SPECTROPHOTOMETRIC DETERMINATION OF NIMESULIDE IN PURE AND IN PHARMACEUTICAL FORMULATIONS USING ION-ASSOCIATE COMPLEX FORMATION

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ABSTRACT
Simple, rapid, and sensitive direct spectrophotometric methods for the determination of nimesulide (NIM) in pure form and pharmaceutical formulations have been developed. The methods depend on the formation of colored ion-pair complexes between NIM and three different reagents, bromocresol green (BCG), bromocresol purple (BCP) and brilliant blue G (BBG) in Britton-Robinson (B-R) universal buffer solutions. The colored complexes were measured directly exhibiting λ_max at (554, 437 and 643 nm) for BBG, BCP and BCG respectively. The analytical parameters and their effects were investigated. The ion-pair complexes are intensely colored and very stable at room temperature. The calibration graphs were linear over the concentration range of 2 - 12 µg/mL for BCP, 2-14 µg/mL for BCG and 2-13 µg/mL BBG. The stoichiometry of the reaction was found to be 1: 1 in all cases. The proposed methods were successfully extended to pharmaceutical preparations in tablet dosage form.

KEYWORDS
Nimesulide determination, Spectrophotometry, Dosage forms and Ion-associate complex formation.

INTRODUCTION
Nimesulide is a relatively cyclo-oxygenase-2 (COX-2) selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoea in adolescents and adults above 12 years old to decrease the risk of hepatotoxicity¹-³. It may be used orally or rectally or topically. It has the potential to reduce the incidence
of formation of gastrointestinal ulcers. It is also well tolerated in comparison to aspirin and is known to exhibit better efficacy than diclofenac and piroxicam. Nimesulide’s pKa value of 6.5 is very important for gastric tolerability, as it avoids the back diffusion of hydrogen ions responsible for tissue damage. The chemical structure of NIM is shown in Figure No.1.

There are several chromatographic analytical methods for the determination of NIM in pharmaceutical dosage forms such as LC, HPLC, HPTLC, and reversed-phase HPLC. Analytical methods reported for NIM also include adsorptive voltammetry. A few spectrophotometric methods were reported for the quantification of NIM in literature. The purpose of the present work was to develop simple, rapid, and sensitive direct spectrophotometric methods for the determination of NIM in bulk drug and pharmaceutical formulations.

EXPERIMENTAL

Apparatus
All spectral measurements were made using JASCO V-530 (UV-VIS) spectrophotometer (Japan) with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10-mm matched quartz cells. The pH measurements were performed by using (HI 8014, HANNA Instruments) pH-meter (Italy).

Materials and Reagents
All solvents and reagents used were of analytical grade and double distilled water was used throughout the work. NIM reference standard was kindly provided by (Sigma Group, Quessna, Egypt) Nimesulide, Bromocresol purple, Bromocresol green and Brilliant blue G were provided by BDH Chemicals Ltd., Poole, England and used without further purification. A series of Britton-Robinson (B-R) universal buffer solutions were prepared according to the standard method. A stock solution of NIM (100 µg/mL) was prepared by dissolving 0.01 g of the reference standard in a 100-mL measuring flask and diluting up to the mark with ethanol 99.5%. Standard solutions of the reagents (1.0 × 10^{-3} M) were prepared by dissolving accurately weighed acid dyes in a few drops of ethanol and then diluting, separately, to the mark with water in a 100-mL measuring flask.

Assay Procedure for Pure Drug
Aliquots of NIM solution containing up to 100 µg/mL were transferred into a series of 100-mL separating funnels. Buffer solutions (5.0 mL) of various pH values (2.16, 3.5 and 5.5) were added to various volumes (3.5, 2.5 and 1.0 ml) of a fixed concentration (1.0 × 10^{-3} M) of BBG, BCP, and BCG, respectively. The absorbencies of the blue-colored species for all reagents (except BCP, which forms a yellow-colored product) were measured against a reagent blank at the values of λ_{max} as shown in Table No.1. The calibration curves for the three proposed methods were constructed by plotting the absorbance of the colored product against the final concentration of NIM.

Assay Procedure for Nimesulide Formulations
Ten commercial tablets of Sulide ® (100 mg/tablet) were crushed and a weight equivalent to one tablet of NIM was taken, dissolved in ethanol, filtered into 100-ml measuring flask and then completed to volume with methanol. Then follow the same procedures described for determination as in authentic sample. A standard addition technique was also used to confirm the accuracy and precision of the methods.

RESULTS AND DISCUSSION
Anionic dyes form ion-association complexes with the positively charged pharmaceutical drug. The pharmaceutical drug- dye complex, with two oppositely-charged ions, behaves as a single unit held together by an electrostatic force of attraction. Therefore, NIM form ion-pair complexes in acidic medium with acidic dyes such as BBG, BCP and BCG. These colored complexes measured directly. The absorption spectra of the complexes were measured between 350 and 800 nm against blank solution containing the same reagent concentration as shown in Figure No.2. The maximum absorption values (λ_{max}) of the different complexes are shown in Table No.1.
Optimization of the Reaction Conditions
The influence of each of the following variables on the reaction was tested to reach the maximum color intensity.

Effect of pH
The effect of pH was studied by measuring the colored complex directly using a series of B-R buffer solutions in the pH range from 2 to 9 as shown in Table No.1 and Figure No.3.

2- Effect of amount of Buffer
The effect of amount of buffer was studied by adding (5.0 mL) of various pH values (2.16, 3.5 and 5.5) to NIM then added dyes stuff (BBG, BCP and BCG) and then complete the volume with distilled water to 10 ml then shaken well. The maximum absorbance was measured against the blank solution similarly prepared, as shown in Figure No.4.

3- Effect of Reagent Concentration
The effect of the concentration of the reagents on the color intensities of the different ion pair complexes was examined at constant concentration (1.0 × 10^{-3} M) using different reagent amounts at the optimum pH values. The \( \lambda_{\text{max}} \) obtained at (3.5, 2.5 and 1.0 ml) for BBG, BCP, and BCG, respectively as shown in Figure No.5.

4- Effect of Sequence of Addition
The most appropriate sequence of NIM with the three proposed methods was (drug solution, buffer and dye).

5- Effect of Standing Time
The color produced is stable for at least two hours, while maximum time was after (5-6) minutes for three reagents.

6- Effect of Temperature
The effect of temperature on the colored complexes was studied at different temperatures (25, 30, 35, 40, 45, 50 and 55°C). It was found that the colored species were stable up to 40°C. At higher temperatures, the concentration of NIM was found to increase due to the volatile nature of the organic solvent, which resulted in an increased absorbance of the products. The colored species were found to be stable for at least 2 hours at room temperature.

Stoichiometric Ratio
The stoichiometric ratio was determined by Job’s method as shown in Figure No.6. It was found to be 1:1 for the three proposed methods.

Quantification
The limits of the Beer-Lambert law showed in Figure No.7, molar absorptivity, \( S \) and \( \varepsilon \), sensitivity, regression equations and correlation coefficients obtained by linear square treatment of the results are given in Table No.1. In order to determine the accuracy and precision of the three methods, three different concentrations of NIM were prepared and analyzed in six replicates and satisfactory results were obtained as shown in Table No.2.

Analytical Applications
The proposed methods have been successfully applied for the determination of NIM in pharmaceutical formulations. For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed methods as shown in Table No.3, since the ion-pair complexes are stable for at least 24 h. The high percentage recoveries indicate that the excipients in pharmaceutical dosage forms of NIM such as (talc, glucose, starch, lactose, sulfate, dextrose, and acetate) were not found to exhibit any interference in the analysis.
Table No.1: Quantitative parameters for determination of Nimesulide

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>BBG</th>
<th>BCP</th>
<th>BCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</td>
<td>554</td>
<td>437</td>
<td>643</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>2.16</td>
<td>3.5</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>Volume of reagent (1x10⁻³ ml)</td>
<td>3.5</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Beer’s law limits µg/ml</td>
<td>2-13</td>
<td>2-12</td>
<td>2-14</td>
</tr>
<tr>
<td>5</td>
<td>Ring bom range µg/ml</td>
<td>3.5-12</td>
<td>2.8-11</td>
<td>3.2-13</td>
</tr>
</tbody>
</table>

Linear regression equation*  

| 7    | Intercept (a)              | -0.01              | 0.038              | -0.009             |
| 8    | Slope (b)                  | 0.042              | 0.076              | 0.046              |
| 9    | Correlation coefficient (r) | 0.9990             | 0.9992             | 0.9990             |
| 10   | Molar ratio                | 1:1                | 1:1                | 1:1                |
| 11   | RSD                        | 1.87008            | 0.60274            | 1.7445             |
| 12   | Molar absorptivity (L.mol⁻¹ cm⁻¹) | 1.2949 x 10⁴       | 2.3431 x 10⁴       | 1.4182 x 10⁴       |
| 13   | Sandell’s sensitivity (ng cm⁻²) | 0.02380         | 0.013157           | 0.021739           |
| 14   | LD, µg/ml                  | 0.305              | 0.286              | 0.406              |
| 15   | LQ, µg/ml                  | 0.93               | 0.86               | 1.23               |
| 16   | Stability constant         | 10.3643            | 10.9912            | 10.2457            |

* A = a + b C, where C = concentration of drug in µg mL⁻¹, A = absorbance, a = intercept, b = slope.

Table No.2: Evaluation of the accuracy and precision of the proposed methods

<table>
<thead>
<tr>
<th>S.No</th>
<th>Method</th>
<th>Drug taken (µg mL⁻¹)</th>
<th>Drug found (µg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>RE (%)</th>
<th>Confidence&lt;sup&gt;b&lt;/sup&gt; Limits</th>
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<tbody>
<tr>
<td>1</td>
<td>BCP</td>
<td>3</td>
<td>3.1</td>
<td>100.33</td>
<td>1.6208</td>
<td>1.169</td>
<td>3.1 ± 0.0352</td>
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<tr>
<td></td>
<td></td>
<td>5</td>
<td>5.2</td>
<td>100.04</td>
<td>0.6939</td>
<td>0.940</td>
<td>5.2 ± 0.0472</td>
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<tr>
<td></td>
<td></td>
<td>7</td>
<td>6.97</td>
<td>99.57</td>
<td>0.3191</td>
<td>0.4246</td>
<td>6.97 ± 0.0296</td>
</tr>
<tr>
<td>2</td>
<td>BCG</td>
<td>2</td>
<td>1.97</td>
<td>99.50</td>
<td>0.6604</td>
<td>0.6294</td>
<td>1.97 ± 0.0124</td>
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<td></td>
<td>4</td>
<td>3.98</td>
<td>99.50</td>
<td>0.8594</td>
<td>0.8191</td>
<td>3.98 ± 0.0326</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>6.1</td>
<td>100.10</td>
<td>1.2429</td>
<td>1.1846</td>
<td>6.1 ± 0.0712</td>
</tr>
<tr>
<td>3</td>
<td>BBG</td>
<td>2</td>
<td>2.2</td>
<td>101.00</td>
<td>0.6284</td>
<td>0.2020</td>
<td>2.2 ± 0.0121</td>
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<tr>
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<td></td>
<td>4</td>
<td>4.2</td>
<td>100.50</td>
<td>1.2318</td>
<td>0.8300</td>
<td>4.2 ± 0.0332</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>5.99</td>
<td>99.83</td>
<td>0.5185</td>
<td>0.7762</td>
<td>5.99 ± 0.0465</td>
</tr>
</tbody>
</table>

* a= Relative standard deviation for six determinations. *b=95% confidence limits and five degrees of freedom.

Table No.3: Evaluation of the accuracy and precision of the standard addition methods

<table>
<thead>
<tr>
<th>S.No</th>
<th>Method</th>
<th>Drug taken (µg mL⁻¹)</th>
<th>Drug added (µg mL⁻¹)</th>
<th>Drug found (µg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>RE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BCP</td>
<td>3</td>
<td>0</td>
<td>3.1</td>
<td>100.33</td>
<td>0.730</td>
<td>0.0033</td>
</tr>
<tr>
<td>2</td>
<td>BCG</td>
<td>4</td>
<td>0</td>
<td>3.98</td>
<td>99.50</td>
<td>0.710</td>
<td>-0.0050</td>
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<tr>
<td>3</td>
<td>BBG</td>
<td>6</td>
<td>1</td>
<td>7.05</td>
<td>100.70</td>
<td>0.540</td>
<td>0.0007</td>
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</table>

* a= Relative standard deviation for six determinations.
Figure No.1: Chemical structure of Nimesulide

Figure No.2: Absorption spectra of (a) 10 µg ml\(^{-1}\) Nimesulide-BBG complex, (b) 10 µg ml\(^{-1}\) Nimesulide-BCG complex and (c) 10 µg ml\(^{-1}\) Nimesulide-BCP complex

Figure No.3: Effects of pH values on the absorption of Nimesulide-dye complexes
Figure No.4: Effect of ml added of universal buffer on the absorption of Nimesulide-dye complexes

Figure No.5: Effects of ml added of reagents on the absorption of Nimesulide-dye complexes

Figure No.6: Job's method of continuous variation of (1x10^-3 M) Nimesulide with (a) BCP, (b) BBG and (c) BCG dyes (1x10^-3 M) system
CONCLUSION
The proposed methods are simple, precise, accurate and sensitive. Therefore, they can be used for routine analysis and quality control assay of NIM in raw material and tablets without interference caused by the excipients expected to be present in tablets.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

BIBLIOGRAPHY


