ELSEVIER

Journal of Chromatography A, 868 (2000) 101–107

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of diclofenac sodium by capillary zone electrophoresis with electrochemical detection

Wenrui Jin[a,*], Jie Zhang[b]

[a]*Laboratory of Analytical Science, School of Chemistry, Shandong University, Jinan 250100, China*
[b]*Fundamental Section, Qigihaer Medical College, Qigihaer 161042, China*

Received 15 March 1999; received in revised form 20 October 1999; accepted 2 November 1999

Abstract

Capillary zone electrophoresis was employed for the determination of diclofenac sodium using an end-column amperometric detection with a carbon fiber microelectrode, at a constant potential of 0.83 V vs. saturated calomel electrode. The optimum conditions of separation and detection are $4.90\cdot10^{-3}$ mol/l Na_2HPO_4–$3.10\cdot10^{-3}$ mol/l NaH_2PO_4 (pH 7.0) for the buffer solution, 10 kV for the separation voltage, 5 kV and 10 s for the injection voltage and the injection time, respectively. The limit of detection is $2.5\cdot10^{-6}$ mol/l or 5.2 fmol ($S/N=2$). The relative standard deviation is 0.8% for the migration time and 4.7% for the electrophoretic peak current. The method was applied to the determination of diclofenac sodium in human urine.

Keywords: Electrochemical detection; Detection, electrophoresis; Electrodes; Diclofenac sodium; Nonsteroidal anti-inflammatory drugs

1. Introduction

Diclofenac sodium, sodium [*o*-(2,6-dichloroanilino) phenyl] acetate (Fig. 1), is a relatively safe and effective non-steroidal drug with pronounced antirheumatic, antiinflammatory, analgesic and antipyretic properties [1], which is widely used in the treatment of degenerative joint diseases and other arthritic conditions [2,3]. It has been determined by a variety of analytical techniques, the most commonly used being spectrophotometry [4,5], thin-layer chromatography [6,7], gas chromatography [8,9] and liquid chromatography [10–15]. Spectrophotometry requires extensive sample preparation by chemical

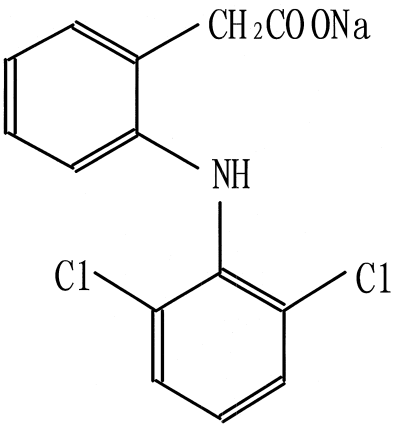

Fig. 1. Structure of diclofenac sodium.

*Corresponding author. Fax: +86-531-8565-167.
E-mail address: wenruijin@jn-public.sd.cninfo.net (W. Jin)

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01149-8

reaction or extraction. Thin-layer chromatography lacks the sensitivity and accuracy. Gas chromatography needs extraction and derivatization prior to separation and detection. Liquid chromatography has been used for the determination of diclofenac sodium alone or together with its metabolites in body fluids. Nevertheless, some procedures have to employ the complex instrumentation and installation or use fluorimetric detector, if higher separation efficiency and sensitivity are obtained.

Recently, capillary zone electrophoresis (CZE) has emerged as a powerful new method for rapid separation and detection of charged analytes [16,17]. This technique has been applied for the analysis of a variety of compounds (e.g., proteins, amino acids, nucleotide, etc.). Amperometric detection provides excellent sensitivity for the small dimensions associated with CZE, while offering a high degree of selectivity toward electroactive species and low cost [18]. In our laboratory this technique has been applied to cysteine [19], glutathione [20], purine bases [21–23], bovine serum albumin [24] and cytochrome *c* [25]. The theory concerning the current for the end-column amperometric detector in CZE has been investigated [26,27].

In this work we developed a method for the detection of diclofenac sodium with the end-column amperometric detection at a carbon fiber microelectrode. The separation was performed in a 25 μm I.D. fused-silica capillary. The detection was carried out by using potentiostatic control of the electrode potential by means of a three-electrode system. The method has been used to determine diclofenac sodium in human urine.

2. Experimental

2.1. Apparatus

2.1.1. Cyclic voltammetry

A cyclic voltammetric analyzer (Model 79-1, Jinan Fourth Radio Factory, China) coupled with an *x–y* recorder (Model 3086-11, Yokogawa Hokuskin, Japan) was used. It was used in connection with a cell, using potentiostatic control of the electrode potential by means of a three-electrode system, which consisted of a carbon fiber array electrode as the working electrode, a Pt wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. The reference electrode connected to the analyte via a salt bridge filled with the same supporting electrolyte as in the cell.

2.1.2. Capillary zone electrophoresis

A high-voltage power supply (Model GDY, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China) provided a variable voltage of 0~30 kV across the capillary with the outlet of the capillary at ground potential. Fused-silica capillaries (360 μm O.D.×25 μm I.D.) were purchased from Yongnian Optical Conductive Fiber Plant, China. They were cut to a length of 30.5 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 8~10 kV.

The electrochemical detection at a constant potential with CZE was performed using the end-column amperometric approach with a micro-current voltammeter (Model 901-pA, Ningde Analytical Instruments, China). The detection cell and detector were housed in a Faradaic cage in order to minimize the interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system. It consisted of a carbon fiber microelectrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the ground for the high potential drop mentioned above across the capillary and a SCE as the reference electrode. The arrangement of the electrochemical detection cell was illustrated in Ref. [24] in detail.

2.1.3. Carbon fiber electrodes

In cyclic voltammetry carbon fiber array electrodes were used. We took a glass capillary (ca. 5 cm×0.5 mm I.D.×1 mm O.D.) with a funnel-shaped inlet at an end. A mercury droplet was put into the glass capillary from the funnel-shaped inlet. Then the ''funnel'' was removed. About 40 carbon fibers of 8-μm diameter soaked with acetone were carefully inserted into the glass capillary at the other end. The carbon fiber array was connected to a copper wire (0.4 mm diameter, 12 cm length) via the mercury junction by pushing a copper wire down. After

drying the other end of the copper wire and the carbon fiber array were bonded to the glass capillary using a low viscosity ethyl α-cyanoacrylate adhesive. The carbon fibers and the adhesive were lightly touched with a glass bar. A glass tube (8 cm×1.5 mm I.D.×8 mm O.D.) was put outside the glass capillary, in order to protect the glass capillary. The copper wire was bonded to the glass tube using epoxy resin. The carbon fiber array was bonded at the other end of the glass tube and protruded approximately 1 cm from the end. Then the carbon fibers were cut to 5 mm.

For CZE, the single carbon fiber microelectrodes were constructed using 8-μm carbon fiber instead of the carbon fiber array. The manufacturing process was the same as the carbon fiber array electrode described above. A copper wire of 0.1 mm diameter as the electric connection was used instead of the copper wire of 0.4 mm diameter and no the glass tube was outside the capillary. The carbon fiber protruded was cut to about 300 μm.

Before use all carbon fiber electrodes were washed with alcohol and immersed in water for 4~5 h.

2.2. Reagents and solutions

A $1.00 \cdot 10^{-2}$ mol/l stock solution of diclofenac sodium was prepared by dissolving an appropriate amount of diclofenac sodium (pharmaceutical grade, Yanzhou Pharmaceutical Factory, China) in water and stored at 4°C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with water. All reagents were of analytical grade. All solutions were prepared with double distilled water.

2.3. Procedure

For cyclic voltammetry the carbon fiber array electrode must be pre-scanned 4~5 times between 0 to 1 V vs. SCE in the 0.120 mol/l Na_2HPO_4–0.080 mol/l NaH_2PO_4 buffer until a steady cyclic voltammogram was obtained. The carbon fiber array electrode was directly inserted in the experimental solution containing diclofenac sodium, and a cyclic voltammogram was recorded. The electrode must be cleaned in water for 2 min with the supersonic wave cleaner before each detection. All potentials were measured vs. SCE.

In CZE, the carbon fiber microelectrode was cemented onto a microscope slide, which was placed over a laboratory-made *xyz* micro-manipulator and glued in place. The position of the carbon fiber microelectrode was adjusted (under a microscope) against the end of the capillary, so that the electrode and the capillary were in contact. This arrangement allowed one to easily remove and realign both the capillary and the electrode. The other end of the capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, 0.1 mol/l NaOH and double distilled water, respectively, and then filled with the corresponding separation electrolyte by means of a syringe. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electroosmotic current reached a constant value (after 10 min), the electromigration injection was carried out and the electropherogram was recorded. The separation electrolyte in the capillary was replaced after five or six runs.

3. Results and discussion

3.1. Cyclic voltammogram of diclofenac sodium

The voltammetric characteristics of diclofenac sodium on glassy carbon electrode have been investigated by several researchers [11,28]. We found that diclofenac sodium can also be oxidized at the carbon fiber array electrode in Na_2HPO_4–NaH_2PO_4 buffer of pH 7. Fig. 2 shows its typical cyclic voltammogram in this solution. An oxidation peak of diclofenac sodium at ca. 0.70 V is observed and no reduction peak appears (curve 2).

3.2. Optimum conditions of CZE with end-column amperometric detection

Fig. 3 shows the relationship between the detected peak current, i_p, and the applied potential, E_d. When

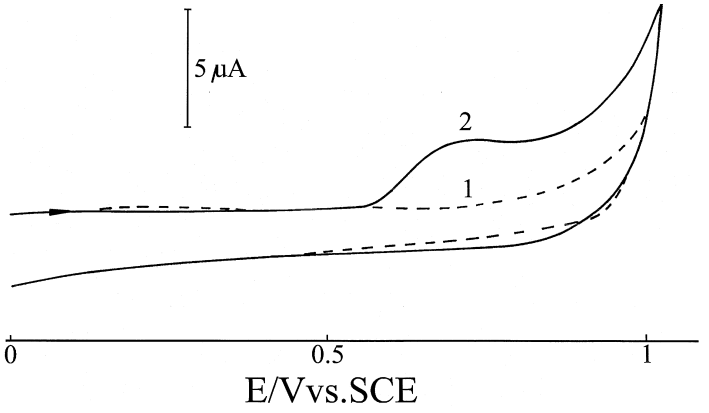

Fig. 2. Typical cyclic voltammogram of diclofenac sodium at the carbon fiber array electrode: 1, 0.120 mol/l Na_2HPO_4–0.080 mol/l NaH_2PO_4; 2, (1)+$1.00\cdot10^{-4}$ mol/l diclofenac sodium. v=20 mV/s.

E_d<0.7 V, i_p increases slowly with increasing E_d. When E_d>0.7 V, i_p increases rapidly. When E_d>0.8 V, i_p increases slowly again. When an E_d of 0.83 V is applied, the baseline of detection current is improving, noise is getting lower and the shape of the peak on the electropherogram also improves. An E_d of 0.83 V is suitable for the detection because of the smooth baseline and fine shape of the electropherograms.

In pH range of 6.5~7.5, the change of peak current, i_p, migration time, t_m, and the number of theoretical plates, N, is very small. i_p is slightly high at pH 7. Therefore pH 7 is selected. Effect of the concentration of the buffer, C_B, on t_m, i_p and N in Na_2HPO_4–NaH_2PO_4 is described in Table 1. In Table 1, C_B only indicates the value of the concentration of Na_2HPO_4, the ratio of the concentration of NaH_2PO_4 to the concentration of Na_2HPO_4 is

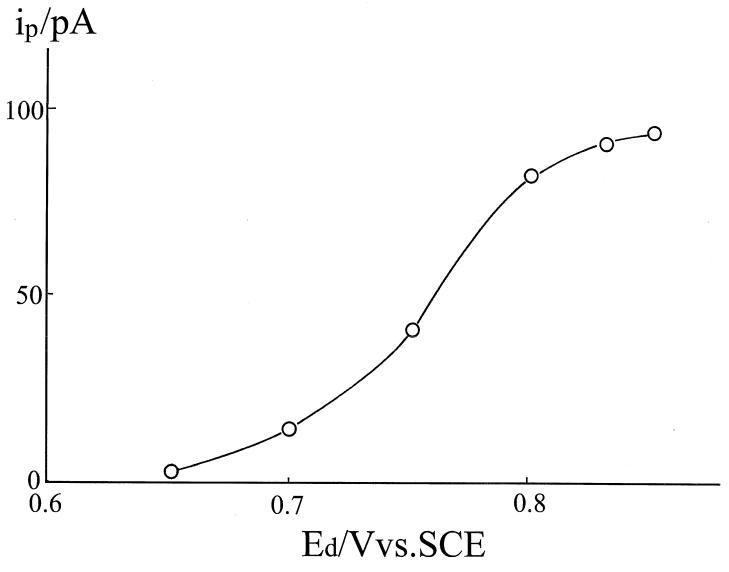

Fig. 3. Relationship between detected peak current and detected potential. $4.90\cdot10^{-3}$ mol/l Na_2HPO_4–$3.10\cdot10^{-3}$ mol/l NaH_2PO_4 (pH 7.0); $9.90\cdot10^{-5}$ mol/l diclofenac sodium; capillary: 30.5 cm×25 μm I.D.; injection, 5 kV for 10 s; separation voltage, 10 kV.

Table 1
The values of t_m, N and i_p at different concentrations of C_B (E_d = 0.83 V, other conditions as in Fig. 3)

C_B (mol/l)	t_m (s)	10^{-3} N	i_p (pA)
$6.1\cdot10^{-4}$	244	6.73	99
$1.2\cdot10^{-3}$	297	8.80	99
$2.4\cdot10^{-3}$	310	1.09	104
$3.1\cdot10^{-3}$	340	1.31	106
$4.9\cdot10^{-3}$	384	1.69	106

1:1.16. N was calculated according to the following equation:

$$N = 5.54 \cdot \left(\frac{t_m}{W_{1/2}}\right)^2 \quad (1)$$

where $W_{1/2}$ is the width at the half-peak on the electropherograms.

t_m, N and i_p increase slowly with increasing C_B. In our experiments $4.90\cdot10^{-3}$ mol/l Na_2HPO_4–$3.1\cdot10^{-3}$ mol/l NaH_2PO_4 was used.

The separation voltage, V_s, exerts an influence on t_m and N [29]. Fig. 4 shows the dependence of $1/t_m$, i_p and N on V_s. $1/t_m$ is proportional to V_s. N decreases with increasing V_s. There is a maximum for i_p at V_s = 8 kV. Therefore 8 kV for V_s was chosen.

Fig. 5 shows the typical electropherograms of $5.00\cdot10^{-4}$ mol/l and $9.90\cdot10^{-6}$ mol/l diclofenac sodium at optimum conditions. Small peak width and little tailing of the peak were obtained.

3.3. Reproducibility, limit of detection and linear range

The response for a series of six injections of $9.90\cdot10^{-5}$ mol/l diclofenac sodium resulted in a relative standard deviation of 0.8% for t_m and 4.7% for i_p, respectively. The limit of detection is $2.5\cdot10^{-6}$ mol/l (signal-to-noise ratio of 2), which was estimated from the electropherograms obtained for $9.90\cdot10^{-6}$ mol/l diclofenac sodium (see Fig. 5, curve 2), or 5.2 fmol for the injected volume calculated.

A linear relationship holds between the peak current detected and concentration in the range of $9.90\cdot10^{-6}$ to $5.00\cdot10^{-4}$ mol/l. Least-squares treat-

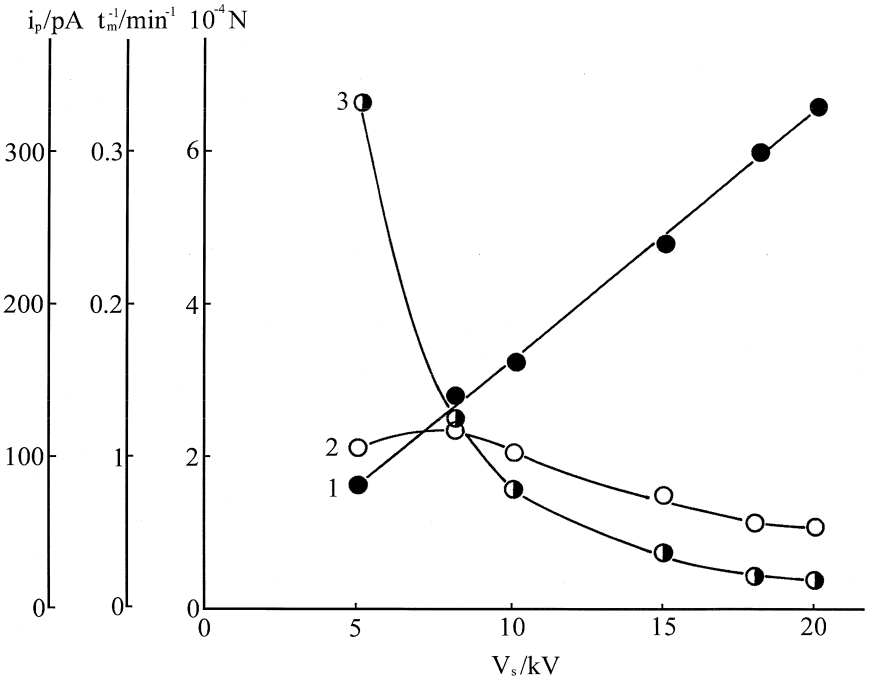

Fig. 4. Dependence of the reciprocal migration time (1), the number of theoretical plates (2) and the peak current detected (3) on the separation voltage. E_d = 0.83 V, other conditions as in Fig. 3.

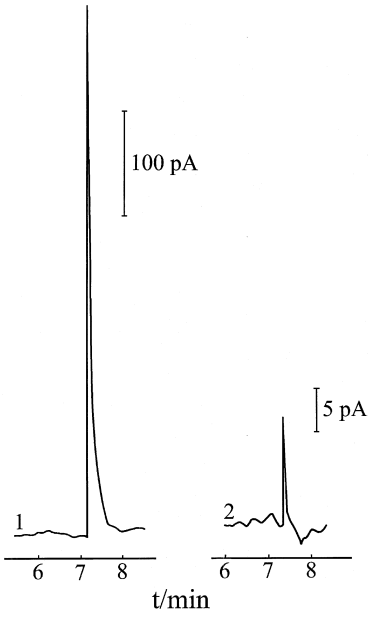

Fig. 5. Typical electropherograms of diclofenac sodium. Concentration of diclofenac sodium: 1, $5.00\cdot10^{-4}$ mol/l; 2, $9.90\cdot10^{-6}$ mol/l. $E_d=0.83$ V, other conditions as in Fig. 3.

ment of these data yielded a slope 1.27 pA μmol^{-1} l and a correlation coefficient of 0.998.

3.4. Determination of diclofenac sodium in human urine

A synthetic human urine sample containing $4.00\cdot10^{-3}$ mol/l diclofenac sodium was used to verify the possibility of the standard addition method. The electropherograms of human urine sample without and with the standard solution of diclofenac sodium are shown in Fig. 6. Peaks A and B in the electropherograms are unknown. The peak of diclofenac sodium appears between peak A and peak B. The average concentration of diclofenac in the human urine samples obtained by the standard addition method is $4.15\cdot10^{-3}$ mol/l, which agrees with the value in the human urine sample. The recovery is between 96% and 104%.

Acknowledgements

This project was supported by the National Science Foundation of China, the Science Foundation of Shandong Province and Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

References

[1] R.E. Small, Clin. Pharm. 8 (1989) 545.
[2] N. Gostick, I.G. James, T.K. Khong, P. Roy, P.R. Shepherd, A.J. Miller, Curr. Med. Res. Opin. 12 (1990) 135.
[3] B. Crowley, J.J. Hamill, S. Lyndon, J.F. Mckellican, P. Williams, A.J. Miller, Med. Res. Opin. 12 (1990) 143.
[4] A. Agatonovic-Kustrin, Lj. Zivanovic, D. Radulovic, M. Vasiljevic, Analyst 116 (1991) 753.
[5] B.V. Kamath, K. Shivram, Anal. Lett. 26 (1993) 903.
[6] S.W. Sun, H. Fabre, J. Liq. Chromatogr. 17 (1994) 433.

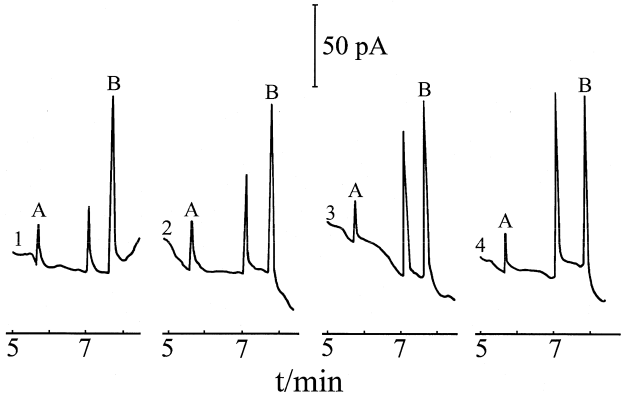

Fig. 6. Electropherograms of diclofenac sodium in the sample of human urine. The concentration of diclofenac sodium (mol/l): 1, sample; 2, (1)+$3.96\cdot10^{-5}$; 3, (1)+$7.92\cdot10^{-5}$; 4, (1)+$1.19\cdot10^{-4}$. $E_d=0.83$ V, other conditions as in Fig. 3.

[7] S.W. Sun, H. Fabre, H. Maillols, J. Liq. Chromatogr. 17 (1994) 2495.
[8] W. Schneider, P.H. Degen, J. Chromatogr. 383 (1986) 412.
[9] D. Lansdorp, T.L. Janssen, P.M.J. Guelen, T.B. Vree, J. Chromatogr. 528 (1990) 487.
[10] B. Wiese, J. Hermansson, J. Chromatogr. 567 (1991) 175.
[11] L. Zecca, P. Ferrario, P. Costi, J. Chromatogr. 567 (1991) 425.
[12] L.A. Brunner, R.C. Luders, J. Chromatogr. Sci. 29 (1991) 287.
[13] J. Moncrieff, J. Chromatogr. 577 (1992) 185.
[14] M. Riegel, P.P. Ellis, J. Chromatogr. B 654 (1994) 140.
[15] K. Li, F.-L. Zhao, Y.-S. Yuan, L. Tan, J. Liq. Chromatogr. 18 (1995) 2205.
[16] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[17] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[18] P.D. Voegel, R.P. Baldwin, Electrophoresis 18 (1997) 2267.
[19] W. Jin, Y. Wang, J. Chromatogr. A 769 (1997) 307.
[20] W. Jin, Y. Wang, Anal. Chim. Acta 343 (1997) 231.
[21] W. Jin, H. Wei, X. Zhao, Anal. Chim. Acta 347 (1997) 269.
[22] W. Jin, H. Wei, W. Li, X. Zhao, Anal. Lett. 30 (1997) 771.
[23] W. Jin, H. Wei, X. Zhao, Electroanalysis 9 (1997) 770.
[24] W. Jin, Q. Weng, J. Wu, Anal. Chim. Acta 342 (1997) 67.
[25] W. Jin, Q. Weng, J. Wu, Anal. Lett. 30 (1997) 753.
[26] W. Jin, H. Chen, J. Electroanal. Chem. 425 (1997) 155.
[27] W. Jin, H. Chen, J. Chromatogr. A 765 (1997) 307.
[28] Y. Yang, H. Shi, Fenxi Shiyanshi 14 (4) (1995) 5.
[29] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.