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Analysis of diclofenac sodium and derivatives

M.E. Palomo, M.P. Ballesteros, P. Frutos *

Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

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Abstract

There are two reasons explaining why several researchers have carried out the in vitro release studies of diclofenac sodium (DFNa) using pH media of above 6.5. Firstly the pH dependence of solubility, and secondly the intramolecular cyclization suffered under acidic conditions which causes the salt to become inactivated. Nevertheless, many commercially available pharmaceutical dosage forms have no protective coat to avoid the inactivation in the gastric juices. A possible explanation may be found if reconstitution of the cyclated form takes place. It is therefore necessary to study the behaviour of diclofenac sodium when it is submitted to the action of different solutions in a wide pH range. To perform this study five analytical methods have been employed: UV-vis spectrophotometry, differential scanning calorimetry (DSC), infrared analysis (IR), X-ray diffractometry (DRX) and energy dispersive X-ray analysis (EDS). © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Diclofenac; Cyclization; DSC; DRX; EDS; IR

1. Introduction

There are many stages involved in drug development: for example, the technological stage, where the research focuses on getting a dosage form of the active substance. This involves different techniques being used to look for the most appropriate substance. However, some of these can damage the active substance, which can loose its effectiveness totally or partially. Therefore, once a dosage form is obtained it has to be analyzed.

The great variety of analytical techniques available provides valuable information which makes it easier to interpret the behaviour of a drug.

Some years ago, one or two analytical techniques were enough to study an active substance, nowadays more than two are required. Of all of these, the most popular is UV-vis spectrophotometry. This technique is based on the absorbance capability of a substance at a specific wavelength. Interference with another substance that absorbs at the same wavelength limits the use of this technique.

In recent years DSC (differential scanning calorimetry) has gained popularity since it is easy and quick. It is based on the detection of endothermic and exothermic peaks that appear as a

* Corresponding author.

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consequence of small changes in temperatures. The number, location and shape of these peaks are used to identify a substance [1].

Other analytical techniques include infrared and X-ray analysis. The first is used commonly to detect the presence of functional groups, whilst the second depends on the crystalline properties of the sample.

SEM (scanning electron microscopy) gives information about the shape of the compound due to the interactions of electrons on the sample, which creates a map of the surface. As a consequence of this interaction the atoms that constitute the compound can loose an electron. Another electron, from a more energetic stage, migrates to the free place generating an X-ray whose energy is characteristic for every atom. Hence it is possible to perform a qualitative analysis (EDS).

If all analytical techniques were employed in the study of an active substance then a great deal of information about its chemical structure would be obtained. Thus the aim of this work is to study what happens to an active substance (diclofenac sodium) when it is treated with acidic solutions and then treated with neutral and basic solutions.

Diclofenac is a non-steroidal anti-inflammatory drug that it is specially indicated in rheumatoid arthritis [2]. Due to its low solubility it is commercially available as diclofenac sodium [3]. This substance, sodium(*O*-((2,6-dichlorophenyl)-amino)-phenyl)-acetate is a weak acid with a pK_a of 4 and a partition coefficient (*n*-octanol/aqueous buffer, pH 7.4) of 13.4.

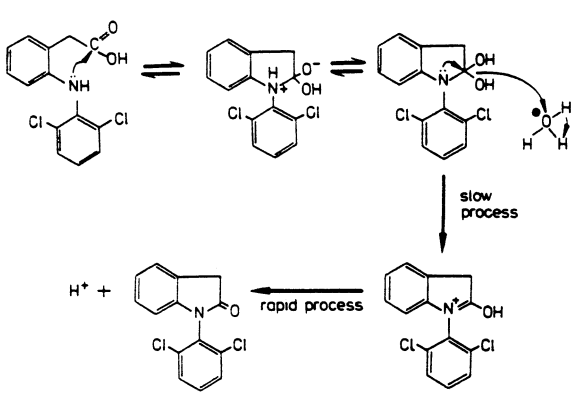

Fig. 1. Cyclization reaction of diclofenac sodium in acidic conditions [4].

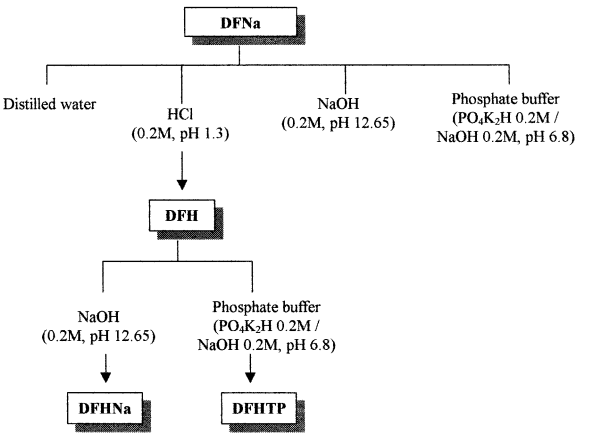

Fig. 2. Schematic representation of diclofenac sodium treatment procedure.

Two important characteristics are linked to this substance. Firstly, its solubility, which depends on the pH of the surrounding solution; in acidic solutions the solubility is lower than 1 mg ml^{-1} [4]. Nevertheless, the solubility increases with pHs of above 6.5 [5–7]. This fact explains why 'in vitro' dissolution tests have to be performed using buffered solutions with those pHs [8]. Secondly, diclofenac sodium undergoes an intramolecular cyclization under the acidic conditions (Fig. 1) found in gastric juices, which can cause its inactivation, so it is recommended to take it after meals [9]. As a consequence of the intramolecular cyclization Na^+ is lost hence the solubility of the compound decreases.

If cyclization under acidic conditions is be reversible when the surrounding solution changes into basic or neutral solutions with a high proportion of Na^+ then a reconstitution of the original structure would take place. Therefore, it would not be necessary to protect it from the action of gastric juices. In fact, some authors [10] suggest that the acid–base reaction takes place only on the external surface of the monolithic form, the low solubility of the acidic form prevents further dissolution and release at low pH; once the pH increases, the thin layer enables release to start again.

Considering that the structure of the cyclated form proposed by Racz [9] is the same structure

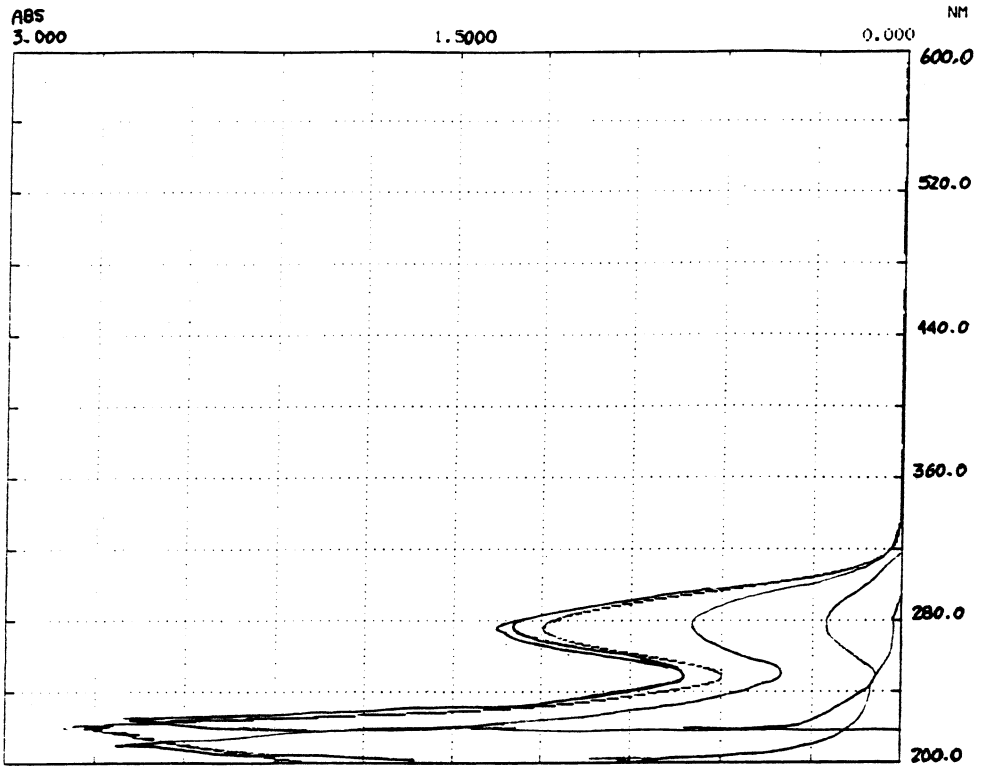

Fig. 3. UV-vis spectrophotometry scans. From left to right: DFNa in buffer solution; DFNa in NaOH solution; DFNa in distilled water; DFHTP (previous crystallization); DFHNa (previous crystallization); DFH (previous filtration).

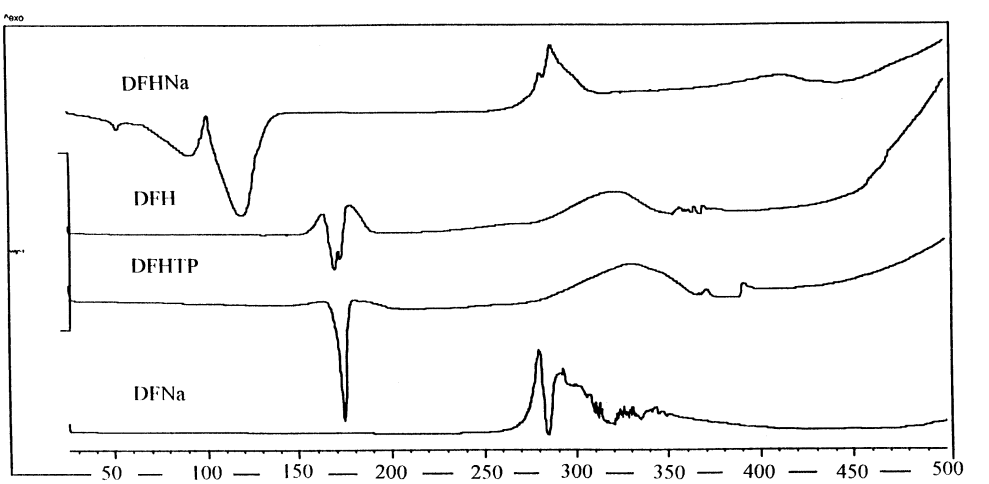

Fig. 4. Results of DSC analysis.

used by Tamura [11] to synthesize diclofenac sodium by reaction with NaOH, then it can be assumed that the reconstitution of the active substance takes place.

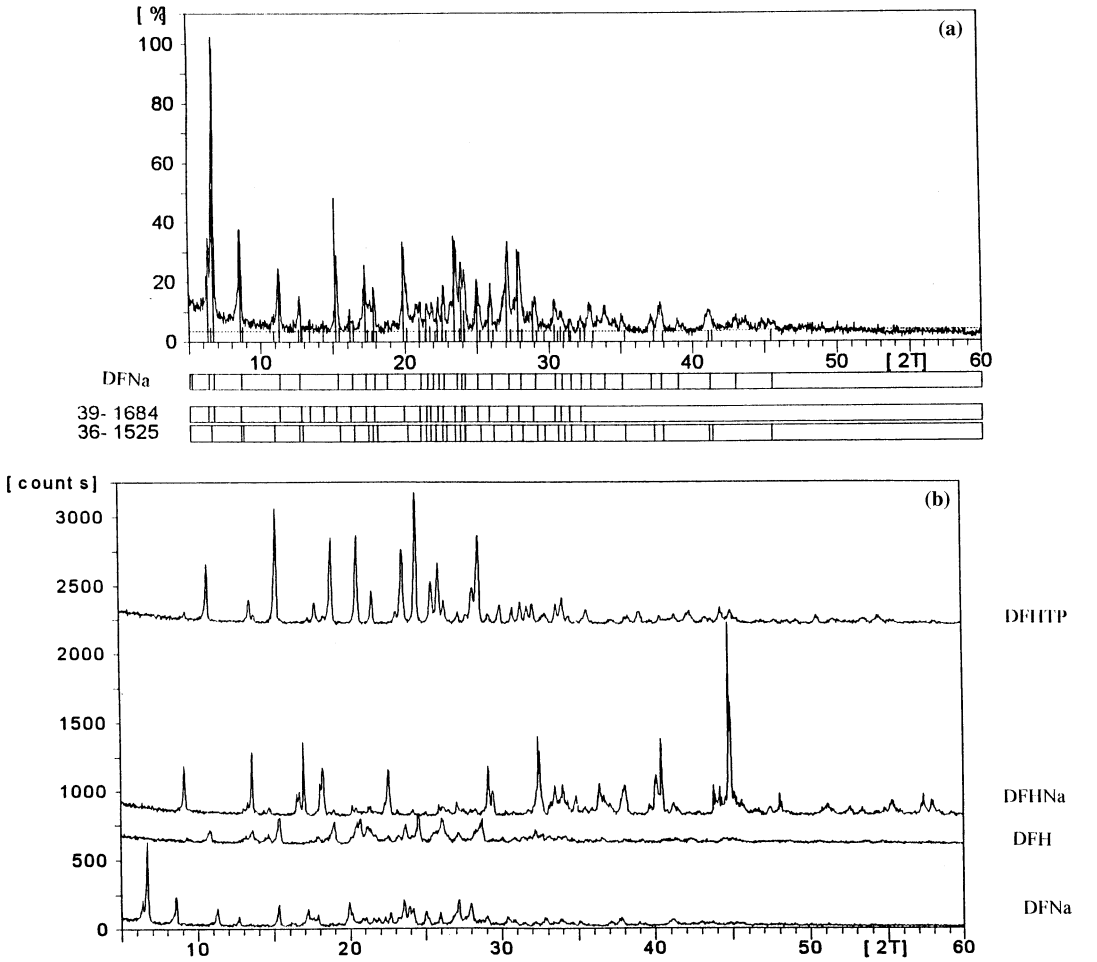

Fig. 5. (a) DRX of DFNa compared to main peaks of two technical files. (b) Results of DRX of all substances assayed.

Nevertheless, there is no evidence to confirm this fact. It is therefore necessary to study the behaviour of this substance and it is helpful to establish the best conditions to avoid its inactivation in order to obtain an appropriate and effective dosage form from a therapeutical point of view.

To perform this study the active substance (diclofenac sodium) will undergo to the action of different solutions in a complex procedure. The resultant compounds will be analyzed using the analytical techniques described above which were selected because of the results obtained in a previous study with diclofenac sodium and in accordance with the literature [4].

2. Materials and methods

The active substance diclofenac sodium (DFNa) was purchased from Impex Química. Equal amounts of diclofenac sodium were treated independently with the next solutions: NaOH 0.2M, pH 12.65; HCl 0.2 M, pH 1.3; distilled water; phosphate buffered solution (PO_4K_2H 0.2 M; NaOH 0.2M; pH 6.8).

As expected, diclofenac sodium was freely soluble in all solutions mentioned above except in HCl. With this last solution and after 24 h at a constant stirring rate (100 rpm), a precipitate (DFH) was obtained. This precipitate was filtered and equal parts of it were submitted to a second

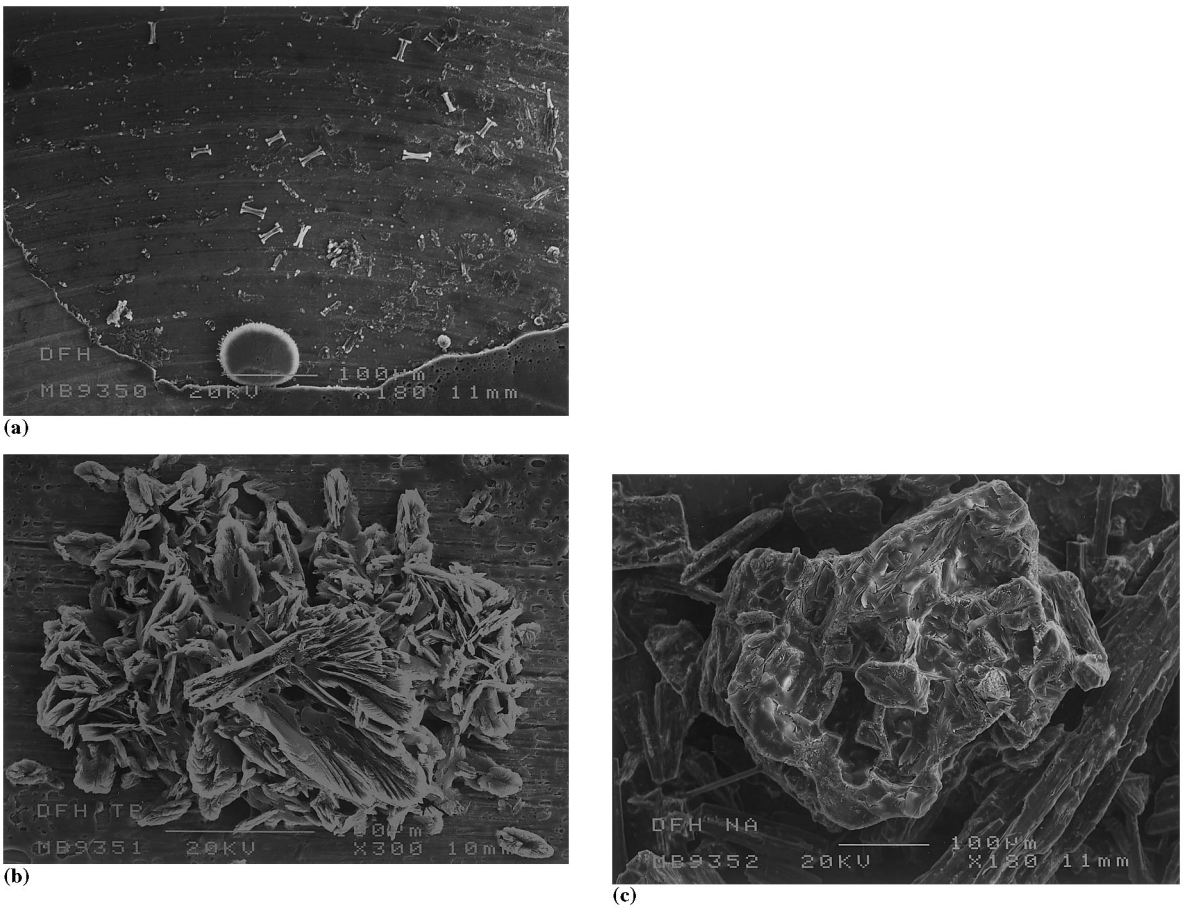

Fig. 6. SEM photographs: (a) DFH; (b) DFHTP; (c) DFHNa.

treatment with the first solution (NaOH 0.2 M, pH 12,65) and another part of the precipitate (DFH) was treated with the last solution (PO_4K_2H 0.2 M; NaOH 0.2 M; pH 6.8) used previously.

In both cases the precipitate (DFH) was dissolved and crystallized in a crystallizer until evaporation at room temperature without stirring; the two solids products were obtained: DFHNa (after the second treatment with NaOH) and DFHTP (after the second treatment with phosphate buffer). Fig. 2 shows the whole procedure.

In order to perform a qualitative study, the analytical techniques mentioned previously were employed:

1. UV-vis spectrophotometry: a Beckman DU-6 was used. The scans were carried out from 210–600 nm at a rate of 60 nm min^{-1}.
2. Differential scanning calorimetry (DSC): Mettler Toledo TA8000 equipment with a DSC-820 furnace was used. Samples of 3–10 mg were weighed directly into aluminium samples pans. The thermal analyses were conducted in a flow of air at atmospheric pressure. Scans were carried out from 30–500°C at a heating rate of 10°C min^{-1}.
3. X-ray diffractometry (DRX): Philips X-Pert MPD equipment was used with Cu–K_α radiation from 5–40°2θ, with a wide angle of 0.04° at a rate of 1 s.

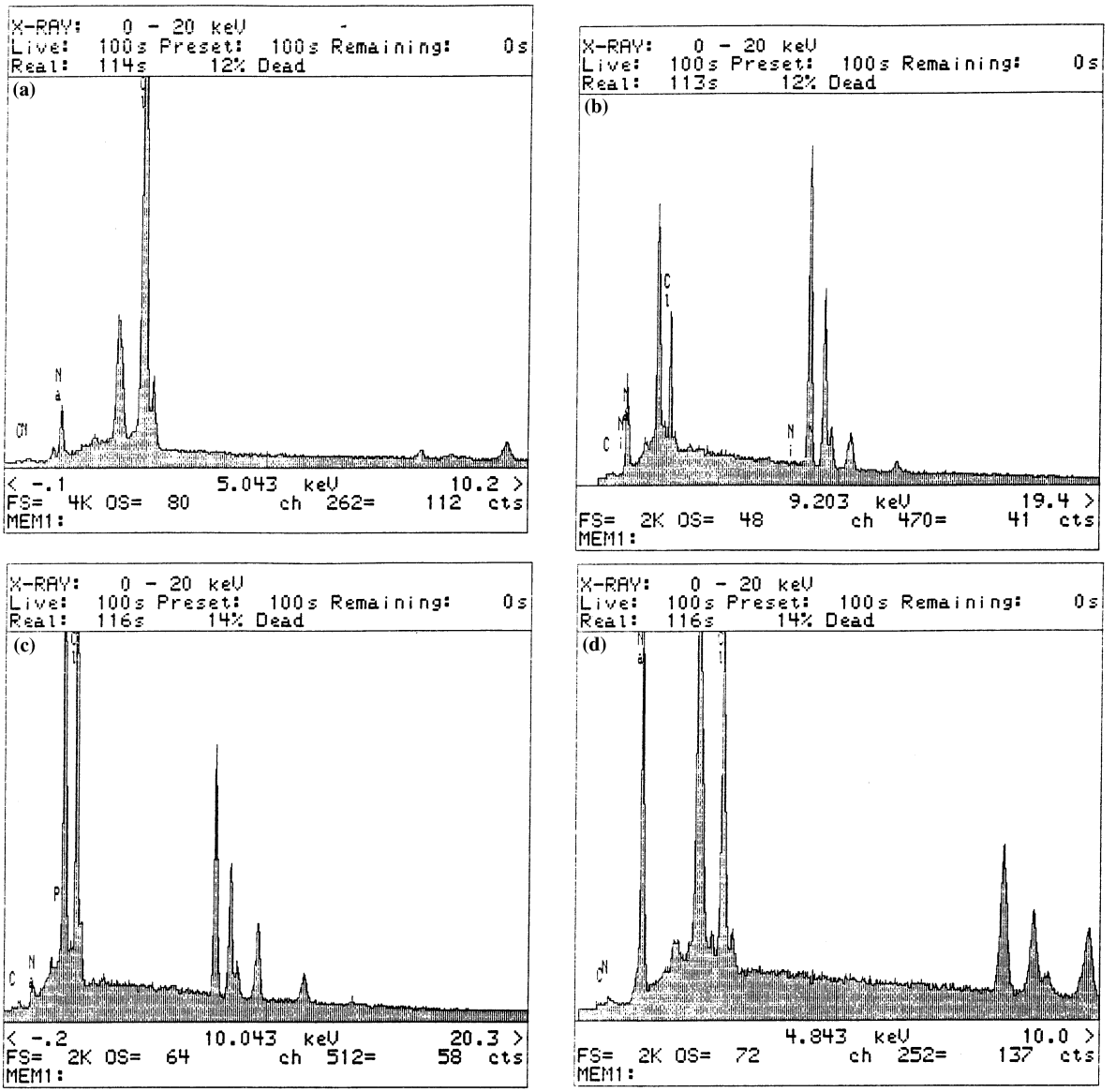

Fig. 7. EDS results: (a) DFNa; (b) DFH; (c) DFHTP; (d) DFHNa, elongated crystals; (e) DFHNa, agglomerates.

4. Energy disperse X-ray analysis (EDS): a LINK eXL coupled to a scanning electron microscope (SEM) JSM-6400 was used for a qualitative analysis of Na and Cl in all compounds.
5. Infrared analysis: tablets of a mixture of the compounds and KBr at a concentration of 4% were analysed with a Perkin Elmer Paragon 100. Each IR was the result of four rounds.

3. Results and discussion

3.1. UV-vis spectrophotometry analysis

In the spectrophotometry analysis (Fig. 3) all solutions showed absorbances between 270–276 nm. No significant differences were found between them except that the reduction of the intensity of absorbance, which is due to the fact that

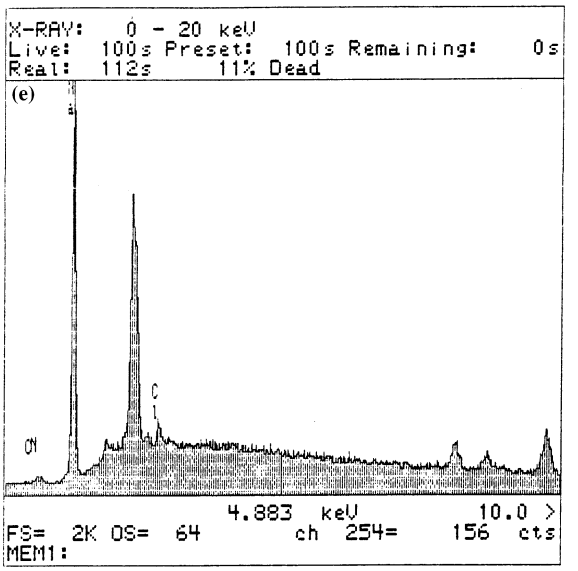

Fig. 7. (*Continued*)

just only the salt (diclofenac sodium), can dissolve in all the solutions. Meanwhile the acidic compound cannot dissolve. These results were confirmed in the literature [4–7,12].

3.2. DSC analysis

The results are shown in Fig. 4. The DSC curve of DFNa (active substance) showed an exothermic peak of melting at 280°C followed by an endothermic peak of decomposition as mentioned in the literature [4]. An accurate measurement was performed and the exothermic peaks were found in the interval from 280.45 to 349.96°C.

The DSC curve of DFH showed two exothermic peaks and an endothermic one from 160–190°C, these peaks corresponded to the melting point (156–158°C) of the acidic compound [13]. In comparison to the DSC curve of DFNa, the peaks were shifted to lower temperatures, and when a temperature of 270°C was reached the decomposition of the product started. Again, an accurate measurement of the thermogram was performed and the main exothermic peak was found at 321.87°C. Meanwhile the secondary exothermic peaks were found in the range of temperatures from 356.85 to 366.55°C.

The DSC curve of DFHNa showed endothermic peaks below 130°C due to the evaporation of the water of crystallisation. This fact was confirmed later when the sample was heated from 25 to 140°C, cooled to 30°C and finally heated again to 400°C. In the new thermogram the endothermic peaks that were located below 130°C disappeared. After heating, two exothermic peaks appeared at 281.98 and 288.48°. A soft exothermic peak was also located at 411.34°C. The thermogram of DFHNa was compared with the those of solid NaOH and a 0.2 M NaOH solution (previously crystallized in a crystalliser at room temperature). Neither of these last two thermograms showed exothermic peaks at 280°C. Hence, the exothermic peaks exhibit between 281–288° by DFHNa substance probably correspond to the presence of DFNa.

The thermogram of DFHTP is quite similar to that of DFH and in an accurate measurement the main exothermic peak was found at 329.631°C, while two secondary exothermic peaks were found at 370.59 and 390.64°C.

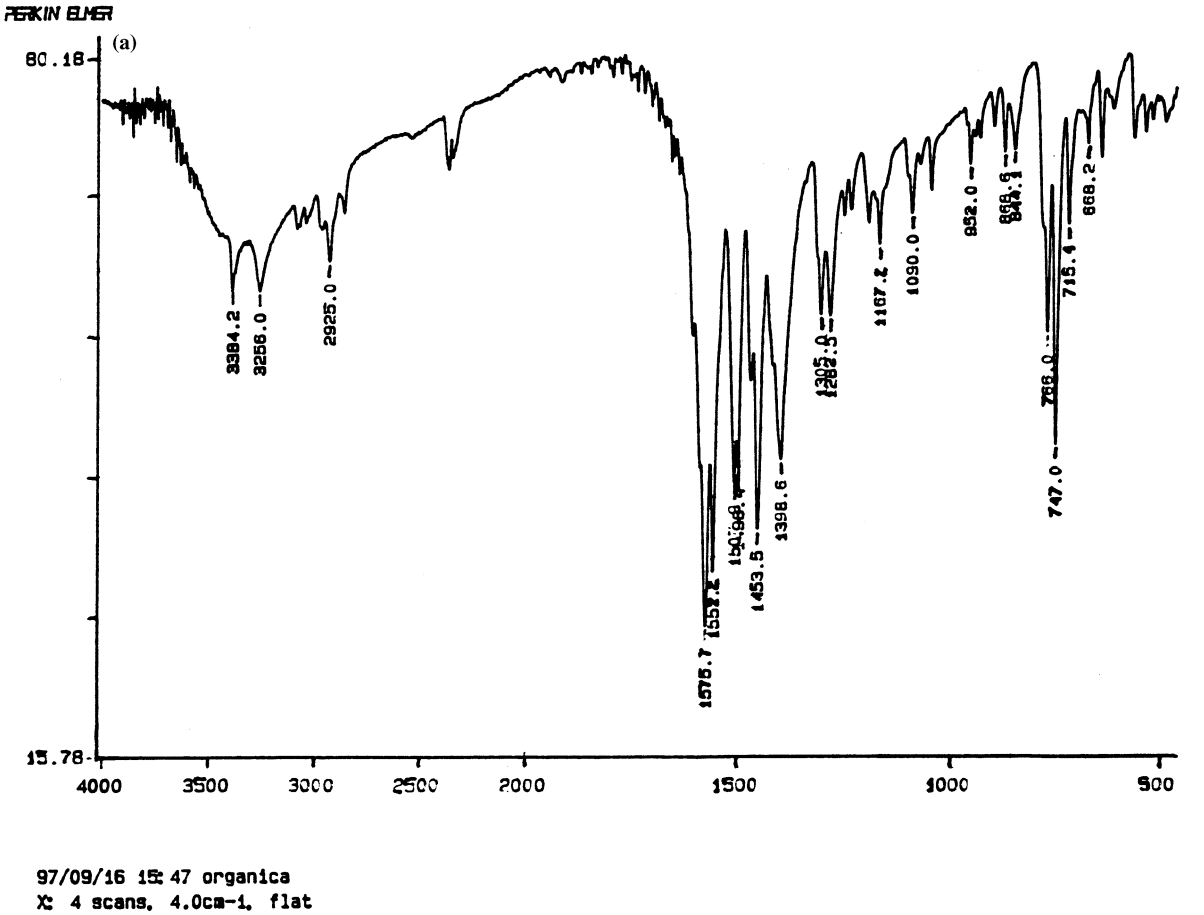

Fig. 8. IR results: (a) DFNa; (b) DFH; (c) DFHTP; (d) DFHNa.

3.3. DRX analysis

The diffractogram of DFNa was compared with two technical files (39-1684) and (36-1525). A high degree of similarity was found between them (Fig. 5(a)). The diffractogram of DFH was quite different (Fig. 5(b)) in comparison to DFNa.

The diffractogram of DFHNa (Fig. 5(c)) showed the presence of DFNa, although the peaks showed lower intensity, nevertheless, other peaks appeared. In comparison with the technical files, it could be observed that the peaks had moved to the right. Thus hypotheses thus arise.

1. The crystals show a preferential disposition. The peaks in the diffractogram of DFNa that appeared with lower intensity are now exalted and vice versa.
2. Some ions have been replaced by other ions so that a variation of distance occurred which corresponds to a displacement of the peaks. The displacement towards the right means the distances were reduced, hence large atoms (Na^+) were substituted by atoms (H^+) of smaller size.

Finally, in the diffractogram of DFHTP (Fig. 5(d)) there were some peaks that corresponded to DFNa, however, more peaks appeared. In comparison with the technical file a displacement of the peaks towards the right can be observed. The peaks on the left have disappeared, which meant that a reduction of the distances took place due to the substitution of some atoms by others of smaller size.

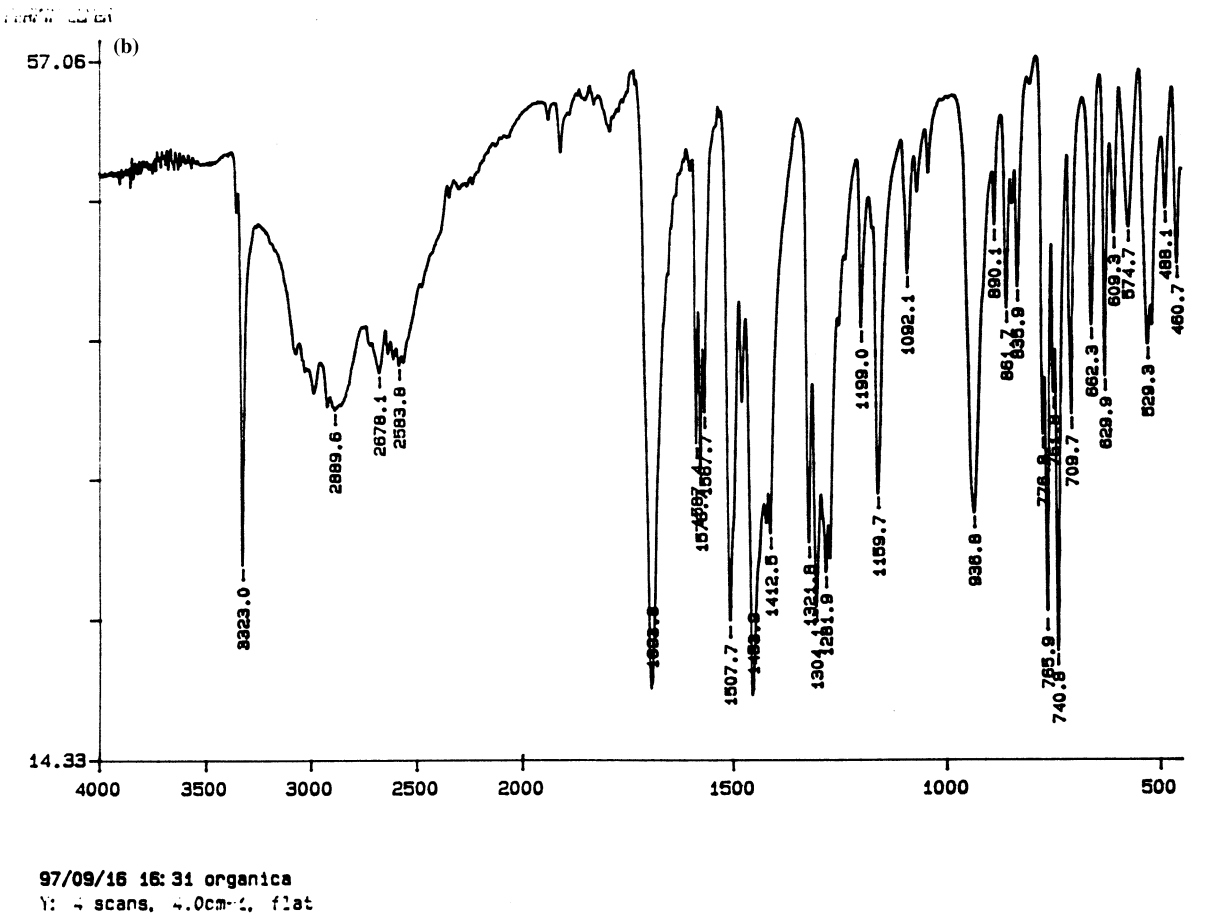

Fig. 8. (*Continued*)

3.4. EDS analysis and SEM photographs

All SEM photographs and EDS results are shown in Figs. 6 and 7, respectively. Fig. 7(a) corresponds to EDS of DFNa. A high proportion of Cl in relation to Na can be observed. In the EDS analysis of DFH, two different structures were observed, one of them has an X shape (Fig. 6(a)) and its EDS (Fig. 7(b)) reveals a lower proportion of Cl and a higher proportion of Na compared to the EDS of DFNa. This probably means that Cl was lost. The second structure has a round shape and its EDS shows also a low proportion of Cl.

With respect to the results obtained with the second treatment, it can be observed that the EDS of DFHTP (Fig. 7(c)) showed increased levels of Cl and P while the proportion of Na decreased.

Finally, in the analysis of DFHNa (Fig. 6(d)) two types of crystals were observed. The elongated one revealed the presence of Cl (Fig. 7(d)). The other structure (Fig. 7(e)), which was an agglomeration of the previous one, revealed a high proportion of Na compared to Cl, which was probably due to the presence of NaOH.

3.5. IR analysis

Fig. 8(a) shows the IR of DFNa, at 1600 a signal of a carbonyl group appeared. Fig. 8(b) corresponds to DFH. In this IR three types of signals were obtained: at 3323 the signal of the

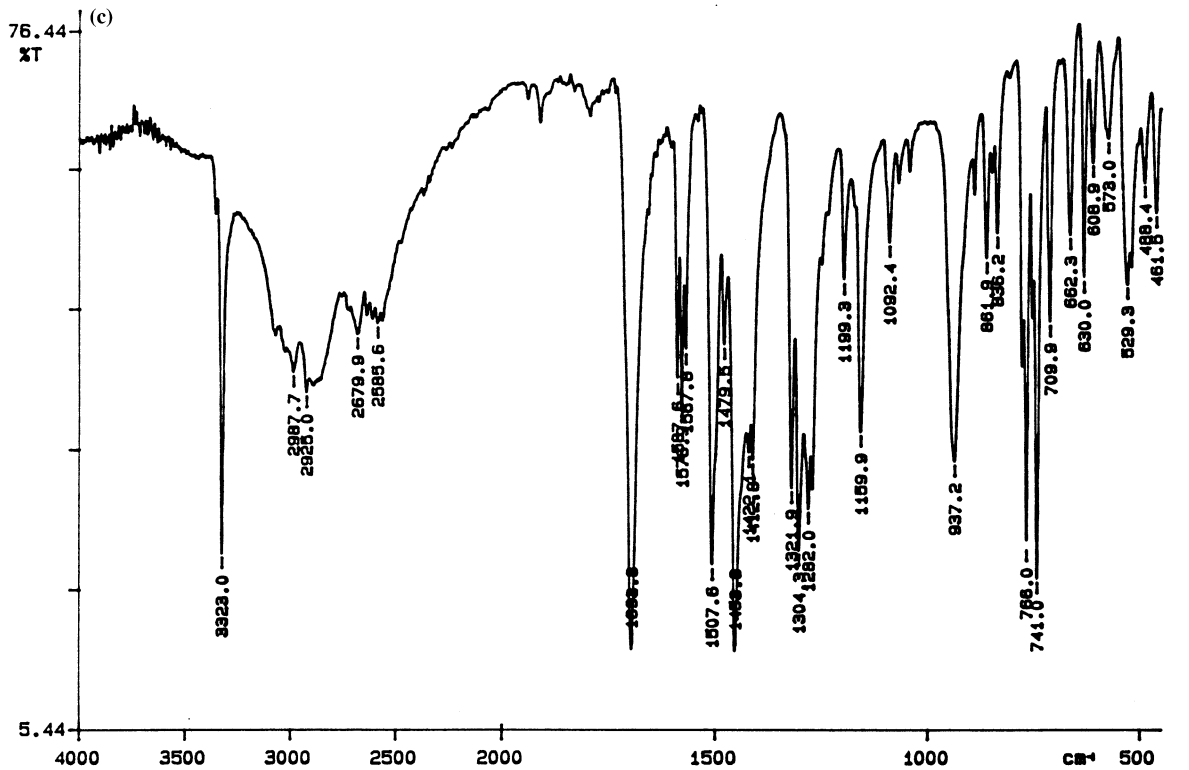

Fig. 8. (*Continued*)

–NH– group appeared, the signals between 2580–2900 corresponded to acidic compounds and finally the signal of the carbonyl group appeared at 1693. All these results suggested that this compound did not have a lactamic structure, hence this compound was not cyclated.

When DFH was treated with phosphate buffer, the structure of the acidic compound remained. The IR (Fig. 8(c)) was quite similar to the IR of DFH. This suggested that the treatment with phosphate solution was not enough to recover the salt.

On the other hand the treatment of DFH with NaOH produced a compound (DFHNa) whose IR (Fig. 8(d)) showed evidence of a NaOH residual in a high proportion. In consequence the spectrum of NaOH overlapped the spectrum of DFHNa, nevertheless a significant difference regarding IR of acidic compound was observed. The signal at 1693 of carbonyl group disappeared and there were signals of DFNa, hence it was possible to assume that the salt was recovered.

4. Conclusions

From the analytical study of Diclofenac sodium and its derivatives, the following conclusions can be drawn.

(1) UV-vis spectrophotometry: no significant differences were observed.

(2) DSC: in the second treatment with phosphate buffer no reconstitution of the DFNa was appreciated. Nevertheless, the treatment with NaOH showed a possible reconstitution of DFNa.

(3) DRX: the treatment of the acidic compound with phosphate buffer or NaOH promoted the exchange of large atoms with smaller ones, hence the distances between planes of crystallization were reduced.

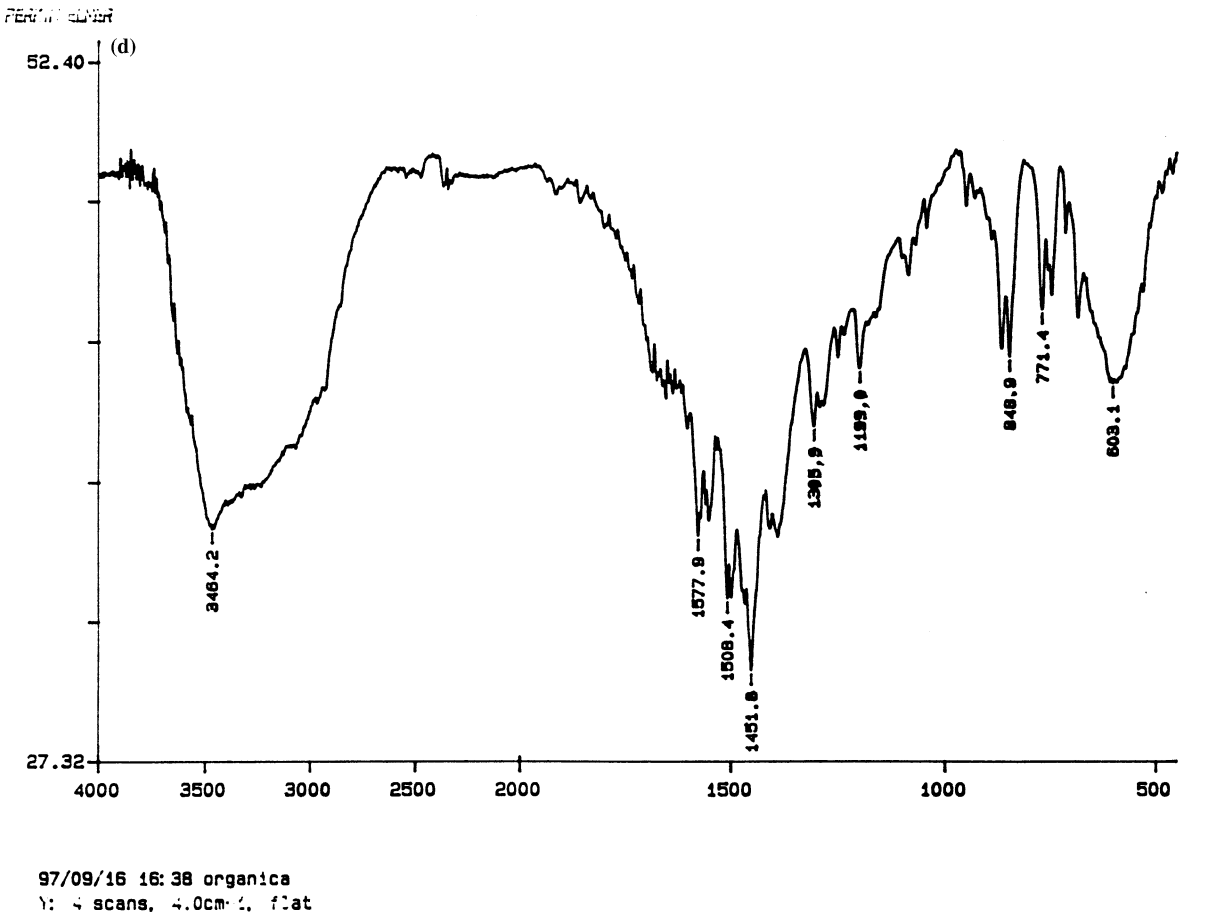

Fig. 8. (*Continued*)

(4) EDS and SEM: the SEM photographs of DFH revealed strange shapes. Its EDS gave a low level of Cl, and the same result was obtained in the analysis of DFHTP. Conversely, the EDS of DFHNa showed high levels of Cl and very high levels of Na, due to saturation with NaOH.

(5) IR analysis: the results obtained in the analysis of acidic compound (DFH) revealed that it was not a lactamic structure, hence it was not cyclated. The treatment with phosphate buffer did not modify the structure. However treatment with NaOH could recover DFNa, although the spectrum was overlapped by NaOH residuals.

Contrary to the literature [9], diclofenac sodium did not undergo intramolecular cyclization in acidic conditions; in fact this substance loose Na^+ in acidic solutions decreasing its solubility. The analytical techniques employed (DSC, DRX, EDS, IR) give evidence for the chemical structure modification of diclofenac sodium (DFNa) once it has been treated with an acidic solution.

When the new compound (DFH) is analysed by IR the spectrum obtained (Fig. 8(b)) does not correspond to a cyclated structure in opposition to what was expected [9]. The acidic solution only takes away the NA ion from the DFNa structure giving rise to a compound (DFH) with a rather low water solubility but not cyclated.

Moreover the original salt (DFNa) can be recovered with an appropriate solution and this means again that the DFH is not a cyclated structure (a lactamic structure does not react as

easily because it has a rather high chemical stability).

Acknowledgements

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