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Novel coupling reagents for the sensitive spectrophotometric determination of nimesulide in pharmaceutical preparations

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Abstract

Novel coupling reagents are used for the sensitive spectrophotometric determination of nimesulide (NIME) in either pure form or in its pharmaceutical preparations. The methods are based on the diazotisation of reduced NIME, followed by either coupling with alcoholic iminodibenzyl (IDB) in acid medium to give a deep blue coloured product (λ_{max} of 600 nm) or coupling with 3-aminophenol (AP) in acid medium to produce an orange red coloured product (λ_{max} of 470 nm). Both the methods are highly reproducible and have been applied to a wide variety of pharmaceutical preparations and the results compare favourably with the reported method. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed methods.

Keywords: Nimesulide; Spectrophotometry; Iminodibenzyl; 3-Aminophenol; Diazotisation; Coupling; Pharmaceuticals

1. Introduction

Nimesulide (NIME), chemically *N*-(4-nitro-2-phenoxyphenyl) methanesulphonamide is a relatively new non-steroidal antiinflammatory (NSAID), antipyretic and analgesic drug [1]. It is widely used for the treatment of inflammatory conditions associated with rheumatoid arthritis, respiratory tract infections, soft tissue and oral cavity inflammations. The therapeutic effect of NSAIDs is the result of their ability to inhibit prostaglandin synthesis via inhibition of cyclo-oxigenase. The analgesic potency of NIME is similar to that of ibuprofen and indomethacin. Nonetheless, NIME has shown a higher antipyretic potency than indomethacin, ibuprofen, aspirin and paracetamol. Davis et al. [2] have given an excellent review on pharmacodynamic and pharmacokinetic properties and therapeutic efficacy of NIME. This drug is not yet official in Indian Pharmacopoeia, British Pharmacopoeia or United States Pharmacopoeia. A survey of literature reveals that there are a very few methods available for the determination of NIME. The methods include voltammetry [3], HPLC [4], fluorimetry [5], calorimetry [6] and spectrophotometry [7–16]. One of the spectrophotometric methods which is already reported [15,16] makes use of $KMnO_4$

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and an organic dye which has serious limitations. Lakshmi et al. [11] have reported the spectrophotometric methods for the determination of NIME using *p*-*N*,*N*-dimethylphenylenediamine in presence of chloramine-T, 3-methylbenzothazolin-2-one hydrazone (MBTH) in presence of $FeCl_3$, cresyl fast violet or metol in presence of $K_2Cr_2O_7$, which have certain drawbacks. Other spectrophotometric methods suffer from disadvantages like extraction, long time required for the reaction to complete, narrow range of determination and lack of sensitivity.

In continuation of our work on the spectrophotometric determination of organic compounds of pharmaceutical importance [17–20], the present paper reports elegant methods for the spectrophotometric determination of NIME using either iminodibenzyl (IDB) or 3-aminophenol (AP) as novel coupling reagents. The reactions reported are sensitive than most of the spectrophotometric methods reported in literature.

2. Experimental

2.1. Instrument and materials

A JASCO Model UVIDEC-610 UV–vis spectrophotometer with 1.0 cm matched cells was used for electronic spectral measurements. NIME (gift sample from Recon Health Care, Bangalore), IDB (Sigma, USA), AP (Sigma, USA), $NaNO_2$ (BDH), HCl (AR) and H_2SO_4 (AR) were used for the experiment. All other chemicals and solvents used were of analytical reagent grade. Deionized water was used to prepare all solutions and in all experiments. Commercial dosage forms were purchased from local sources.

2.2. Solutions

Accurately weighed NIME (100 mg) was transferred to a 100 ml beaker containing 4.0 ml of methanol. Zinc dust (0.5 g) and 4.0 ml of concentrated hydrochloric acid were added and the mixture is left for 30 min till the reaction ceases. Solution was filtered into 100 ml standard flask and made up to the mark. The working standard solution of reduced NIME was prepared by further dilution. A 1% solution of freshly prepared IDB in alcohol, a 1% aqueous solution of freshly prepared AP, an aqueous solution of 1% $NaNO_2$, 2% aqueous solution of sulphuric acid, 10 mol dm^{-3} HCl solution and 1:1 H_2SO_4 solution were used.

2.3. Procedure

Aliquots of the working standard solution of reduced NIME (2.5–187.5 μg for IDB; 10–300 μg for AP) were transferred into series of 25 ml calibrated flasks. For both the methods, 1 ml of 1% $NaNO_2$ was added, cooled in an ice bath and 2 ml of 2% sulphuric acid was added and cooled. For IDB method, 3 ml of 1% IDB solution was added to the diazotised solution of the drug. The solution was swirled and heated for 5 min on a boiling water bath, cooled to room temperature (27 °C), followed by the addition of 3 ml of alcohol and diluted to the mark with 1:1 H_2SO_4. After mixing the solution thoroughly, the absorbance was measured at 600 nm against the corresponding reagent blank with in 60 min and calibration graph was constructed. For AP method, 2 ml of 1% AP was added to the diazotised solution of the drug. The solution was mixed well and heated for 5 min on a boiling water bath, cooled, followed by the addition of 2 ml of 10 mol dm^{-3} HCl solution and diluted to the mark with water. The solution was mixed thoroughly and the absorbance was measured at 470 nm against the corresponding reagent blank and the calibration graph was constructed.

2.4. Procedure for the assay of NIME in commercial samples

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 50 mg of NIME was dissolved in 4 ml of methanol and the substance was subjected to reduction using zinc and HCl. The solution was filtered and the filterate was made up to 100 ml and an aliquot of this solution was treated as described above for the pure sample.

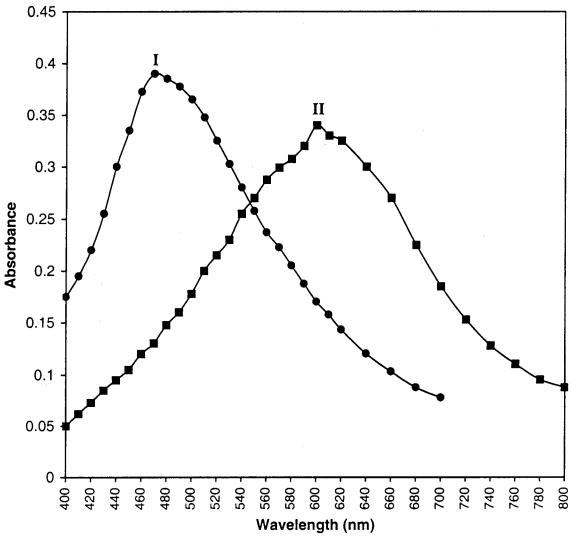


Fig. 1. Absorption spectra of NIME–AP (I) and NIME–IDB (II) reaction products. Initial concentration of NIME = 6 μg ml^{-1} for AP and 4 μg ml^{-1} for IDB.

Table 1
Optical characteristics and precision data

Parameters/characteristics	IDB	AP
Colour	Deep blue	Orange blue
λ_{max} (nm)	600	470
Stability (h)	01	72
Beer's law range (μg ml^{-1})	0.1–7.5	0.4–12.0
Limit of detection (μg ml^{-1})	0.0752	0.2141
Limit of quantification (μg ml^{-1})	0.2508	0.7138
Molar absorptivity (1 mol^{-1} cm^{-1})	0.298×10^5	0.209×10^5
Sandell's sensitivity (μg cm^{-2})	0.01036	0.01477
Optimum photometric range (μg ml^{-1})	0.5–6.9	1.0–11.0
Regression equation (Y)[a]		
Slope (b)	0.0868	0.0673
Intercept (a)	−0.0065	−0.0146
Correlation coefficient (r)	0.9923	0.9980
R.S.D.[b] (%)	0.2407	0.1508
Range of error	±0.3341	±0.2093

[a] $Y = bx + a$, where x is the concentration in μg ml^{-1}.
[b] Five replicates.

3. Results and discussion

3.1. Spectral characteristics

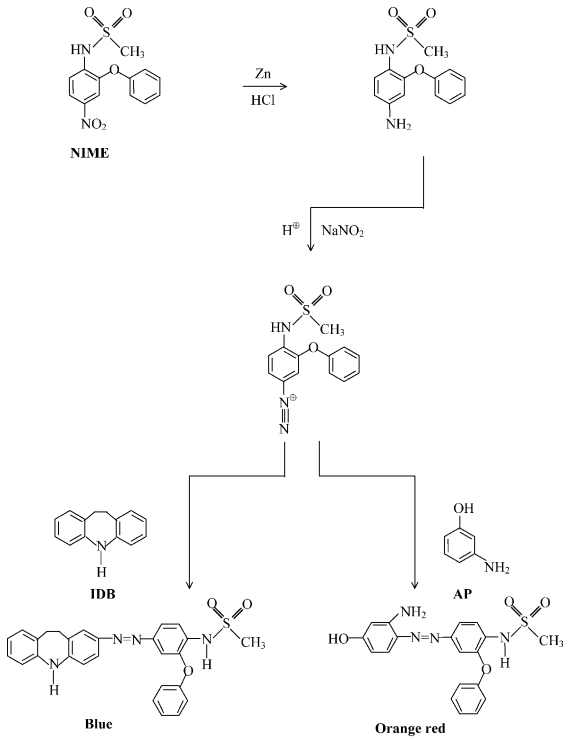
The reduced NIME was diazotised in acidic medium and coupled with IDB in alcohol medium to form a blue coloured product of λ_{max} equal to 600 nm or coupled with AP in acid medium to get a orange red coloured product of λ_{max} equal to 470 nm. These wavelengths were used for all measurements. The absorption spectra of NIME reaction products are shown in Fig. 1. The corresponding reagent blanks have practically negligible absorbance at these wavelengths. The unknown concentration of the drug can be calculated by knowing the absorbance at the λ_{max}, using the regression equation.

3.2. Optimum reagents concentration

It was found that a 1% solution of $NaNO_2$ in the range of 0.5–2.0 ml, a 2% solution of sulphuric acid in the range of 1.0–3.0 ml, 2.0–4.0 ml of 1% alcoholic IDB solution (2.0–4.0 ml of 1% AP), 2.0–4.0 ml of alcohol (2.0–4.0 ml of 10 mol dm^{-3} HCl) were necessary to get the maximum colour intensity. In both the methods, the excess of nitrite could be removed by the addition of sulphuric acid solution. Addition of excess of sulphuric acid solution has no effect on the absorbance values. In the IDB method, excellent results were obtained using 1:1 H_2SO_4 for dilution compared with other acids and solvents. In case of AP as a coupling agent, dilution of the coloured solution with different solvents like water, methanol, ethanol, acetic acid and acetonitrile have been tested. Results showed that water gives maximum intensity and stability of the colour.

3.3. Quantification and reaction sequence

Beer's law is obeyed over the NIME concentration range of 0.1–7.5 μg ml^{-1} for IDB and 0.4–12 μg ml^{-1} for AP as coupling agents. The optical characteristics and precision data are given in Table 1. In an acidic medium, nitrite reacts with reduced NIME to form diazonium salt which is then coupled with either IDB in presence of



Scheme 1. Reaction sequence for the formation of coupled products.

alcohol to give a blue coloured product or with AP in presence of acid to produce an orange red coloured product. The reaction sequence for the formation of the products are shown in Scheme 1.

3.4. Stability

The diazotisation of reduced NIME is complete in 5 min at room temperature. The stability of the blue coloured product formed from NIME–IDB interaction is 60 min. After this time interval, the colour intensity slowly decreases. Attempts to increase this time interval, by changing the reaction conditions were unsuccessful. However, a time interval of 60 min was sufficient enough to record the measurements. In contrast, the orange red coloured product formed by the coupling reaction between NIME and AP was stable for 3 days. An increase of temperature up to 40 °C, did not affect the stability of colour in both the methods.

3.5. Interference

Under the diazotisation reaction conditions used, other amines such as morphine, aniline, piperidine, etc. give a positive reaction. However, the problem of interference does not arise in the analysis of commercially available NIME tablets. The effect of additives associated with the NIME in its formulations were investigated using the developed methods. The methods does not suffer any interference from common excipients and

Table 2
Determination of NIME* in presence of excipients

Excipients	Amount of excipient added (mg)	Percent recovery of NIME ± % R.S.D.[a]	
		IDB method	AP method[b]
Talc	40	99.7 ± 0.30	99.6 ± 0.25
Gumacacia	40	99.6 ± 0.28	99.7 ± 0.18
Starch	40	99.8 ± 0.25	99.8 ± 0.18
Sodiumchloride	50	99.8 ± 0.30	99.7 ± 0.25
Dextrose	30	99.7 ± 0.25	99.6 ± 0.25
Glucose	30	99.6 ± 0.30	99.7 ± 0.20
Lactose	30	100.2 ± 0.25	100.2 ± 0.20
Carboxymethylcellulose	50	100.3 ± 0.30	100.2 ± 0.20
Magnesium stearate	40	99.6 ± 0.30	99.7 ± 0.20
Sodium alginate	40	99.7 ± 0.25	99.8 ± 0.25

*, 4 μg ml^{-1} of NIME taken.
[a] Average of five determinations.
[b] 6 μg ml^{-1} of NIME taken.

Table 3
Determination of NIME* in pharmaceutical preparations

Tablet	Label claim (mg)	Amount of NIME found* in mg		
		IDB method	AP method	Reported method [9]
Emsulide[a]	100	99.6 ± 0.30	99.7 ± 0.35	99.6 ± 0.30
Maxiflam[b]	100	99.7 ± 0.40	99.8 ± 0.25	99.5 ± 0.40
Nelsid[c]	100	99.8 ± 0.35	99.8 ± 0.30	99.5 ± 0.50
Neosaid[d]	100	99.6 ± 0.40	99.7 ± 0.35	99.4 ± 0.50
Nimbid[e]	100	100.2 ± 0.35	100.3 ± 0.35	100.6 ± 0.60
Nimegesic[f]	100	100.3 ± 0.40	100.3 ± 0.35	100.7 ± 0.65
Nimfast[g]	100	100.4 ± 0.40	100.3 ± 0.35	100.5 ± 0.45
Nimind[h]	100	100.2 ± 0.35	100.4 ± 0.40	100.6 ± 0.55
Nimulid[i]	100	99.6 ± 0.40	99.7 ± 0.30	99.4 ± 0.60
Nise[j]	100	99.5 ± 0.40	99.6 ± 0.40	99.3 ± 0.60
Novolid[k]	100	99.4 ± 0.50	99.6 ± 0.40	99.2 ± 0.50
Orthobid[l]	100	99.6 ± 0.40	99.7 ± 0.30	99.5 ± 0.60
Pronim[m]	100	99.8 ± 0.45	99.7 ± 0.25	99.6 ± 0.60
Remulide[n]	100	99.7 ± 0.30	99.6 ± 0.25	99.5 ± 0.60

*, Average of five determinations ± %R.S.D.
[a] Marketed by: Emcure.
[b] Karnataka Antibiotics.
[c] End-Swift.
[d] Blue cross.
[e] Astra-IDL.
[f] Alembic.
[g] Indon.
[h] Indoco.
[i] Panacea.
[j] Dr. Reddy's.
[k] Brown and Burk.
[l] Nicholas-piramal.
[m] Unichem.
[n] Recon.

other substances. The results are given in Table 2 for various excipients and the percentage recovery of the drug ranged from 99.6 to 100.3.

3.6. Applications

The reproducibility of the method was checked by five replicate determinations at the 4 μg ml^{-1} level of NIME for IDB (6 μg ml^{-1} level of NIME for AP) and the relative standard deviation was found to vary between 0.18 and 0.30%. The applicability of the method for the assay of pharmaceutical preparations was examined. The results of the assay of available tablets of NIME are summarized in Table 3. The results are highly reproducible and the assay of tablets were cross checked by the reported method [9] which involves the coupling of diazotised NIME and *N*-(1-naphthyl)ethylenediaminedihydrochloride (NEDA). The results agree favourably with one another. The robustness of the method is that it does not involve extraction. The experiment for the proposed methods were conducted by the second analyst on different days and the results of recovery and reproducibility justified the raggedness of the proposed methods.

4. Conclusions

The present methods are found to be economical and more sensitive than the few reported

methods. The methods are economical, in the sense that the reagents IDB and AP are economically cheaper than the universal reagents MBTH or NEDA. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing NIME showed no interference from common additives and excipients. Hence, these methods could be considered for the determination of NIME in pharmaceutical preparations.

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