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Chemical characterization with XPS of the surface of polymer microparticles loaded with morphine

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Abstract

Hydrophilic matrices are a potentially useful option for the development of oral controlled–release formulations. The porous surface of these particles makes it possible to control or modify release of the active principle after administration. As a result, such formulations can be used in liquid controlled–release pharmaceutical formulations. We investigated a method of spontaneous drug encapsulation to prepare ethylcellulose polymer microparticles (since the polymer is synthetic rather than natural the final suspension is called pseudolatex) filled with morphine hydrochloride. Morphine is incorporated to water during the synthesis process and thus it is microencapsulated inside the micelles that give rise to the final microparticles. X-ray photoelectron spectroscopy (XPS), a technique that can identify elements in a sample without destroying it, was used for the chemical analysis of the surface of these microspheres. The results demonstrated the complete absence of morphine from the microsphere surface, which was taken as evidence that the drug had been completely encapsulated.

Keywords: X-ray photoelectron spectroscopy; Ethylcellulose; Drug encapsulation

1. Introduction

Microencapsulation of the active principle is one of the most widely used methods to obtain controlled–release dosage systems (Robinson and Manger, 1991; Banerjee and Robinson, 1991). In this connection, colloidal particles have attracted considerable interest because of their high loading capacity for the drug as a function of the size and surface characteristics of the particle. Microparticles or microcapsules may in themselves constitute a pharmaceutical formulation, or may be used as a component in secondary formulations. This makes it possible to prepare suspensions of the drug, if this route of administration is suitable (Gallardo et al., 2000; Puisieux, 1985).

Microparticle systems reduce the number of doses needed, as plasma concentrations of the drug are maintained within the therapeutic range with no fluctuations (Goldman, 1982). This is especially useful for active principles that undergo intense first-pass metabolism or that have a short half-life. Morphine hydrochloride is an example of a drug that meets both of these conditions (Florey, 1985). We therefore developed an encapsulated form of the drug with ethylcellulose polymer microspheres for the oral administration of a controlled–release pharmaceutical formulation. We chose this cellulose derivative because of its versatile properties and applications (Rekhi and Jambherkar, 1995).

Interest in the surface properties of particles has increased considerably in the last 20 years (Duke, 1994; Duke and Plumier, 2002). One of the most sensitive and therefore informative techniques (Briggs and Seah, 1996; Beamson and Briggs, 1992) is X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA). With this technique, the surface of the sample is bombarded with X-rays, and the kinetic energy of the ejected photoelectrons is measured. The difference in energy between incident X-rays and the kinetic energy of the photoelectrons is used to determine the binding energy and oxidation states of different atoms in the system. In the case of a solid, the free short distance covered by the electrons, limits the analyzed zone to a superficial layer with a depth between 1 and 10 nm. The values of binding energy are used to clarify the chemical environment of the atom or to quantify a

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determined element in the sample. In other words, the technique makes it possible to identify and quantify all elements in the sample without destroying it (Chen and Gardella, 1998; Leinen et al., 1999).

In this study we used XPS to determine the success of the encapsulation process by the chemical analysis of the particles in the polymer surface. XPS is also used to verify that no drug molecules are adsorbed in the polymer surface. This technique is not particularly sensitive when applied to a large, homogeneous sample, since the detection limit is approximately 1–0.1% depending on the analytical conditions. In contrast, in monolayer analysis the limit of sensitivity can reach 1% (∼1013 atoms cm^{-2} and $\sim 10^{-9}$ g cm^2). Although there are other techniques used for the same purpose, such as thermal analysis or infrared analysis, XPS is highly useful in assays to determine the extent of encapsulation, in spite of its relatively uncommon use (Oliva et al., 2002, 2003).

2. Experimental

2.1. Materials

Pseudoatex was prepared in accordance with the technique proposed by Vanderhoff et al. (1979), with some modifications. Specifically, we omitted the stabilizing emulsifier (cetyl alcohol) and increased the amount of sodium lauryl sulfate. The ethylcellulose polymer (9004-57-3) was supplied by ICN Ohio (Aurora, OH, USA).

All chemicals were of analytical grade, and were from Panreac (Barcelona, Spain). Water used to prepare the solutions and suspensions was of Milli-Q quality (Milli-Q Academic, Millipore, Gif-sur-Yvette, France).

2.2. Ethylcellulose pseudolatex preparation and particle analysis

Because the cellulose polymer is not soluble in aqueous media, it was prepared by polymer emulsification. The first step in synthesis is dissolving ethylcellulose in a suitable mixture of solvents (ethanol and benzene at a proportion of 15:85) and emulsifying the monomers in water, with sodium lauryl sulfate at a concentration of 0.4%. Emulsification was achieved by mechanical stirring. Then organic solvents were also removed by mechanical stirring, and the final pseudolatex was washed by dialysis with distilled water.

The resulting suspension had a solid content of about 4.5% (w/v). Shape, surface characteristics, size and size distribution of the final polymer particles were investigated by scanning electron microscopy (SEM). Two mean particle diameters were found: most particles were 3–5 μm, and about 10% were approximately 20 μm (Fig. 1). All particles were spherical and had a porous surface (Blankenship et al., 1996). These polymers possess the characteristics of a true latex in terms of colloidal stability, particle size uniformity, film forming properties, etc., and pH was 6.5 ± 0.1.

A 30% ethylcellulose pseudolatex is commercially available to the industry (Aquacoat, FMC Corp., Philadelphia, PA, USA).

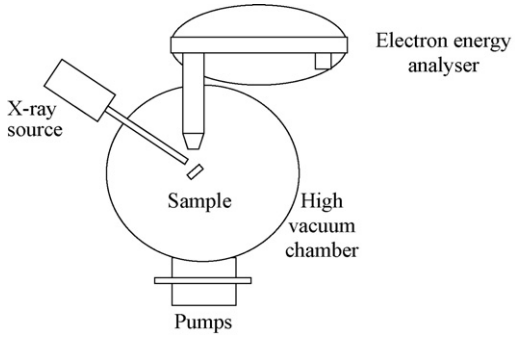

Fig. 1. Scheme of the X-ray photoelectron spectroscope.

The differences between Aquacoat® and our latex lie mainly in solid concentration and particle size (Aquacoat® < 1 μm).

Our formulation has advantages over other pseudolatexes (Banker and Rhodes, 1990; Vera et al., 1996), the most important of which may be its easy synthesis in laboratory. Accordingly, different formulations can be prepared with the active drug. Concretely, morphine is added to water and thus, in the process of micelles formation, drug molecules are located inside these micelles (Morales et al., 2004). In previously published works we have demonstrated that the concentration of morphine in the aquose supernatant of the ethylcellulose suspension was lower than 10% of the total morphine (Morales et al., 2004).

2.3. X-ray photoelectron spectroscopy

For XPS we used a Physical Electronics 5500 spectroscope (Fig. 1), a model able to detect all elements in the periodic table except hydrogen, at a standard working depth of 10 nm. For all samples, spectra were recorded over a range of binding energies from 0 to 1100 eV, with a pass energy of 180 eV for the wide scan survey spectrum and a pass energy of 23.5 eV for high energy resolution spectra for regions of C 1s, N 1s, O 2s, Cl 2p and S 2p. The sample chamber was kept under ultra-high vacuum at a base pressure of 5×10^{-9} Torr.

The resolution at which all spectra were obtained was calculated by taking the center of the peak, at half-height, for a known, unique assignment. This width was used to estimate the resolution for binding energy measurements for carbon 1s.

3. Results and discussion

Colloidal microparticles were prepared with an emulsion–polymerization process (Couvreur et al., 1995; Douglas et al., 1997). For spontaneous encapsulation of the drug to occur, the drug must be added to the water used during synthesis of the formulation. This ensures that when the micelles that give rise to the polymer microparticles are formed, the morphine is trapped inside the microspheres.

We compared our spectra and binding energies with values reported in earlier studies of similar compounds (Chan, 1994; Garbassi et al., 1994; Ferraria et al., 2003).

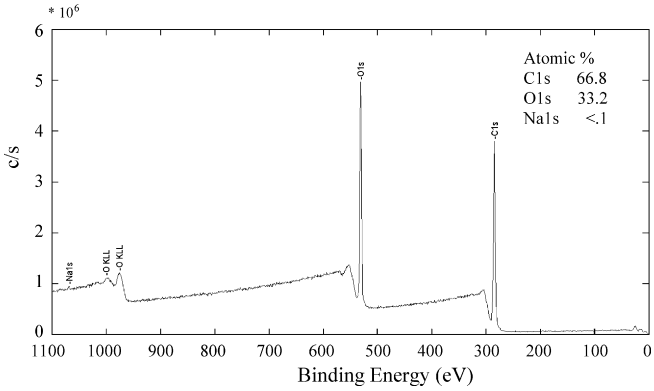

Fig. 2. Wide scan spectrum for ethylcellulose.

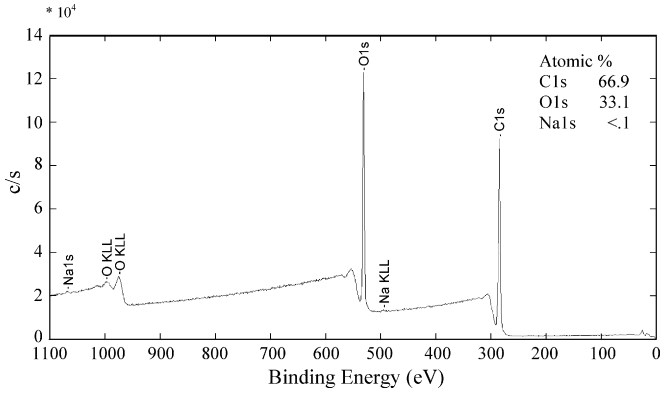

Fig. 3. Wide scan spectrum for latex particles.

3.1. Wide scan spectra

Figs. 2 and 3 show the wide scan spectra for the ethylcellulose polymer and latex particles. A notable feature of both spectra is the high carbon content, reflecting the organic origin of ethylcellulose polymer. The only clear difference between the spectra is a small peak for sodium in the latex spectrum, reflecting the emulsifier (sodium lauryl sulfate) used during synthesis.

The spectrum for morphine hydrochloride (Fig. 4) shows a characteristic nitrogen atom in the pyrimidine ring.

The spectrum for particles loaded with the active principle is similar to the spectrum for latex (Fig. 5). The absence of a peak

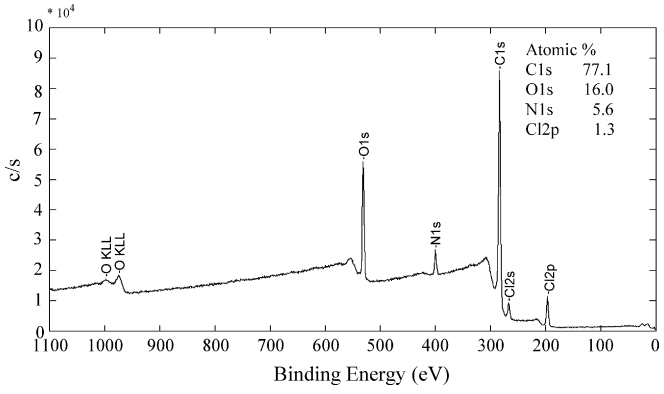

Fig. 4. Wide scan spectrum for morphine hydrochloride.

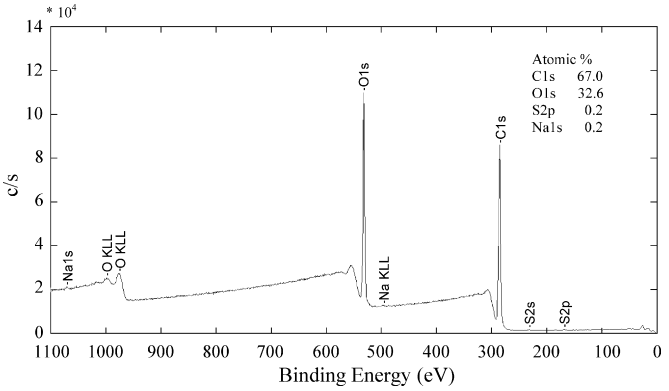

Fig. 5. Wide scan spectrum for morphine-loaded particles.

for nitrogen indicates that none of the drug was present on the particle surface.

3.2. High energy resolution C 1s spectra

Because the energy of the core electrons is modified if the chemical environment of the atoms changes, different chemical states of the atom within the compound can be identified, as can the presence of different chemical compounds in a heterogeneous sample.

We used the adventitious carbon reference technique to assign a binding energy of 284.8 eV to saturated hydrocarbons. The C 1s spectra show that the resolution used to measure energy at the 1s carbon level was 1.1 eV, a value considered appropriate for our analytical purposes.

The percentage compositions obtained with XPS are atom percentage values. These percentage compositions differed substantially from the values for bulk samples, evidence that the surface composition differed between the interior pores and the surface.

In the ethylcellulose molecule, carbon atoms can be grouped into three components: C–C/C–H, C–O and O–C–O. As noted in Section 2, to manually fit the peak the number of components needs to be defined. In the present case, the spectrum can be deconvolved into three components with binding energies of 284.59, 286.18 and 287.81 eV. A binding energy of 284.59 eV was assigned to carbon atoms bound only to other carbon atoms or to a hydrogen atom, and this value was used as a reference to determine all other shifts. For the spectra we obtained, this procedure yielded values of 27.55% for C–C/C–H, 64.64% for C–O and 7.82% for O–C–O.

In the morphine molecule (Fig. 6), carbon atoms can be grouped into four components. A reference binding energy of 284.74 eV was assigned to the carbon atoms at C13, C14 and C15. The carbon atoms at C7 and C8, as well as those at C9, C16 and C17, had a binding energy of 285.75 eV. Aromatic carbons, which are unaffected by oxygen induction, were assigned a binding energy of 286.58 eV. Carbon atoms bound to oxygen were then assigned a binding energy of 287.48 eV. This shift, which is greater than that used to deconvolve the ethylcellulose spectrum, can be explained by the inductive effect of the cyclic structures within which they are located.

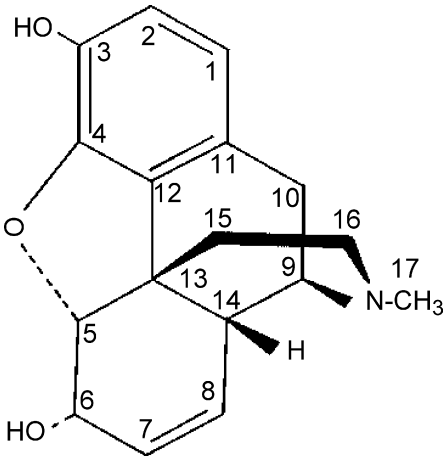

Fig. 6. Chemical structure of morphine.

Table 1
Binding energies and chemical shifts as a result of deconvolution of the spectra for latex and microspheres with morphine hydrochloride

Species	Binding energy (eV)	Shift	Area (%)
Latex	284.60	0.00	27.13
	286.25	1.65	64.92
	287.90	3.30	7.95
Microspheres with morphine	284.38	0.00	28.18
	285.89	1.50	64.36
	287.37	2.98	7.46

Deconvolution of the spectrum for complex species of latex and of the microspheres yielded three binding energies, as shown in Table 1. Comparison of these values shows that the surface of the particles is similar in composition to latex, and to ethylcellulose.

To refine the analysis, we compared the difference spectra with point-by-point substraction. The addition spectrum for the combination of latex and morphine was first obtained, then the difference spectra were found for microspheres without latex and morphine. The difference spectrum for morphine particles (Fig. 7) was not negative, a result that suggests that components

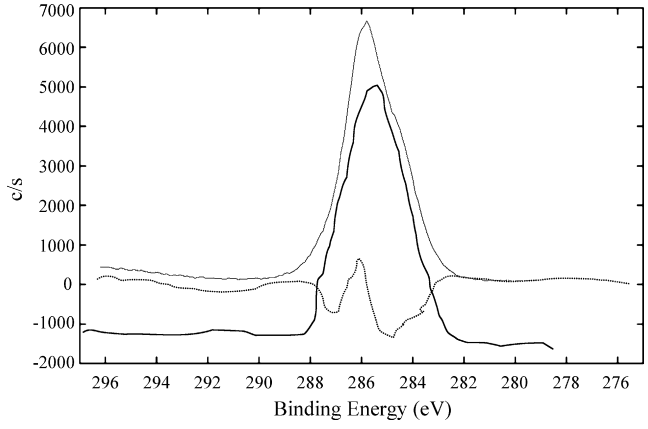

Fig. 7. Difference spectrum (discontinue line) for morphine-loaded microspheres (continue line) and morphine alone (continue black line).

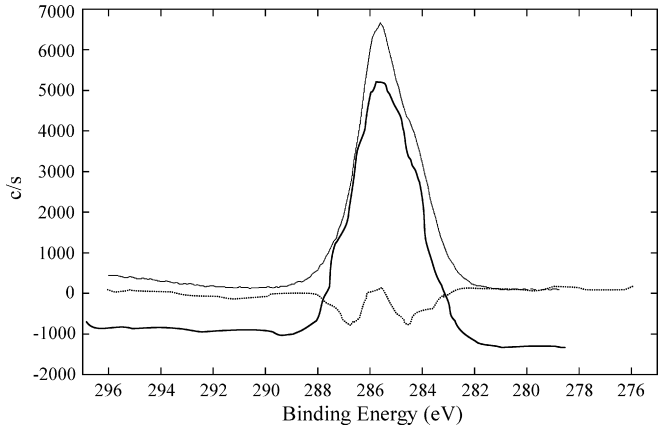

Fig. 8. Difference spectrum (discontinue line) for morphine-loaded microspheres (continue black line) and morphine + latex (continue line).

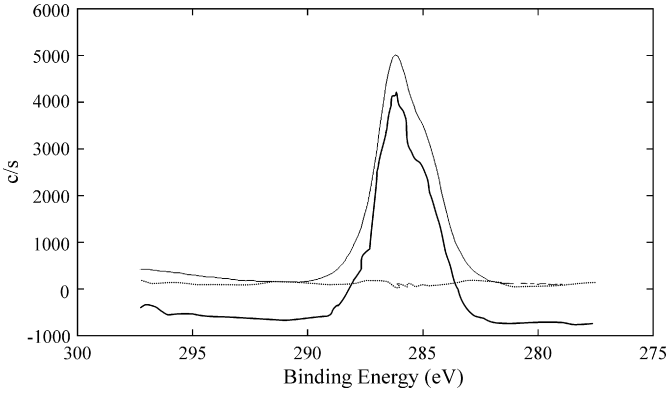

Fig. 9. Difference spectrum (discontinue line) for morphine-loaded microspheres (continue line) and latex alone (continue black line).

associated with binding energies of 285 and 287 eV, if present in the interior of the microspheres, were present in very low amounts. The resulting spectrum matches neither that of latex nor that of ethylcellulose; overlap between the spectra would be evidence of the presence of morphine on the surface.

Fig. 8 shows the difference spectrum for the microspheres, and the addition spectrum for morphine and latex. The resulting spectrum is not flat, and does not match the spectrum of any of the components in the system. Fig. 9 shows the difference spectrum for polymer microspheres and latex. The resulting flat spectrum is strong evidence that the surface of the microspheres consisted exclusively of latex, and that no morphine was present on the surface.

4. Conclusion

The most significant conclusion suggested by the wide scan spectra is that the absence of a peak for nitrogen in the particle spectra is clear evidence of the absence of morphine molecules adsorbed on the particle surface. This result was confirmed by subtracting the difference spectra obtained for "pure" microspheres and for their components from spectrum for bulk sample microspheres after synthesis, and by deconvolution analysis of these spectra. These data together with the low concentration

of morphine in the aquose supernatant of the ethylcellulose suspension (data not shown), strongly suggest that morphine hydrochloride was completely encapsulated inside the ethylcellulose polymer microspheres assayed in these experiments. Since XPS is not a destructive technique, we did not evaluate the presence of morphine inside the particles. Further studies are needed to elucidate this aspect.

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