Acid–Base Indicator Properties of Synthesized Phenylhydrazones: 4-(2-(2,4-Dinitrophenyl) Hydrazono) Methyl)-2 Methoxy Phenol and 4-(2-(2,4-Dinitrophenylhydrazono) Methyl) Benzene-1…

Article *in* Journal of Chemistry · September 2022

DOI: 10.1155/2022/3667814

CITATIONS
2

READS
359

11 authors, including:

Cedric Dzidzor K. Amengor
University of Health and Allied Sciences
29 PUBLICATIONS **230** CITATIONS
SEE PROFILE

Abena Brobbey
Kwame Nkrumah University Of Science and Technology
24 PUBLICATIONS **72** CITATIONS
SEE PROFILE

Isaac Ayensu
Kwame Nkrumah University Of Science and Technology
48 PUBLICATIONS **789** CITATIONS
SEE PROFILE

Emmanuel Orman
University of Health and Allied Sciences
51 PUBLICATIONS **244** CITATIONS
SEE PROFILE

Hindawi
Journal of Chemistry
Volume 2022, Article ID 3667814, 9 pages
https://doi.org/10.1155/2022/3667814

Research Article

Acid-Base Indicator Properties of Synthesized Phenylhydrazones: 4-(2-(2,4-Dinitrophenyl) Hydrazono) Methyl)-2 Methoxy Phenol and 4-(2-(2,4-Dinitrophenylhydrazono) Methyl) Benzene-1,3-diol

Cedric D. K. Amengor,[1] **Abena Brobbey**,[2] **Isaac Ayensu**,[1] **Emmanuel Orman**,[1] **Paul Peprah**,[1] **Billy Mawunyo Abudey**,[1] **Seth Junior Ayihi**,[1] **Claudia Naa Dedei Attoh**,[3] **Alberta Ade**,[2] **Paul Kwesi Sodzi**,[4] **and James Sarpong Aboagye**[3]

[1]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Health and Allied Sciences, Ho, Ghana*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[3]*Center for Laboratory Services and Research Unit (Drug Physicochemical Unit), Food and Drugs Authority, Accra, Ghana*
[4]*Department of Pharmaceutical Sciences, Faculty of Applied Science and Technology, Sunyani Technical University, Sunyani, Ghana*

Correspondence should be addressed to Cedric D. K. Amengor; camengor@uhas.edu.gh

Received 16 February 2022; Accepted 20 July 2022; Published 20 September 2022

Academic Editor: Mohd Sajid Ali

4-(2-(2,4-Dinitrophenyl) hydrazone) methyl)-2 methoxy phenol (IND-X) and 4-(2-(2, 4-dinitrophenylhydrazono) methyl) benzene-1,3-diol (IND-Y) were synthesised by condensation reactions with 2,4-dinitrinophenylhydrazine and were observed to possess acid-base indicator properties. The study was conducted to explore and verify their use in different analytical situations and, consequently, validate them. Analysis of their use in acid-base titrations between strong acids/strong bases and weak acids/strong bases as well as pharmaceutical applications in the assay of ibuprofen pure powder and batch of ibuprofen tablets (400 mg) using standard methods in the British Pharmacopoeia (2020) was all considered. The validations were performed by the International Conference on Harmonisation of Technical Requirements for the registration of Pharmaceuticals for Humans Q2 (*R*1) guidelines. The outcomes were statistically compared with existing conventional analytical approaches. Titrimetric data obtained were analyzed statistically by Student's *t*-test and one-way ANOVA at a 95% confidence level. IND-X and IND-Y were shown to be similar in behaviour to phenolphthalein, methyl orange, and methyl red as visual indicators after preliminary evidence of sharp colour changes in acid, alkaline, and neutral pH solutions. The use of the two indicators in the assay of ibuprofen powder and tablets has also been established. The validation parameters considered were found to be consistent with respective acceptance criteria for analytical purposes. IND-X and IND-Y are therefore proposed as alternative acid-base indicators to methyl red, methyl orange, and phenolphthalein for routine volumetric analysis in students' laboratory experiments. They also serve as alternative indicators to phenolphthalein for the titrimetric analysis of ibuprofen-based samples and products.

1. Introduction

The technique of titrimetric quantitative analysis is broadly based on volumetric, gravimetric analysis, and biomedical 2 analytical chemistry [1]. The titrimetric analysis is one of the most used and relevant analytical techniques in pharmaceutical analysis. The speed of analysis, the high precision determination of the purity of compounds in the absence of reference standards, instantaneous completion of reactions, and monitoring of endpoints through indicators and electrometric methods have made titrimetry still useful in several applications, including pharmaceutical analysis [1].

An indicator as used in titrimetry is a chemical substance sensitive enough to display a clear change in colour very close

to the point in the ongoing titration process at which equimolar quantities of the analyte and titrant have almost virtually reacted with each other [2, 3]. Generally, chemical indicators may be classified into acid-base, oxidation-reduction, complexometric, adsorption, and chemiluminescent indicators [4]. The pH indicators are acid-base indicators, mostly weak acids such as methyl orange, phenolphthalein, and phenol red, and are coloured differently in their dissociated and associated forms. Acid-base titrations depend on the neutralization between an acid and a base when mixed in solution. The acid-base indicator indicates the endpoint of the titration by changing colour. The colour change of the indicator is pH-dependent, as it affects the dissociation of the indicator compound. Consider an indicator which is a weak acid, with the formula HIn. At equilibrium, the following equilibrium equation is established with its conjugate base: $HIn + H_2O \rightleftharpoons H_3O{+}{+}In$, where HIn refers to the indicator colour in an undissociated state and In refers to the indicator colour in dissociated condition. The indicator exhibits different halochromic properties under different dissociation conditions, and this tends to affect its colouration in the presence of analytes being investigated. The indicator endpoint and the equivalence point are not the same because the equivalence point is determined by the stoichiometry of the reaction, while the endpoint is just the colour change from the indicator. However, the choice of a suitable indicator for titration depends on its ability to minimise the difference (or indicator error) between the endpoint and the nominal equivalence point [5, 6]. Earlier works show that p-nitrophenylhydrazones do possess halochromic properties, and this makes them good candidates as indicators. For example, the potassium salt of benzaldehyde p-nitrophenylhydrazone was observed to change colour from yellow to red as pH increased from below 11.3 to 11.7 and above [7]. Also, salicylaldehyde phenylhydrazones have been used as colour indicators in the titration of organometallic agents including Grignard reagents [8]. Similarly, p-nitrophenylhydrazone of pyridinealdehyde has also been proposed as an alkali indicator [9]. The halochromic effect of nitrophenylhydrazones is partly attributed to the presence of the nitro group and partly to the nature of the electrophilic substituents attached, which tend to reduce the electron density of the molecule, making it possible for the release of proton [9]. As a result, several derivatives of nitrophenylhydrazone have been explored for their acid-base indicator properties, and these include but are not limited to crotonaldehyde- and acrylaldehyde p-nitrophenylhydrazones, furfurylidene- and 5-nitro-2-furfurylidene, p-nitrophenylhydrazones, and picolinealdehyde [9].

Following up on the previous synthesis and antimicrobial studies on phenylhydrazones [10], we hereby report the validation of the strong colour indicator property of two of those compounds in acid-base titrations.

2. Experimental

2.1. Materials and Chemicals. The materials used in the study included silica gel (70 : 230 mesh size) (Merck, US), silica gel precoated TLC plates (Merck, Germany), ibuprofen BP powder (98% w/w), and ibuprofen tablets (400 mg) (Ernest Chemists, Ghana). The chemicals employed were ethyl acetate, petroleum ether, concentrated hydrochloric acid (36% w/v), sodium hydroxide pellets, 2,4-dinotrophenylhydrazine, vanillin (4-hydroxy-3-methoxy benzaldehyde), 2,4-dihydroxy benzaldehyde, Analar anhydrous sodium carbonate (99.5% w/w), methanol, Analar sulphamic acid (99% w/w), and commercial acetic acid (36% w/v) which were all sourced from Fisher Scientific, United Kingdom.

2.2. Synthesis of the Phenylhydrazones. The two compounds, 4-(2-(2,4-dinitrophenyl) hydrazone) methyl)-2 methoxy phenol (IND-X) and 4-(2-(2,4-dinitrophenyl) hydrazone) methyl) benzene-1,3-diol (IND-Y), were synthesised following the synthetic schemes as reported [10] and as illustrated in Figure 1.

2.2.1. Synthesis of 4-(2-(2,4-Dinitrophenyl) Hydrazono) Methyl)-2 Methoxy Phenol. IND-X was prepared from the reaction between 2,4-dinitrophenylhydrazine (0.78 g, 3.93 mmol, 1 eq.) dissolved in methanol (10 mL) with 4-hydroxy-3-methoxy benzaldehyde (vanillin) (0.50 g, 1.04 eq., 4.09 mmol) in an acidified medium (concentrated H_2SO_4, 98% v/v, 2 mL) and maintained in an iced bath (0°C) with stirring for at least 24 hours. The reaction was monitored with TLC plates, and upon completion, the product was purified by recrystallization, and the identity of the compound was confirmed by comparing its melting point and spectroscopic data with the literature [10].

2.2.2. Synthesis of 4-(2-(2,4-Dinitrophenyl) Hydrazono) Methyl) Benzene-1,3-diol. IND-Y on the other hand was produced from the reaction between 2,4-dinitrophenylhydrazine (0.69 g, 3.48 mmol, 1 eq.) dissolved in methanol (10 mL) with 2,4-dihydroxy benzaldehyde (0.50 g, 1.04 eq., 3.62 mmol) in an acidified medium (concentrated H_2SO_4, 98% v/v, 2 mL) and maintained in an iced bath (0°C) with stirring for at least 24 hours. The reaction was also monitored with TLC plates, and upon completion, the product was purified by recrystallization, and the identity of the compound was confirmed by comparing its melting point and spectroscopic data with the literature [10].

2.3. Characterisation of Synthesised Phenylhydrazones

2.3.1. 4-(2-(2,4-Dinitrophenyl) Hydrazone) Methyl)-2 Methoxy Phenol. IND-X (0.89 g, 75%) presented as an orange solid. Rf (Pet. ether 70%: EtOAc 30%): 0.63. Mpt: 267–270°C. UV-Vis (MeOH) λmax: 218 nm and 394 nm. Infrared (neat) υmax cm^{-1}: 3360 (OH), 3253 (NH), 3080 (C=CH); 1H NMR (400 MHz, CDCl3) δH 11.50 (1H, s, NH), 10.02 (1H, H-C4′, s, ArOH), 9.62 (1H, H-C3, s, ArH), 8.75 (1H, H-C5, s, ArH), 8.68 (1H, H-C7, s, N=CH), 8.30 (1H, H-C6, d, $J = 4.0$, ArH), 7.90 (1H, H-C2′, d, $J = 4.0$, ArH), 7.60–7.80 (1H, H-C6′, d, $J = 8.0$, ArH), 6.35–6.38 (1H, H-C5′, d, $J = 12.0$, ArH), (3H, H-C4′, Ar-OCH3). 13C NMR (400 MHz, CDCl3) δc 150.0, 150.2, 148.5, 144.6, 136.9, 130.2,

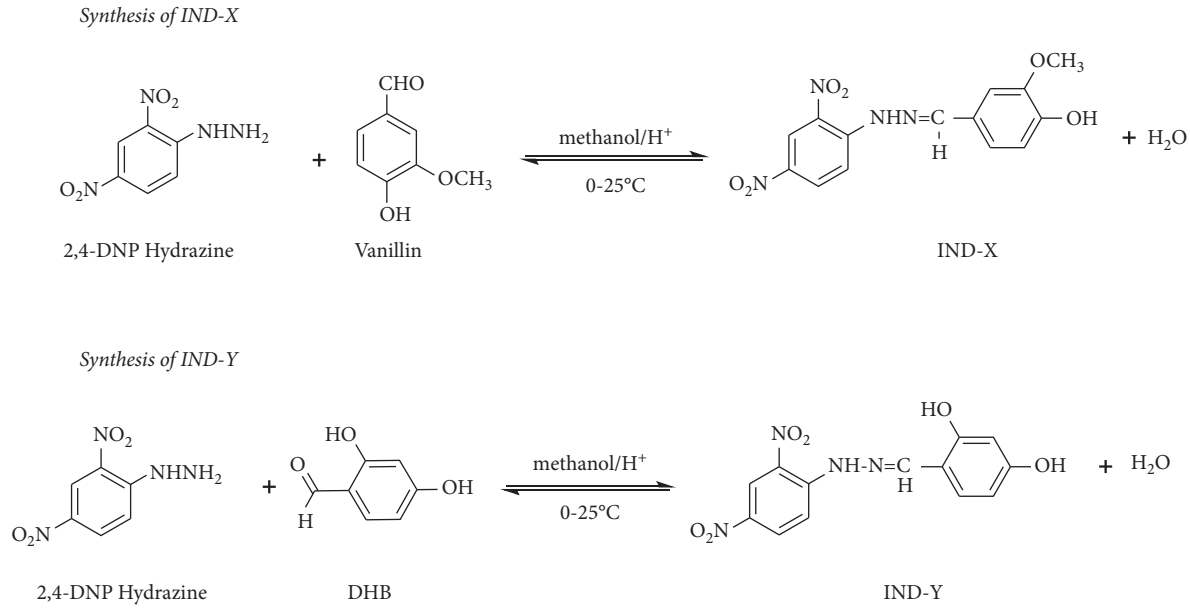

Figure 1: Synthetic schemes for the compounds 4-(2-(2,4-dinitrophenyl) hydrazone) methyl)-2 methoxy phenol (IND-X) and 4-(2-(2,4-dinitrophenyl) hydrazone) methyl) benzene-1,3-diol (IND-Y).

125.5, 123.2, 123.1, 117.3, 116.2, 110.2, 54.0 ppm. These parameters were observed to be consistent with the literature [10].

2.3.2. 4-(2-(2,4-Dinitrophenylhydrazono) Methyl) Benzene-1,3-diol. IND-Y (0.83 g, 75%) was produced as a dark red solid. Mpt: 270–272°C; UV-Vis (MeOH) λmax: 218 nm and 403 nm; Infrared (neat) υmax: 3350, 3090, 1620, 1570 cm^{-1}. dH (400 MHz; CDCl3): 11.52 (1H (C1′), s, ArOH), 10.02 (1H (C1), s, NH), 9.96 (1H (C3′), s, ArOH), 8.80 (1H (C3), s, ArH), 8.78 (1H, s, N=CH), 8.30–8.36 (1H (C5), d, $J = 12.0$, ArH), 7.92–7.96 (1H (C6), d, $J = 8.0$, ArH), 7.50–7.56 (2H (C2′, C5′, m, ArH), 6.35 (1H (C4′), d, $J = 8.0$, ArH). dc 160.2, 157.2, 148.0, 145.7, 136.2, 130.2, 129.2, 127.8, 122.4, 116.0, 112.2, 106.0, 102.2 (Ar). These parameters were also consistent with the literature [10].

2.4. Screening of the Synthesised Compounds for Indicator Property. The two indicators, IND-X and IND-Y (0.05 g), were each dissolved completely in ethanol (50 mL) and diluted to the 100 mL mark with the same solvent in a volumetric flask to obtain a final concentration of 0.05% w/v. Indicator solutions of different pHs were prepared using acetate (pH = 3.0), phthalate (pH = 7.00), and phosphate buffers (pH = 10.0). Five drops of each indicator were added to the prepared solutions at room temperature, and observations were made for colour changes.

2.5. IND-X and IND-Y as Alternative Visual Indicators for Conventional Titrations. The titrimetric analyses considered included strong base vs. strong acid and strong base vs. weak acid titrations. In the strong base vs. strong acid titrimetric investigations, standardised 0.5 M NaOH (10 mL) was titrated with standardised 0.5 M HCl using IND-X and IND-Y as test visual indicators. Methyl orange, methyl red, and phenol red as standard indicators were also used, and their results were compared with that of the test indicators. In the strong base vs. weak acid titrations, standardised 0.5 M NaOH (10 mL) was titrated with standardised 0.5 M CH_3COOH using IND-X and IND-Y also as test visual indicators. Phenolphthalein as a standard indicator was used, and its results were compared with that of the test indicators.

The titrimetric endpoints and potentiometric equivalence points from the indicators were determined, and their respective indicator errors were calculated. Replicate determinations were made, and the data obtained were statistically analyzed by one-way ANOVA. The same analyses were repeated with titrations involving 0.5 M NaOH (solution (10 mL) and 0.5 M CH_3COOH (strong base/weak acid) using phenolphthalein as the standard indicator and the two test indicator solutions in triplicate.

2.6. IND-X and IND-Y as Alternative Visual Indicators for Titrimetric Assay of Ibuprofen BP Powder and Tablets. The British Pharmacopoeia method was used to assay ibuprofen BP starting material (0.4000 g) and formulated tablets (equivalent weight containing 400 mg ibuprofen) [11]. The prepared ibuprofen solutions were titrated with a standardised solution of 0.1 M NaOH (titrant) ($f = 0.9980$), using IND-X, IND-Y, and phenolphthalein as indicators. Replicate analyses were carried out, and the results were analyzed statistically.

2.7. Validation of Indicator Property. The use of IND-X and IND-Y in titrations involving NaOH/HCl (strong base/strong acid) and NaOH/CH_3COOH (strong base/weak acid), and in the assay of ibuprofen, BP powder and tablets

(400 mg) were validated according to the International Conference on Harmonisation (ICH) Q2 (R1) guidelines [12].

3. Results and Discussion

3.1. Spectroscopic Characterisation of the Compounds. There were two absorption peaks in the UV-visible spectrum at a wavelength maximum of 218 nm and 394 nm as also reported in the literature [10]. The infrared spectrum revealed the presence of a broad band at 3360 cm^{-1} representing a hydroxyl moiety. Bands at 3253 cm^{-1} and 3080 cm^{-1} indicated the presence of a secondary amine group and an sp^2-hybridised carbon, respectively. In the ^1H NMR spectrum, the formation of the imine bond was confirmed by the presence of a singlet resonating at 8.68 ppm, whilst the other peaks also corroborated with that reported by [10].

3.2. Acid-Base Indicator Properties of the Compounds. The condensation reactions between 4-hydroxy-3-methoxy benzaldehyde and 2,4-dihydroxy benzaldehyde, with 2,4-dinitrophenylhydrazine, produced orange and dark red coloured products which absorbed light in the UV-Vis region. This phenomenon is due to their extended conjugations as a result of the introduction of extra chromophores after the reactions, which result in their wavelengths of absorption being in the visible region of the electromagnetic spectrum.

In an alkaline medium, both IND-X and IND-Y are ionised or deprotonated, and this is illustrated in Figure 2. The presence of the hydroxy groups in both compounds provides the chemistry behind their ionisation. The extra electron gained as a result of the ionisation increases the electron density of the benzene-bearing hydroxy group, and this results in a bathochromic shift to 394 and 403 nm, respectively (Figure 3). Consequently, this translates into changes in colour for the ethanolic solutions of the compounds in acid, neutral, and alkaline media (Table 1). In an acidic medium, IND-X and IND-Y become the phenolic hydroxyl group tethered to the benzene ring and are protonated, resulting in a decreased electron cloud density and exhibiting a yellow colour associated with a hypsochromic shift at 218 and 210 nm, respectively.

3.3. Titrimetric Analysis with IND-X and IND-Y. Titrimetric analyses were carried out with the test indicators, IND-X and IND-Y, at 0.05% (w/v) concentration of each in ethanol. In the titration involving 0.5 M NaOH and 10.00 mL of 0.5 M HCl, IND-X was observed to transition from a yellowish colour when added to the analyte (0.5 M HCl) to pink as the solution becomes alkaline with the addition of 0.5 M NaOH. The endpoint was determined to be 10.69 ± 0.07 mL. In the case of IND-Y, the colour transition was also from yellow in 0.5 M HCl to deep pink after the end of the titration in an alkaline medium, with an endpoint of 9.92 ± 0.08 mL (Table 2). In the titration involving 0.5 M NaOH and 0.5 M CH_3COOH, with the indicator in the analyte solution (0.5 M NaOH), the colour transition for both IND-X and IND-Y was from pink to colourless, with the end points occurring at 10.20 ± 0.16 mL and 11.18 ± 0.13 mL respectively.

Similarly, ibuprofen is a weak acid and when titrated with a strong base, 0.5 M NaOH, there is the formation of the sodium salt of ibuprofen. The sodium salt hydrolyses to produce excess hydroxyl ions which makes the solution alkaline and causes the colour change of IND-X and IND-Y which is observed as a final pink solution (Figure 4). Though the phenylhydrazones are structurally different from phenolphthalein, this also is the explanation for the use of phenolphthalein in strong base/weak acid and the pharmacopoeia recommended assay of ibuprofen.

3.4. Validation of Use of Indicators. In validating the use of the indicators in the above-described titrimetric applications, the parameters, specificity, accuracy, precision, and robustness were considered following the ICH Q2 (R1) guidelines [12].

3.4.1. Specificity. Specificity is the ability to unequivocally assess an analyte in the presence of expected components [12]; in this case, the presence of IND-X and IND-Y in the indicator solutions and being responsible for the colour changes in the titrimetric analysis [1, 11]. In the test for specificity, five drops of 0.05% w/v ethanolic solutions of the test indicators were added to 10 mL of 0.1 M·NaOH, 0.1 M·HCl, and 0.1 M·CH_3COOH in separate beakers, and the colour changes were noted. Titrations were then carried out using 0.1 M HCl and 0.1 M NaOH, respectively, and colour changes were again noted and recorded [1, 11]. Similarly, five drops of ethanol were also added to similar volumes of the abovementioned standard solutions, and similar investigations were carried out. Ethanol was observed not to have affected the original colours of the standard solutions. There were also no observable colour changes in the titrations of such solutions with their respective titrant solutions. However, in the case of IND-X and IND-Y, colour changes were observed when added to the standard solutions, and changes in these colours occurred upon their titrations. This showed that the IND-X and IND-Y compounds were responsible for the observed effects and as such were selective ad specific.

3.4.2. Accuracy. The accuracy of an analytical method is the closeness of the test results obtained to the mean or the theoretical true value [12]. In this study, the accuracy associated with the use of the indicators was defined as the closeness of their titrimetric endpoints with that of conventional indicators; and this was determined by comparing results obtained from the use of IND-X and IND-Y with that of methyl orange and methyl red for titrations involving 0.1 M·HCl/0.1 M·NaOH, phenol red, and phenolphthalein for titrations involving 0.1 M·CH_3COOH/0.1 M·NaOH and phenolphthalein for the titrimetric assay of ibuprofen [1, 11].

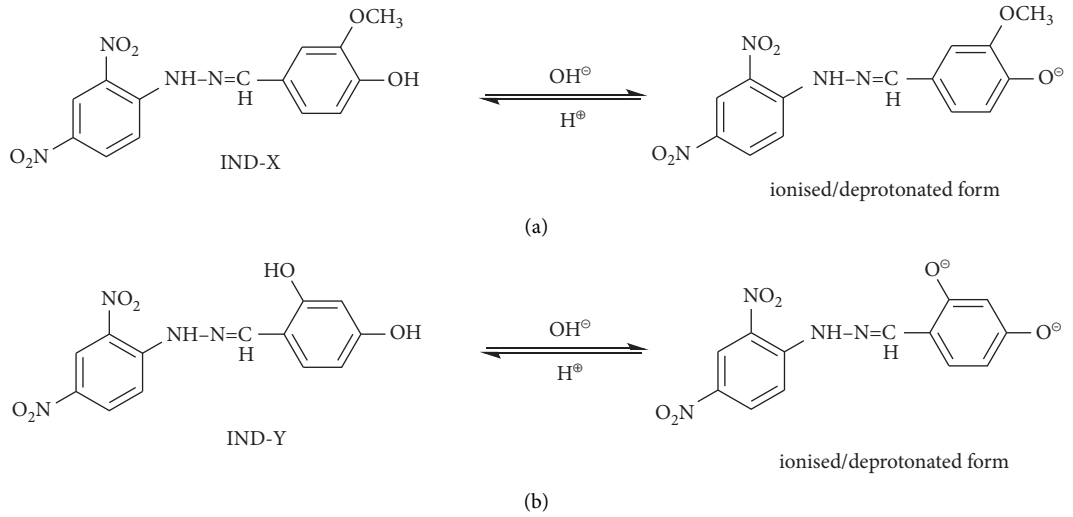

Figure 2: The ionisation of IND-X and IND-Y in an alkaline medium (bathochromic shift with pink colour). (a) UV-Vis spectrum of IND-X. (b) UV-Vis spectrum of IND-Y.

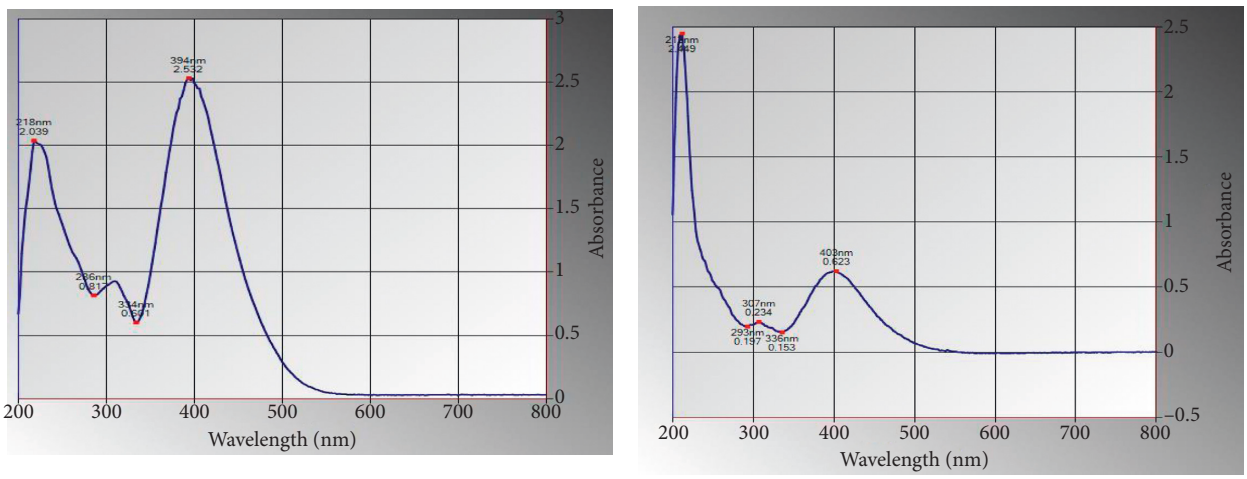

Figure 3: UV-Vis spectra of synthesised indicators (a: IND-X; b: IND-Y) in ethanolic/0.5 M NaOH solution. (a) UV-Vis spectrum of IND-X. (b) UV-Vis spectrum of IND-Y.

Table 1: Indicator colour changes in different conditions.

Compound	Colour in alkaline medium	Colour in neutral medium	Colour in acidic medium
IND-X	Pink	Orange	Yellow
IND-Y	Pink	Red	Yellow

Table 2: Colour changes and pH ranges were observed during titrimetric analysis.

Indicator	NaOH vs. HCl		NaOH vs. CH_3COOH		Assay of ibuprofen	
	Colour	pH range	Colour	pH range	Colour	pH range
Methyl red	Yellow to red	4.40–6.20	Yellow to red		—	—
Methyl orange	Yellow to orange	3.00–4.4	Yellow to orange		—	—
Phenolphthalein	Pink to colourless	8–10	Pink to colourless		Pink to colourless	
IND-X	Yellow to pink	8.02–10.20	Pink to colourless	8.02–10.20	Pink to colourless	8.02–10.20
IND-Y	Yellow to deep pink	8.00–10.20	Pink to colourless	8.00–10.20	Pink to colourless	8.00–10.20

—: not applicable.

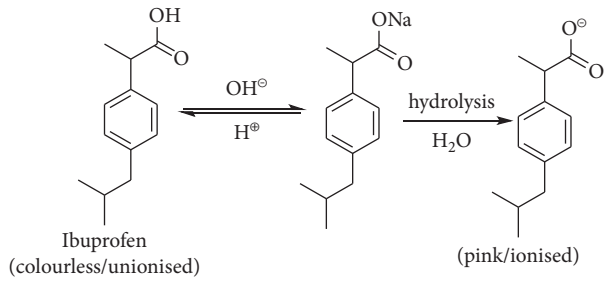

Figure 4: Various proposed forms of ibuprofen under acidic and alkaline conditions.

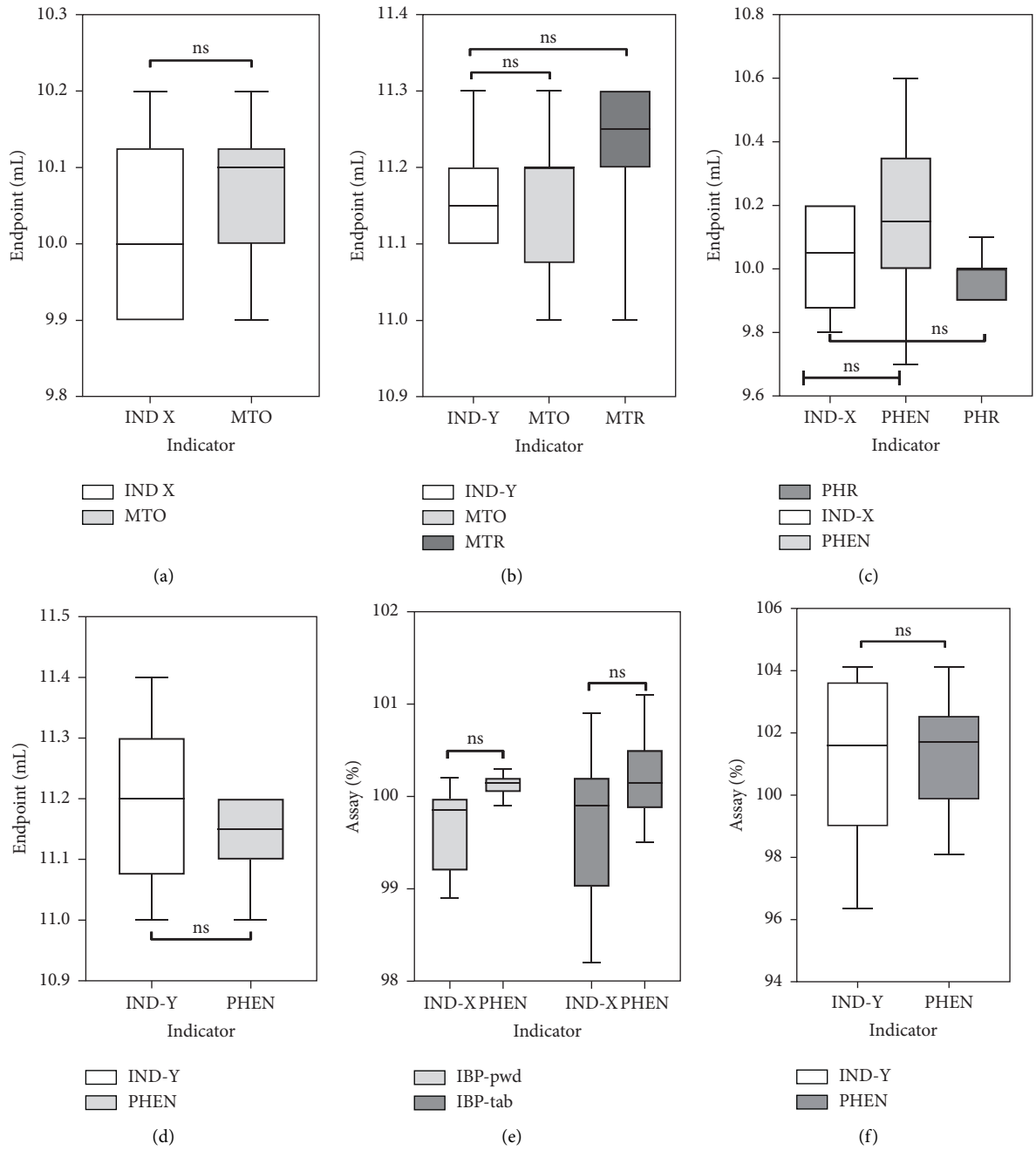

Figure 5: Accuracy investigations involving IND-X and IND-Y. (a) Comparing results from the use of IND-X with methyl orange (MTO) in HCl/NaOH titrations ($n = 10$); (b) comparing results from the use of IND-Y with MTO and methyl red (MTR) in HCl/NaOH titrations ($n = 10$); (c) comparing results from the use of IND-X with phenolphthalein (PHEN) and phenol red (PHR) in CH_3COOH/NaOH titrations ($n = 10$); (d) comparing results from the use of IND-Y with phenolphthalein (PHEN) in CH_3COOH/NaOH titrations ($n = 10$); (e) comparing results from the use of IND-X with phenolphthalein (PHEN) in the titrimetric assay of ibuprofen powder (IBP-PWD) and ibuprofen tablets (IBP-tab) ($n = 10$); (f) comparing results from the use of IND-Y with phenolphthalein (PHEN) in the titrimetric assay of ibuprofen powder.

Table 3: Results showing precision of outcomes from the use of the proposed indicators.

Precision parameter		IND-X (mean ± SD; RSD)			IND-Y (mean ± SD; RSD)		
		HCl vs. NaOH	CH_3COOH vs. NaOH	Assay of ibuprofen	HCl vs. NaOH	CH_3COOH vs. NaOH	Assay of ibuprofen
Repeatability	Replicate titrations ($n = 6$)	10.01 ± 0.120; 1.20%	9.88 ± 0.140; 1.42%	99.66 ± 0.826; 0.83%	10.69 ± 0.074; 0.69%	10.49 ± 0.120; 1.14%	101.0 ± 1.890; 1.87%
	Different conc.						
	1. 0.05% w/v	10.04 ± 0.158; 1.57%	10.03 ± 0.082; 0.82%	99.68 ± 0.554; 0.56%	10.82 ± 0.045; 0.41%	10.64 ± 0.089; 0.84%	101.0 ± 1.446; 1.43%
	2. 0.10% w/v	10.02 ± 0.162; 1.62%	9.80 ± 0.103; 1.04%	99.32 ± 0.841; 0.85%	10.64 ± 0.055; 0.51%	10.50 ± 0.071; 0.67%	101.0 ± 1.687; 1.67%
	3. 0.15% w/v	10.00 ± 0.141; 1.41%	10.02 ± 0.079; 0.79%	99.86 ± 0.410; 0.41%	10.70 ± 0.071; 0.66%	10.48 ± 0.045; 0.43%	100.6 ± 0.909; 0.90%
Intermediate precision	Different days						
	(1) Day 1	10.01 ± 0.120; 1.20%	9.98 ± 0.092; 0.92%	100.3 ± 0.585; 0.58%	10.82 ± 0.045; 0.41%	10.44 ± 0.055; 0.52%	101.0 ± 0.477; 0.47%
	(2) Day 2	9.78 ± 0.114; 1.16%	10.08 ± 0.123; 1.22%	100.1 ± 0.288; 0.29%	10.84 ± 0.089; 0.83%	10.52 ± 0.084; 0.80%	100.2 ± 1.822; 1.82%
	(3) Day 3	9.94 ± 0.178; 1.79%	9.90 ± 0.125; 1.26%	100.1 ± 0.187; 0.19%	10.76 ± 0.114; 1.06%	10.50 ± 0.123; 1.17%	102.4 ± 1.243; 1.21%

Table 4: Results showing precision of outcomes from the use of the proposed indicators.

Indicator	Type of titration	No. of drops	Mean ± SD, RSD	One-way ANOVA
IND-X	NaOH vs. HCl	2 drops	10.02 ± 0.162, 1.62%	$F_{(2,\ 27)} = 1.105$; $p = 0.3456$
		3 drops	10.01 ± 0.120, 1.20%	No significant difference in the endpoint when a different number of drops are used
		4 drops	9.92 ± 0.204, 2.06%	
	NaOH vs. CH_3COOH	2 drops	9.91 ± 0.129, 1.30%	$F_{(2,\ 27)} = 1.058$; $p = 0.3612$
		3 drops	9.90 ± 0.149, 1.51%	No significant difference in the endpoint when a different number of drops are used
		4 drops	9.98 ± 0.123, 1.23%	
IND-Y	NaOH vs. HCl	2 drops	11.19 ± 0.120, 1.07%	$F_{(2,\ 27)} = 2.241$; $p = 0.1258$
		3 drops	11.16 ± 0.097, 0.87%	No significant difference in the endpoint when a different number of drops are used
		4 drops	11.10 ± 0.067, 0.60%	
	NaOH vs. CH_3COOH	2 drops	10.49 ± 0.120, 1.14%	$F_{(2,\ 27)} = 0.2520$; $p = 0.7791$
		3 drops	10.47 ± 0.095, 0.91%	No significant difference in the endpoint when different numbers of drops are used
		4 drops	10.50 ± 0.067, 0.63%	

In 0.1 M·HCl/0.1 M·NaOH titrations, it was observed that the endpoints produced from the use of IND-X were comparable with that of methyl orange ($t = 1.233$, df = 18, $p = 0.2336$; Figure 5(a)). When IND-Y was used, the endpoints were also comparable with that of methyl orange and methyl red (F (2, 27) = 2.110, $p = 0.1408$; Figure 5(b)). In titrations involving 0.1 M CH_3COOH and 0.1 M·NaOH, similar comparable endpoints were observed from the use of IND-X and phenol red and phenolphthalein (F (2, 27) = 2.057, $p = 0.1474$; Figure 5(c)), as well as that from the use of IND-Y and phenolphthalein ($t = 0.8585$, df = 18, $p = 0.4073$; Figure 5(d)). Also, in the titrimetric assay of ibuprofen, both indicators proved to produce similar results as phenolphthalein, which is the recommended indicator in the compendial method (Figures 5(e) and 5(f)) [13]. In all, the proposed indicators demonstrated comparable analytical qualities, in terms of the accuracy of their outcomes when compared to already established and widely used indicators.

3.4.3. Precision. Precision is defined as the closeness of agreement of results under defined conditions [12]. In testing for precision, both repeatability and intermediate precision were considered. For repeatability, replicate determinations of the endpoints with the use of 0.5% (w/v) concentrations of IND-X and IND-Y were carried out under the considered titrimetric applications. Titrations with different concentrations of the indicators were also considered. In each of the instances, the relative standard deviations calculated on the replicate endpoints and assays were compliant with the acceptance criteria of <2.00% (Table 3). In effect, the use of the two indicators resulted in precise results.

3.4.4. Robustness. tIn the test for robustness, different number of drops of the 0.05% (w/v) concentrations were considered in the titrimetric analysis, and these were 2, 3, and 4 drops. The results as shown in Table 4 indicate that irrespective of the number of drops used, and the endpoints in each titrimetric scenario were comparable for both indicators. Also, the RSDs determined were mostly compliant with the acceptance criteria of ≤2%, except for using 4 drops of IND-X in titrations involving NaOH and HCl (Table 4).

4. Conclusion

The acid-base indicator property of IND-X and IND-Y have been evaluated and validated according to the ICH standards. Both indicators were found to serve as an alternative indicator to methyl orange, methyl red, phenol red, and phenolphthalein in titrations involving strong acids/strong bases and weak acids/strong bases. Also, both indicators have demonstrated suitable alternatives to phenolphthalein in the titrimetric assay of ibuprofen-related products. Hence, 4-(2-(2,4-dinitrophenyl) hydrazone) methyl)-2 methoxy phenol and 4-(2-(2,4-dinitrophenyl) hydrazone) methyl) benzene-1,3-diol can be employed as suitable indicators for conventional titrimetric analysis in student demonstration laboratories and the quality control of ibuprofen-related products. In terms of sustainability, a starting material like vanillin for IND-X are readily available and can be obtained from a natural source without much cost compared to the standard indicators mentioned. In terms of precision, IND-X is generally the best for the assay of ibuprofen, whilst IND-X is more suitable for the NaOH/HCl and NaOH/CH_3COOH titrations.

Data Availability

Characterisation and analytical data are available at the Synthetic Chemistry Laboratory, Department of Pharmaceutical Chemistry, School of Pharmacy, University of Health and Allied Sciences, Ho.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

C. D. K. A, E. O, A. B, I. A, A. A developed the concept. C. D. K. A synthesised the compounds, and S. A., B. M. A and E. O carried out the titrimetry. C. N., D. A and J. S. A carried out the spectroscopic analysis and pH determinations. C. D., K. A and E. O wrote the draft manuscript. A. B, I. A, and P. K. S reviewed and edited the draft manuscript.

Acknowledgments

The authors are very much grateful to the School of Pharmacy, UHAS, for providing the equipment and facilities for the research. The authors also appreciate the immense contribution of the Institute for Health Research, UHAS, for scrutinising the draft protocol for the research. Funding was provided by the collaborating institutions.

References

[1] E. B. A. Adusei, R. K. Adosraku, J. Oppong-Kyekyeku, and C. D. K. Amengor, "Investigation of acid-base indicator property of plumbagin from plumbago zeylanica Linn," *International Journal of Analytical Chemistry*, vol. 2019, Article ID 4061927, 13 pages, 2019.

[2] K. C. Joshi, P. Singh, and G. Singh, "Juglone and lawsone as acid-base indicators," *Zeitschrift für Naturforschung B*, vol. 32, no. 8, pp. 890–892, 1977.

[3] S. K. Kyei, O. Akaranta, and G. Darko, "Synthesis, characterization and antimicrobial activity of peanut skin extract-azo-compounds," *Scientific African*, vol. 8, Article ID e00406, 2020.

[4] F. A. El-Seify and F. I. El-Dossoki, "Spectrophotometric, pH-metric and conductometric studies on some 3-arylhydrazone derivatives of (2-phenyl) ethyl pyruvate)," *Journal of the Korean Chemical Society*, vol. 50, no. 2, pp. 99–106, 2006.

[5] C. A. De Caro, A. Aichert, C. M. Walter, and Efficient, "precise and fast water determination by the Karl Fischer titration," *Food Control*, vol. 12, no. 7, pp. 431–436, 2001.

[6] S. I. R. Okoduwa, L. O. Mbora, M. E. Adu, and A. A. Adeyi, "Comparative analysis of the properties of acid-base indicator of rose (*Rosa setigera*), Allamanda (*Allamanda cathartica*), and hibiscus (*Hibiscus rosa-sinensis*) flowers," *Biochemistry Research International*, vol. 2015, Article ID 381721, 6 pages, 2015.

[7] R. O'Connor, W. Rosenbrook, and G. Anderson, "A new indicator for pH 11 to 12," *Analytical Chemistry*, vol. 33, no. 9, 1961.

[8] B. E. Love and E. G. Jones, "The use of salicylaldehyde phenylhydrazone as an indicator for the titration of organometallic reagents," *Journal of Organic Chemistry*, vol. 64, no. 10, pp. 3755-3756, 1999.

[9] A. G. Asuero, "Analytical uses of phenylhydrazines and phenylhydrazones I," *Microchemical Journal*, vol. 24, no. 2, pp. 217–233, 1979.

[10] A. Ade, C. D. K. Amengor, A. Brobbey, I. Ayensu, B. K. Harley, and Y. D. Boakye, "Synthesis and antimicrobial resistant modulatory activity of 2,4-dinitrophenylhydrazone derivatives as agents against some ESKAPE human pathogens," *Journal of Chemistry*, vol. 2020, Article ID 2720697, 9 pages, 2020.

[11] B. Pharmacopoeia, *Her Majesty's Stationery, vol. II, Her Majesty Stationary Office*, University Press, Cambridge, UK, A326-327, 2020.

[12] ICH, *Validation of Analytical Procedures: Text and Methodology Q2 (R1)*, ICH, Geneva, Switzerland, 2005.

[13] British Pharmacopoeia Commission, "Ibuprofen," in *British Pharmacopoeia 2020*, pp. 1298-1299, Stationery Office, London, UK, 2019.