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In-line reaction monitoring of entacapone synthesis by Raman spectroscopy and multivariate analysis

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ABSTRACT

In-line Raman spectroscopy and multivariate analysis were used to monitor Knoevenagel condensation reaction, the final step in preparation of drug entacapone. By applying a fiber optical Raman probe immersed into a reaction vessel Raman spectra of the reaction mixture were recorded *in situ* during the entacapone synthesis in toluene, heptane and isobutyl acetate. Due to the complexity of the measured spectra, the obtained data were analyzed and interpreted by means of principal component analysis.

It has been shown that progress of this reaction can be monitored in real-time and reaction end points can be determined in different solvents. The reaction was found to be the fastest in heptane due to the lower loss of the catalyst. For a comparison the reaction was independently monitored by *off-line* Raman spectroscopy and liquid chromatography which confirmed the results obtained *in-line*.

The results presented here have shown that this *in-line* approach can be used as a fast, non destructive and reliable method to monitor the Knoevenagel reaction in real time. The knowledge gained in this study can further be exploited for the industrial process control.

1. Introduction

In recent years, it has become obvious that for the production of drugs new challenges have to be dealt with including the control of parameters defining the quality of particles during the preparation process as well as isolation of the active substances. The use of sophisticated, advanced and efficient analytical methods and techniques is of utmost importance in modern pharmaceutical manufacturing processes [1–3]. The standard monitoring procedures usually include analytical measurements with samples taken out of the chemical reactor and hence do not provide information on chemical reactions in real time. The transport of sample may cause many dynamic processes to occur which can alter the composition of the reaction mixture and lead to significant measurement errors. Major limitations to improve the control of physical and chemical processes arise from the lack of versatile, accurate and reliable *in-line* sensors. Hence, new approaches and methodologies are needed for a better quality control and higher product efficiency. The main advantages of *in-line* methods are that they are not time-consuming and offer a non-destructive, non-invasive and real time detection of different physical and chemical transformations of the reactants and products during the laboratory and production processes. It is very important to monitor the chemical reaction in real time to ensure that it is proceeding as expected and to change the reaction conditions if deviations of the expected reaction profile occur or even to stop the reaction to prevent the waste of a batch.

Vibrational spectroscopy (IR and Raman) has been applied extensively for the characterization of pharmaceuticals. Drug crystallization process, polymorphism, production of dosage forms and chemical reactions of drugs have recently been studied by *in situ* or *in-line* FTIR and Raman spectroscopies [4–15]. The physicochemical properties of bioactive compounds such as, solubility and permeability depend on their crystallographic forms which may have a dramatic impact on the therapeutic efficacy and on the manufacturing of the final dosage forms. Hence, it is crucial to develop tools that will enable understanding and better control of both chemical reaction and crystallization process in real time. The main prerequisites for using Raman spectroscopy are that reactants and product should be Raman active and present in adequate concentrations in order to detect start and end points of chemical reactions or to obtain kinetic data [10–15]. Also, possible strong scattering properties of solvents can make spectral data interpretation very difficult and statistical methods are often necessary.

In this paper we report on *in-line* monitoring of a chemical reaction of the drug entacapone formation by Raman spectroscopy. Entacapone (1), 2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-

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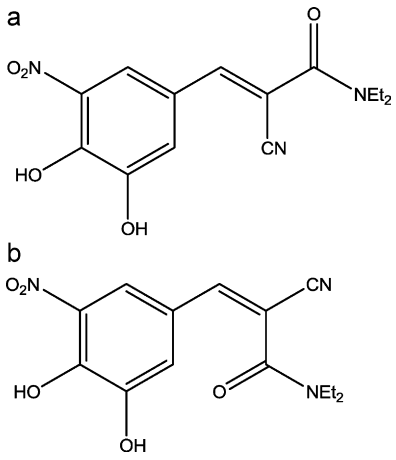

Scheme 1. Structures of (a) *E*- and (b) *Z*-isomer of entacapone.

5-nitrophenyl) propenamide, is a selective inhibitor of catechol-*O*-methyltransferase, an enzyme responsible for the metabolism of L-dopa being a precursor to dopamine. The lack of dopamine is connected to Parkinson's disease. Entacapone is clinically used and prescribed for the treatment of Parkinson's disease [16,17].

Raman spectroscopy has the advantage that it can easily be used for the remote detection through fiber optics facilitating the *in-line* monitoring of chemical reactions and crystallization processes thus generating data in real time. Hence a combination of *in-line* Raman spectroscopy and multivariate analysis [6,17–19] was used to monitor Knoevenagel condensation reaction which is the final step in preparation of entacapone. The reaction gave two isomers of entacapone *E* and *Z* (Scheme 1) in the relative ratio of 2:1 [16]. It was also reported that *E*-isomer was slowly converted to *Z* in human plasma *in vitro* until the ratio 2:1 was reached [20]. We were particularly interested to see whether a combination of Raman spectroscopy and chemometrics could be used to determine entacapone and the reaction end point in the reaction mixture. For comparison the reaction was monitored by the *off-line* Raman spectroscopy and liquid chromatography.

2. Materials and methods

2.1. Materials

3,4-Dihydroxy-5-nitrobenzaldehyde (DHNBA), 2-cyano-*N*,*N*-diethylacetamide (CDEAA) and isobutyl acetate were obtained from

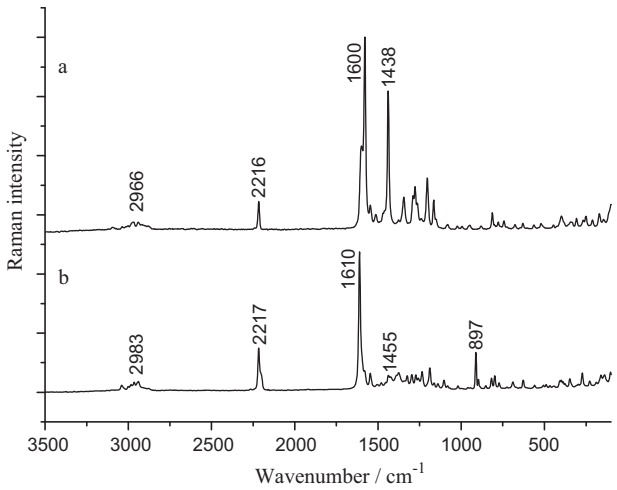

Fig. 1. Raman spectra of (a) *E*- and (b) *Z*-isomer of entacapone.

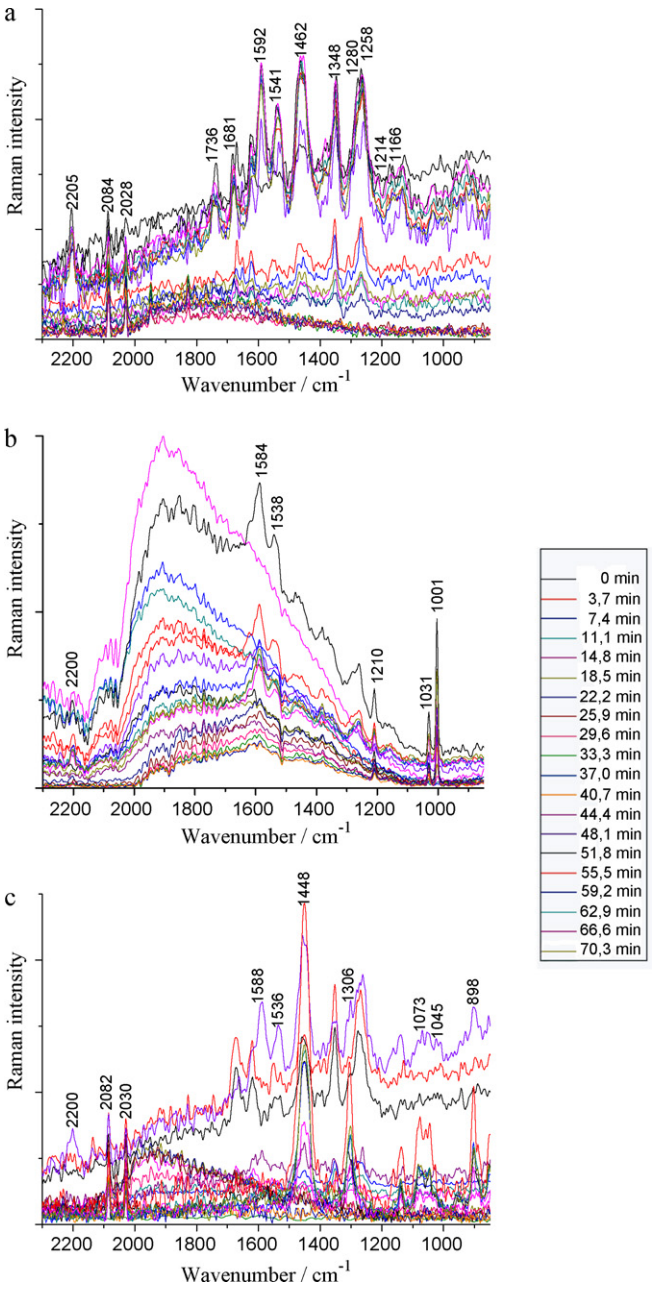

Fig. 2. *In-line* Raman spectra of the Knoevenagel condensation reaction in (a) isobutyl acetate, (b) toluene, and (c) heptane.

PLIVA (Zagreb, Croatia). Methanol (min. 99.5%), heptane (min. 99.8%) and hydrochloric acid (36.5%) were purchased from Kemika (Zagreb, Croatia). Acetic acid (min. 99.8%), piperidine (min. 99%) and toluene (min. 99.5%) were purchased from E. Merck (Darmstadt, Germany), Acros Organics (NJ, USA), Carlo Erba Reagents SpA (Rodano, Italy) and J.T. Baker (Deventer, Holland), respectively.

2.2. Sample preparation

2-Cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)propenamide (entacapone) was prepared by the Knoevenagel condensation of 3,4-dihydroxy-5-nitrobenzaldehyde (DHNBA) and 2-cyano-*N*,*N*-diethylacetamide (CDEAA) [1].

Prior to condensation reaction a catalyst, e.g. piperidine acetate was prepared. In a solution of toluene (41 ml) and acetic acid (1.03 ml), piperidine (1.78 ml) was