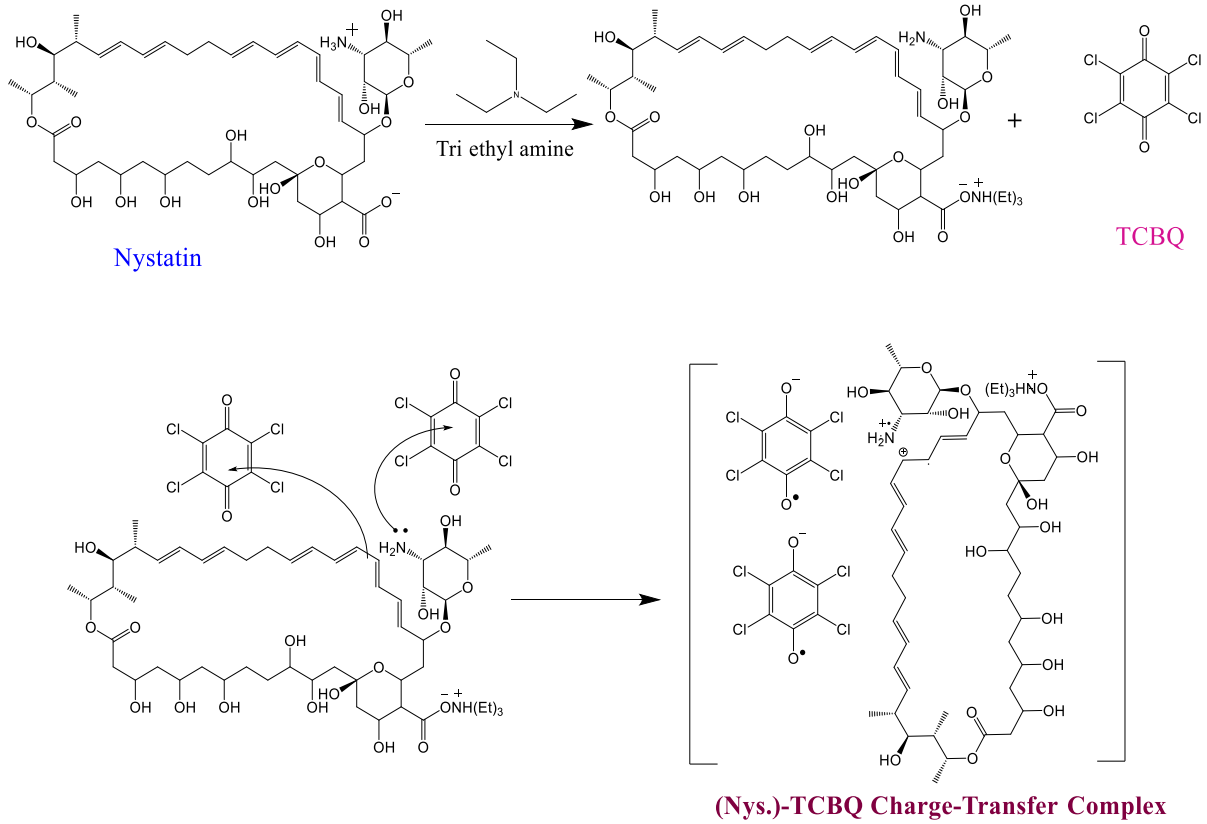


Figure 7: Mole-ratio method of continuous variations plot Charge-transfer complex of Nys. =5.3990×10^{-4} at 544 nm.

Given this result, a reaction mechanism proposed that two of (TCBQ) molecules interact with the (Nys.) molecule. The first connection was proposed as a result of the transfer of free non-bonding electrons on the nitrogen atom of the Primary amino group existing in a molecule of the (Nys.) to the charge-deficient center of the (TCBQ) molecule to form n-π^* interaction. Much literatures data are corresponding with these experimental results. [(25)- (32)].

The second connection was proposed as a result of the transfer of electrons from one of four conjugated double bonds in the (Nys.) as a type of polyenes [33] to the charge-deficient center of the (TCBQ)molecule to form π-π^* interaction. [34,35,36& 37]

(Nys.) is thus shown to form Charge-Transfer complex with (TCBQ) through the reaction.



Scheme (2): The proposed mechanism of (Nys.)- TCBQ Charge-Transfer complex formation

k) Association constant of Charge-Transfer complex

The equation Benesi-Hildebrand [38,39] was used for the determination of Association constant of the complex, which presented as below:

$$\frac{[Reagent]}{Ac} = \frac{1}{\varepsilon_c} + \frac{1}{\varepsilon_c K_{C.T}} \times \frac{1}{[Drug]}$$

Where:

[regent] and [Drug]: refers to the total concentration of (TCBQ) and (Nys.), respectively.

Ɛc: refers to complex molar absorptivity.

Ac: refers to complex absorbance.

$K_{C.T}$: The formation constant of (Nys.)-TCBQ complex.

The value for $K_{C.T}$ was calculated from the slope of the line attained by plotting **[**(TCBQ)]/Ac versus 1/[Nys.]**]**. The relation was linear as shown in (Fig 8) and the result is demonstrated in Table (3).

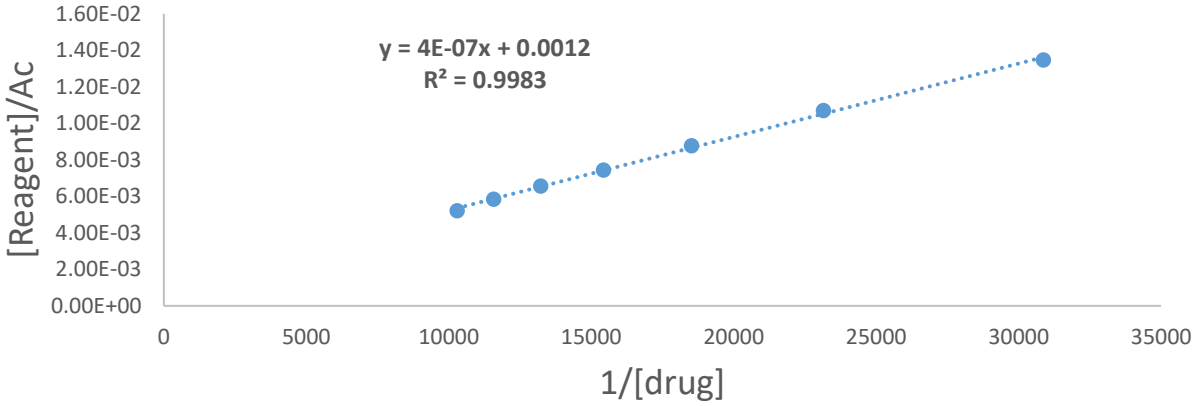


Figure 8: Benesi-Hildebrand plot for (Nys.)- TCBQ complex.

Table 3: Formation constant of Nys. - TCBQ charge-transfer complex

Parameter	Observation
Intercept	0.0012
ε_c (L.mol^{-1}.cm^{-1})	833.3333
Slope	4.00E-07
$K_{c.p}$ (L.mol^{-1})	3.00E+03
ΔG (KJ.mol^{-1})	-22.8417
Correlation Coefficient (r)	0.9991

I) Method Validation

a) Linearity

The parameters (molar absorptivity, Beer's law range, regression equation, Sandell's sensitivity, and correlation coefficient) were listed in Table (4). A linear relationship was construct between the concentration of the drug in the range 10-90 µg. mL^{-1} (Fig. 9) at λmax 544 nm. The calibration curve linearity was affirmed by a high correlation coefficient (r)value.

Table 4: The statistical information & optical Characteristics and the regression equation for Nys. - TCBQ complex

Parameter	The proposed method
λ_{max} (nm)	544
Beer's limit (µg. mL^{-1})	10-90
Slope (mL. µg^{-1})	0.0076
Molar absorptivity (L.mol^{-1}cm^{-1})	7038.2840
LOD (µg. mL^{-1})	0.5661
LOQ (µg. mL^{-1})	1.71558
Intercept	0.0659
Sandell's sensitivity (µg.cm^{-2})	0.01315
Correlation Coefficient (r)	0.9998

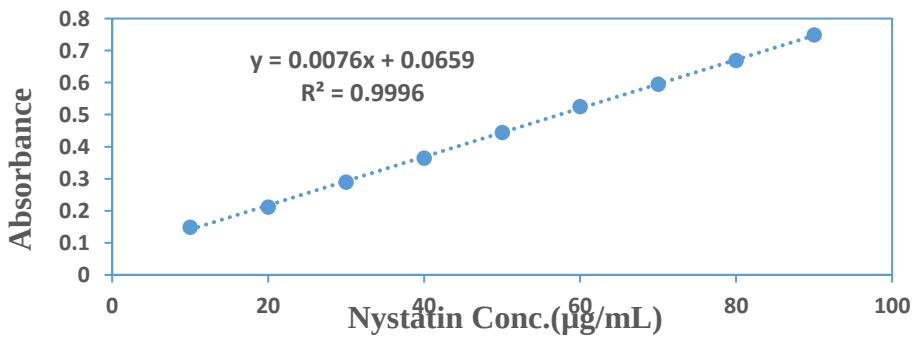


Figure 9: Calibration Curve of Nystatin.

b) Sensitivity

The values of the limit of detection (LOD) and the limit of quantification (LOQ) for this procedure were determined [40]. The limit of detection (LOD) value for the suggested procedure was calculated by the subsequent equation:

LOD=3.3 s/slope

Where:

s: is the standard deviation of repeating results in similar circumstances as in the sample analysis with no analyte.

Slope: is the calibration curve slope.

Depending to this method, the limit of detection was found to be 0.5661µg. mL^{-1}.

The limit of quantification, LOQ is defined as:

LOQ=10 s/slope

for this method, LOQ was found to be 1.71558 µg. mL^{-1}.

c) Precision and Accuracy

The suggested procedure was applied successfully for (Nys.) quantitative determination in its pure solutions. Solutions with Three various concentrations of (Nys.) were prepared and analyzed with five replicate measurements. The validation of the proposed procedure was attained by the accuracy and precision test expressed with percent relative standard deviation and percent relative error for intraday measurements of the prepared solutions. The data are demonstrated in (table 5). The recoveries of (Nys.) of each concentration were also determined. The outcomes have exhibited good accuracy and precision.

Table 5: The values of (Nys.) Precision and accuracy are achieved by the proposed method.

Nominal Conc. (µg. mL^{-1})	Found* Conc. (µg. mL^{-1})	RSD %	Er %	Recovery %
20.000	19.9728	0.5444	0.3704	99.8642
40.000	39.8854	0.2621	-0.2863	99.7136
80.000	79.9648	0.3608	-0.0438	99.9561

*Average of five determinations.

d) Analysis of Pharmaceutical Preparation

The direct method was used for the determination of (Nys.) in a type of pharmacological formulation; Belarusian (Nys.) 500.000 IU coated tablets. The results acquired are recorded in (table 6). The procedure indicated that RSD% ranged between (0.1820-0.3642) and Rec% ranged between (100.1557-100.2666). The results obtained are listed in (table 6). This method exhibited that the RSD% ranged (from 0.1820-0.3642) and Rec% ranged (from 100.1557-100.2666). The documented percent recoveries of Nys. in (table 6) were estimated using the data documented by the British Pharmacopoeia.

Table 6: Analysis of Pharmaceutical Preparation

Nominal Conc. µg. mL^{-1}	Found* Conc. µg. mL^{-1}	RSD %	Er %	Recovery %
20.000	20.0311	0.2970	0.0192	100.1557
40.000	40.1066	0.3642	0.2666	100.2666
60.000	60.1046	0.1820	0.1308	100.1744

*Average of five determinations.

e) Conclusion

This procedure is built on the formation of a charge-transfer reaction between the basic nitrogen of the Nys. as n &π-donor with π-acceptor of the tetrachloro-1,4-benzoquinone (TCBQ) which produced a Tyrian purple complex. The obtained results showed good values of precision, accuracy, and recovery of Nys. which proved the sensitivity, reproducibility, and simplicity of this procedure to determine the drug (Nys.) in pure form and its pharmaceutical formulations.

References

Bronwen Bryant and Kathleen Knights" PHARMACOLOGY FOR HEALTH PROFESSIONALS", 6e,6th edition, Mosby, Elsevier Australia CAN 001002357,ISBN:978-0-7295-4390-P:(322), (2022).

Haveles, Elena Bablenis "Applied Pharmacology for the Dental Hygienist" Elsevier Health Sciences, St. Louis, Missouri 63043, ISBN: 978-0-323-59539-1,p:(330), (819),(2020).

Linda E. McCuistion , Kathleen Vuljoin DiMaggio , Mary B. Winton , Jennifer J. Yeager "Pharmacology A Patient-Centered Nursing Process Approach"10th edition, Elsevier, St. Louis, Missouri 63043, ISBN: 978-0-323-64247-7,p:(1043), (2021).

R.S. Vardanyan and V.J. Hruby" Synthesis of Essential Drugs" Elsevier, ISBN: 978-0-444-52166-8 p:(537), (2006).

WILLIAM E. DISMUKES ,PETER G. PAPPAS& JACK D. SOBEL" CLINICAL MYCOLOGY" Oxford University Press, Inc. ,P:(65-75), (2003).

M. LINDSAY GRAYSON, SARA E. COSGROVE, JAMES S. MCCARTHY, SUZANNE M. CROWE, JOHN MILLS, JOHAN W. MOUTON, WILLIAM HOPE & DAVID L. PATERSON" Kucers' THE USE OF ANTIBIOTICS: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs" Taylor & Francis Group, LLC , p:(2647),(2018).

Charles D. Ciccone" Pharmacology in Rehabilitation" 5th, A.F Davis Company, p:(551), (2022).

J.M. Lemus Gallego & J. Pérez Arroyo" Spectrophotometric determination of hydrocortisone, nystatin and oxytetracycline in synthetic and pharmaceutical preparations based on various univariate and multivariate methods" Analytica Chimica Acta , Vol. 460, Issue 1, Pages 85-97, 28 May 2002, https://doi.org/10.1016/S0003-2670(02)00138-1

I Ia Bershteĭn "Spectrophotometric determination of nystatin based on Ashton and Tootill's method" PMID: 7396443,;Vol.25, Issue 6, p:(444-9), (1980) Jun

Bahram hemmateenejad, mohsen nekoeinia, Ghodratollah aBsalan , mehdi ansari "Photodegradation Study of Nystatin by UV-Vis Spectrophotometry and Chemometrics Modeling" Journal of AOAC International, Vol. 97, Issue 4, p: (1206–1212), 1 July 2014. https://doi.org/10.5740/jaoacint.13-110

S. RODINO, M. BUTU, C. NEGOESCU, A. CAUNII, R. T. CRISTINA, M. BUTNARIU "SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF NYSTATIN ANTIFUNGAL AGENT IN PHARMACEUTICAL FORMULATIONS" Digest Journal of Nanomaterials and Biostructures, Vol. 9, No. 3, p: (1215 – 1222), July – September 2014.

Nickos A. Botsoglou & and Dimitrios J. Fletouris "Rapid Spectrophotometric Method for the Assay of Nystatin in Feeds" J. Agric. Food Chem., Vol. 44, p:(1271−1274),1996.

Marjan Rahimi Fard & Zeinab Pourghobadi "The Spectrophotometric Determination of Nystatin in Real Samples Using Solid Phase Extraction Based on Sodium Dodecyl Sulphate-Coated Magnetite Nanoparticles" Anal. Bioanal. Chem. Res., Vol. 5, No. 2, p:(249-259), December 2018.

Intisar A. Shihab & Theia'a N. Al-Sabha "Spectrophotometric Determination of Ibuprofen and Nystatin Via Ion Pair Complex Formation Using Chromotrope 2R" J. Edu. & Sci., Vol. 28, No. 2 ,p: (289-299) , 2019.

Patel Ankita Dipak & Madhuri Hinge "Development and validation of Two UV Spectrophotometric methods for simultaneous estimation of Nystatin and Triamcinolone acetonide in synthetic mixture" International Journal of All Research Education and Scientific Methods (IJARESM), Vol.9, Issue 6, p: (97-105), June 20121, Impact Factor :7.429, ISSN: 2455-6211.

Luma Ismail Ibrahem & Qabas Naji "Rashid Spectrophotometric determination of nystatin in its pharmaceutical preparations" AIP Conference Proceedings 2450, 020034 (2022); https://doi.org/10.1063/5.0094147.

Juan M. Llabot, Daniel A. Allemandi, Ruben H. Manzo, Marcela R. Longhi "HPLC method for the determination of nystatin in saliva for application in clinical studies" Journal of Pharmaceutical and Biomedical Analysis, Vol. 45 p: (526–530), (2007). www.Elsevier.com

Ana Paola Prata Cione, Márcio José Liberale & Paulo Marcos da Silva "Development and validation of an HPLC method for stability evaluation of nystatin" Brazilian Journal of Pharmaceutical Sciences, vol. 46, No. 2, abr./jun., (2010).

Hala M. Heneedak, Ismail Salama, Samia Mostafa & Mohamed El-Sadek "HPLC and Chemometric Methods for the Simultaneous Determination of Miconazole Nitrate and Nystatin" Journal of Chromatographic Science, Vol.50, p:(855–861), (2012).

M Herold, I May & A Nicolas "Development and validation of three methods: liquid chromatography, flow injection analysis and UV spectrophotometry for the routine control of nystatin capsules" Eur J Hosp Pharm, Vol.22, P:(46–50) ,(2015) ,doi:10.1136/ejhpharm-2014-000494.

Farnaz Shokraneh, Ramin Asgharian, Assem Abdollahpour, Mehdi Ramin, Ali Montaseri and Arash Mahboubi "A Novel High Performance Liquid Chromatographic Method for Determination of Nystatin in Pharmaceutical Formulations by Box–Behnken Statistical Experiment Design" Iranian Journal of Pharmaceutical Research ,Vol.14 ,p: (43-49) (Supplement), (2015).

Frank J. Dowd, Bart Johnson & Angelo Mariotti "Pharmacology and Therapeutics for Dentistry - E-Book" Elsevier Health Sciences, September 3, 2016, ISBN: 9780323445955, 0323445950,p:(490)

Dhruba Charan Dassh "Analytical Chemistry" 2nd edition , Prentice Hall India Pvt., Limited, New Delhi, India-110001, p:(400-404), August 2017

Na Li, John J Hefferren & Ke'an Li "Quantitative Chemical Analysis" World Scientific Publishing Company, p: (232-233),April 26, 2013. ISBN: 9789814452311, 9814452319.

HAMSA M. YASEEN, RUAA M. MAHMOOD &ROKAYIA S. SADEQ "Spectrophotometric Determination of Olanzapine by Charge-Transfer Complex Formation in its Pure Form and Pharmaceutical Formulations" International Journal of Pharmaceutical Research, Vol 12, Issue 1, pages (700-709), Jan - March 2020.
https://doi.org/10.31838/ijpr/2020.12.01.139

NAHLA. A. ALASSAF & FAEZA H. ZANKANAH "Sensitive Spectrophoto metric Determination of Doxycycline in Pure and Dosage Forms using p-Bromanil" International Journal of Pharmaceutical Research, Vol 11 , Issue 2,pages :(90-97), April-June 2019.
https://doi.org/10.31838/ijpr/2019.11.02.016

Hashim Jaafar Abd-alaah & Azhar Sadeq Hamody Design of experiments model for optimization of spectrophotometric determination of phenylephrine hydrochloride in pure and pharmaceutical formulations using p-Bromanil" J. Pharm. Sci. & Res. Vol. 11(2), pages:(501-507), 2019.

Mouyed Q. Al-Abachi, Sadeem S. Abed, Malik H. Alaloosh Alamr "Charge Transfer Spectrophotometric Determination of Metronidazole in Pharmaceutical Formulations by Normal and Reverse Flow Injection Analysis Coupled with Solid-Phase Reactor Containing Immobilized FePO4" Iraqi Journal of Science, Vol. 61, No. 7, p: 1541-1554, 2020.

Sarmad B. Dikran, Alaa K. Mohammed & Ali Kh. Mahmood "Uni and Multivariate Optimization for the Spectrophotometric Determination of Cimetidine Drug via Charge-Transfer Complex Formation" Ibn Al-Haitham Jour. for Pure & Appl. Sci., Vol. 28 (3) 2015.

Hind Hadi "Spectrophotometric Determination of Clonazepam in Pure and Dosage forms using Charge Transfer Reaction" Iraqi J Pharm Sci, Vol.24(1), p (25-32),2015.

Tariq Y. Mahmoud ,Sarmad B. Dikran ,Alaa K. Mohammed "Spectrophotometric Determination of Sulfamethoxazole Based on Charge-Transfer Complexation with Sodium Nitroprusside" Ibn Al-Haitham Jour. for Pure & Appl. Sci., Vol. 30 (3) 2017

Farha Khalaf Omar "Spectrophotometric determination of cefixime by charge transfer complex formation" Baghdad Science Journal, Vol.10(3)2013.

Zuzanna Kowszyk, Wlodzimierz Kurylowicz & Tadeusz Korzybski "ANTIBIOTICS Origin, Nature and Properties" Volume I, PAŃSTWOWE WYDAWNICTWO NAUKOWE (PWN-Polish Scientific Publishers) ,WARSZAWA,p:(771)

Prabir Pal & T N Misra "Charge transfer complexes of polyenes: spectroscopic, dark and photoconductive studies" J. Phys. D: Appl. Phys., Vol.23, p:218-222, (1990).

Mustafa Arslan & John Masnovi "Charge transfer complex formation between p-chloranil and 1,n-di(9-anthryl)alkanes" Spectrochimica Acta Part A , Vol.64 , p:(711–716), (2006).

Hirotada Iida, Tetsuo Nogami, Takashi Sawahata, Akio Noguchi & Kazutoshi Yamada "CHARGE TRANSFER COMPLEXES OF POLYENE WITH CHLORANIL" Tetrahedron Letters No. 27, p:(2495-249),1971.

Biswanath MALLIK, K. M. JAIN & T. N. MISRA "Charge-transfer complexes of some linear conjugated polyenes" Biochem. J., Vol.189, p:(547-552),1980.

Benesi H. A. & Hildebrand J.H." A Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons" Journal of the American Chemical Society, Vol. 71, No.8, p: (2703-2707), (1949).

Robert A. Copeland "ENZYMES A Practical Introduction to Structure, Mechanism,and Data Analysis" 3th edition, John Wiley & Sons, Inc.,p:(83-92),2023.

Christain G.D., Dasgupta P.K. & Schug K.A. "Analytical Chemistry" 7th edition, John Wiley & Sons, p:(62-120), (2014).