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Preparation and Characterization of Inclusion Complex of Norflurazon and β-Cyclodextrin To Improve Herbicide Formulations

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The formulation of inclusion complexes of the herbicide norflurazon as guest and β-cyclodextrin (β-CD) as host has been studied as a first step in the use of cyclodextrins to obtain improved formulations of this herbicide. The interaction of norflurazon with β-CD produced the formation of an inclusion complex in solution and in solid state. The inclusion of norflurazon in β-CD in solution was studied by phase solubility, and an apparent stability constant of 360 M^{-1}, a 1:1 stoichiometric ratio for the complex, and up to 5-fold increase in norflurazon solubility were determined. Three processing methods (kneading, spray drying and vacuum evaporation) were used to prepare norflurazon−β-CD solid inclusion complexes. X-ray diffraction, infrared spectroscopy, differential scanning calorimetry, and scanning electron microscopy techniques were used to study the solid complexes. From the different solid systems, an increase of norflurazon aqueous dissolution rate was obtained in comparison to the uncomplexed herbicide. This finding is a first step to obtain controlled release and/or protective formulations of norflurazon, which allow a more rational application of norflurazon, diminishing the use of organic solvents and increasing its efficacy.

KEYWORDS: Norflurazon; β-cyclodextrin; inclusion complex; stoichiometry.

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides, containing 6 (α-CD), 7 (β-CD) or 8 (γ-CD) α-(1,4)-linked glucose units, formed from the enzymatic degradation of starch by bacteria. The most important structural feature of these compounds is their toroid shape, with hydrophobic interior cavity and hidrophilic faces (*1*). It is well-known that they are capable of forming inclusion compounds both in solution and in solid state with a variety of guest molecules, which are placed in their hydrophobic interior cavity (*2*). CDs are widely used in pharmaceutical science (*3*) to improve the chemical stability, absorption, bioavailability, and controlled release of some drugs and the dissolution of nonpolar compounds. However, in the past decade, cyclodextrins have aroused considerable attention in many other fields (agriculture, nanocomposite technologies, chromatography, biotechnology, etc.), due to the low-cost productions of some of them.

Many synthetic pesticides can form inclusion complexes with cyclodextrins (*4*), often resulting in improvements in their chemical and physical properties, such as enhancement of solubility and bioavailability (*5*−*8*), increase in stability of photodegradable and/or unstable pesticides (*9*, *10*), catalytic effects on the degradation of pesticides (*11*), diminution of unpleasant taste and odor and controlled release (*12*), reduction of contact toxicity to humans (*13*), influence on pesticides adsorption, and leaching on soils (*14*−*16*). Some publications confirm the prediction that, in the next years, a rapid development can be expected in the application of CDs to pesticides formulations (*17*−*19*). In addition, cyclodextrins, as biodegradable enzyme-modified starch derivatives, are natural, nontoxic compounds, harmless to microorganisms, and hence not noxious for the environment (*20*, *21*).

Norflurazon was first registered as an herbicide in the U. S. in 1974. It is a selective preemergent herbicide used to control germinating annual grasses and broadleaf weeds in fruits, vegetables, nuts, cotton, peanuts, and soybeans. Norflurazon blocks carotenoid biosynthesis (by inhibition of phytoene desaturase) and chlorophyll accumulation. The typical application rate is from 3 to 5 kg/ha. Norflurazon has a poor water solubility (28 mg/L) and a vapor pressure of 2.0×10^{-8} mmHg at 20 °C (*22*). Norflurazon generally has been shown to be of low acute toxicity. It is a persistent and mobile compound (*23*); it presents a moderate to long residual persistence in soils, with a half-life of 45−180 days, depending on the type of soil, with CO_2 and desmethyl norflurazon as major metabolites (*23*).

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Norflurazon is mobile to highly mobile in soil, especially in sandy soils, with the subsequent losses of active ingredient and the possibility of groundwater contamination, and it may also contaminate surface water due to runoff (*24*, *25*). To prevent norflurazon leaching in soil, Boydston (*26*), Undabeytia et al. (*27*), and El-Nahhal et al. (*28*) prepared controlled release formulations of norflurazon, to avoid this herbicide from reaching deep soil layers and injuring perennial crops grown on sandy soils under sprinkler and deep irrigation. Photodegradation contributes significantly to field dissipation when norflurazon remains on the soil surface (*29*), with a half-life of about 41 days, and volatilization is also important on the surface of the soil (*23*).

Therefore, the aim of this study is to investigate the possibility of obtaining inclusion complexes of norflurazon with β-CD, as a first step to obtain formulations that supply a more rational use of this herbicide, improving its behavior in relation to photodegradation, leaching, and/or persistence in soils.

MATERIALS AND METHODS

Norflurazon (purity 97.8%), was kindly supplied by Novartis (Barcelona, Spain), and β-CD (99%) by Roquette (Lestrem, France). Organic solvents employed were of analytical reagent grade.

The phase solubility studies were performed according to the method reported by Higuchi and Connors (*30*). Norflurazon (5 mg) was added to aqueous solutions (10 mL) containing different concentrations of β-CD (0, 2, 4, 6, 8, 10, 11, and 12 mM). The experiments were carried out in triplicate. Solutions were shaken at 25 °C for one week. This time of reaction was chosen from preliminary kinetic studies (not shown). After that, the suspensions were filtered through a 0.22-μm Millipore glass fiber membrane filter, and the concentration of norflurazon in the filtrate was determined by HPLC equipped with fluorescence detector. β-CD was not detected using this analytical method, and therefore it did not interfere with the assay. The conditions were as follows: mobile phase, acetonitrile/water (60:40); flow rate, 0.6 mL/min; temperature, 30 °C; chromatographic column, Kromasil C18 reverse phase; fluorescence detector (Shimadzu RF-535); excitation wavelength 310; and emission 405 nm.

The apparent stability constant K_c was calculated from the straight line obtained in the phase solubility diagram, following the equation proposed by Higuchi and Connors (*30*).

$$K_c = \text{slope}/S_0\,(1 - \text{slope}) \qquad (1)$$

where S_o is the norflurazon equilibrium concentration in aqueous solution in the absence of β-CD (0.1 mM), and slope is the slope of the phase solubility diagram.

The 1:1 stoichiometric ratio employed for the preparation of the solid complexes was deduced from the phase solubility diagram. Norflurazon/β-CD physical mixture (1:1) has also been prepared to use it as reference.

Spray Drying Method. Spray drying was performed in a Büchi 190M mini Spray-Dryer equipment. For this purpose, 660 mg of norflurazon was dissolved in 400 mL of 96% ethanol. The required amount of β-CD to obtain 1:1 stoichiometry ratio was dissolved in 200 mL of distilled water. Both solutions were mixed for 15 min by sonication, to produce a clear solution, which was spray dried. The drying conditions were as follows: flow rate 10 mL s^{-1}; inlet temperature, 157 °C; outlet temperature, 90 °C; and airflow rate, 400 L h^{-1}.

Kneading Method. β-CD and norflurazon were mixed in a mortar and kneaded for 45 min. During this process, 2 mL of ethanol were added to the mixture to maintain a suitable consistency. The product was dried at 37 °C for 48 h. The dried residue was gently ground into a fine powder (<125 μm).

Vacuum Evaporation Method. β-CD and norflurazon were mixed in a 1:1 stoichiometry proportion in a hydro alcoholic mixture, where 240 mg of the herbicide was dissolved in 26.5 mL of ethanol (solution 1), and 760 mg of β-CD in 9.8 mL of distilled water (solution 2). The solutions 1 and 2 were mixed for 20 min by sonication to obtain a clear dissolution, which was evaporated under vacuum for 20 min. Finally, the product was heated at 37 °C for 48 h.

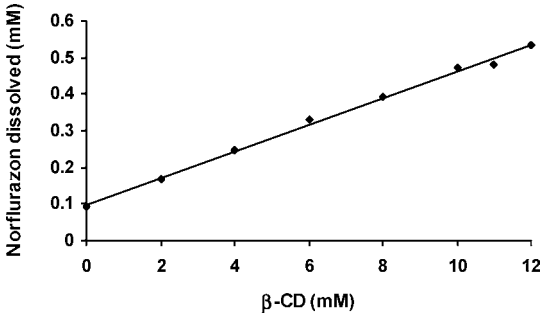

Figure 1. Phase solubility diagram of norflurazon in the presence of β-CD.

Study of Solid Complexes. The Fourier Transformed Infrared (FTIR) spectra were recorded from 4000 to 250 cm^{-1}, using a Nicolet 510-P FTIR spectrophotometer on samples prepared as KBr disks (1 mg of sample and 100 mg of KBr).

Thermal Analysis by Differential Scanning Calorimetry (DSC) was carried out using a Mettler apparatus equipped with a FP85 furnace. Samples of about 10 mg were put into aluminum pans and covered with lids which were pierced to permit the gas release during the heat process, which was performed under static air atmosphere. The conditions were as follows: heating rate, 10 °C min^{-1}; temperature range from 40 to 400 °C.

X-ray Powder Diffraction Diagrams. X-ray powder diffraction diagrams of different samples were obtained using an X-ray diffractometer Siemens model Kristallofex D-5000. The conditions used were as follows: Ni-filtered CuKα radiation, 36 kV, 26 mA; scanning speed 1° (2Θ) min^{-1}; chart speed, 1 cm min^{-1}; and adequate sensibility, usually 4×10^4 counts per second.

The morphological features of norflurazon and β-CD and norflurazon-β-CD systems (physical mixture, kneaded, spray dried, and vacuum evaporated), were studied by Scanning Electron Microscopy (SEM), using JSM-5400 equipment, employing 20 kV tension, after sputtering the samples with a thin layer of gold to make the surfaces conductive.

Dissolution Rate Study of Solid Complexes. The dissolution rate studies of solid complexes were performed according to the USP 25 paddle method using Turu Grau equipment, model D-6. The amount of each binary system employed was 21.68 mg, containing 5 mg of the herbicide, in according to stoichiometry obtained (1:1). The dissolution medium was deionized water (1000 mL), the stirring speed was 50 rpm, and the temperature was maintained at 37 ± 5 °C. Aliquots (1 mL) were withdrawn at various time intervals using an automatic pipet and analyzed by HPLC.

RESULTS AND DISCUSSION

The phase solubility diagram of norflurazon in the presence of different β-CD concentrations is shown in **Figure 1**. A linear increase up to 5-fold in norflurazon solubility is observed when increasing β-CD concentration, and a solubility limit is not obtained in the range of β-CD concentrations used. This is in agreement with an A_L classification according to Higuchi and Connors (*30*). The diagram is a straight line with a slope less than 1, and it may be ascribed to the formation of a 1:1 complex stoichiometry in solution. The apparent formation constant (K_c) was calculated according to eq 1. In our case, a value of K_c= 360 M^{-1} was obtained.

The DSC curve of β-CD shows a broad endothermic peak in the range 140−150 °C (**Figure 2a**), corresponding to a dehydration process. Norflurazon DSC-thermogram shows a characteristic single endothermic fusion peak about 180 °C (**Figure 2b**). DSC thermogram of the physical mixture (**Figure 2c**) shows the two peaks corresponding to β-CD and norflura-

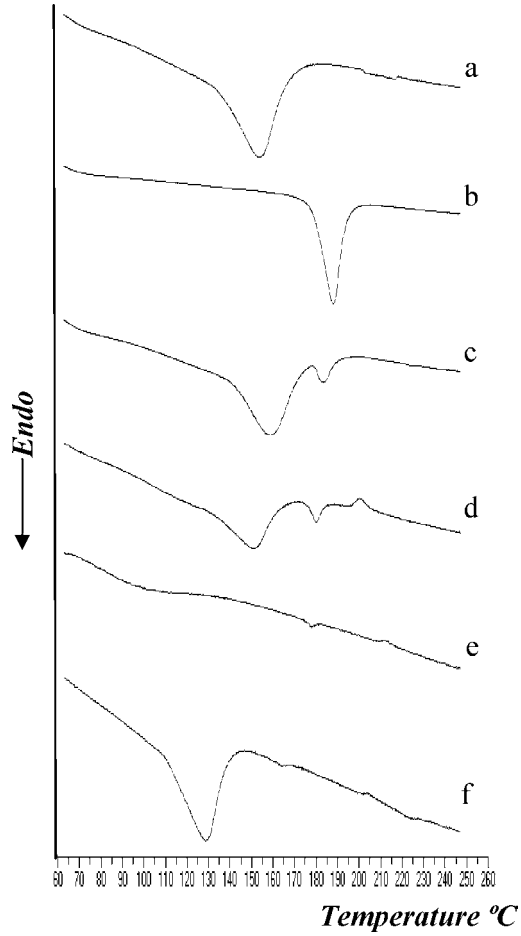

Figure 2. DSC-thermograms of β-CD (a), norflurazon (b), and 1:1 norflurazon–β-CD systems; physical mixture (c), kneaded sample (d), spray dried (e), and vacuum evaporated samples (f).

zon, indicating the absence of interaction between both components. When the kneeding method is used to prepare the complex (**Figure 2d**), the thermogram obtained is similar to that of the physical mixture (**Figure 2c**), although a small decreasing of norflurazon endothermic peak and a new exothermic peak about 205 °C, probably due to a crystalline transition, are also observed. There is also a small diminution of the dehydration effect of β-CD, indicating that a lower amount of water molecules are present at the internal cavity of β-CD. The diminution of the endothermic peak corresponding to norflurazon, and β-CD dehydration besides the appearance of a new exothermic peak, could be indicative of complexation of a small percentage of the pesticide used in the CD cavity, with the formation of a new crystalline structure. These results must be corroborated by other techniques.

In the thermogram obtained using the spray drying processing method (**Figure 2e**) a dramatic decreasing of the peaks can be observed. This fact could indicate a complexation of the herbicide into the cavity of β-CD, although it also could be attributed to the amorphization of the sample, due to the processing methods employed. In the case of using the vacuum evaporation processing method to obtain the complex (**Figure 2f**), only an endothermic peak at 120 °C corresponding to the dehydration of CD is observed in the thermogram, causing the norflurazon endothermic effect to dissappear. That the endothermic peak corresponding to dehydration peak is displaced to lower temperatures (from 150 to 120 °C) indicates that the bindings between the hydration water and the CD are weaker,

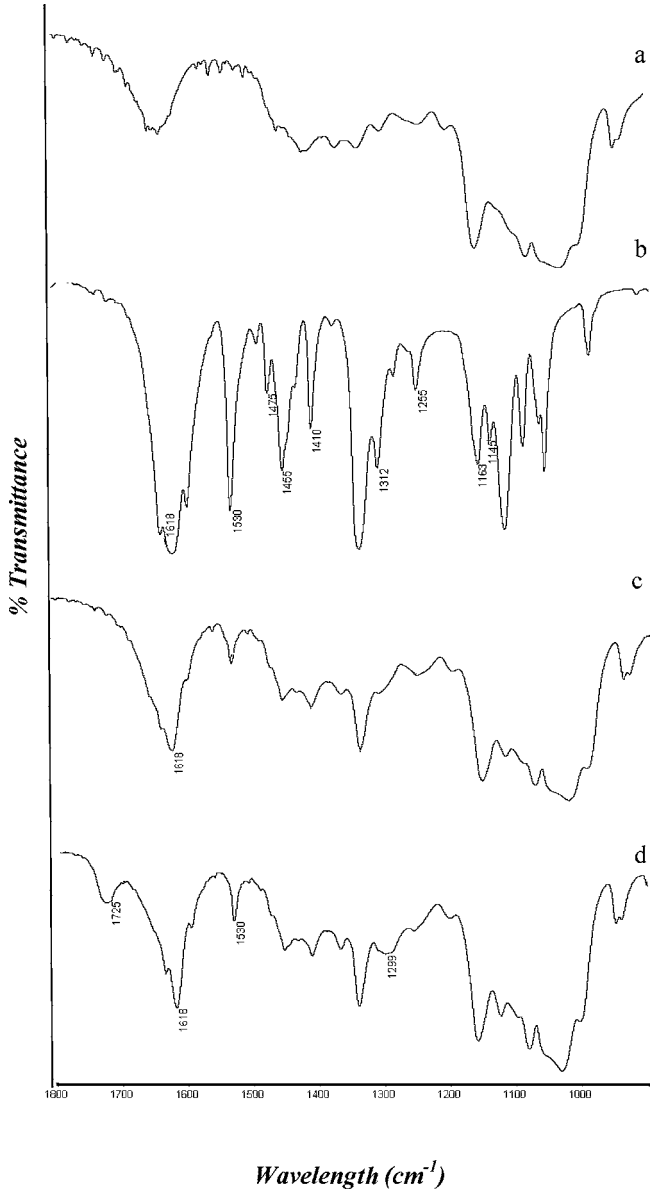

Figure 3. FTIR spectra of β-CD (a), norflurazon (b), and 1:1 norflurazon–β-CD systems; physical mixture (c), and vacuum evaporated sample (d).

and it is probably due to the interaction with norflurazon molecules that is taking place in the cavity of the cyclodextrin.

Figure 3b shows the norflurazon IR spectrum in which some absorption bands are observed due to the vibration modes of the pyridazinone ring at 1410 and 1530 cm^{-1}, the aromatic C−N stretching frequency at 1339 cm^{-1}, the aliphatic C−N streching at 1255 cm^{-1}, and the C−F stretching frequency at 1312, 1163, and 1145 cm^{-1} (*31*)**.** The absorption bands at 1475 and 1455 cm^{-1} can be attributed to skeletal ring breathing modes.

Although the carbonyl group in ketones exhibits a strong band at about 1725−1705 cm^{-1} (*32*), in α, β-unsaturated β-amino ketones (as is the case of norflurazon), a chelation effect has been described that gives rise to a very considerable shift of the carbonyl frequency. Instead, a very broad band, estimated to be more than a hundred times as strong as the normal carbonyl vibration, is observed in the range 1639−1538 cm^{-1} and in the case of norflurazon is about 1618 cm^{-1}. This absorption arises from a carbonyl group, which has its double-bond character reduced by resonance between the amino and the ketonic groups, in which N and O can act as electron donors or aceptors. The bands produced are very much stronger than normal hydrogen

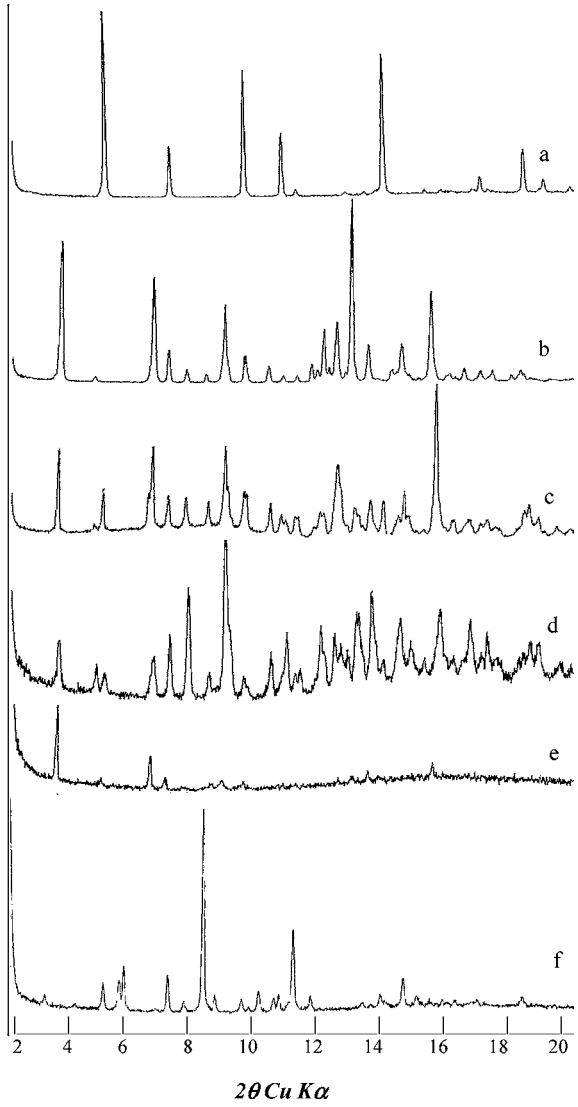

Figure 4. X-ray diffraction patterns of norflurazon (a), β-CD (b), and 1:1 norflurazon–β-CD systems; physical mixture (c), kneaded (d), spray dried (e), and vacuum evaporated samples (f).

bridges. For this reason, the NH stretching frequency (3335 cm^{-1}, not shown) is lower than is usual for secondary amines.

In the β-CD spectrum (**Figure 3a**), many intensive and sharp bands can be observed in the range 1030−1160 cm^{-1}, which can be associated with the primary and secondary C−OH groups stretching frequency.

Little or no observable changes occur in many cases, due to the overlapping of the herbicide characteristic bands with those of β-CD, which is the main component. For this reason, IR spectroscopy is not very useful in the caracterization of cyclodextrin complexes. Among the different processing methods used in the present paper, only the FTIR study of the complex obtained by the vacuum evaporation method gave the clearest evidence of complex formation with β-CD (**Figure 3d**). The spectrum of the sample obtained clearly shows a new absorption band at 1725 cm^{-1}, which could correspond to the formation of the inclusion complex, because its presence indicates that the chelation between carbonyl and amine groups does not exist, due to the fact that the secondary amine group is involved in the formation of a complex with β-CD, thus the vibration frequency of the carbonyl group changes to its normal value (1725 cm^{-1}).

However, the band at 1618 cm^{-1} does not disappear completely, which would indicate that only a part of norflurazon employed in the system is complexed into β-CD cavity, confirming that the product obtained is not homogeneous. A new band appears at 1299 cm^{-1}, which would indicate the formation of the inclusion complex through CH_3−N or CF_3 groups, because their corresponding bands (1255 and 1312 cm^{-1}, respectively) have disappeared, and only the new band at 1299 cm^{-1} is present.

X-ray diffraction (XRD) studies have been carried out because the XRD pattern of the complexes should be different from those corresponding to isolated β-CD and norflurazon. XRD patterns of pure norflurazon, β-CD, and the binary systems obtained under different processing methods are included in **Figure 4**. The XRD patterns for pure norflurazon and β-CD show the high crystallinity of both solids (**Figure 4**, parts **a** and **b**). The XRD pattern corresponding to the physical mixture shows an overlaping effect of the diffractions of both isolated compounds (**Figure 4c**). In the XRD pattern of the kneaded sample (**Figure 4d**), the diffractogram is similar to that of the physical mixture.

Figure 4e, corresponding to the XRD pattern of the sample obtained by the spray drying processing method, exhibits a large decrease in the number of diffraction peaks, suggesting that the sample is less crystalline than the isolated compounds. This result could confirm the formation of a new amorphous inclusion complex, because the application of the spray drying processing technique over the isolated compounds (norflurazon and β-CD)

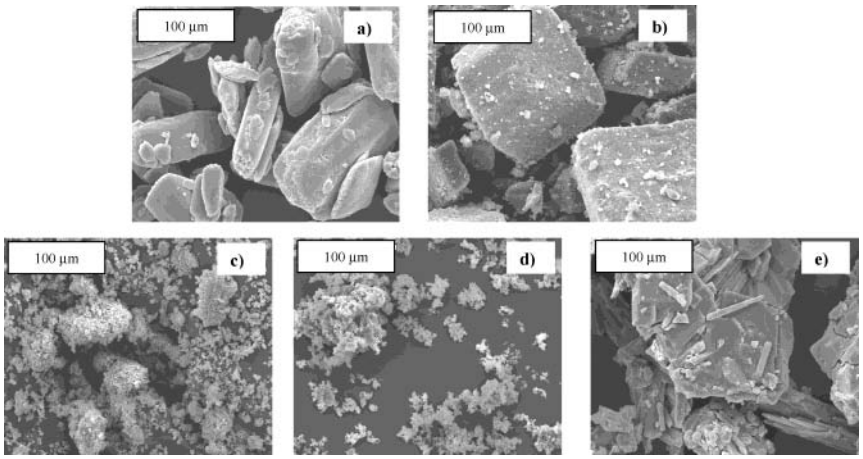

Figure 5. SEM micrographs (×200) of norflurazon (a) and 1:1 norflurazon–β-CD systems; physical mixture (b), kneaded sample (c), spray dried sample (d), and vacuum evaporated sample (e).

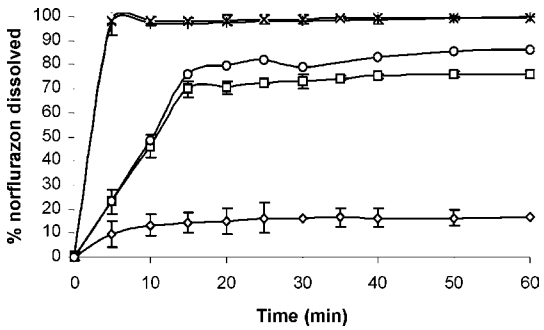

Figure 6. Dissolution profiles in water of norflurazon (◇) and 1:1 norflurazon–β-CD systems; physical mixture (□) kneaded sample (△), spray dried sample (×), vacuum evaporated sample (+), and physical mixture spray dried (○).

did not provoke a diminution of their respective diffraction peaks. In the sample obtained by vacuum evaporation (**Figure 4f**), new diffraction peaks corresponding to a new cristalline compound can be observed, indicating the formation of the inclusion complex. This result is according to those obtained by IR and supports the theory that norflurazon and β-CD form a true inclusion complex in solid state.

Examination by SEM of the samples obtained using different processing methods revealed that β-CD crystallizes in polyhedral form (not shown) and pure norflurazon appears as crystal particles with large dimensions (**Figure 5a**). The physical mixture shows both crystalline components (**Figure 5b**). In the micrographs of the samples prepared by different processing methods (**Figure 5**, parts **c**, **d**, and **e**) a dramatic reduction of the particle size can be observed in the case of using kneading and spray drying methods, which would provoke a high agglomeration of the particles, increasing the wettability of norflurazon. This decreasing of particle size was reflected in X-ray diffraction study, where a loss of crystallinity or amorphization of the samples could be observed. However, a new crystalline product is obtained when the vacuum evaporation processing method was employed, corroborating those results obtained by XRD.

Dissolution Rate Studies. The dissolution profiles in water of pure norflurazon and norflurazon-β-CD binary systems are reported in **Figure 6**. A strong increase of the dissolution profile was observed for the physical mixture with respect to the free herbicide, reaching a plateau after 15 min. The rapid dissolution of the herbicide observed in the physical mixture can be explained by the formation of an inclusion complex with β-CD in solution, corroborating the high K_c value obtained for this complex (360 M^{-1}). However, for the systems obtained by different processing methods (kneading, spray drying, vacuum evaporation), 100% of norflurazon was dissolved after only 5 min (in comparison to the maximum percentage dissolved of pure norflurazon, 15% after 30 min), and a stronger increase of the dissolution profile can be observed (Figure 6).

For the kneaded sample, a strong increase of the dissolution profile is observed, despite the small percentage of solid complex obtained by this processing method, as was demonstrated previously by DSC. This can also be due to the high reduction in particle size obtained by this method, as could be observed by SEM studies. To test if the dissolution profile obtained for the spray dried sample is only due to the particle size decreasing of the binary system (as in the case of kneaded sample) or to the complexation of the herbicide, every isolated compound (Norflurazon and β-CD) was spray dried to reduce their particle size, and subsequently, a new physical mixture was prepared with both spray dried compounds. The dissolution profile obtained is slightly higher than that obtained with the physical mixture but lower than those obtained by spray drying. It indicates that the improvement of the dissolution profile for the spray dried sample (**Figure 6**) is due to the complexation of the herbicide into the cavity of the β-CD. In the case of using the vacuum evaporation method, the increase of the dissolution rate is clearly in relation to the formation of the inclusion complex with β-CD, in agreement with the results obtained by DSC, XRD, and IR techniques, where a new crystalline product after vacuum evaporation was observed.

Among the three methods used for preparing β-CD-norflurazon complexes, probably the preferable method for real life application would be the kneading method, because it is less expensive than the others and leds to the same dissolution results, despite the small percentage of solid complex obtained.

The formation of inclusion complexes between norflurazon and β-CD gives the possibility of using cyclodextrins to remove herbicides, such as norflurazon, or other toxic substances, from contaminated soils, leaving these toxic substances more readly available for degradation by microorganisms. In this case, cyclodextrins can be viewed as microscopic organic-phase extractants, as it occurs in the case of other organic pollutants in soils (*20*, *21*, *33*, *34*). On the other hand, the application of herbicides-CDs solid complexes to soils could improve some physicochemical properties of encapsulated molecules. Application to the soils of the solid and solubilized inclusion complexes norflurazon-β-CD are in progress, to improve its behavior on leaching processes through soils and diminish its losses due to photodegradation and volatilization.

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