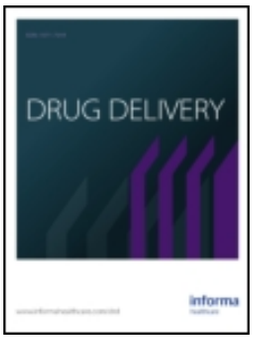


Drug Delivery

ISSN: 1071-7544 (Print) 1521-0464 (Online) Journal homepage: informahealthcare.com/journals/idrd20

# Preparation and Analgesic Activity of Eudragit RS100® Microparticles Containing Diflunisal

Rosario Pignatello, Davide Amico, Santina Chiechio, Concetta Spadaro, Giovanni Puglisi, Paolo Giunchedi

**To cite this article:** Rosario Pignatello, Davide Amico, Santina Chiechio, Concetta Spadaro, Giovanni Puglisi, Paolo Giunchedi (2001) Preparation and Analgesic Activity of Eudragit RS100® Microparticles Containing Diflunisal, Drug Delivery, 8:1, 35-45, DOI: 10.1080/107175401300002748

**To link to this article:** https://doi.org/10.1080/107175401300002748

Published online: 29 Sep 2008.

Drug Delivery, 8:35–45, 2001

1071-7544/01 $12.00 + .00

# Preparation and Analgesic Activity of Eudragit RS100® Microparticles Containing Diflunisal

**Rosario Pignatello, Davide Amico, Santina Chiechio, Concetta Spadaro, and Giovanni Puglisi**
*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Catania, Catania, Italy*

**Paolo Giunchedi**
*Dipartimento di Scienze del Farmaco, Università degli Studi di Sassari, Sassari, Italy*

**Two different techniques, the quasi-emulsion solvent diffusion method and spray drying that provide polar and nonpolar preparation environments, were used to prepare microspheres from Eudragit RS100® (RS) (acrylic/methacrylic copolymer) incorporating the nonsteroidal anti-inflammatory drug diflunisal. The effects of pH on the preparation medium and drug/polymer ratio on production yield and drug incorporation, as well as on the in vitro drug release at pH 1.2 and 6.8 from tabletted microparticles, were evaluated. The drug-polymer interactions and the effect of diflunisal incorporation in the polymer matrix on drug crystallinity have been evaluated by using differential scanning calorimetry, IR and ultraviolet spectroscopy, x-ray diffraction, and microscopy analysis. A preliminary biological assay confirmed that diflunisal maintains its analgesic activity after intraperitoneal administration to rats.**

**Keywords** Analgesic Activity, Diflunisal, Eudragit RS100®, Microparticles, Quasi-Emulsion Solvent Diffusion, Spray-Drying

Eudragit RS100® (RS) is a polymer commonly used for the coating of tablets and preparation of controlled-release oral pharmaceutical forms. It is a copolymer of poly(ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate) containing an amount of quaternary ammonium groups between 4–8%. Its composition makes the RS polymer insoluble at physiologic pH values but able to swell and become permeable to water. Thus it represents a good material for the controlled oral administration of drugs [1–6]. In fact, solid dispersion technology and microparticle formulations are widely used to improve the dissolution of poorly water-soluble drugs (by using water-soluble carriers), as well as to regulate the dissolution rate and bioavailability of hydrophilic compounds, by dispersing them in water-insoluble polymers [7, 8]. In particular, microparticles (both microspheres and microcapsules) can widely distribute throughout the entire gastrointestinal tract, improving the absorption of drugs and reducing the possible irritating effects against the stomach or enteric mucosae.

In our work, some microparticle formulations were prepared and characterized in which diflunisal (DIF) was dispersed into an RS matrix. DIF (2′,4′-difluoro-4-hydroxy -[1,1′-biphenyl]-3-carboxilic acid) (Figure 1) is a widely used anti-inflammatory agent that is chemically derived from aspirin; it has a molecular weight of 250.2, and a $pK_a$ of 3.3 [9]. Its acidic nature and solubility are strongly dependent on the pH and, along with its typical side effects such as gastric irritation (ulceration), suggest the validity of microencapsulation in controlled-release polymeric systems. The dispersion of such a slightly water-soluble drug in a polymer can improve its efficacy by prolonging the duration of action and reducing the side effects.

The main purpose of the present research was to determine the influence of formulation and preparation variables on microparticle characteristics, such as drug incorporation and in vitro drug release rate.

DIF/RS microspheres were prepared by using two different procedures: the quasi-emulsion solvent diffusion method (QESD), described by Kawashima et al. [10] and the spray-drying (SD) method [5]. The first technique is a particular case of coacervation in which the pseudo-emulsion, a concentrated drug/polymer ethanol solution in water as the external phase, is converted into a submicron suspension after diffusion of water into the ethanol droplets, along with the diffusion of the organic solvent to the external phase. Further evaporation of ethanol leads to the final solid drug-loaded micro- or nanoparticles [10].

Received 18 July 2000; revised 3 October 2000; accepted 10 October 2000.

Address correspondence to Professor Rosario Pignatello, Dipartimento di Scienze Farmaceutiche, viale A. Doria, 6; I-95125 Catania, Italy. E-mail: pignatel@mbox.unict.it

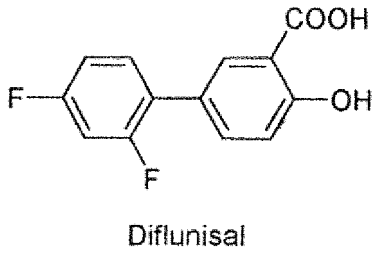



**FIG. 1.** Molecular structure of diflunisal.

Microparticle preparations were characterized in the solid state (IR and ultraviolet spectrophotometry, differential scanning calorimetry, scanning electronic microscopy, x-ray diffractometry) to investigate the interactions occurring between the drug and the polymer during particle formation.

Selected preparations were assayed in vivo on rats using the formalin test to determine the possible variations of DIF analgesic activity after its dispersion into the polymeric matrix.

## MATERIALS AND METHODS

### Materials

Eudragit RS100® was kindly gifted by Rofarma Italia S.r.l. (Gaggiano, Italy); DIF was purchased from Sigma-Aldrich Chimica S.r.l. (Milan, Italy); both compounds were used as received. Absolute ethanol and methanol were analytical or superior grade products; Mg stearate was a Carlo Erba product (Milan, Italy). The dispersing agent of Tween 80, lactose, and Avicel PH 101 were purchased from Fluka (Milan, Italy).

### Microsphere Preparation

In the QESD method (Table 1), DIF and RS (at 1:1, 1:2, 1:5, or 1:10 weight ratio) were dissolved in absolute ethanol (~5 ml) at room temperature. The solution was slowly poured into 50 ml water (pH ~6.5–6.8) or the same volume of a pH 2.0 or 4.0 phosphate buffer, all containing Tween 80 (0.02% w/v). It was stirred at 13,500 rpm for 15 min (Ultra-Turrax T25) in a cylindrical container maintained at low temperature by an iced-water bath to avoid excessively rapid evaporation of ethanol. The resulting dispersion was then stirred for 30 min at room temperature to complete the solvent elimination. Such a process leads to hardening of the emulsion drops into microspheres that were collected by filtration with paper, washed with water, dried at 30°C under reduced pressure for 24 hr, and sieved (40 mesh).

For the spray-drying procedure (Table 2), the drug and polymer (1:1, 1:2, 1:5, or 1:10 weight ratio) were slowly dissolved in dichloromethane. The spray-dryer used was a Mini Spray HO Pabish in the following operative conditions: inlet air temperature, 43 ± 1°C; outlet air temperature, 40 ± 1°C; feed rate, 10 ml/min; and spray pressure, 2 atm. The microspheres, collected in the manifold of the device, were maintained under vacuum for 24 hr and then sieved (40 mesh).

### Differential Scanning Calorimetry (DSC)

Aluminium pans (Mettler ME-26763) of 40 $\mu$l were filled with DIF, the pure polymer, or the microparticle samples (10–15 mg) and sealed. The experiments were carried out with a Mettler DSC 12E calorimeter, linked with a Haake D8-G thermocryostat. An indium standard was used for the temperature and enthalpy change ($\Delta$H) calibration. The scan speed was set at 5°C $min^{-1}$, between 25°C and 240°C (determined by DIF melting point: 211–213°C). An empty pan was used as the reference.

All the samples to be tested were stored overnight in a Büchi T-50 oven at 30°C to ensure identical thermal history. To investigate the interactions between the DIF and polymeric matrices, RS was tested in the pure form as well as after microparticle formation, both in the presence and absence of DIF (drug-free empty microparticles) (Figure 2).

**TABLE 1**
Properties of DIF/RS microparticles prepared by the quasi-emulsion solvent diffusion technique

| Formulation | pH of the dispersing medium | Drug/polymer ratio (w/w) | Loading efficiency: Theoretical drug content (% w/w) | Loading efficiency: Actual drug content (% w/w) | Drug incorporation (%) | Production yield (%) |
|---|---|---|---|---|---|---|
| DS16QE | 6.8 | 1:1 | 50 | 40.5 | 80.2 | 76.6 |
| DS26QE | ” | 1:2 | 33 | 27.0 | 80.3 | 69.0 |
| DS56QE | ” | 1:5 | 16 | 15.6 | 92.0 | 54.0 |
| DS06QE | ” | 1:10 | 9 | 6.0 | 67.5 | 35.0 |
| DS24QE | 4.0 | 1:2 | 33 | 29.5 | 87.5 | 77.2 |
| DS54QE | ” | 1:5 | 16 | 15.8 | 90.6 | 74.3 |
| DS04QE | ” | 1:10 | 9 | 8.2 | 90.0 | 70.5 |
| DS12QE | 2.0 | 1:1 | 50 | 40.0 | 79.5 | 78.0 |
| DS22QE | ” | 1:2 | 33 | 26.6 | 79.5 | 76.8 |
| DS52QE | ” | 1:5 | 16 | 16.0 | 100 | 55.0 |

**TABLE 2**
Properties of DIF/RS microparticles prepared by spray-drying

| Formulation | Drug/polymer ratio (w/w) | Loading efficiency: Theoretical drug content (% w/w) | Loading efficiency: Actual drug content (% w/w) | Drug incorporation (%) | Production yield (%) |
|---|---|---|---|---|---|
| DS1SD | 1:1 | 50 | 35.15 | 70.4 | 85 |
| DS2SD | 1:2 | 33 | 21.2 | 63.5 | 90 |
| DS5SD | 1:5 | 16 | 12.0 | 70.9 | 89 |
| DS0SD | 1:10 | 9 | 8.7 | 85.6 | 87 |

### X-Ray Diffractometry

Diffraction patterns were recorded with a Philips PW 1050/25 diffractometer for powders. A voltage of 40 kV and a current of 30 mA for the generator were used, with Cu as the tube anode material. The solids were exposed to a Cu-$K_\alpha$ radiation ($\alpha_1$ = 1.54060 Å and $\alpha_2$ = 1.54439 Å, with an $\alpha_1/\alpha_2$ ratio of 0.5), over a range of $2\theta$ angles from 3°–30°, at an angular speed of 1° ($2\theta$) per min, using divergence and receiving slits of 1.5° and 0.2°, respectively. Typical x-ray patterns are reported in Figure 3.

### FT-IR Spectroscopy

Samples were analyzed after dispersion in KBr (about 10–15 mg microparticles per 20–45 mg KBr) with a Perkin-Elmer 1600-series instrument.

### Scanning Electron Microscopy (SEM) Analysis

Microsphere samples (DS24QE and DS52QE) were diluted with PBS (5 mg for 25 ml of PBS). The microsphere suspension was sonicated for 30 sec by a probe, with intervals of 30 sec between each cycle. The resulting microsuspension was subjected

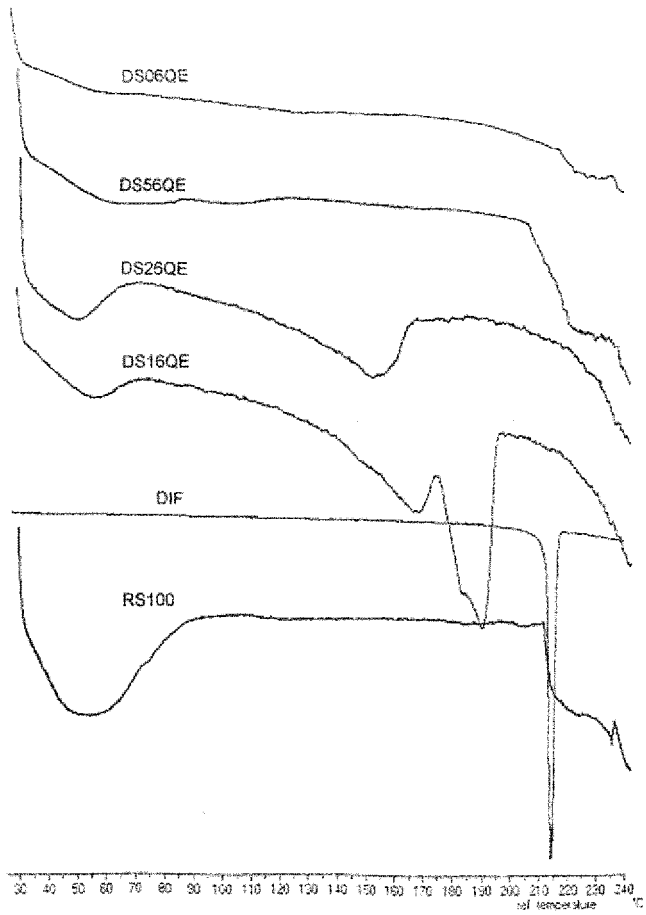



**FIG. 2.** Comparison among DSC thermograms of pure RS, DIF, and a QESD microparticles series prepared at different DIF-RS weight ratios.

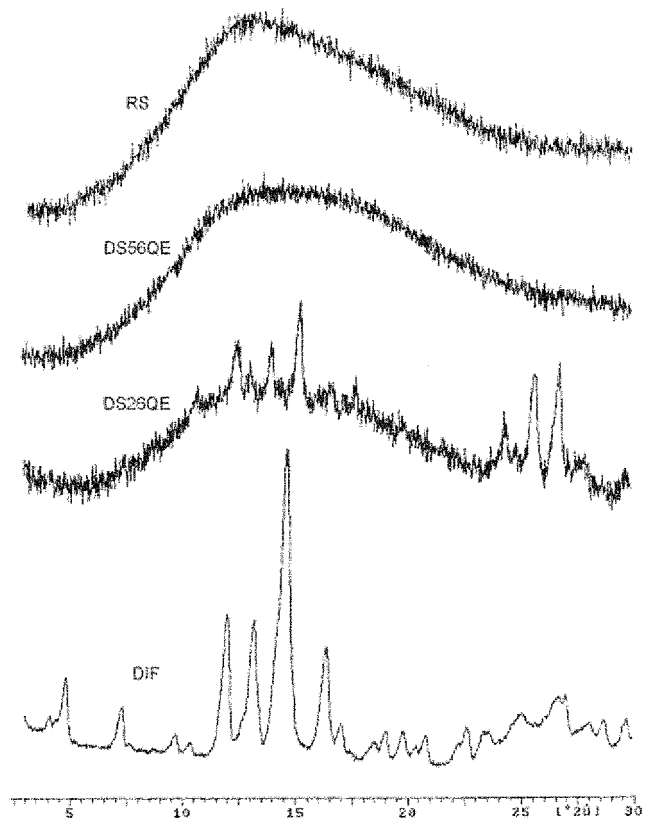
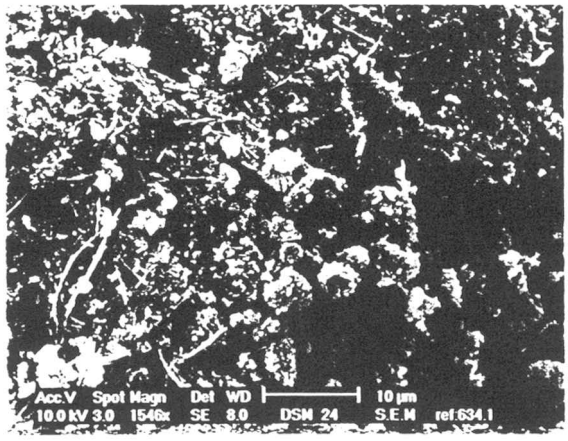



**FIG. 3.** X-ray diffraction patterns of DIF, RS, and two QESD microparticles prepared at different DIF-RS ratios.



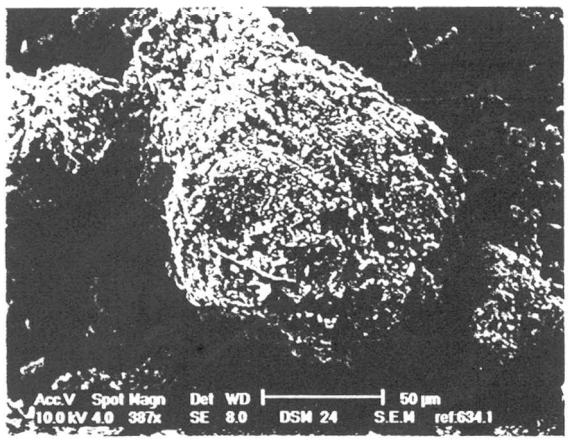
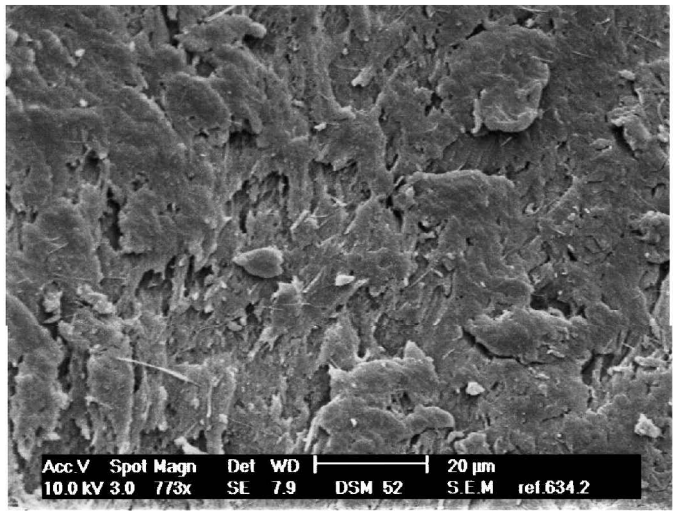




**FIG. 4.** SEM analysis of DS24QE microparticles.



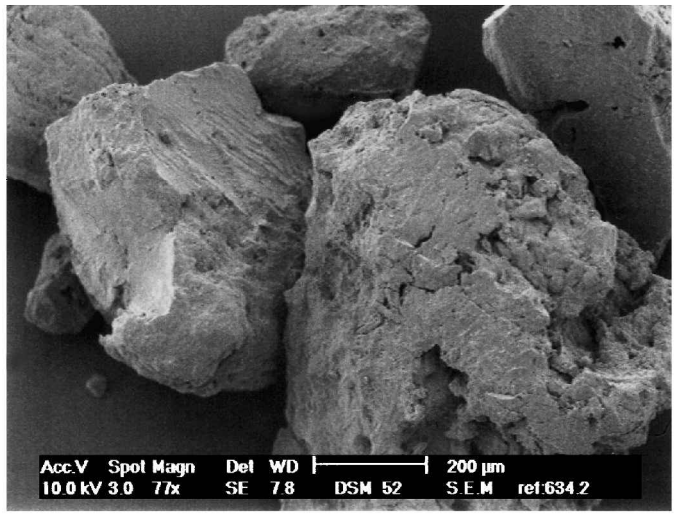




**FIG. 5.** SEM analysis of DS52QE microparticles.

to SEM analysis by a Philips XL 20 scanner, after fixation with a 1% phosphotungstic acid solution (Figures 4 and 5).

## DIF Content Determination

Microsphere samples (~100 $\mu$g) were accurately weighed and dissolved in 5 ml methanol. The solution was analyzed by a Shimadzu UV-1601 spectrophotometer. DIF content was calculated at 254 nm by comparison with a calibration curve of the drug in methanol. Each determination was performed at least in duplicate. Results are expressed as percent incorporation of DIF (Tables 1 and 2).

RS displays a slight absorption at this wavelength, but the high dilution used limited their effect on the drug concentration calculation (less than 3% increase of sample absorbance).

## Tabletting of Microparticles

Microparticle powders (100–150 mg) were mixed for 15–20 min in a porcelain mortar with 10–50 mg of spray-dried lactose, 50–100 mg of Avicel PH 101, and 2 mg of magnesium stearate. The resulting mixture reached a total weight of 300 mg (400 mg in the case of 1:10 DIF/RS microparticles). The mixture was then compressed into a disk using a 1.3-cm diameter flat-faced die in an IR press and a force of 1–3 tons. Tablets were stored over a desiccant until used.

## Drug Release Studies

The F.U.I. (Farmacopea Ufficiale Italiana, X Ed.) basket method was adopted at 37°C and 100 rpm to follow the release profile of DIF from the particulate systems. Sink conditions were assured during the entire dissolution process. The Method A for delayed-release (enteric-coated) materials was used (USP XXIII). 750 ml of 0.1 N HCl were equilibrated at 37 ± 0.5°C in a 1-l vessel. Each microsphere tablet was placed in the vessel and 1 ml aliquots of the medium were withdrawn at preset times

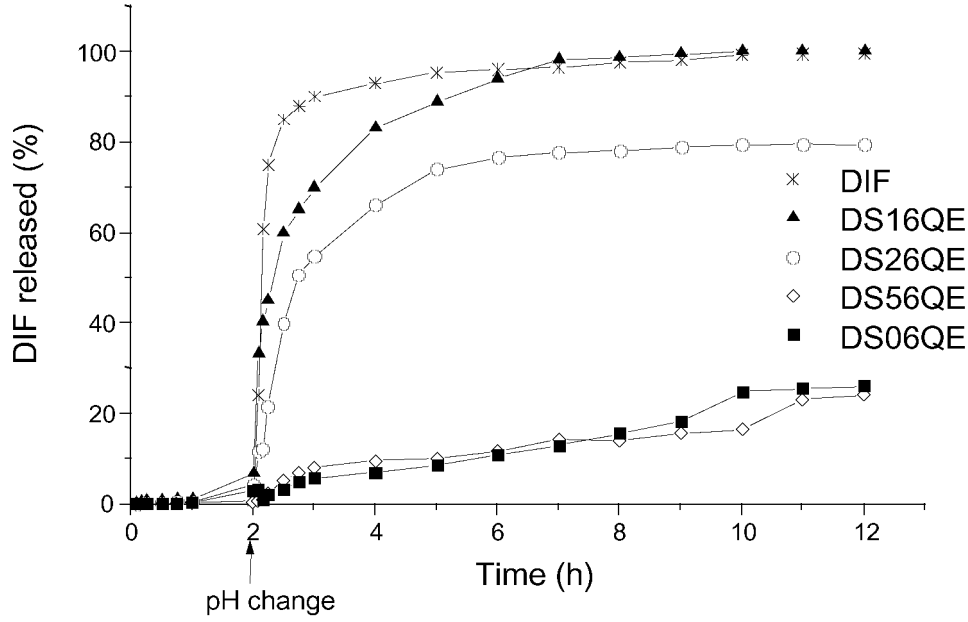



**FIG. 6.** In vitro release pattern of DIF, at pH 1.2 and 6.8, from QESD microparticles prepared in distilled water.

over 2 hr and replaced by 1 ml of prewarmed medium. The collected samples were filtered through 0.45 $\mu$m filters and used for the spectroscopic determination of DIF, diluting with water if necessary. After 2 hr, 250 ml of 0.2 M tribasic sodium phosphate pre-equilibrated at 37°C were added to the dissolution vessel. The pH was immediately adjusted, if necessary, with 2 N HCl or 2 N NaOH to pH 6.8, and the dissolution profile of DIF was followed as above up to 8–24 hr (Figures 6, 7, and 8). Drug concentration in the samples was measured by ultraviolet analysis at 300 nm or 252 nm for the acid and intestinal buffers,

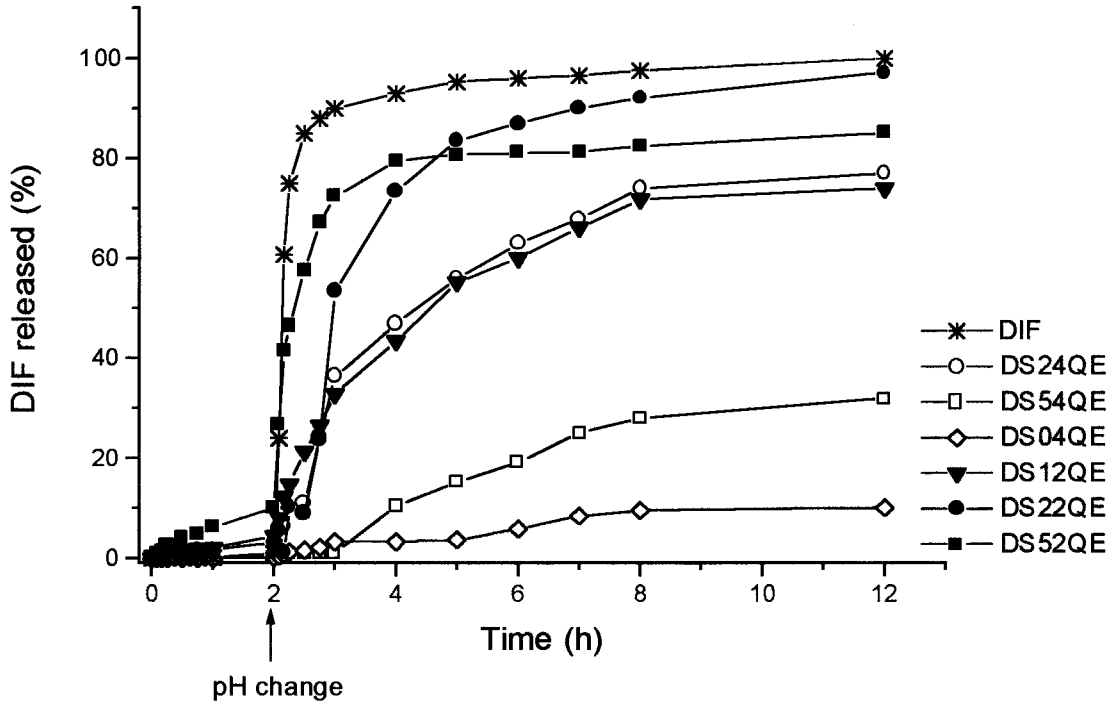



**FIG. 7.** In vitro release pattern of DIF, at pH 1.2 and 6.8, from QESD microparticles prepared in the pH 4.0 or pH 2.0 phosphate buffer.

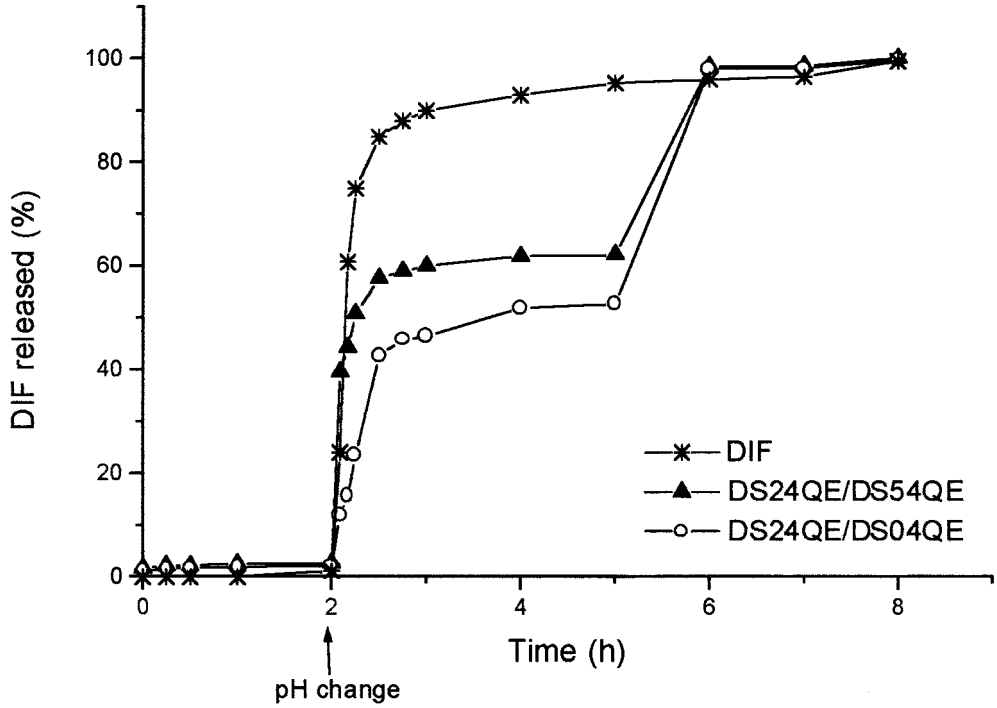



**FIG. 8.** In vitro release pattern of DIF, at pH 1.2 and 6.8, from a 1:1 mixture of two QESD microparticles batches prepared in a pH 4.0 phosphate buffer.

respectively. Each experiment was repeated two or three times, and a close reproducibility of results were obtained.

For the pH-dependent release tests (Figure 9), the same assay was performed but using a 0.1 N HCl solution (pH 1.2), or pH 5.5 and 7.4 phosphate buffer solutions.

## Mathematical Evaluation of Drug Release Data

The experimental release results obtained from the different batches were fitted to the following semiempirical equations, describing dissolutive (equation 1) and Fickian diffusional release mechanism (equation 2) of the drug from microparticles

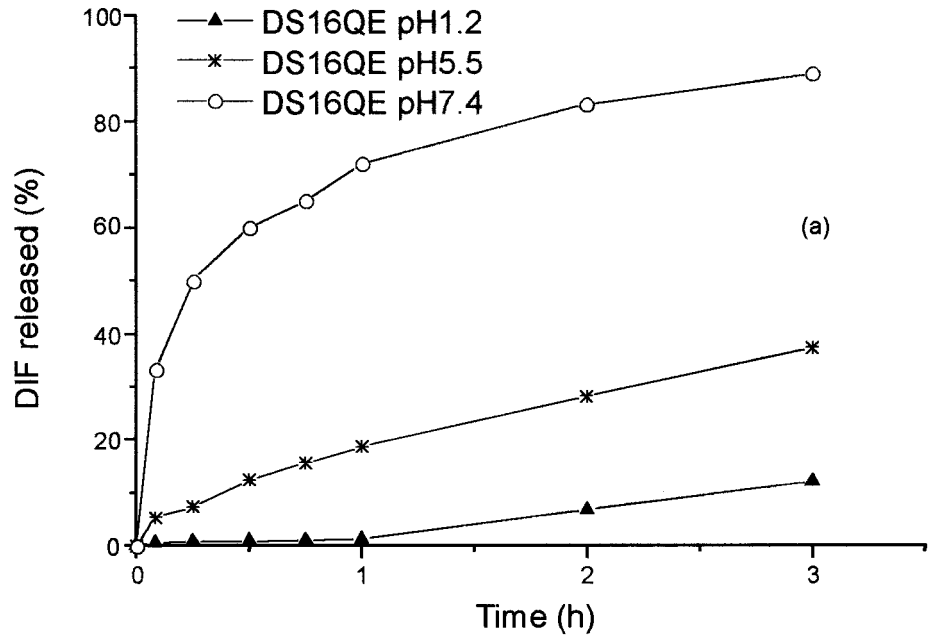



**FIG. 9.** In vitro release pattern of DIF at different pH from QESD microparticles prepared in distilled water and at an 1:1 DIF-RS ratio.

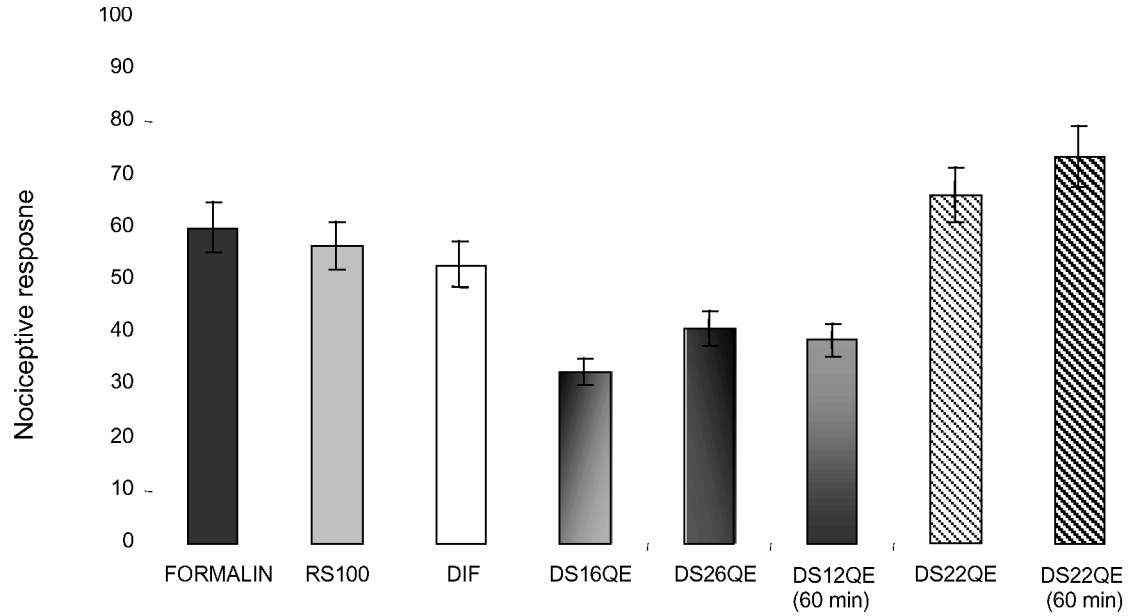



**FIG. 10.** Antinociceptive activity (formalin test) of selected QESD microparticle systems.

respectively [11]:

$$\left(1 - \frac{M_t}{M_\infty}\right) = e^{-K_{dlsst}} + c \qquad [1]$$

$$\frac{M_t}{M_\infty} = K_{diff} t^{0.432} + c' \qquad [2]$$

where $M_t/M_\infty$ is DIF fraction released at time $t$ in respect to the total content in the analyzed microparticles ($M_\infty$), and $K$, $c$, and $c'$ are coefficients calculated by plotting the linear forms of these equations.

### In Vivo Biological Tests

Experiments were conducted on male Sprague-Dawley rats (Charles River, Italy) of variable weights (150–200 g). Groups of 2–4 animals were housed in a single cage at a constant temperature (22 ± 1°C) with 12-hr alternating light/dark cycles. Animals had free access to a normal diet for laboratory rats and water. On the day of experiment, the rats were housed individually in a 28 × 28 × 28-cm observation chamber, 20 min before testing, to allow them to adapt to the environmental conditions.

### Formalin Test

After the acclimatation period, DIF (100 mg/kg) or microparticles (containing an equivalent drug concentration) were intimately dispersed in an aqueous Tween 80 solution (0.02% w/v). Pure RS microparticles (without drug) also were used as a control to assess the absence of toxicity or effects by the polymer. The obtained suspensions were administered intraperitoneally 30 or 60 min before the subcutaneous injection of a 5% (w/v) solution of formalin (50 $\mu$l) into the dorsal surface of the right hind paw of the rat. Rats were then re-housed into the observation chamber and the number of flinches counted in 1-min periods at 5-min intervals up to 60 min postinjection.

Results shown in Figure 10 describe the cumulative response of phase II in which the inflammatory process is prevalent [12–15].

## RESULTS AND DISCUSSION

### Effects of Formulation Variables on the Physical Properties of Microparticles

DIF-loaded microparticles were prepared using a pH-independent copolymer, Eudragit RS100®, by the QESD and SD methods [16–18] and at different drug-to-polymer ratios (1:1, 1:2, 1:5, and 1:10). Both preparative techniques gave high drug incorporation and production yields (Tables 1 and 2).

Results of particle size analyses did not indicate significant differences between the various batches. Microspheres showed a median diameter ($d_{50}$) ~280 $\mu$m and a $d_{90}$ (diameter corresponding to 90% of particles) between 356 and 385 $\mu$m. Microparticles prepared by SD were smaller than QESD ones ($d_{50}$ = 210 and $d_{90}$ = 350 $\mu$m).

Because of the acidic nature of DIF, electrostatic interactions between drug carboxyl group and the charged ammonium groups in the polymer are expected to occur [19–22]. To verify this aspect, microparticles were prepared both in distilled water (6.5–6.8 pH range) or in acidic phosphate buffers, at pH 2.0 or 4.0, that is, at a little lower or higher pH value than the $pK_a$ of drug (3.3). The importance of pH of the dispersing environment

on the dissociation/crystallization of the active compound during the emulsification of the polymer solution and the following particle formation is well known [23].

When different pH values of the dispersing medium were tested, incorporation of DIF was not significantly influenced. Therefore, although DIF has a solubility profile strongly related to the pH, the determining factor for drug dispersion in the polymer matrix did not seem to be its solubility into the dispersing aqueous solution, rather the solubility into the polymer network itself.

The production yields were lower with increasing polymer amounts (Table 1). Such behavior relates to the ability of drug microcrystals to act as seeds for the polymer deposition, as well as to the relative smaller size of the microparticles obtained at higher polymer ratios, which led to a partial loss of the product during recovery and filtration. Drug encapsulation efficiency values always approached the theoretical ones (Tables 1 and 2). In this case, the presence of higher RS ratios increased the percentage of retained drug [24].

## Solid State Characterization of DIF-Loaded Microparticles

The interactions between the drug and RS in microparticles were studied by means of IR spectroscopy, DSC, x-ray diffractometry, and scanning electron microscopy (SEM) analysis (Figures 2–5).

The drug appears to be homogeneously dispersed in a crystalline form into the polymeric matrix of the particles prepared by both the QESD and SD methods. The IR patterns (not shown) are in fact the simple overlapping between the pure drug and RS (with main signals at 3444, 1736, 1460, 1390 $cm^{-1}$).

In the DSC analysis (Figure 2), DIF shows a sharp endothermic fusion peak at 212°C. Empty RS microparticles only display a broad endothermic signal around 55°C, corresponding to the glass polymer transition ($T_g$), from a more fragile state (glassy state) to a rubbery one. In DIF/RS microparticles, the endothermic signal (fusion peak) of the drug disappeared, whereas a less defined and broader peak appeared at a lower temperature. Such behavior suggests a partial loss of drug crystallinity and the formation of zwitterionic adducts between the drug and polymer reactive centres. The same endothermic signal is attenuated with increasing polymer concentrations (1:5 and 1:10 ratios), indicating a complete and homogeneous dispersion of the drug in the RS matrix.

The x-ray diffraction patterns are shown in Figure 3. The RS diffractogram shows a typical profile for an amorphous material. QESD microparticles diffraction profiles (DIF/RS 1:2 and 1:5 ratios) showed a progressive disappearance of drug signals, proportional to the increasing polymer amount. DIF seemed then able to crystallize within the polymeric network, when the drug concentration exceeded its solubility in the polymer itself.

SEM analysis, performed on the DS24QE and DS52QE samples, confirmed the previous results (Figures 4 and 5). In fact, drug crystals on their surface are visible in the system prepared with a 1:2 drug-to-polymer ratio, but not in the microparticles obtained with a higher polymer ratio (DS52QE).

**TABLE 3**
Kinetic parameters obtained from in vitro release tests of the diflunisal-loaded microparticles

| Formulation | $t_{50}$[a] (h) | $t_{pl}$[b] (h) | Released drug[c] $\%_{max}$ | $AUC_{0\rightarrow12}$[d] (% × h) |
|---|---|---|---|---|
| DIF | 2.2 | 4.0 | 100 | 944.6 |
| DS16QE | 2.4 | 7.0 | 100 | 906.3 |
| DS26QE | 2.5 | 6.0 | 79.5 | 715 |
| DS56QE | NC | 11 | 24.1 | 137.0 |
| DS06QE | NC | 10 | 26 | 144.0 |
| DS24QE | 4.8 | 25 | 96.9 | 598.5 |
| DS54QE | 21.5 | 25 | 49.8 | 211.5 |
| DS04QE | NC | 25.5 | 18.9 | 72.7 |
| DS12QE | 4.6 | 8.0 | 71.8 | 595.6 |
| DS22QE | 3.0 | 8.0 | 95.1 | 817.8 |
| DS52QE | 2.3 | 5.0 | 82.5 | 797.9 |
| DS1SD | 2.4 | 5.0 | 100 | 895.0 |
| DS2SD | 2.4 | 7.0 | 91.1 | 825.5 |
| DS5SD | 2.4 | 8.0 | 91.7 | 758.5 |
| DS0SD | 4.1 | 6.0 | 60.6 | 508.7 |

[a,b] Time needed for 50% and plateau release of the loaded drug, respectively.
[c] Highest percentage released.
[d] Area under the percent/time release curve, calculated by trapezoidal rule.
NC = not calculable.

## Drug Release from Microparticles

The in vitro DIF release profiles from tabletted microparticles are plotted in Figures 6 and 7. Repeated tests gave good reproducibility (s.d. $\leq$ 7%). The relative kinetic parameters are listed in Table 3.

All the microparticles prepared in distilled water showed a pH-dependent release of the drug (Figure 6). During the first 2 hr at pH 1.2, only a small amount of DIF was released; after the pH change to 6.8, while tablets containing the free DIF showed an almost instantaneous and complete dissolution of the drug, the other microparticle batches displayed a controlled release pattern, strongly related to the drug-polymer ratio (Figure 6). Increasing the amount of polymer seemed to hinder the penetration of the dissolution medium into the microparticles and the subsequent drug dissolution and diffusion.

As already observed, DIF release at equilibrium often was not complete: the released drug, becoming ionized at the pH of the final dissolution medium, can be re-adsorbed from the latter back on microparticle surface [4, 20–22, 26].

The QESD batches prepared at pH 4 showed a slower drug release pattern ($t_{eq}$ > 24 hr, Table 3), but with the same dependence

on the polymer ratio (Figure 7). For microparticles prepared at pH 2 (DS12QE, DS22QE, and DS52QE), the drug release increased with the polymer amount (Figure 7). The effect of the pH of the preparation medium on the DIF release patterns can be observed in Table 3. The drug is released at a lower rate from the microparticles prepared at pH 4, while the other two preparations showed higher release rates. Thus, at pH values higher or lower than the $pK_a$ of the drug (=3.3), the electrostatic interaction with the polymer active sites is hampered because of the greater dissociation state of the drug (at pH > 6) or of the polymer carboxyl groups (at pH 2). At pH 4 an optimal interaction between the two components occurs, limiting the diffusion/dissolution of the drug from the microparticle network [25–27].

Spray-dried microparticles showed a different drug release profile. The DS1SD, DS2SD, and DS5SD systems (Table 2) gave a rapid drug leakage (data not shown), releasing almost all the incorporated drug in 5, 7, and 8 hr, respectively (Table 3). The SD microparticles had a smaller size than QESD ones, with a greater contact surface with the dissolution medium and a favored electrostatic interactions between DIF and RS. Furthermore, the nonpolar environment used in the SD technique made such interactions stronger between the drug and the active sites in the polymer.

### Kinetic Analysis of the In Vitro Release Profile

The resulting experimental data from the in vitro release curves were examined under the two kinetic equations discussed. Equation (1) fits better with a dissolutive release mechanism, whereas equation (2) describes a diffusion-type process; a 0.432 value as the $t$ exponent corresponds to a Fickian release from a nonswelling spherical matrix system [28].

The experimental release data were subdivided into two phases: from 0–60% and from 60–100% of the initially dispersed drug. From the linear regression coefficients $r$, a diffusional process seemed to be prevalent in the first portion of the release ($r > 0.930$), whereas a dissolutive process characterized the final phase, following the wetting and swelling of the particles ($r > 0.989$).

On the contrary, a diffusional mode of release fits better for the formulations prepared by dispersing the DIF/RS ethanol cosolution in the pH 2 buffer (DS12QE, DS22QE, and DS52QE) ($r > 0.990$). DIF is incorporated in the microparticles in an undissociated and less soluble form at this pH.

However, the observed higher values for the coefficient $r$, obtained for both the kinetic equations, confirmed that drug release from RS microparticles is complex and not associated with only one mechanism.

### Drug Release from Co-Tabletted Microparticles with Different Drug-to-Polymer Ratios

To investigate the potential use of DIF-RS microparticles not only as a prolonged but also as a pulsatile drug release formulation, the in vitro release studies used tablets of two different but equivalent particle batches: an 1:2 and 1:5 DIF/RS ratio, or an 1:2 and 1:10 DIF/RS ratio. The systems prepared in a pH 4 dispersing medium were chosen, since they showed a slower drug release (Figure 7).

As Figure 8 displays, both mixed systems gave a triphasic release pattern: after the initial step (2 hr) at pH 1.2, where no significant drug release occurred, the pH change to 6.8 caused a rapid dissolution of a part of the drug within the next 30 min and up to ~5 hr. At this time, a further drug leakage phase started, allowing a complete dissolution of the incorporated drug within the next hour. In this case, we assumed that the two systems co-mixed in each tablet were "activated" at different times. It is noteworthy that in these as well as in the previous dissolution tests, all tablets disgregated in a short time (5–12 min), and the observed drug release must then be ascribed to the single microparticle polymer matrixes. The 50–60% DIF release observed during the first part of the dissolution curve (Figure 8) can be ascribed mainly to the fast-releasing system (DS24QE, common to both the tested mixtures), with only a lower rule from the other co-mixed particles. On the contrary, the microparticles containing higher polymer amounts (DS54QE and DS04QE) participated to the overall drug release only after a longer "lag-time," during which polymer hydration and swelling took place. It is also noteworthy that the two latter systems did not release more than 40% of DIF after 12 hr, when tabletted alone (Figure 7); in the co-mixed tablets complete drug release occurred after 6 hr, suggesting that the progressive drug leakage from DS24QE particles allowed a deeper penetration of the dissolution medium inside the microparticles containing higher RS amounts.

### DIF Release Studies at Different pH Values

Drug-polymer solid systems have often been proposed as useful pharmaceutical formulations to ensure a controlled release and absorption of the active compound in selected areas of the gastroenteric tract. Some commercial products containing NSAIDs also recently have become available for clinical use.

To evaluate the pH-dependent DIF release from QESD microparticles, two of the formulations prepared in distilled water with different drug-RS ratios (DS16QE and DS26QE, Table 1) were subjected to the in vitro dissolution test using three different media: at pH 5.5 (i.e., buccal pH), 1.2 (gastric environment), and 7.4 (intestinal pH). Both formulations displayed a similar drug release profile, strongly dependent on the pH of the dissolution medium: the behavior of DS16QE system is reported in Figure 9. After 3 hr at gastric pH, only a little amount of DIF was released, whereas drug dissolution at pH 5.5 reached a 20–30% of the initial amount. At pH 7.4 the dissolution of DIF was more evident, showing a rapid initial phase (30–60 min), followed by an almost complete dissolution of the drug in the external solution. These findings suggest using this kind of DIF-RS solid dispersions to ensure a delayed and a pH-dependent targeted

release of the active compound after oral administration, e.g., as a fast-disgregating buccal tablet.

### Antinociceptive Activity

The formalin test evaluated the biological efficacy of DIF after its encapsulation in RS microparticles. The subcutaneous injection of a diluted aqueous solution of formalin into the hind paw dorsal surface of the rat evoked quantifiable nociceptive behavior of the injected paw [12, 15].

Figure 10 shows the results obtained for some microparticle formulations. The test samples were chosen on the basis of their different in vitro drug release profiles, hence, the evaluation of their pharmacologic behavior allows a better comparison between the two sets of data.

The intraperitoneal administration of empty microparticles (pure RS) did not interfere with the pain stimulation induced by formalin; furthermore, the polymer did not induce any irritation or local lesion in the treated animals. The parent drug was administered as a suspension for comparison purposes (suspended in 0.02% w/v Tween 80 containing distilled water) and did not show significant effects.

As Figure 10 shows, 3 of the assayed formulations gave an important reduction of the nociceptive response in the rat (phase II), when the formulations were administered 30 min (DS16QE and DS26QE) or 1 hr (DS12QE) before the formalin injection. The DS22QE formulation showed no evident activity, whether administered 30 min or 1 hr before the formalin. The administration times of the formulations were chosen by their in vitro drug release patterns to have a significant drug release at the observation time.

Results clearly show that DIF maintains its analgesic and anti-inflammatory activity even after dispersion into the RS matrix. The microcrystalline state of the dispersed active compound seemed to favor a more rapid dissolution and then a significant higher biologic activity, as compared with the pure drug dispersion.

## REFERENCES


1. Goto, S., Kawata, M., Nakamura, M., Maekauwa, K., and Ayoama, T. 1986. Eudragit RS and RL (acrylic resins) microcapsules as pH insensitive and sustained release preparations of ketoprofen. *J. Microencapsul*. 3:293–304.
2. Kawata, M., Nakamura, M., Goto, T., and Ayoama, T. 1968. Preparation and dissolution pattern of Eudragit RS microcapsules containing ketoprofen. *Chem. Pharm. Bull.* 34:2618–2623.
3. Lehmann, K. 1989. Chemistry and application properties of polymethacrylate coating systems. In *Aqueous Polymeric Coating for Pharmaceutical Dosage Forms*, ed. J. W. McGinity, p. 153. New York: Marcel Dekker.
4. Oth, M. P., and Möes, A. J. 1989. Sustained release solid dispersions of indomethacin with Eudragit RS and RL. *Int. J. Pharm.* 55:157–164.
5. Palmieri, G. F., Lovato, D., and Martelli, S. 1988. New controlled-release ibuprofen tablets. *Drug Dev. Ind. Pharm.* 25:671–677.
6. Vachon, M. G., and Nairn, J. G. 1995. Physiochemical evaluation of acetylsalicycal acid-Eudragit RS100 microspheres prepared using a solvent partition method. *J. Microencapsul*. 12:287–305.
7. Kawashima, Y., Niwa, T., Handa, T., Takeuchi, H., Iwamoto, T., and Itoh, Y. 1989. Preparation of prolonged-release spherical micro-matrix of ibuprofen with acrylic polymer by the emulsion-solvent diffusion method for improving bioavailability. *Chem. Pharm. Bull.* 37:425–429.
8. Kawashima, Y., Iwamoto, T., Niwa, T., Takeuchi, H., and Itoh, Y. 1991. Preparation and characterisation of a new controlled release ibuprofen suspension for improving suspendibility. *Int. J. Pharm.* 75:25–36.
9. Cotton, M., and Hux, R. A. 1985. Diflunisal. In *Analytical Profiles of Drugs Substances*, ed. K. Florey, vol. 14, 491–526. Orlando, FL: Academic Press.
10. Kawashima, Y., Niwa, T., Handa, T., Takeuchi, H., Iwamoto, T., and Itoh, Y. 1989. Preparation of controlled-release microspheres of ibuprofen with an acrylic polymer by novel quasi-emulsion solvent diffusion method. *J. Pharm. Sci.* 78:68–72.
11. Nastruzzi, C., Esposito, E., Cortesi, R., Gambari, R., and Menegatti, E. 1993. Kinetics of bromocriptine release from microspheres: comparative analysis between different in vitro models. *J. Microencapsul*. 11:565–574.
12. Meunier, C. J., Burton, J., Cumps, J., and Verbeeck, R. K. 1998. Evaluation of the formalin test to assess the analgesic activity of diflunisal in the rat. *Eur. J. Pharm. Sci.* 6:307–312.
13. Rosland, J. H., Tjølsen, A., Maehle B., and Hole, K. 1990. The formalin test in mice—effect of formalin concentration. *Pain* 42:235–242.
14. Sawynok, J., Reid, A., and Poon, A. 1998. Peripheral antinociceptive effect of adenosine kinase inhibitor, with augmentation by an adenosine inhibitor, in the rat formalin test. *Pain* 74:75–81.
15. Tjølsen, A., Berger, O. G., Hunskaar, S., Rosland, J. H., and Hole, K. 1992. The formalin test: an evaluation of the method. *Pain* 51:5–17.
16. Kawashima, Y., Niwa, T., Takeuchi, H., Hino, T., and Itoh, Y. 1992. Controlled of prolonged drug release and compression properties of ibuprofen microsponges with acrylic polymer, Eudragit RS, by changing their intraparticle porosity. *Chem. Pharm. Bull* 40:196–201.
17. Palmieri, G. F., Wehrlè, P., and Stamm, A. 1994. Evaluation of spray drying as a method to prepare microparticles for controlled drug release. *Drug Dev. Ind. Pharm.* 20:2859–2879.
18. Pignatello, R., Vandelli, M. A., Giunchedi, P., and Puglisi, G. 1997. Properties of tolmetin-loaded Eudragit RL100 and Eudragit RS100 microparticles prepared by different techniques. *S.T.P. Pharma Sci.* 7:148–157.
19. Heun, G., Lambov, N., and Gröning, R. 1989. Experimental and molecular modelling studies on interactions between drugs and Eudragit® RL/RS resins in aqueous environment. *Pharm. Acta Helvetiae* 73:57.
20. Jenquin, M. R., Liebowitz, S. M., Sarabia, R. E., and McGinity, J. W. 1990. Physical and chemical factors influencing the release of drugs from acrylic resin film. *J. Pharm. Sci.* 79:811–816.
21. Otsuka, M., Onoe, M., and Matsuda, Y. 1993. Hygroscopic stability and dissolution properties of spray-dried solid dispersions of furosemide with Eudragit. *J. Pharm. Sci.* 82:32–38.
22. Pignatello, R., Consoli, P., and Puglisi, G. 2000. In vitro release kinetics of tolmetin from tabletted Eudragit microparticles. *J. Microencapsul*. 17: 373–383.
23. Kawashima, Y., Iwamoto, T., Niwa, T., Takeuchi, H., and Hino, T. 1993. Size control of ibuprofen microspheres with an acrylic polymer by changing the pH in an aqueous dispersion medium and its mechanism. *Chem. Pharm. Bull.* 41:191–195.
24. Perumal, D., Dangor, C. M., Alcock, R. S., Hurbans, N., and Moopanar, K. R. 1999. Effect of formulation variables on in vitro drug release and

micrometric properties of modified release ibuprofen microspheres. *J. Microencapsul.* 16:475–487.

25. Okor, R. S. 1989. Solute adsorption and concentration-dependent permeability in certain polymer films. *J. Pharm. Pharmacol.* 41:483–484.
26. Jenquin, M. R., and McGinity, J. W. 1994. Characterization of acrylic resin matrix films and mechanisms of drug-polymer interactions. *Int. J. Pharm.* 101:23–34.
27. Khalil, E., and Sallam, A. 1999. Interaction of two diclofenac acid salts with copolymers of ammoniomethacrylate: effect of additives and release profiles. *Drug Dev. Ind. Pharm.* 25:419–427.
28. Ritger, P. L., and Peppas, N. A. 1987. A simple equation for description of solute release. I: Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *J. Cont. Release* 5:23–26.