ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 257–262

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Polarographic determination of benzaldehyde in benzyl alcohol and sodium diclofenac injection formulations

Amir G. Kazemifard [a], Douglas E. Moore [b,*], A. Mohammadi [a]

[a] *College of Pharmacy, Medical Sciences University of Tehran, Tehran, Iran*
[b] *Faculty of Pharmacy, University of Sydney, Sydney NSW 2006, Australia*

Received 11 March 2002; received in revised form 17 April 2002; accepted 28 April 2002

Abstract

A rapid and sensitive polarographic method was developed for qualitative as well as quantitative analysis of the neurotoxic contaminant benzaldehyde in Na-diclofenac injections and in benzyl alcohol used for parental formulations. A well-defined differential pulse (DP) polarographic peak or a sampled direct current (SDC) wave was obtained at -1.39 V (vs. silver–silver chloride reference electrode) in Britton–Robinson buffer (pH 9.15) and at -1.41 V in 0.1 M LiCl solution. The reduction step involves a two-electron process, corresponding to the formation of benzyl alcohol. No peaks were observed in the anodic branch of the cyclic voltammogram, emphasizing the occurrence of an irreversible process. The peak current versus concentration relationship was found to be linear up to 50 μg/ml with the detection limit of 10 ng/ml and quantitation limit of 30 ng/ml. The relative standard deviations (S.D.) obtained for concentration levels of benzaldehyde as low as 25 μg/ml with the SDC and DP methods were 1.5 and 0.78% ($n = 10$), respectively. Benzyl alcohol and Na-diclofenac are not electrochemically active, and metabisulfite reductions at -0.664 and -1.240 V do not interfere with the benzaldehyde reduction peak. The proposed methods (DP and SDC polarography) have been applied satisfactorily to the determination of benzaldehyde traces in benzyl alcohol and in different pharmaceutical products such as Na-diclofenac injectable formulations.

Keywords: Na-diclofenac injection; Benzyl alcohol; Benzaldehyde; Differential pulse polarography; Sampled direct current polarography

1. Introduction

Benzyl alcohol is frequently used as a bacteriostatic agent or co-solvent in a variety of liquid pharmaceutical preparations. The main toxic oxidation product is benzaldehyde, occurring as a result of oxidation on long-term storage or heat sterilization of parenteral dosage forms containing benzyl alcohol, if oxygen is not excluded by nitrogen flushing [1]. Sonication of solutions containing benzyl alcohol also generates benzaldehyde [2]. The presence of this potential impurity needs to be monitored owing to its reactivity and toxicity.

* Corresponding author. Tel.: +61-2-9351-2334; fax: +61-2-9351-4391
E-mail address: demoore@pharm.usyd.edu.au (D.E. Moore).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00340-0

It is known that prolonged exposure of nerve fiber (either in isolation or in experimental animals) to benzyl alcohol results in extensive degeneration and demyelination, though the causative agent has not been positively identified [3–5]. Evidence to date suggests that benzaldehyde might also be a possible offender, since the benzaldehyde containing sting of the Apheloria arthropods (millipedes) causes a similar neurotoxic symptom.

Commercial pharmaceutical preparations of Na-diclofenac include injection formulations that contain benzyl alcohol as a preservative or co-solvent in the concentration range up to 5%. In Iran a warning was issued by the Ministry of Health to medical practitioners and hospitals that in a single year (2000) nearly 200 cases of paraplegia had resulted from the use of generic brands of Na-diclofenac injections. This prompted us to examine these preparations and we found that many contained very high concentration levels (up to 140 μg/ml) of benzaldehyde, which is potentially the cause of the paraplegia symptoms and other reported adverse neurotoxic side-effects.

Benzyl alcohol intended for use in the manufacture of parenteral dosage forms should not contain more than 0.05% of benzaldehyde quantifiable by gas chromatography [6]. The United States Pharmacopoeia does not contain a monograph on injectable benzyl alcohol solutions, but limits the presence of benzaldehyde in benzyl alcohol to levels of 0.2%, with quantification by HPLC [7]. Several other gas [8–10] and liquid [11–14] chromatographic methods, as well as derivative UV spectrometry [15] and polarography [16] have been employed for identification and quantitation of benzaldehyde. Some of these methods are time-consuming and clearly not satisfactory for handling large numbers of samples. In addition, no report has been published yet for electrochemical determination of benzaldhyde in injectable formulations such as Na-diclofenac. The injection forms of this non-steroidal anti-inflammatory agent are not as yet detailed in the various pharmacopoeias, but are widely used in countries such as Iran, and, to a lesser extent, in the European community and the USA.

Consequently a need exists for methodology capable of rapid monitoring of parental dosage forms and injectable formulations potentially containing benzaldehyde arising from the use of benzyl alcohol as a preservative.

In this work different electrochemical techniques were used to study benzaldehyde in aqueous solution, and a direct method was developed for a rapid and sensitive quantitation of this toxic impurity at low concentration in Na-diclofenac injectable formulation and in benzyl alcohol.

2. Experimental

2.1. Apparatus

Polarograms and voltammograms were obtained with a Princeton Applied Research (PAR) (Princeton, NJ) Model 394 polarographic analyzer combined with a PAR Model 303A electrode system [static mercury drop electrode (SMDE), dropping mercury electrode (DME), hanging mercury drop electrode (HMDE) serving as the working electrode, a Ag/AgCl electrode as reference electrode and a platinum wire as auxiliary electrode]. A PAR Model 305 magnetic stirrer, a MMX Pentium computer, using M394 software, and a HP LaserJet 6L printer were used. The PAR Model 9301 thermostatic cell was maintained at 21 °C in all experiments. A Pye Unicam PW 9418 pH meter (Philips England) was used for the pH measurements.

2.2. Materials

All chemicals were of analytical-reagent grade and deionized, double distilled water was used for preparing the solutions. Various batches of Na-diclofenac injections were donated by several Iranian pharmaceutical manufacturing companies (Alborz Daru, Aburaihan, Chimidaru, Zahravi, IPDIC and LPC). The reference formulation was Voltaren (Novartis Pharma GmbII, Nuremberg, Germany, Batch Numbers: 366200 and 367200).

2.3. Procedure

A 3 ml aliquot of supporting electrolyte solution (0.2 M LiCl or Britton–Robinson buffer, pH 9.15) was placed in the polarographic cell and a 3 ml aliquot of analyte solution with the required concentration of benzaldehyde or benzyl alcohol was added. The solution was deoxygenated for 3 min with a stream of pure nitrogen and then maintained in a nitrogen atmosphere. A calibration plot obtained with known concentrations of benzaldehyde was used to convert peak height into sample concentration.

For the analysis of benzaldehyde in the injectable formulations, the contents of ten Na-diclofenac vials were mixed and a 3 ml aliquot of the mixed solution was transferred into the polarographic cell and diluted to 6 ml with the supporting electrolyte solution. The determinations by differential pulse (DP) polarography or by sampled DC or cyclic voltammetry were carried out with optimum instrumental parameters (drop times of 0.8 s, pulse amplitude of 20 mV and scan rate of 6–20 mV/s). The potentials of the working electrode were scanned in the negative direction over the range of -400 to -1700 mV.

3. Results

Under the experimental conditions, benzaldehyde is easily reducible at the dropping mercury electrode. When the reduction of benzaldehyde is carried out in an acidic medium, two successive polarographic peaks (or waves) are observed. The first peak corresponds to the reversible addition of one electron and one proton, which is associated with the formation of a free radical. This radical is reduced to benzyl alcohol and the second peak appears at a more negative potential [16,17]. The complexity of the polarographic behavior at pH values below 7.0 leads to a non-linear relationship between diffusion current and concentration, which is unsuitable for a sensitive and robust assay procedure.

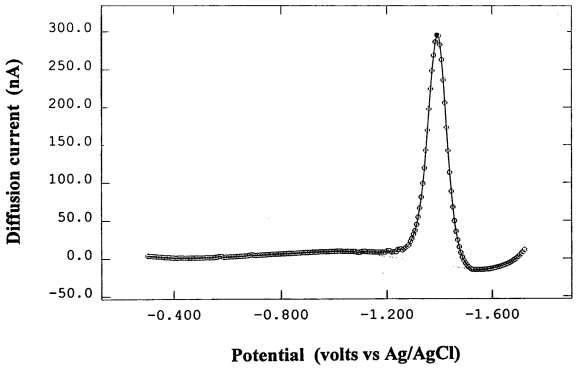

Fig. 1. Differential pulse polarogram of 20 μg/ml benzaldehyde in Britton–Robinson buffer at pH 9.15.

At pH values higher than 7.0, or in aqueous 0.1 M LiCl, the two peaks are gradually replaced by only one peak, associated with an electrode reaction of two electrons, which corresponds to the formation of benzyl alcohol (Fig. 1). No peak is observed when extra pure benzyl alcohol is examined under the same conditions. The effect of pH, operating with 50 μg/ml benzaldehyde and adjusting the pH between 7.0 and 12.0 with Britton–Robinson buffer was studied. It was found that the benzaldehyde peak potential varies linearly with pH between pH 7.0 and 11.0, with a slope of -60 ± 2 mV/pH, close to the theoretical value of -59 mV/pH for a reduction process in which the same number of protons and electrons is involved. That process, responsible for this polarographic behavior, must be the reduction of the aldehyde group in the benzaldehyde molecule:

$$C_6H_5CHO + 2e^- + 2H^+ \rightarrow C_6H_5CH_2OH$$

The effect of pH on the polarograms of benzaldehyde leads to the conclusion that an alkaline medium is suitable for analytical studies. The peak at pH 9.15 or in 0.1 M LiCl is well suited for a sensitive and precise quantification of benzaldehyde in benzyl alcohol and Na-diclofenac injection solutions. To elucidate further the electrode reaction of benzaldehyde in the media used, repetitive cyclic voltammograms were recorded using the HMDE. Cyclic voltammograms show that, under the conditions used, benzaldehyde reduction at the HMDE is an irreversible process, because no anodic peak appears.

The relation between the diffusion current i_d (in nA), and the concentration (in μg/ml) was found

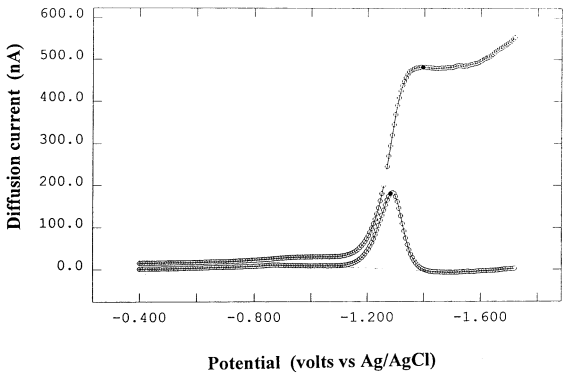

Fig. 2. Differential pulse and Sampled DC polarogram of a benzyl alcohol sample in Britton–Robinson buffer at pH 9.15.

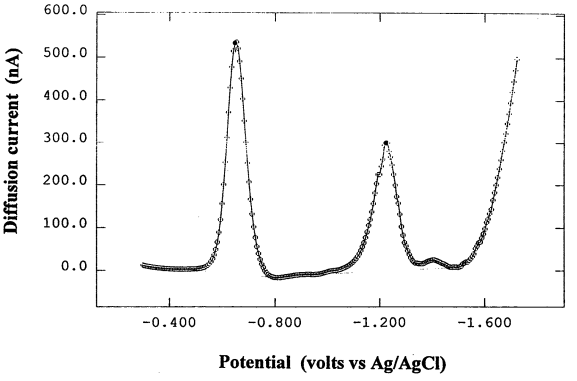

Fig. 3. Differential pulse polarogram of Voltaren® injection in Britton–Robinson buffer at pH 9.15.

to be linear over the concentration range of 0.03–50 μg/ml. Linear regression analysis of the above data gave the equation of best fit as:

$$y = (11.68 \pm 0.18)x + (8.6 \pm 4.0) \quad (r = 0.9989)$$

The precision of the electrochemical procedure was tested by repeated analysis ($n = 10$) of a mixture containing 25 μg/ml of benzaldehyde, 40 mg/ml of benzyl alcohol and 25 mg/ml of Na-diclofenac. The concentration of benzaldehyde (mean ± S.D.) determined was 24.8 ± 0.2 μg/ml. Recovery tests were carried out on nearly pure benzyl alcohol and Na-diclofenac injectable formulations each spiked with 25 μg/ml benzaldehyde, to evaluate the reproducibility and accuracy of the proposed methods. The mean percentage recovery of benzaldehyde was $99.2 \pm 0.8\%$ and the RSD derived from the replication ($n = 10$) all fell in the range of 0.67–1.5%, indicating that the polarographic methods used here have good precision. The high percentage recovery obtained indicates that the method is not influenced by other components (common exipients and additives, and the therapeutically active substance) and does not require a specific clean-up or extraction of the analyte from the samples.

The limits of detection and quantitation of benzaldehyde in the presence of benzyl alcohol and Na-diclofenac, were determined to be 10 and 30 ng/ml, respectively. The specificity of the method was checked by adding, in turn, each of the known benzyl alcohol oxidation products and other injection components such as Na-diclofenac, propylene glycol and Na-metabisulfite to the benzaldehyde samples. The response of the analyte in these mixtures was evaluated by the proposed polarographic methods and showed accurate and precise results without interference. Specificity was also checked by stressing pure benzyl alcohol and benzaldehyde by heat and UV-irradiation, then applying the proposed methods. The clear resolution of benzaldehyde from its degradation products and other injection ingredients was attained.

In order to test the application of the developed method for quality control in manufacture, the concentration of benzaldehyde was measured in several different brands of commercial benzyl alcohol samples designed for use in the manufacture of parental formulations (Fig. 2). As can be seen from this figure, some benzyl alcohol as used in parenterals, contains considerable amounts of benzaldehyde.

By way of illustrating the application of the polarographic method developed here, eight generic brands of Na-diclofenac injection solution were analyzed in comparison with Voltaren® as reference. The results showed that five of the generic brands contained considerable amounts of the toxic benzaldehyde. The difference in benzaldehyde content can be observed from the polarograms in Fig. 3 (reference) and Fig. 4 (rejected brand). The less cathodic peaks at -0.664 and -1.240 V in these figures are due to the reduction of Na-metabisulfite, which is present as an antiox-

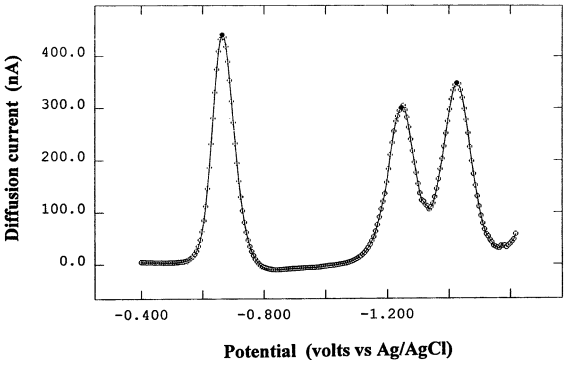

Fig. 4. Differential pulse polarogram of a generic brand of Na-diclofenac injection in Britton–Robinson buffer at pH 9.15.

idant in all Na-diclofenac injections. Metabisulfite reduction peaks do not interfere with the benzaldehyde peak under the conditions used, but a possible reaction between these two reactive compounds [15] may cover (and therefore decrease) the real aldehyde concentration by producing a lower diffusion current. Nevertheless, benzaldehyde levels well above the pharmacopoeia limit values were found in most samples analyzed, with the exception of three samples, including the Voltaren reference samples, as can be observed from Table 1.

The results achieved using the polarographic assay have been compared with those obtained by two independent assay methods, which are modifications of the pharmacopoeia gas [6] and liquid chromatographic [7] procedures. Details of these systems will be presented separately because of their application to a wider range of formulations (manuscript in preparation). As shown in Table 1 there is statistically significant agreement ($P = 0.01$) between these methods according to the paired t-test.

4. Discussion

The reduction of benzaldehyde in Britton–Robinson buffer (pH 9.15) or in 0.1 M aqueous LiCl was characterized as being an irreversible process and controlled mainly by diffusion. The peak current is proportional to the concentration in the range of 0.05–50 μg/ml. The DPP or SDC polarography described here, with a mean recovery of benzaldehyde of 99.2% and a relative standard deviation of nearly 1.0, is rapid, accurate, sensitive, and simple to perform. It is thus suitable for the qualitative and quantitative analysis of benzaldehyde in benzyl alcohol and in Na-diclofenac injection formulations.

Taking into account the results obtained for the calibration graphs, DPP is more sensitive than SDCP, with a greater linear range and similar coefficients of variations. The detection limit value obtained for DPP (10 ng/ml) is lower than those reached using the SDCP technique (24 ng/ml). Although the peaks corresponding to the DPP technique are more distant from the metabisulfite reductions and background discharge, the DPP technique is preferable for quan-

Table 1
Determination of benzaldehyde in Na-diclofenac injection solutions by different analytical methods

Preparation	Supplier	Amount of benzaldehyde found (μg/ml) ± S.D. ($n = 6$)		
		By DPP	By GC	By HPLC
Voltaren (Batch number:366200)	Novartis, Germany	0.5 ± 1.3	0.5 ± 1.7	0.5 ± 2.8
Voltaren (Batch number: 367200)	Novartis, Germany	1.2 ± 1.2	1.2 ± 1.7	1.4 ± 2.0
Na-diclofenac	Alborz Daru, Iran	25.2 ± 1.0	24.6 ± 1.3	25.7 ± 1.7
Na-diclofenac	Aburaihan, Iran	23.6 ± 0.9	23.8 ± 1.1	24.3 ± 1.0
Na-diclofenac	Chimidaru, Iran	37.5 ± 0.8	36.9 ± 1.9	37.9 ± 1.2
Na-diclofenac	Zahravi, Iran	48.9 ± 0.8	48.1 ± 1.0	50.2 ± 1.0
Na-diclofenac	IPDIC, Iran	17.6 ± 1.0	18.1 ± 1.6	17.5 ± 1.5
Na-diclofenac	LPC, Iran	25.1 ± 1.0	25.0 ± 0.9	25.4 ± 1.3

titative purposes, for all the reasons given above as well as their high scan rate, which provides a great economy of time. Once the instrument is set, the amount of benzaldehyde can be determined in 3–4 min, including deoxygenating time. The developed method can be used to quantify benzaldehyde in Na-diclofenac injection without interference from other ingredients. In conclusion, the developed polarographic method has been successfully used on a routine basis and allows the quantitation in pharmaceutical preparation in a very short analytical time. It is possible to use this method as an official method for benzaldehyde assay in Na-diclofenac injection and in routine quality control analysis. The results obtained by the polarographic method reported here compared favorably with those obtained by the validated standard chromatographic methods, and the polarographic method has the added advantage of specificity for benzaldehyde in the presence of the other ingredients of the formulation without the requirement of an extraction step. However, it should be pointed out that more complex injection formulations may have overlapping polarographic peaks.

The finding that some injection formulations of Na-diclofenac contain higher than acceptable levels of benzaldehyde, illustrates the need for stringent quality control in the manufacture of these solutions. In particular, the use of especially pure benzyl alcohol, is most important, together with the avoidance of heat sterilization and exposure to light.

Acknowledgements

This work has been financially supported by the Research Council of the Medical Sciences University of Tehran.

References

[1] Handbook of Pharmaceutical Excipients, third ed., American Pharmaceutical Association and Pharmaceutical Press, Washington, 2001, p. 41.
[2] K. Urakami, C. Kobayashi, Y. Miyazaki, K. Nishijima, Y. Yoshimura, Chem. Pharm. Bull. 48 (2000) 1299–1303.
[3] J.H. Saiki, S. Thompson, F. Smith, R. Atkinson, Cancer 29 (1972) 370–374.
[4] D. Duncan, W.H. Jarvis, Anesthesiology 4 (1943) 465–474.
[5] A.F. Hahn, T.E. Feasby, J.J. Gilbert, Neurology 33 (1983) 1032–1038.
[6] The British Pharmacopoeia, HMSO, London, 1998, p. 161.
[7] The United States Pharmacopoeia 24 and The National Formulary 19, USP Commission, Rockville, MD, USA, 2000, 2420.
[8] C.J. Lindemann, A. Rosolia, J. Pharm. Sci. 58 (1969) 118–119.
[9] I.V. Pakhomova, N.S. Evtushenko, Farmatsiya 37 (1988) 38–40.
[10] C.E. Hignite, D.D. Shen, D.L. Azarnoff, Cancer Treatment Rep. 62 (1978) 13–18.
[11] D. Gormand, J.L. Brazier, F. Comet, D. Lecompte, J. Chromatogr. 335 (1986) 345–349.
[12] A. Rego, B. Nelson, J. Pharm. Sci. 71 (1982) 1219–1223.
[13] B.J. Floor, A.E. Klein, N. Muhammad, D. Ross, J. Pharm. Sci. 74 (1985) 197–200.
[14] A.M. Di Pietra, V. Cavrini, M.A. Raggi, Int. J. Pharm. 35 (1987) 13–20.
[15] I.I. Hewala, Talanta 40 (1993) 919–923.
[16] P. Zuman, D. Barnes, A. Ryvolova-Kejharova, Discuss Faraday Soc. 45 (1968) 202–226.
[17] M.H. Kim, Anal. Chem. 59 (1987) 2136–2144.