Construction of a Diflunisal Ion Sensor and Its Use in Automated Flow-Injection Methods for Assay, Content Uniformity, and Dissolution Studies of Formulations

PETR SOLICH[†§], PANOS E. MACHERAS[‡], AND MICHAEL A. KOUPPARIS[†x]

Received October 6, 1994, from the [†]*Department of Chemistry, and* [‡]*Department of Pharmacy, University of Athens, Panepistimiopolis, Kouponia, Athens 15771, Greece.* Accepted for publication March 17, 1995[⊗]. [§]Present address: Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, 50165 Hradec Kralove, Czech Republic.

Abstract □ A diflunisal ion selective electrode of the PVC membrane type with an ion-exchanger consisting of the tetraheptylammonium–diflunisal ion pair is described. The sensor exhibits a rapid, near-Nernstian, selective response to diflunisal anion in the pH range 7–10, with a (batch) detection limit of 1×10^{-5} M. The ion sensor was used as a flow detector in an automated flow-injection analyzer to develop routine methods for assays (concentration range $1–50 \times 10^{-4}$ M, (flow) detection limit 2.6×10^{-5} M), content uniformity, and dissolution studies of diflunisal formulations. No serious interference from common ions and tablet excipients was found, and the drug can be directly determined in colored samples without separation steps. Fourty measurements can be performed automatically per hour with a precision of 0.5–1.8% relative standard deviation. The automated method for the dissolution test provides a complete dissolution profile by the end of the experiment. Using the constructed ion sensor, the intramolecular hydrogen bonding of the diflunisal anion was studied, thereby revealing a new application of ion sensor potentiometry.

Diflunisal [5-(2,4-difluorophenyl)salicylic acid] is a nonsteroidal anti-inflammatory drug. Formulated in tablets (capsule-shaped, film-coated), it is indicated for acute or long-term use for symptomatic treatment of mild to moderate pain, osteoarthritis, and rheumatoid arthritis. It is a sparingly soluble in aqueous acid with a pK_a of 3.3.

The extensive use of diflunisal formulations requires the development of a rapid, selective, and accurate method that can be used in routine quality control. Diflunisal in commercial formulations has been determined by colorimetry using ferric ion to form a colored complex[1] and fluorimetry after extraction with methanol.[2] The United States Pharmacopoeia (USP) XXII[3] recommends an HPLC method with UV detection at 254 nm, while the British Pharmacopoeia[4] (BP) uses a simple UV spectrophotometric method after extraction in acidified methanol. The disadvantages of these methods are the lack of selectivity and the enormous consumption of toxic methanol (350 mL per sample) for the UV spectrophotometric method and a time consuming procedure for the HPLC method. For the determination of diflunisal in biological specimens, several HPLC methods using UV,[5] fluorimetric,[6] or amperometric[7] detection have been described. Other methods are based on GC[8] or second-derivative synchronous fluorescence spectroscopy.[9]

Ion-selective electrodes, recently known as ion sensors (IS),[10] found many applications in pharmaceutical analysis.[11–13] The inherent advantages of ion sensors [i.e. simplicity; short measurement time; low cost; adequate precision, accuracy, and detectability; wide analytical range; and, particularly, the ability to measure the activity (concentration) of the ionic drug directly, selectively, and, in most instances, without prior separation from the formulation matrix in colored or cloudy sample solutions] make this potentiometric technique very useful for pharmaceutical analysis. The potential of an IS measured against an external reference electrode (e.g. Ag/AgCl) is related to the logarithm of the analyte activity according to the well-known Nernst equation:

$$E = E_{const} + 2.303RT/z \log \alpha = E_{const} + S \log \alpha \quad (1)$$

where E_{const} is a sum of constant potentials, z the charge of the analyte with its algebraic sign, and S the so-called slope of the electrode. When the slope is equal to its theoretical value (59.16/z mV at 25 °C) we say that the electrode exhibits a Nernstian response. At low concentrations of the analyte ($C < 10^{-2}$ M) or at constant ionic strength of the measured solutions (e.g. using a buffer) the concentration can be used instead of activity in the Nernst equation, since α and C are related proportionally, $\alpha = f\ C$, where f is the activity coefficient dependent on the ionic strength of the measured solution; in this case the constant term ($\log f$) is incorporated in the constant term E_{const} of the Nernst equation. It is clear from eq 1 that the relationship between the electrode's potential and the analyte's concentration is logarithmic.

Automated flow injection (FI) is an attractive technique for the manipulation of samples and reagents, signal processing, and data treatment. Its application to routine pharmaceutical analysis has been proven to be very useful, attractive, and of great potential.[14–16] Ion sensors have been used as successful detectors for FI using various flow-through configurations (dip-type, with flow-through cap, tubular, wall-jet, etc.)[17–22] FI–IS methods have the combined advantages of flow injection and ion-sensor potentiometry. Applications in which FI has shown tremendous potential are the routine tests of dissolution[23] and content uniformity.[15] Dissolution testing is one of the most important quality controls performed in pharmaceutical laboratories to assess the rate of release of active ingredient with respect to time. The determination of the concentration of dissolved drug in the dissolution medium (as well as in sample solutions obtained during the content uniformity test) can be accomplished by either HPLC or UV spectrophotometry. HPLC with the advantages of low quantitation limits and high selectivity has become the method of choice for dissolution testing. However, chromatographic procedures for dissolution testing are time-consuming and not fully automated. The conventional UV spectrophotometric method (adopted for diflunisal tablets by the British Pharmacopoeia) is more economical and rapid, but suffers from interferences caused by UV-absorbing excipients of the formulations, such as coloring and coating agents.[24] The interfacing of a FI analyzer with a dissolution apparatus leads to a fully automated dissolution monitoring system capable of providing real time analysis and a multipoint dissolution profile.[23, 25]

In this paper, the construction of a diflunisal flow ion sensor, based on the use of the tetraheptylammonium–diflunisal ion pair as the ion exchanger in a polyvinyl chloride

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matrix, is described. A conventional diflunisal selective electrode constructed in our laboratory has already been used successfully for binding studies with proteins and cyclodextrins under batch conditions.[26] The flow sensor is used as a potentiometric detector of the wall-jet type in an automated FI system to develop routine methods for assays, content uniformity, and dissolution studies of diflunisal formulations.

Experimental Section

Reagents—All solutions were prepared in deionized water. Diflunisal was obtained from Sigma (St. Louis, MO) and used without any further purification. Its exact purity was found using the official USP HPLC method. Polyvinyl chloride (PVC) of high molecular weight was obtained from Janssen Chimica (Beerse, Belgium), and 2-nitrophenyl octyl ether (2-NPOE) and tetraheptylammonium bromide were from Fluka (Buchs, Switzerland). The various excipients and other drugs used in the interference study were obtained from various commercial sources and used as received. All other chemicals used were of analytical grade.

A 0.050 M Tris buffer (pH 8.0) was prepared by dissolving 6.057 g of tris(hydroxymethyl)aminomethane in deionized water, adjusting the pH to 8.0 using 12 M sodium hydroxide solution, and diluting the mixture to 1 L.

Diflunisal standard stock solution (0.0100 M) was prepared by dissolving 250.2 mg of diflunisal in 100 mL of 0.010 M sodium hydroxide solution. This solution was stable for at least 2 months when stored in a dark place (it was tested by comparison with a fresh solution using the diflunisal IS). Working standard solutions in the range from 4×10^{-5} to 5×10^{-3} M were prepared by appropriate dilution with 0.010 M sodium hydroxide.

A dissolution medium of a 0.10 M Tris buffer (pH 7.20) was prepared according the procedure described in the USP XXII.[3] Working standard solutions of diflunisal for dissolution study in the range from 1×10^{-4} to 4×10^{-3} M were prepared by dilution of diflunisal standard stock solution in 0.10 M Tris buffer (pH 7.20).

Sensor Construction—The diflunisal sensor was of the PVC membrane type[27] and constructed by attaching the electroactive PVC membrane (diameter of 6 mm) to a used sensing module of a commercially available nitrate electrode (Orion model 93-07) using a cyanoacrylate glue. The sensing module was prepared by careful removing its old membrane, emptying the internal reference solution, and washing and air-drying the internal compartment.

The internal reference solution for the diflunisal IS was 0.0100 M diflunisal in 0.050 M Tris buffer (pH 8.0) in 0.010 M sodium chloride, saturated with silver chloride. A portion of 250 μL of this solution was used for filling the internal compartment of the sensor.

The electroactive PVC membrane was constructed by entrapping the corresponding liquid ion exchanger in a PVC matrix according the method of Craggs et al.[28] The liquid ion exchanger for the diflunisal IS consisted of its ion pair with tetraheptylammonium cation in 2-NPOE at a 0.01 M concentration and was prepared as follows: 5 mL of a 0.010 M tetraheptylammonium bromide solution in 2-NPOE was shaken five times with the 0.0100 M diflunisal solution in order to exchange Br^- with diflunisal anion, and every time the aqueous phase was separated by centrifugation and decanted. The organic phase was then dried with anhydrous sodium sulfate to remove any trace of water. A small amount (0.5 mL) of this ion exchanger was used for the preparation of the PVC membrane with approximately 0.3 mm thickness (measured with a vernier caliper).

After its construction, the sensor was conditioned for 24 h before use by immersing it in a stirred 0.0100 M diflunisal solution in 0.050 M Tris buffer, pH 8.0. The same solution was used for the storage of the sensor when not in use.

Apparatus—Any commercial FI analyzer capable for potentiometric measurements can be used for these applications. The FI analyzer used was a laboratory-built automated system consisting of a multichannel peristaltic pump (Ismatec MPN-8), a Rheodyne 5001 injection valve equipped with a pneumatic actuator (Rheodyne 5003) controlled by a microcomputer through solenoid valves, a mixing and reaction coil made from PTFE tubing (0.8 mm i.d.), an IBM compatible personal computer associated with the necessary interface card (PLCD-718, Advantech), an electrometer unit (Health-Schlumberger EU-200−30), and an optional chart recorder (Radiometer REC 61).

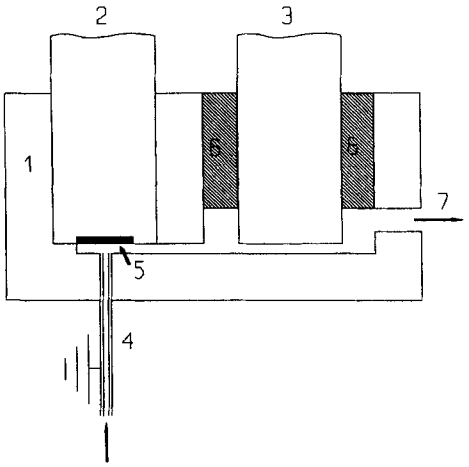

Figure 1—Flow-through potentiometric detector: (1) Plexiglas block, (2) diflunisal ion sensor, (3) reference electrode, (4) stainless tube (ground), (5) sensor's membrane, (6) rubber O-ring, (7) waste.

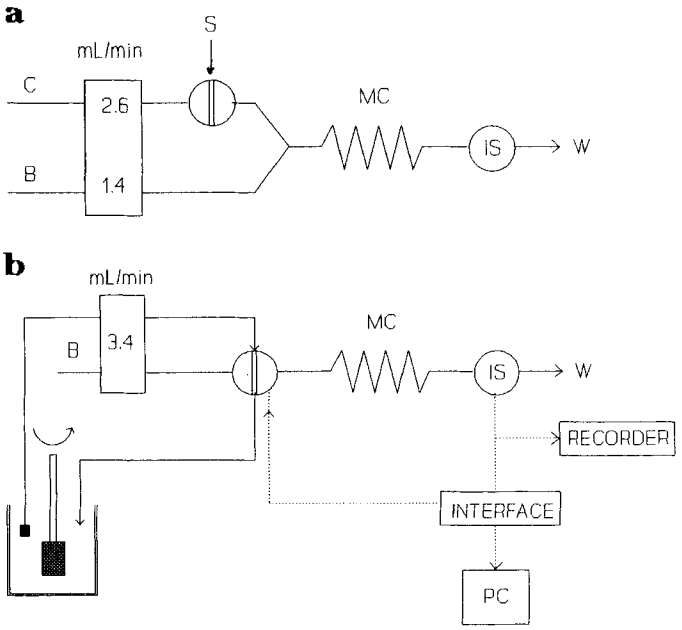

Figure 2—Flow injection manifolds designed for (a) potentiometric determinations for assays and content uniformity tests and (b) dissolution studies. C, carrier (0.01 M NaOH); B, buffer (0.05 M Tris, pH 8.0, for assays and 0.1 M Tris, pH 7.2, for dissolution studies); S, sample (85 μL); MC, mixing coil (50 cm); IS, flow-through potentiometric detector; W, waste.

The potentiometric flow-through cell, of the wall-jet type, was constructed from Plexiglas (Figure 1). The external reference electrode was a single junction Ag/AgCl electrode (Orion 90-04) filled with 4 M KCl electrolyte solution. A piece of string was immersed in the exit of the reference electrode compartment and dipped in the waste solution to ensure a pulse-free flow. To overcome problems from the static electricity generated by the liquid flow, the flow stream was ground just before the sensor compartment using a 10 mm long stainless steel tube (0.8 i.d.). All measurements, except those in dissolution studies, were performed in a room with a controlled temperature of 25 ± 1 °C.

The FI manifold optimized for the determination of diflunisal in formulations is depicted in Figure 2a. A 85 μL aliquot of standard or sample solution prepared in 0.010 M NaOH is injected in the carrier solution (C) (0.010 M NaOH) and mixed with the Tris buffer (B), pH 8.0, in the 50 cm mixing coil (MC). In dissolution studies the dissolution medium was used as the carrier and the one-channel FI manifold shown in Figure 2b was constructed.

Procedures—*Measurements for Assay and Content Uniformity Test*—The manifold shown in Figure 2a was constructed and the pump

was started. In order to obtain a stabilized potential baseline, a concentration of 1×10^{-5} M of diflunisal was added to both the carrier and buffer solutions. After stabilization of the baseline to within ±0.1 mV, the program for the routine measurements was run. In each sequence the injection valve is turned by the microcomputer to its "load" position for a preselected load time (30 s) during which the sample loop (85 μL) is filled with the solution under measurement. Then the injection valve is turned to its "injection" position for a preselected measurement time (30 s), and the loaded sample solution is transferred by the carrier solution, mixed with the buffer, and measured by the flow potentiometric detector. During this period the microcomputer stores the potential readings of the obtained FI peak and determines the potential at the peak maximum. After an additional washing time of 30 s the operation sequence is repeated for the preselected number of measurements. Prior to the first injection and measurement of either a standard or a sample, the baseline voltage is measured; afterwards, the peak height (ΔE) for each injection is measured automatically. This approach compensates for baseline drift between sets of measurements, a common problem when using potentiometric sensors in flow analyzers. After measuring the standards, the calibration graph was constructed by linear regression of ΔE (mV) versus log C. The sample solutions were then measured in the same way and their concentrations were calculated from the constructed calibration graph, displayed, and printed by the computer. Using 30 s as a measuring time and 60 s as a loading/washing time, 40 measurements can be obtained per hour.

Sample Preparation for Assay—For assay of tablets, the sampling, and treatment, the official USP procedure was followed. From the final fine powder an accurately weighted portion equivalent to about 125 mg of diflunisal was transferred to a 100 mL volumetric flask containing 70 mL of 0.05 M sodium hydroxide. The dissolution of the drug was assisted by sonication (5 min) and mechanical shaking (5 min) and the solution was diluted to the mark with the same NaOH solution. A portion of the solution was centrifuged to remove undissolved excipients, and 10.00 mL of the clear supernatant was transferred to a 50 mL volumetric flask and diluted with water to the mark. By this treatment the concentration of the drug lies in the linear range of the calibration graph ($1-40 \times 10^{-4}$ M) in a solution of 0.01 M NaOH.

For the content uniformity test, one tablet was transferred to a 100 mL beaker containing 50 mL of water and stirred for 30 min for disintegration. The solution was then quantitatively transferred to a 100 mL volumetric flask and sonicated for 2 min. Twenty milliliters of 1 M sodium hydroxide was added, and the solution was sonicated for 5 min and diluted with water to volume. A portion of the solution was centrifuged and 5.00 mL of the clear supernatant was transferred to a 100 mL volumetric flask and diluted with water to volume to yield a final NaOH concentration of 0.01 M.

Dissolution Studies—The rotating basket apparatus of USP XXII was connected with the FI analyzer as shown in Figure 2b. One tablet was placed into the 250 mesh screen basket and the whole basket was immersed in the dissolution vessel containing 900 mL of the dissolution medium thermostated at 37 ± 0.2 °C throughout the dissolution experiment. The basket was rotated at 50 rpm. The sampling channel of the analyzer's pump creates a closed system starting and ending in the dissolution medium and continuously circulates the dissolution medium through the sample loop of 85 μL capacity. In this way the dissolution volume remained constant throughout the test. A small filter (for pipet tips, Centraur Chemical Co.) was attached to the end of the sample probe to provide filtration of the dissolution medium. Every 2.5 min the injection valve introduced a microvolume of the dissolution medium containing an increasing concentration of the dissolved diflunisal into the flow system for measurement. The whole procedure was performed automatically under computer control and after 35 min the complete dissolution profile, consisting of 14 potential peaks and the corresponding diflunisal concentrations, was obtained. For the calibration curve, three diflunisal standards in the concentration range $1-40 \times 10^{-4}$ M, prepared in the dissolution medium and thermostated at 37 °C, were measured just before the dissolution experiment.

Results and Discussion

Construction of the Diflunisal Ion Sensor—From the various bulky quaternary ammonium cations (octadecyltrimethylammonium, hexadecyltriethylammonium, hyamine, 1-hexadecylpyridinium, and tetraheptylammonium) the latter was found to be the most successful to form an ion pair with diflunisal anion, which was then used for the preparation of an electroactive PVC membrane with excellent characteristics (slope and detection limit). 2-NPOE was used as the organic solvent because of its excellent characteristics in the extraction, the plasticizing properties, and the operational life of the membrane. Other solvents tested were *p*-nitrocumene and 4-nitro-*m*-xylol, which showed poor plasticizing properties. By using the commercial sensing module (eight small holes permit contact of the internal reference solution with the membrane which is supported at the module's end) it was possible to use a very thin membrane (0.3 mm) with excellent response characteristics. The flow detector assembly shown in Figure 1 provides low dead volume, fast response, fast washing (therefore high enough sample throughput), stable baseline, and pulse free potential signal.

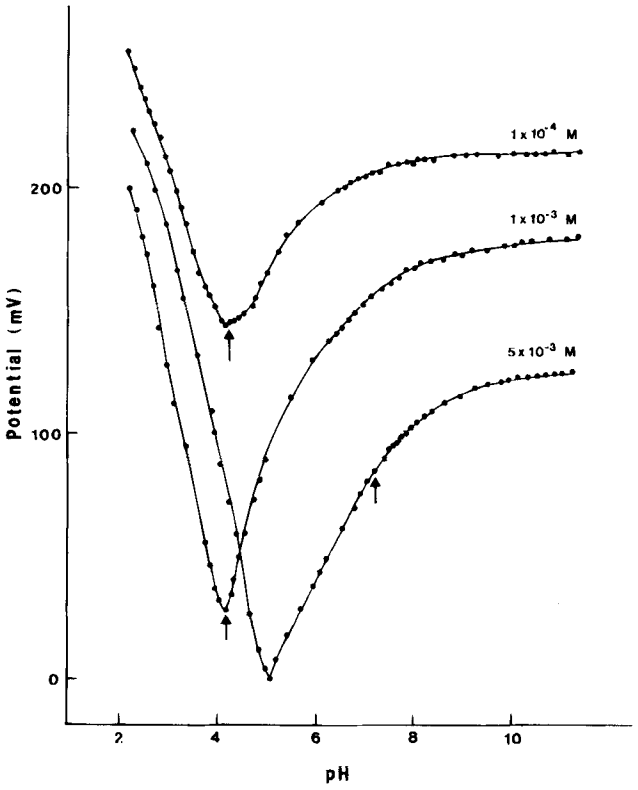

Figure 3—Effect of pH on the sensor's potential. Diflunisal concentration: (a) 1×10^{-4} M, (b) 1×10^{-3} M, and (c) 5×10^{-3} M. The arrows show the pH at which precipitation is observed visually.

Effect of pH on the Sensor's Response—The study of the pH-dependence of the sensor potential was performed under batch conditions, in a thermostated 50 mL cell with stirring. The pH of the initial solution containing various concentrations of diflunisal (1×10^{-4}, 1×10^{-3}, 5×10^{-3} M) was adjusted to 11.0 with NaOH solution, and small portions of 1.0 M sulfuric acid were gradually added using a microsyringe. Both pH and E (mV) were recorded simultaneously after each addition. The E vs pH plots for the three concentrations of diflunisal are shown in Figure 3. The pH effect is not the expected one since an increase of the apparent ion concentration with the decrease of pH in the 8 to 4 range was noticed. This observation is indicative of an intramolecular "salicylate-like" hydrogen bonding for the diflunisal ion in alkaline solutions. Since the "hydrogen-bonded" species dissociates with an increase of the hydronium concentration, we

can consider diflunisal participating in the following equilibria

$$\text{R-COOH} \overset{K_a}{\rightleftharpoons} \text{R-COO}^- + \text{H}^+ \qquad (2)$$

$$\text{R-COO}^- + \text{H}^+ \overset{K_f}{\rightleftharpoons} \text{R-CO}^-\cdots\text{HO} + \text{H}^+$$

The diflunisal ion sensor responds to the $R-COO^-$ and to a certain degree to the $\overline{R-COO^-\cdots HO}$ species. Therefore, a modified Nernst equation can be written (Nicolsky-Eisenman equation):[29]

$$E = E_{const} + S \log[\alpha_1 C + K^{pot}\alpha_2 C] \qquad (3)$$

where

$$\alpha_1 = K_a[H^+]/([H^+]^2 + K_a[H^+] + K_aK_f)$$

$$\alpha_2 = K_aK_f/([H^+]^2 + K_a[H^+] + K_aK_f)$$

and E is the electrode potential at each pH ($[H^+]$), E_{const} the constant term of the Nernst equation, S the slope of the electrode, K^{pot} the potentiometric selectivity coefficient of the electrode for the hydrogen-bonded species, and C the total concentration of the diflunisal. Using the literature[30] value for the dissociation constant of the acid, $K_a = 5.0 \times 10^{-4}$, and the experimentally determined Nernstian slope, $S = -59.0$ mV, the fitting of eq 2 to the experimental data pairs E_i–$(pH)_i$, shown in Figure 3, was accomplished. An estimate for the association constant of the "hydrogen-bonded" species was derived as $K_f = [\overline{R-COO^-\cdots HO}]\,[H^+]/[R-COO^-] = 1.6(\pm 0.3) \times 10^{-4}$, while the estimate for K^{pot} was found to be $2.1(\pm 0.4) \times 10^{-3}$. This means that, in alkaline solutions, the diflunisal anions are practically in the form of the hydrogen-bonded species to which the electrode response is 476 ($1/K^{pot}$) times less than the diflunisal anion. However, the same equilibria are prevalent in the microenvironment of the electrode membrane and therefore the working calibration curves (E (mV) versus log C) are valid in the pH range 7–10 using phosphate, Tris, borate, or mixed buffers. At lower pH, the acid form precipitates due to its low aqueous solubility. The arrows in Figure 3 indicate the pH where visually observed precipitation, which, as expected, is greater for the highest diflunisal concentration.

To the best of our knowledge, a study of the hydrogen-bonding equilibrium with ion selective electrodes has not described previously. The above results reveal a new potential for the technique, and it is evaluated and expanded for other carboxylic acids.

Design and Optimization of the FI Manifold—Diflunisal is a weak acid of low aqueous solubility, and an organic solvent (methanol) is needed for its extraction from formulations in the official assay procedures. In this proposed method, a 0.010 M NaOH solution was found to be adequate for complete drug dissolution and extraction from its formulations. A dual-channel FI manifold (Figure 2a) was designed to ensure adequate pH and ionic-strength buffering of the alkaline solution of the analyte. Several buffers in the pH range 7–10 (Tris, phosphate, and borate) and at various concentrations (0.02–0.2 M) were examined under batch and flow conditions. The finally chosen buffer, 0.05 M Tris, pH 8.0, gave the best overall results (stability of baseline, Nernstian slope of the electrode response, low detection limit, good precision, and good linearity of the calibration curve). In addition, Tris buffer has been adopted as dissolution medium by the USP.

Table 1—Recovery of Diflunisal[a] from Synthetic Mixed Solutions Containing Various Common Salts and Excipients

Interferent	Concn. Ratio[b]	Recovery (%)
(A) Common Salts		
Potassium iodide	6	104.5
	1	100.7
Potassium fluoride	2	100.5
Sodium chloride	2	96.7
Potassium nitrate	4	100.3
Disodium hydrogen phosphate	10	102.6
(B) Common Excipients		
Fructose	7	100.2
Galactose	7	100.5
Glucose	7	99.6
Lactose	7	99.2
Saccharose	7	99.4
Sorbitol	7	99.9
Carbowax 4000	10	90.6
	2	98.3
Carbowax 6000	10	89.9
	2	97.9
Sodium alginate (Protanal TXF 200)	10	96.0
Ludipress (lactose)	10	88.4
	2	98.6
Calcium sulfate	5	75.5
	1	98.9
Red 40	1	101.8
Yellow 5	1	100.6
Green 3	1	101.3
(C) Excipients Existing in the Unisal Tablets[c]		
Starch (0.4)	10	99.0
Hydroxypropylmethylcellulose (Hypromellose) (0.008)	2	98.2
Hydroxypropylcellulose (Hyprollose) (0.008)	10	98.9
Cellulose microcrystalline (0.18)	10	99.1
Magnesium stearate (0.012)	20	98.5
Talc (0.003)	10	101.6
Titanium dioxide (0.012)	10	100.7

[a] Concentration of diflunisal 1×10^{-3} M. [b] Interferent/diflunisal (w/w). [c] The concentration ratio in tablets is shown in parentheses.

The other FI parameters (flow rate, sample volume, and length of mixing coil) were also optimized for high measurement throughput with high precision and wide linear range of the calibration curve. Under the optimized conditions (flow rate, 4.0 mL min^{-1}; sample volume, 85 μL; and mixing coil length, 50 cm), the analytical characteristics of the method are as follows: the linear concentration range (ΔE versus log C) $1-50 \times 10^{-4}$ M ($r = 0.9996$), with a slope ranging from 57 to 60 mV per decade of concentration; the (flow) detection limit, 2.6×10^{-5} M; the precision, 0.38% [relative standard deviation, (RSD), $n = 10$ for 1×10^{-3} M diflunisal]. The good reproducibility and stability of the baseline potential during measurements were ensured by the addition of 1×10^{-5} M diflunisal, corresponding to the (batch) detection limit, into the buffer and carrier streams. Under the optimized conditions the operative life of the sensor was about 3 months with everyday use.

The response time of the chemical sensor in the optimized FI system was studied by creating a stepwise concentration change (by injecting a large volume of 2.6 mL of 1×10^{-3} M diflunisal) and recording the E vs time exponential curve until a steady-state potential was established. The time constant (T) of the system was found to be 2.7 s, which is sufficient for a potentiometric sensor and a flow system.

Interferences—The possible interference from various common salts and excipients was studied by preparing and

Table 2—Interence of Diflunisal Potentiometric Determination for Organic Anions of Similar Structure[a]

Compound	Concn. ratio[b]	Recovery (%)
Benzoate	10	95.2
2-fluorobenzoate	10	95.0
Acetyl salicylate	10	98.7
Salicylate	10	118
Mandelate	10	103.5
Phenolate	10	222

[a] Diflunisal concentration, 1×10^{-3}M. [b] Interferent/diflunisal (w/w); undissolved material was filtered.

measuring synthetic mixed solutions of diflunisal (1×10^{-3} M) and the interferent under study (concentration range $1-10 \times 10^{-3}$ M) in 0.01 M NaOH. As shown in Table 1, most of the excipients showed no effect on the diflunisal potentiometric determination, even at high concentration ratios, unexpected in commercial pharmaceutical formulations. Carbowax, Ludipress (lactose readily compressible containing kollidon 30 and kollidon chloride), and calcium sulfate showed negative interferences only at high concentration ratios, probably because of the association of the diflunisal anion with the excipients. The absence of any effect on the potentiometric determination of diflunisal from various coloring agents, the common problem for spectrophotometric methods, is noticeable. The only excipient which caused positive interference was sodium lauryl sulfate, which must be attributed to the interaction of this lipophilic anion with the counterion (tetraheptylammonium) of the ion exchanger of the PVC membrane.

In order to further examine the selectivity of the diflunisal chemical sensor, the possible interference from anions of similar structure was tested by performing recovery experiments from synthetic mixtures. As shown in Table 2, only salicylate and phenolate at 10-fold concentrations showed positive interference. For these two anions, as well as the lauryl sulfate, the so-called potentiometric selectivity coefficient, K^{pot}, was determined using the mixed solution method[31] in a flow–concentration gradient mode. In these experiments, carried out at pH 8.0, the diflunisal concentration remained constant 10^{-3} M, while the interferent's concentration ranged from 10^{-2} M to 0. Values of K^{pot} found were 0.018 ± 0.011 for salicylate, 0.84 ± 0.02 for phenolate and 9.46 ± 0.13 for lauryl sulfate. The remarkably lower response of the diflunisal sensor to the very similarly structured salicylate is most likely due to differences in their lipophilicity.

Assays in Formulations—The proposed potentiometric method for the determination of diflunisal was further evaluated by direct analysis of commercial formulations (tablets). The results shown in Table 3 compared favorably (t-test) with those obtained with the UV-spectrophotometric method of BP.

Although the Unisal and Analeric tablets were colored, they were accurately analysed by the diflunisal IS, as the potentiometric measurements are not affected by optical interferences. This was achieved by dissolving diflunisal direct in alkaline aqueous solutions (instead of the undesirable methanol of the official BP procedure). The proposed method is characterized by both good accuracy and precision, with RSD, ranging from 0.5 to 1.8% ($n = 3$, measured in triplicate), and relatively high measurement throughput (40 per hour).

Content Uniformity Test—The high measurement throughput of the FI–potentiometric method exploited in the content uniformity test increases considerably the output of the analytical pharmaceutical laboratories. Ten individual Unisal tablets were treated as stated in the corresponding procedure and measured in triplicate in less than 40 min. The results shown in Table 4 give a between sample precision of 2.5%, while the within sample precision of the triplicate measurements ranged from 0.4 to 1.6% RSD.

Table 3—Determination of Diflunisal in Commercial Formulations by Using Flow Injection–Potentiometric (FI–POT) and Reference Methods

Formulation (tablets)	Drug Content (mg) ± Standard Deviation ($n = 3$)[a] Claimed	Found FI-POT	Found Reference[b]	t-Test[c]
Unisal (Merck Sharp & Dohme-Chibret AG, Switzerland)	500	509 ± 2	511 ± 2	0.733
Analeric (Vianex S. A., Greece)	500	486 ± 8	494 ± 5	1.477
Di-flu (Petsiavas, Greece)	500	504 ± 8	515 ± 5	1.902

[a] Average of three samples measured in triplicate. [b] UV spectrophotometry according the BP 1993.[4] [c] Theoretical t-value for 95% confidence level = 2.776.

Table 4—Results of Content Uniformity Test of Unisal[a] Tablets of 500 mg of Diflunisal

Tablet	Found[b] (mg) ± SD	percentage of claimed
1	489.1 ± 3.4	97.8
2	513.7 ± 2.0	102.7
3	478.3 ± 2.8	95.7
4	491.1 ± 4.4	98.2
5	516.8 ± 4.8	103.4
6	496.2 ± 8.1	99.2
7	501.2 ± 5.8	100.2
8	491.1 ± 5.9	98.2
9	511.6 ± 6.6	102.3
10	506.4 ± 5.2	101.3

[a] Merck Sharp & Dohme - Chibret AG. [b] Grand mean: 499.6 mg, RSD = 2.5%. Range (R) = 38.5 mg, relative range = 7.7%. Each sample was measured in triplicate.

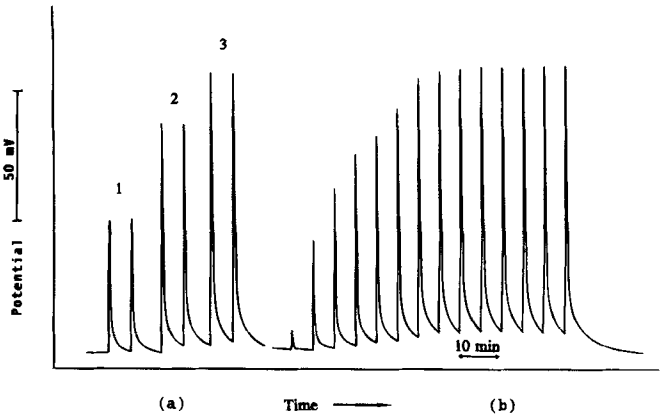

Figure 4—Typical potential peaks obtained during a dissolution test by the FI technique for a 500 mg diflunisal tablet (Unisal): (a) calibration with (1) 2×10^{-4} M, (2) 1×10^{-3} M and (3) 2×10^{-3} M standards; (b) dissolution of a tablet in 0.1 M Tris buffer pH 7.2 (900 mL, 37 °C).

Dissolution Studies—The ability of the FI–potentiometric method to measure directly, rapidly, and accurately the diflunisal concentration in aqueous solutions of tablets was exploited by automation of the dissolution test of commercial formulations. Since the recommended dissolution medium in USP XXII is 0.1 M Tris buffer, pH 7.2, at 37 °C, a one-channel FI manifold (Figure 2b) was designed to pump this medium as a carrier. A calibration curve was constructed just before the experiment. Figure 4 shows typical potential readings obtained during such an automated dissolution test. The average percentage of dissolution as a function of time along

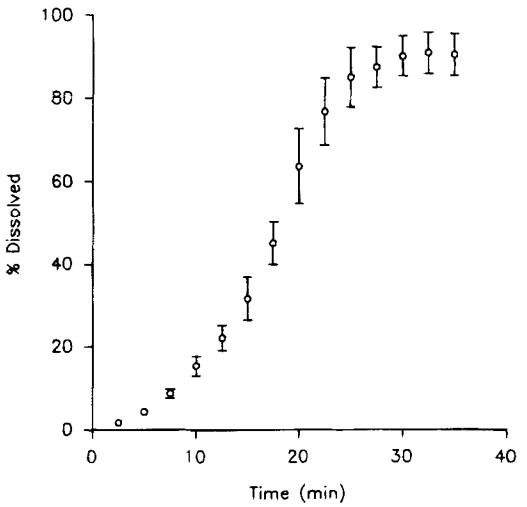

Figure 5—Dissolution profile of diflunisal from Unisal tablets. Bars represent the standard deviation ($n = 6$).

with their standard deviations for six film-coated Unisal tablets tested are shown in Figure 5. The simultaneous test of six tablets is possible by using a six-pot commercial dissolution apparatus and a six-port valve to connect it with the FI potentiometric analyzer.[25] The dissolution profile reflects the film-coated Unisal tablets (a lag time is required for the wetting and disintegration of the film, prior to the actual dissolution of drug). The results satisfy the USP XXII requirement (80% of the diflunisal must be dissolved in 30 min). The long term stability of the FI system was excellent, with a stable baseline, and practically the same calibration curves were obtained at the beginning and the end of the six dissolution tests.

Conclusions

This work shows the potential of potentiometric ion chemical sensors and their usage as flow detectors in automated routine pharmaceutical analysis and quality control of drug formulations. The developed automated potentiometric method does not suffer from optical interferences and provides rapid, accurate, and precise results.

References and Notes

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