ELSEVIER

International Journal of Pharmaceutics 176 (1999) 261–264

international journal of pharmaceutics

Note

Spectrophotometric determination of pK_a of nimesulide

Saranjit Singh *, Nishi Sharda, Lalit Mahajan

National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar 160062, India

Received 21 May 1998; received in revised form 2 September 1998; accepted 8 September 1998

Abstract

The note reports on the determination of pK_a of nimesulide, a potent anti-inflammatory agent, by a spectrophotometric method. The ruggedness of the result was validated by determination by different persons on different days and on different spectrophotometers employing different drug concentrations. Compared to the literature reports of pK_a values of 5.90, 6.46, 6.50 and 6.80, the value calculated in the present study is 6.56 ± 0.01.

Keywords: pK_a Determination; Nimesulide; Spectrophotometric method; Validation

1. Introduction

Nimesulide is a potent anti-inflammatory agent which has a reduced gastric irritancy. The drug is receiving much attention these days due to its significant selectivity towards cyclooxygenase-2 (COX-2) versus COX-1 inhibition. It is a novel compound in the respect that it exhibits an acidic character that is attributed to sulfonanilide rather to a carboxyl group, that is the case with all other acidic anti-inflammatory agents.

The determination of pK_a of nimesulide is of interest from the standpoint of its influence on prostaglandin synthesis (Magni, 1991). The pK_a of the drug is cited in literature by several authors, the value ranging from 5.90 (Hansch et al., 1990), 6.50 (Magni, 1991) to 6.80 (Piel et al., 1997). None of these reports, however, provide source of information and details of methodology. The first real report on the determination of pK_a value of nimesulide appeared only recently (Fallavena and Schapoval, 1997) in which the dissociation constant was determined by a potentiometric method employing mixed solvent techniques using methanol–water mixtures. The reported value is 6.46.

* Corresponding author. Tel.: +91 172 673848; fax: 91 172 677185; e-mail: niper@chd.nic.in

0378-5173/99/$ - see front matter
PII S0378-5173(98)00304-4

Nimesulide is very sparingly soluble in water (~ 0.01 mg ml^{-1}). For such insoluble compounds, the ideal recommended procedure for determination of pK_a is the spectrophotometric method (Albert and Serjeant, 1962). The other advantage of spectrophotometric technique is that it gives precise values, more accurate than those determined by potentiometric or other methods. The only prerequisite is that the two ionic species involved in the equilibrium should show different spectra. Nimesulide in our preliminary investigations was found to meet this prerequisite. Therefore, this method was followed in our laboratory.

There was another intention in carrying out the study. The mixed solvent potentiometric technique for determination of pK_a of insoluble compounds in particular has been expressed to give anomalous results due to, first, the different solvating power of the two components creates new complexities and second, the organic component contributes extra acidic and basic species to the solution (Albert and Serjeant, 1962; Zalipsky et al., 1976). Also, a problem has been expressed of accurate calibration of pH meters, as the standardisation of electrodes is done using aqueous buffers. However, refinements in the technique have been proposed lately (Avdeef et al., 1993) which were employed by Fallavena and Schapoval (1997) in their study on pK_a determination of nimesulide. It was, therefore, our endeavour to test the accuracy of the refined mixed solvent potentiometric technique by comparing the reported results with those obtained through the so considered ideal spectrophotometric method.

An added feature of our study is the validation of the determination of pK_a. This was done keeping into view the absoluteness of the pK_a value and the scope of its universal application. The pK_a values reported in literature find passage into handbooks, treatises and codexes and it is ironical that despite this, the validation of the ruggedness of the determined value is not practised. In our study, the results were validated through determination of the dissociation constant by different persons on different days using different spectrophotometers and different starting drug strengths. The results are presented.

2. Materials and methods

The drug was received as a free gift from M/S Panacea Biotec Ltd., Lalru, Punjab, India. It was used without further purification. Buffer chemicals and all other reagents were of analytical grade. Freshly triple distilled water from an all glass still was employed throughout.

The pH were determined on a pH meter (Control Dynamics, Mumbai, India) equipped with a combined glass electrode. It was standardised at 25°C using standard buffers (Bates, 1962). The absorbance readings and spectra were recorded on two spectrophotometers, model DU640i and diode array model 700 (both Beckman, USA). A thermostatic bath equipped with a precision controller (Model MV, Julabo, Darmstadt, Germany) was used for the control of temperature.

The procedure for determination and calculation of pK_a was essentially the same as described by Albert and Serjeant (1962). The buffers were prepared by mixing predetermined volumes of stock 0.2 M NaH_2PO_4, 0.1 M K_2HPO_4, 0.2 M NaCl solutions and water to give final buffer molarity of 0.01 M and ionic strength 0.02. Aliquots of 5 ml of each buffer were distributed into five tubes each. To each tube 50 μl drug stock solution in methanol (2 mg ml^{-1}) was added to give a final drug strength of 20 μg ml^{-1}. For a validation study in which the effect of drug concentration was looked for, the drug strength was doubled to 40 μg ml^{-1}. The solutions were mixed and the tubes were placed in a thermostatic bath set at 25°C. The absorbance was determined for each solution at 393 nm. Full scans were also taken for selected samples.

The absorbance of neutral and ionic species of the drug were determined in a similar manner employing 0.01 N HCl and 0.01 N NaOH, respectively. The ionic strength of these solutions was also preadjusted to 0.02 by addition of suitable quantity of sodium chloride.

To establish the validity (ruggedness) of the results, the whole procedure starting from the weighing of the buffer salts to measurement of absorbance was repeated independently by two different members of the technical team. While absorbance measurements in three studies were

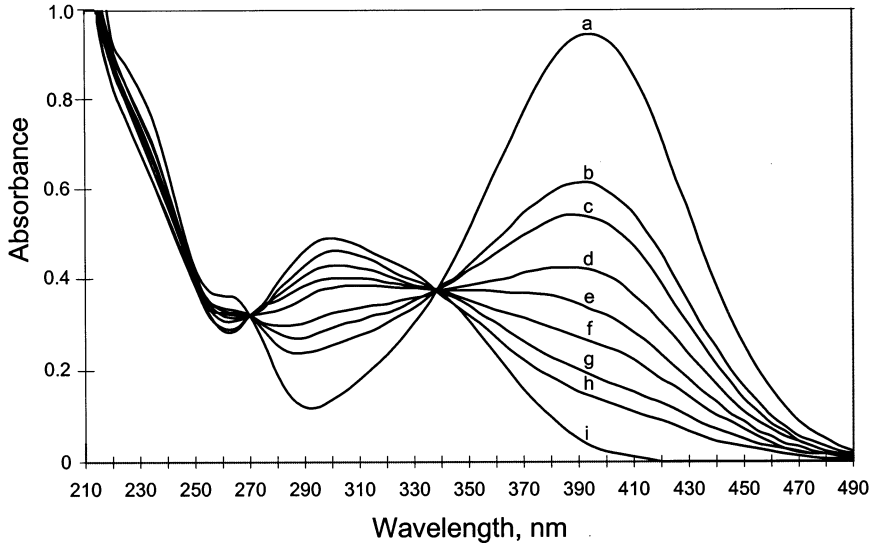

Fig. 1. Spectral changes of nimesulide at various pH conditions. Key: (a), 0.01 N NaOH; (b), pH 5.66; (c), pH 5.80; (d), pH 6.00; (e), pH 6.17; (f), pH 6.43; (g), pH 6.63; (h), pH 6.83; and (i), 0.01 N HCl. Isosbestic points are shown at 270 and 339 nm.

done on one spectrophotometer, the measurement in another study was done on the other instrument. Each study was done on different days.

3. Results

Fig. 1 shows the variation of UV spectrum of nimesulide with pH. Clearly two isosbestic points are shown at 270 and 339 nm. The data of studies where such sharp isosbestic points were observed were only used for calculation of pK_a. Table 1 contains the pH and absorbance data of one of the determinations. It describes the calculations of pK_a and scatter which were done in the manner suggested by Albert and Serjeant (1962). The calculations are based on the following equation:

$$pK_a = pH + \log (d_i - d/d - d_m)$$

where d_i is the absorbance of the ionised species, d = absorbance of the solution tested and d_m = absorbance of the unionised species. The average pK_a was calculated by taking antilogarithm of each individual calculated pK_a value in the table, adding all the antilogarithmic values, dividing by total number of pK_a values and taking logarithm of the averaged value. The scatter is the maximum difference between the average value and any of the tabulated pK_a values at various pH. The same procedure was repeated in all four sets of determinations.

The values of pK_a obtained in different studies are listed in Table 2. Evidently, the scatter for all the determinations is very much below the limit of ± 0.06 prescribed by Albert and Serjeant (1962) which is indicative of the accuracy of the technique. Table 2 shows that despite the use of different equipment and determination by different persons on different days using different start-

Table 1
Typical example of calculation of pK_a from absorbance data at various pH

pH	Absorbance (d)	$pK_a = pH + \log (d_i - d/d - d_m)$
5.66	0.1475	6.5528
5.80	0.1789	6.5598
6.00	0.2361	6.5718
6.17	0.3054	6.5632
6.43	0.4322	6.5558
6.63	0.5415	6.5449
6.83	0.6349	6.5572

Result: $pK_a = 6.5579 \pm 0.0139$.
$d_m = 0.0488$ (in 0.01 N HCl); $d_i = 0.9498$ (in 0.01 N NaOH).

Table 2
Validation of ruggedness of the pK_a value

Instrument 1			Instrument 2
Person 1, day 1, drug strength = 20 μg ml^{-1}	Person 1, day 2, drug strength = 20 μg ml^{-1}	Person 1, day 3, drug strength = 40 μg ml^{-1}	Person 2, day 4, drug strength = 20 μg ml^{-1}
6.5579 ± 0.0139	6.5593 ± 0.0102	6.5584 ± 0.0317	6.5451 ± 0.0251

ing drug concentrations, very close values were obtained suggesting ruggedness of the determinations. The final determined value with the scatter calculated using the antilogarithm method is 6.56 ± 0.01 (after rounding off).

This pK_a value of 6.56 for nimesulide determined spectrophotometrically is about 0.1 unit different from 6.46 obtained by Fallavena and Schapoval (1997) using refined potentiometric technique employing mixed solvents. In one of the earlier studies on methaquolone, a difference of almost 1.02 unit in pK_a values was reported between the values obtained by a spectrophotometric method and a nonrefined potentiometric technique involving mixed solvents (Zalipsky et al., 1976). The present study shows that the refinement proposed by Avdeef et al. (1993) in potentiometric technique employing mixed solvents is useful and as such yields much more accurate pK_a values.

One main disadvantage of spectrophotometric technique is said to be that it requires whole day of work-up against shorter time period required for potentiometric titrations. However, keeping into view the importance of pK_a value as a parameter in general, the use of most accurate method shall always be given an upper hand. Also, the concept of validation which presently does not exist in pK_a determinations, shall be made part of the studies. This would really help in reporting in literature of more accurate, reproducible pK_a values.

References

Albert, A., Serjeant, E.P., 1962. Ionisation Constants of Acids and Bases. Methuen, London.

Avdeef, A., Comer, J.E.A., Thomson, J.S., 1993. pH-Metric log P. 3. Glass electrode calibration in methanol water, applied to pK_a determination of water-insoluble substances. Anal. Chem. 65, 42–49.

Bates, R.G., 1962. Revised standard values for pH measurements from 0–95°. J. Res. Natl. Bur. Stand., Sect A 66, 179–184.

Hansch, C., Sammes, P.G., Taylor, J.B., 1990. Comprehensive Medicinal Chemistry, vol. 6. Pergamon, Oxford, p. 711.

Fallavena, P.R.B., Schapoval, E.E.S., 1997. pK_a Determination of nimesulide in methanol–water mixtures by potentiometric titrations. Int. J. Pharm. 158, 109–112.

Magni, E., 1991. Nimesulide an overview. Drug Invest. 3 (Suppl.), 1–3.

Piel, G., Pirotte, B., Delneuville, I., Neven, P., Llabres, G., Delarge, J., Delattre, L., 1997. Study of the influence of both cyclodextrins and L-lysine on the aqueous solubility of nimesulide; isolation and characterisation of nimesulide-L-lysinecyclodextrin complexes. J. Pharm. Sci. 86, 475–480.

Zalipsky, J.J., Patel, D.M., Darnowski, R.J., Reavey-Cantwell, N.H., 1976. pK_a Determination of methaqualone. J. Pharm. Sci. 65, 460–461.