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Determination of Enantiomeric Excess of Cyclophosphamide by X-Ray Powder Diffraction (XRPD)

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The anticancer agent: cyclophosphamide **1** was studied by X-ray powder diffraction. The linear correlations between intensity/spectra from X-ray powder diffraction/and enantiomeric excess was observed.

Key words: cyclophosphamide, X-ray powder diffraction, enantiomeric excess

Today, the complete characterization of pharmaceutical solids (including bulk drugs, excipients, physical mixtures, formulated product, and placebo) is a requirement for the consistent, reliable and safe development of drug products. The crucial step in the characterization of pharmaceutical solids involves studies at the molecular level. Ideally suited for this task are the various molecular spectroscopy techniques such as infrared, Raman and nuclear magnetic resonance (NMR) spectroscopy, which provides insight into the local environment of each NMR active atoms. Most of drugs are solid, so very useful for these study is solid state NMR /SS NMR/.

Many solids can be prepared only as microcrystalline powders and therefore not suitable for structural characterization by conventional single-crystal diffraction methods. For such materials, it is necessary to tackle structure determination using powder diffraction data.

It's known that two enantiomers gave the different pharmacological response, for instance: only S-enantiomer of propranolol is an antihypertensive and antiarytmic used in the treatment of heart disease and only (–)-enantiomer of alkaloid leverphanol is a potent narcotic analgesic; their enantiomers have none of these activities, so pharmaceutical companies tend to produce chiral drugs in single enantiomeric forms.

The enantiomeric excess (*ee*) can be determined by the following principal methods: (a) chiroptical, (b) chromatography (GS, HPLC, TLC), (c) isotopic dilution, (d)

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kinetics, (e) electrophoresis, (f) calorimetry, (g) nuclear magnetic resonance but any new method able to control the enantiomeric purity are strongly desired.

In our recent paper we shown [1], that ODESSA (One Dimensional Exchange Spectroscopy by Sideband Alteration) technique in SS NMR [2] permits to distinguish enantiomer from racemate for some oxazaphosphorinanes as well as to assess the enantiomeric excess in different compositions of the cyclophosphamide samples. Cyclophosphamide **1**, 2-[bis(2-chloroethyl)amino]-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide (Figure 1) is one of the most frequently used anticancer agents [3].

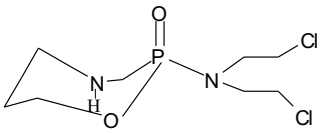

Figure 1. Cyclophosphamide.

It belongs to the group of medicines called alkylating agents and it is used to treat cancer of the ovaries, breast, blood and lymp system, nerves, retinoblastoma (a cancer of the eye), multiple myeloma (a cancer in the bone marrow) or mycosis fungoides (tumors on the skin) [4,5,6]. This alkylating substance is not active itself but requires in the first step an enzymatic activation in the liver to both active antineoplastic alkylating agents and inactive metabolites [7,8,9]. The active metabolites alkylate nucleic acids, thus interfering with the growth of neoplastic and normal tissues. The cytotoxic action is due to cross-linking of strands of DNA and RNA and inhibition of protein synthesis.

Cyclophosphamide is a white crystalline powder. Its intensive structural studies were performed mostly by X-ray crystallography [10–15].

Now we present determination of enantiomeric excess in the mixtures of this compound using XRPD.

RESULTS AND DISCUSSION

X-ray Powder Diffraction. The X-ray powder diffraction is a powerful technique for the identification of powders. The underlying crystal structure of matter will, in principle, give rise to a unique powder diffraction pattern – diffractogram – (which expresses the existence of lattice planes in materials) for each pure compound and for each individual component in a mixture of compounds. It is a unique "fingerprint" of the compound.

Nowaday, when atomic parameters are available from single crystal structure determination, the XRPD pattern expected for samples can be simulated by special computer program, which the main aim is the calculation of the diffraction pattern. Crystal structure of cyclophosphamide (racemate and enantiomers) is known, so we simulated spectra racemate and enantiomers of cyclophosphamide and the mixture of both of them using program PowderCell 2.4 and compared them to these obtained experimentally. They look very similar (Figure 2 and 3).

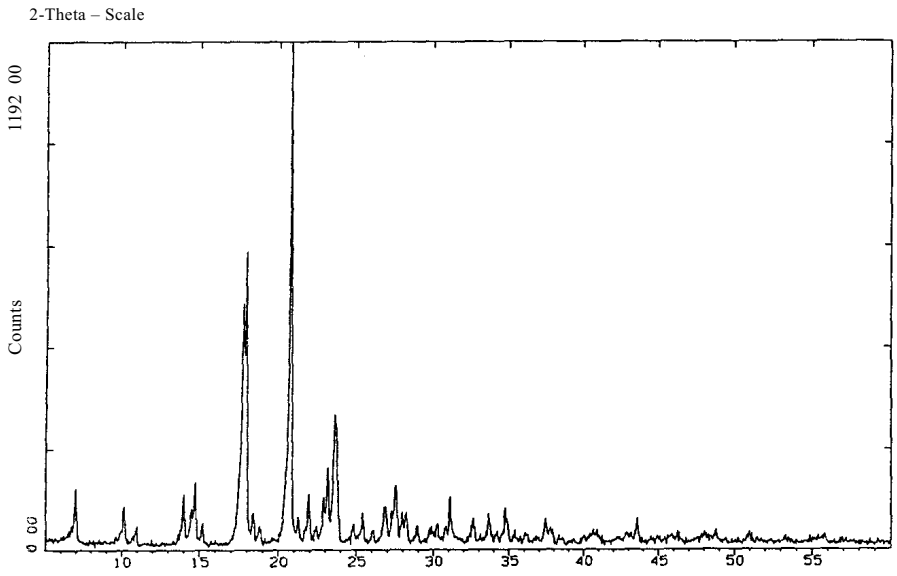

Figure 2. Diffractogram (experimental) of the sample of cyclophosphamide *ee* = 50%.

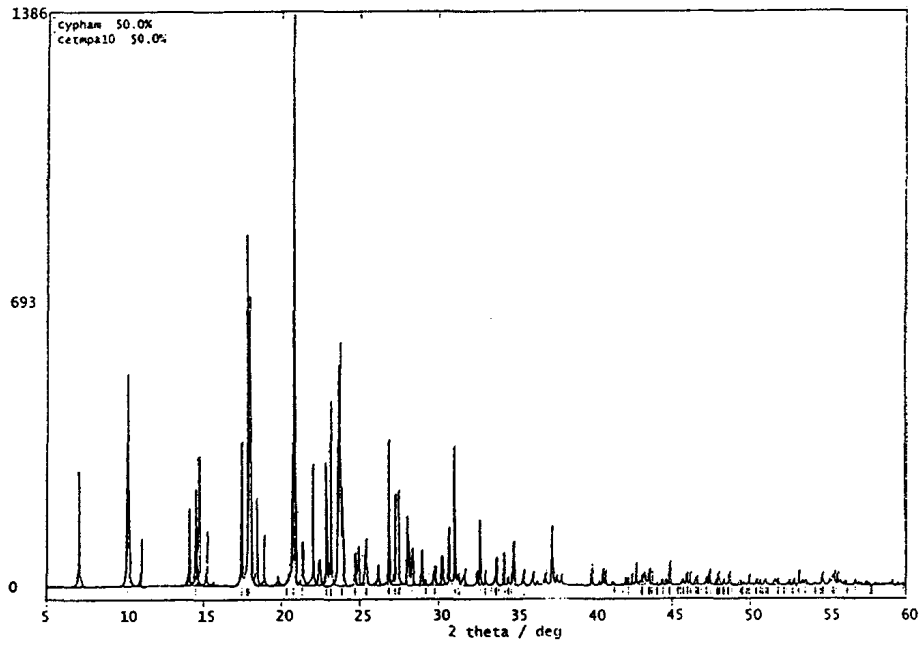

Figure 3. Diffractogram (simulated) of the sample of cyclophosphamide *ee* = 50%.

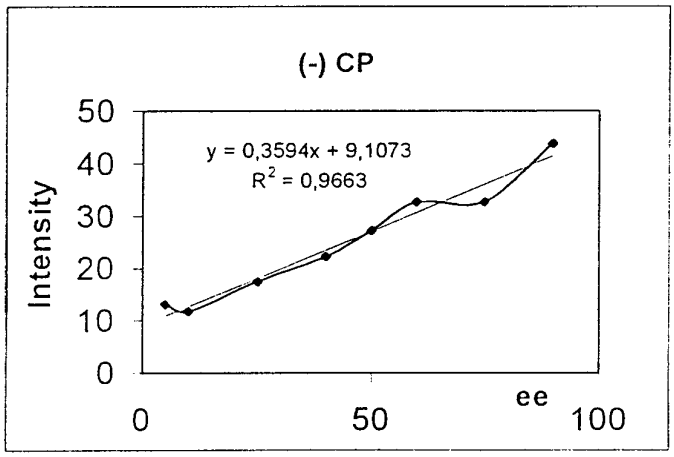

Figure 4. Linear correlation between intensity and *ee* (for enantiomer (–)CP) in samples of cyclophosphamide (reflex: $\theta = 10°$).

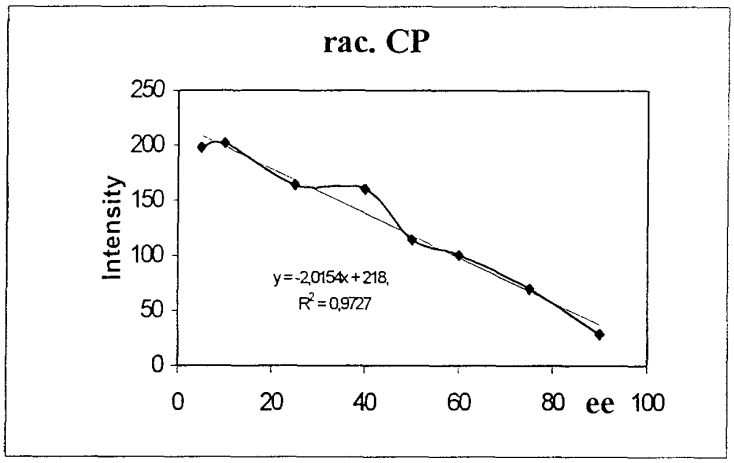

Figure 5. Linear correlation between intensity and *ee* (for racemate CP) in samples of cyclophosphamide (reflex: $\theta = 23°$).

Next we obtained experimental X-ray powder diffraction patterns for the samples of racemate and enantiomer of cyclophosphamide, which were mixed in different proportions. The *ee* values were: 5%, 10%, 25%, 40%, 50%, 60%, 75%, 90%. The X-ray diffractograms were recorded using a D-5000 diffractometer and CuK_α (Ni-filtered) radiation. The measurements were made in the $2\theta = 2–80°$ range. The area of the peak was computed by EVA version 3.09 from diffrac at program PACKAGE with ω 2θ scan mode.

We observed a linear correlation between intensity (the area of the peak) of racemate (decrease) or enantiomer (increase) and *ee* (Fig. 4 and 5).

A satisfactory correlation (with regression coefficient of $R^2 = 0.97$ for racemate and $R^2 = 0.96$ for (–)CP) means that the method may be applied to probe the *ee* values in this case.

Thus, when we have diffractogram a sample of cyclophosphamide with unknown *ee* we may determined *ee* this sample using XRPD.

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