



## DSC, X-ray and FTIR studies of a gemfibrozil/dimethyl- $\beta$ -cyclodextrin inclusion complex produced by co-grinding

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### ABSTRACT

The steps of formation of an inclusion complex produced by the co-grinding of gemfibrozil and dimethyl- $\beta$ -cyclodextrin were investigated by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD) and Fourier transform infrared (FTIR) spectroscopy with curve-fitting analysis. The endothermic peak at 59.25 °C reflecting the melting of gemfibrozil progressively disappeared from the DSC curves of the products on increase of the duration of co-grinding. The crystallinity of the samples too gradually decreased, and after 35 min of co-grinding the product was totally amorphous. Up to this co-grinding time, XRPD and FTIR investigations indicated a linear correlation between the cyclodextrin complexation and the co-grinding time. After co-grinding for 30 min, the ratio of complex formation did not increase. These studies demonstrated that co-grinding is a suitable method for the complexation of gemfibrozil with dimethyl- $\beta$ -cyclodextrin. XRPD analysis revealed the amorphous state of the gemfibrozil–dimethyl- $\beta$ -cyclodextrin product. FTIR spectroscopy with curve-fitting analysis may be useful as a semiquantitative analytical method for discriminating the molecular and amorphous states of gemfibrozil.

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### 1. Introduction

Grinding as a mechanical process can be used as a means of reducing the particle size of solid drugs to improve their dissolution rate and of changing the crystallinity of the active ingredients (APIs), the phase transitions of polymorphs and the molecular interactions in the solid state. The efficiency of grinding depends on the intensity of mechanical stress and the additives applied [1].

Cyclodextrins (CDs) have received considerable interest in consequence of their specific chemical modifications and ability to form inclusion complexes thereby improving the solubility, stability and bioavailability of various drugs [2–5]. The frequently used complexation methods (kneading, spray-drying and precipitation) require the application of a certain amount of solvent [6,7]. Removal of the residual solvent from the solid product often causes problems, and an appropriate particle size can be achieved only in a grinding operation. The industrial-scale use of solvent-free technology is therefore of particular importance.

A number of reports have dealt with the applicability and advantages of co-grinding in CD complexation [8–12]. The co-grinding of ibuprofen with  $\beta$ -CD led to a positive change in its molecular

state, and converted the crystals into an amorphous state, which exhibited an improved solubility and a higher rate of dissolution of ibuprofen. The stability of the amorphous complex was investigated in a range of relative humidities and temperatures. According to these investigations, the stability of the complexes was suitable at 25 °C, 40 °C and 55 °C, below 74% RH [11]. The co-grinding of acetaminophen with  $\alpha$ -CD or  $\beta$ -CD likewise resulted in amorphization of the products. This API could be incorporated in a stepwise manner only into the cavity of  $\beta$ -CD;  $\alpha$ -CD did not form an inclusion complex with acetaminophen, presumably because of the small cavity size. Complexation with  $\beta$ -CD was confirmed by differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopic investigations [1,9]. The optimum co-grinding time for the triamterene- $\beta$ -CD system was determined in an FTIR investigation, the results showing that co-grinding increases the molecular interaction between the drug and the  $\beta$ -CD in the mixtures, and causes good dissolution properties [10]. FTIR and DSC investigation methods were used in the case of the loratadine-hydroxypropyl- $\beta$ -CD system. FTIR curve-fitting analysis revealed that increase of the co-grinding time was accompanied by a progressive increase in complexation. The FTIR spectrum of a mixture co-ground for 15 min was identical with that of the co-evaporated solid product [8].

Gemfibrozil (GEM) decreases the serum levels of triglycerides and very low-density lipoprotein cholesterol, and increases that of high-density lipoprotein cholesterol [13,14]. We earlier

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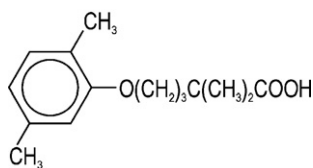


Fig. 1. Chemical structure of GEM.

demonstrated that dimethyl- $\beta$ -CD (DIMEB) was the most suitable CD derivative increasing the aqueous solubility of poorly-water soluble GEM (which belongs in class II of the Biopharmaceutical Classification System). The phase solubility curve of the active agent was linear ( $A_L$  type) in the investigated CD concentration range (0–200 mM). All of the kneaded and spray dried products exhibited better dissolution properties than GEM alone. A more than 500–700-fold solubility increase was observed during saturation concentration measurements. A 3–5-fold increase was measured during *in vitro* membrane diffusion studies on solutions in simulated gastric medium, whereas no significant difference was detected in simulated intestinal medium. Complexation with DIMEB decreased the *n*-octanol/water partition coefficient considerably. Complex formation was proved by thermoanalytical and FTIR measurements in several compositions made by different preparation methods (kneading or spray-drying) [15,16].

In the present study, the effectiveness of mechanical grinding was investigated on the complexation between GEM and DIMEB. Inclusion complex formation was studied by DSC, FTIR spectroscopy and X-ray powder diffraction (XRPD) analysis. Our aim was to establish analytical test methods suitable to shed light on the CD complexation process.

## 2. Experimental

### 2.1. Materials

Gemfibrozil (Fig. 1): 2,2-dimethyl-5-(2,5-xylyloxy)valeric acid (Plantex Chemicals, Israel, API Division Teva Group); heptakis-2,6-di-*O*-methyl- $\beta$ -CD (DIMEB) (degree of substitution: 14.00; molar mass: 1331.0 g mol<sup>-1</sup>) (Cyclolab R&D Laboratory Ltd., Budapest, Hungary).

### 2.2. Preparation of co-ground mixtures of GEM and DIMEB

Co-ground mixtures of GEM and DIMEB in 1:1 molar ratio were prepared in an agate mortar. The co-grinding was started with 0.25 g of GEM and the calculated amount of DIMEB and suitable quantities of samples were removed at prescribed intervals (5, 10, 15, 20, 25, 30 and 35 min) for further examinations.

### 2.3. Differential scanning calorimetry

The DSC analysis was carried out with a Mettler Toledo STAR<sup>®</sup> thermal analysis system, version 9.30 DSC 821<sup>e</sup> (Switzerland), at a heating rate of 5 °C min<sup>-1</sup>, with argon as carrier gas (10 l h<sup>-1</sup>). The sample size was in the range 2–5 mg and examinations were performed in the temperature interval 25–100 °C, in sealed pans with two leaks.

### 2.4. X-ray powder diffractometry

XRPD spectra were recorded with a BRUKER D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with Cu K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) over the interval 5–30°/2. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.010°.

In determining the degree of crystallinity, the total area of the three peaks with largest intensity was examined, after smoothing and background removal.

### 2.5. Fourier transform infrared spectroscopy and curve-fitting analysis

Spectra were recorded on a Bio-Rad Digilab Division FTS-65A/896 FTIR spectrometer between 4000 and 400 cm<sup>-1</sup>, at an optical resolution of 4 cm<sup>-1</sup>, using Harrick's Meridian SplitPea single reflection, diamond, ATR accessory. 256 scans were averaged to achieve a proper signal-to-noise ratio. All spectral manipulations were performed by using Thermo Scientific GRAMS/AI Suite software.

The components and relative compositions of co-ground samples were estimated semiquantitatively in the 1770–1670 cm<sup>-1</sup> (in the case of the physical mixture 1760–1640 cm<sup>-1</sup>) region of the FTIR spectra by a curve-fitting algorithm with a Gaussian–Lorentzian function. The best curve-fitting procedure was performed by iterative fits toward a minimum standard error. The relative proportion of a component was calculated as the fractional area of the corresponding peak, divided by the sum of the areas of the peaks.

## 3. Results

### 3.1. Differential scanning calorimetry

GEM, DIMEB and the co-ground products were investigated by DSC. The curve of the intact GEM displayed one sharp endothermic peak at 59.25 °C, which corresponds to the melting point of the material. After melting, GEM exhibits suitable stability in the investigated temperature interval. It was previously established that a broad endothermic peak at about 230 °C indicates the evaporation and decomposition of GEM. The TG curve of the API demonstrated that GEM did not contain moisture. The mass decrease started at about 200 °C [16].

DIMEB gave a smooth DSC curve in the investigated temperature interval, with no thermal signal. The moisture content of DIMEB was found in our previous TG investigation to be <0.5%. The moisture contents of the products were also very low, which favoured the evaluation of the measurements; as there was no broad endothermic peak below 100 °C (reflecting the water content), this did not disturb the examination of the API with its low melting point [16] (Fig. 2).

The DSC curve of the physical mixture of GEM and DIMEB displayed the endothermic peak of the API. The form of this peak was similar to that for pure GEM, though the area was smaller, in accordance with the mass ratio of the components. The peaks of the co-ground products were somewhat broader and shifted to lower temperatures. The areas of these endothermic peaks gradually decreased with increasing grinding time: after 35 min, the peak had practically disappeared (Fig. 3).

This phenomenon indirectly substantiates the complexation with DIMEB, where the decrease in the endothermic peak may be associated with the amorphization of GEM or a complex formation between GEM and DIMEB molecules. It is not possible to distinguish these two processes through an assessment of the DSC curves. The FTIR investigation can be used for the detection of secondary bond formation between the host and guest molecules.

The difficulties in the correct integration of the peaks were caused by the differences between the pre- and post-baselines of the melting peaks and the peak broadening. The integrated area of the endothermic peak of the physical mixture was smaller than for the products co-ground for 5 or 10 min, and integration of the

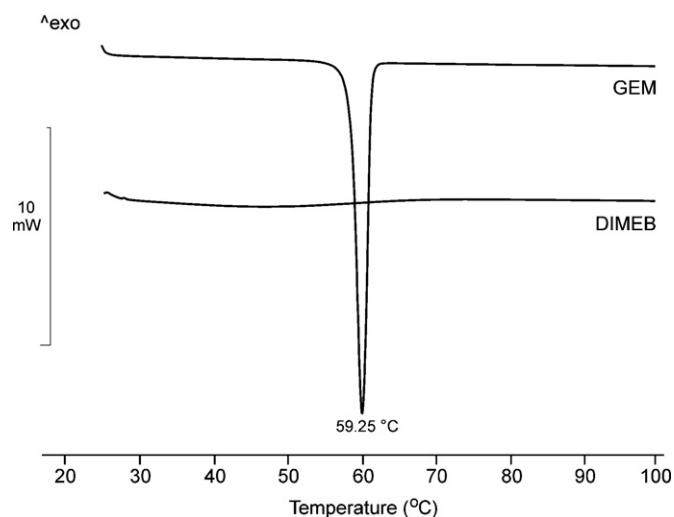


Fig. 2. DSC curves of GEM and DIMEB.

endotherm peaks was impossible for the products co-ground for 25 or 30 min. Our investigation indicates that the DSC test is not applicable for the semiquantitative determination of the complexation process in the case of these materials.

### 3.2. X-ray diffraction

XRPD serves as a suitable method for determination of the molecular state of a complex. Fig. 4 depicts the diffraction patterns of GEM and DIMEB. The X-ray spectrum of the intact GEM presents numerous and sharp reflections, which indicate its high degree of

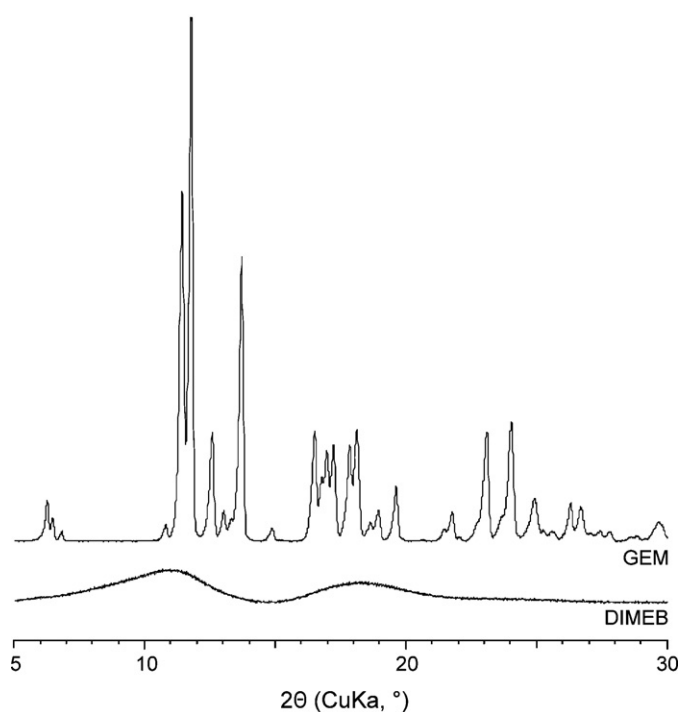


Fig. 4. XRPD diffractograms of GEM and DIMEB.

crystallinity. The DIMEB is practically an amorphous material; its spectrum does not contain any peaks.

The characteristic diffraction peaks of GEM were detected in the co-ground mixtures, but with lower intensity corresponding to the smaller quantity of the API. The intensity of these peaks constantly decreased with increase of the grinding time, which caused a progressive change in the crystalline state of GEM and converted it to an amorphous form. As regards the GEM and DIMEB mixtures, all of the peaks had disappeared after 35 min of co-grinding (Fig. 5). Similar results were obtained when the examination was repeated after 1 month, which demonstrated the appropriate stability of the amorphous form.

The total area of the three peaks with largest intensity, at  $11.413^\circ$ ,  $11.759^\circ$  and  $13.698^\circ$ , were selected for determination of the crystallinity of the co-ground mixtures. The results showed that the crystallinity of the products decreased linearly with increase of the co-grinding time (the regression line equation is  $y = -1.5093 \cdot x + 53.47$   $R^2 = 0.9848$ ). After 35 min of co-grinding the solid phase did not change further, the total amount of the product being non-crystalline, which means that DIMEB functioned as a complexation agent and a crystallization inhibitor, and the amorphous form proved stable in repeated examinations. Similarly to the DSC investigations, the XRPD examinations could not distinguish between the amorphous complexed and non-complexed products, and the method did not detect a small quantity of non-complexed crystalline GEM in the product co-ground for 35 min.

### 3.3. FTIR spectroscopy

The ATR method makes it possible to monitor the slightest changes in the state of the sample, since there is no sample preparation, unlike in the case of the KBr pellet method, when grinding and pressing mostly unifies the samples with weak interactions.

The most obvious changes in the IR spectra of the samples ground for various periods of time were observed in the range of the C=O stretching mode of the carboxylic group, but relatively strong intensity changes could also be identified at  $1510 \text{ cm}^{-1}$ ,

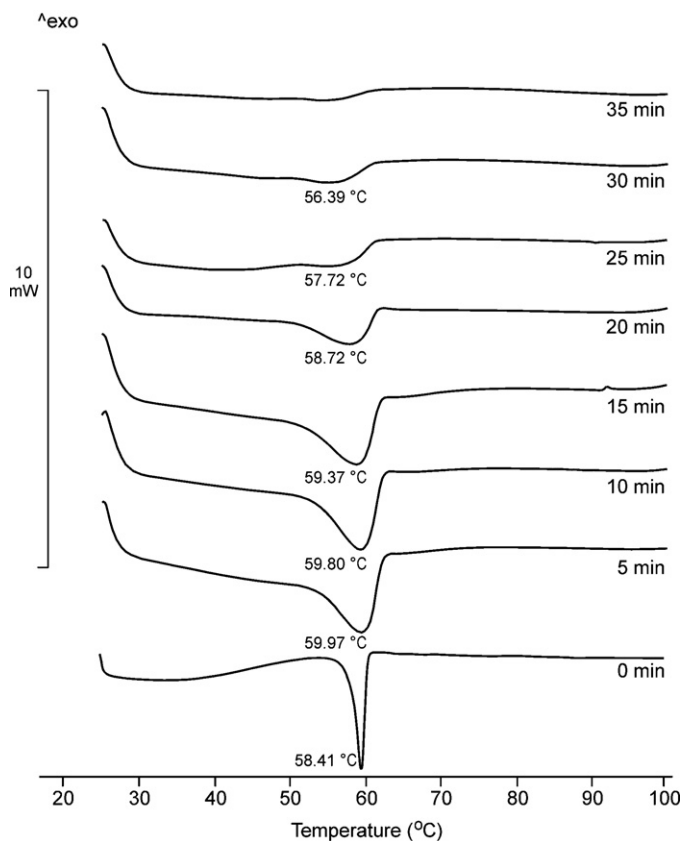


Fig. 3. DSC curves of the physical mixture and co-ground products.

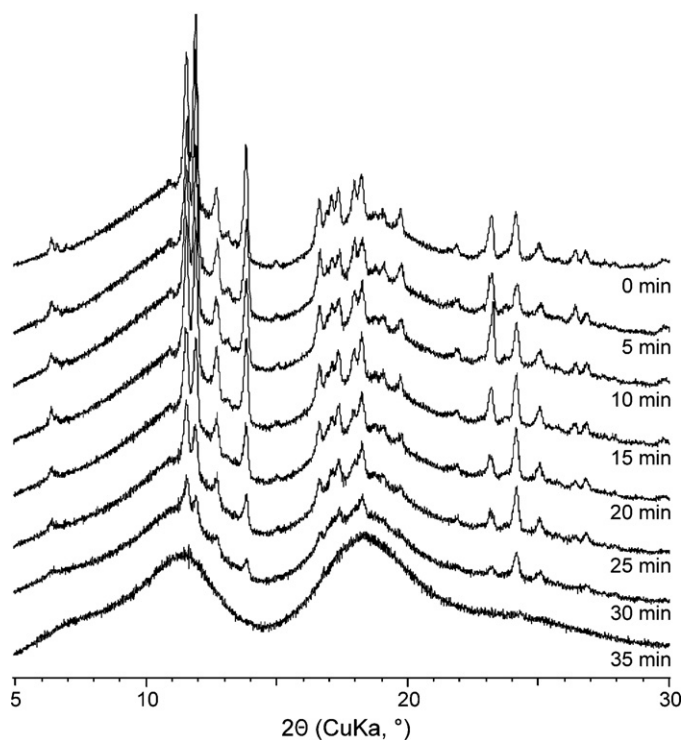


Fig. 5. XRPD diffractograms of the physical mixture and co-ground products.

1402  $\text{cm}^{-1}$ , around 1265  $\text{cm}^{-1}$  and around 805  $\text{cm}^{-1}$  (Fig. 6). New bands were developing in the course of the grinding process in close proximity of the original peaks of GEM, influencing the relative intensity of them. These alterations indicate that the aromatic ring is influenced by the interaction of GEM and the DIMEB molecule, since there are bands in all four ranges which are assigned to certain stretching, in-plane and out-of-plane bending modes of the 1,2,5-trisubstituted benzene ring, respectively [17]. It should be noted that the band at around 810  $\text{cm}^{-1}$  corresponds to the motion of those two vicinal hydrogens. On the other hand, the alterations in the  $\beta(\text{OH}) + \nu_{\text{as}}(\text{O}-\text{C}-\text{C})$  mode and the complemen-

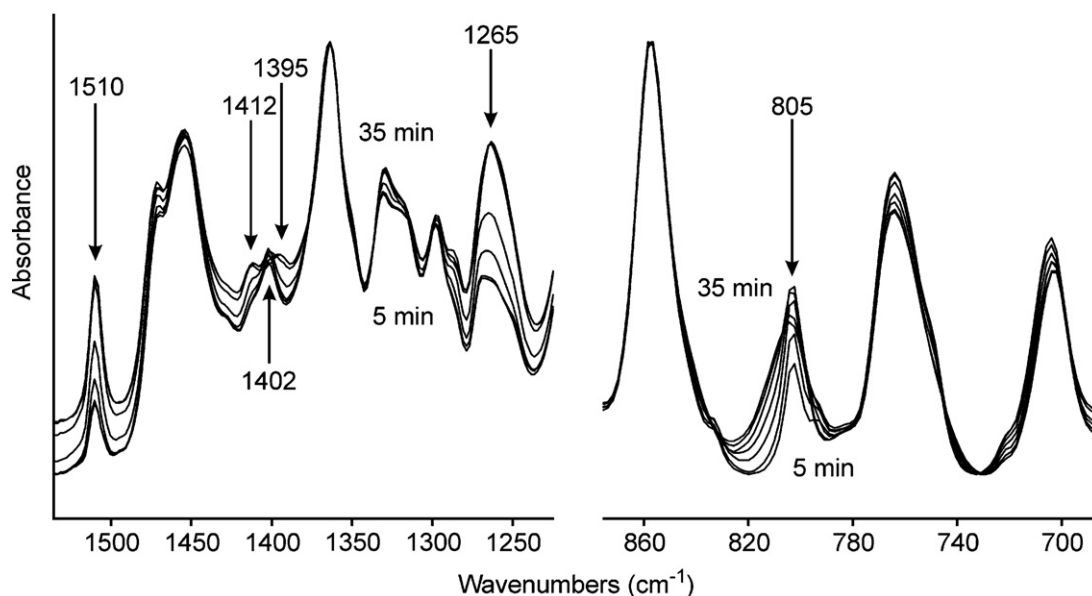


Fig. 6. The 1,2,5-trisubstituted aromatic ring is also influenced by the grinding process, as indicated by the ring stretching modes at 1510 and 1402  $\text{cm}^{-1}$ , the in-plane C-H bending modes at around 1265  $\text{cm}^{-1}$  and the out-of-plane deformation mode at around 805  $\text{cm}^{-1}$ .

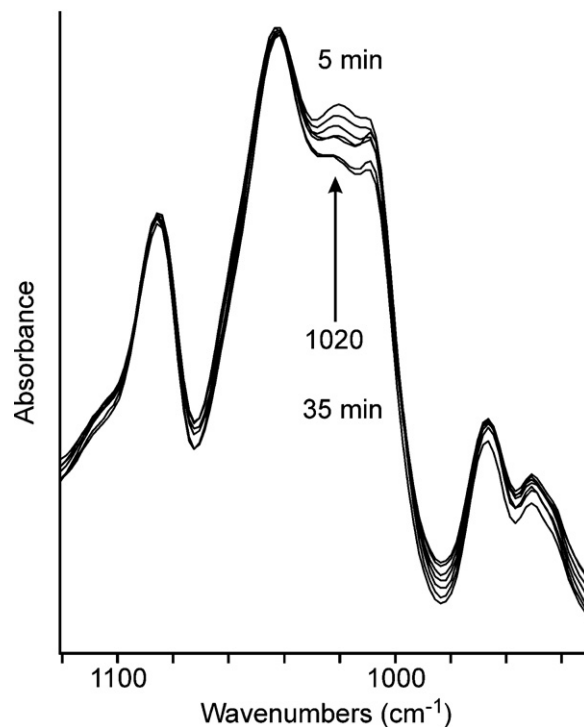


Fig. 7. The alcoholic  $\nu_{\text{as}}(\text{O}-\text{C}-\text{C}) + \beta(\text{OH})$  band of the DIMEB molecule is also influenced by the grinding process.

tary  $\nu_{\text{as}}(\text{O}-\text{C}-\text{C}) + \beta(\text{OH})$  mode can be incorporated into the changes in the bands at around 1412 and 1265  $\text{cm}^{-1}$ , since the carboxyl group is also influenced [18]. The most pronounced changes in the spectrum of DIMEB can be found at around 1025  $\text{cm}^{-1}$ , relating to the  $\nu_{\text{as}}(\text{O}-\text{C}-\text{C}) + \beta(\text{OH})$  mode of the alcohols, indicating that the interaction influences these groups (Fig. 7).

The C=O stretching mode of GEM is well separated from the other bands, and undergoes considerable changes; it can therefore be used as a semiquantitative indicator of the various species present in the samples. This range of the spectrum for pure GEM is characterized by a peak at 1705  $\text{cm}^{-1}$  and a shoulder at around



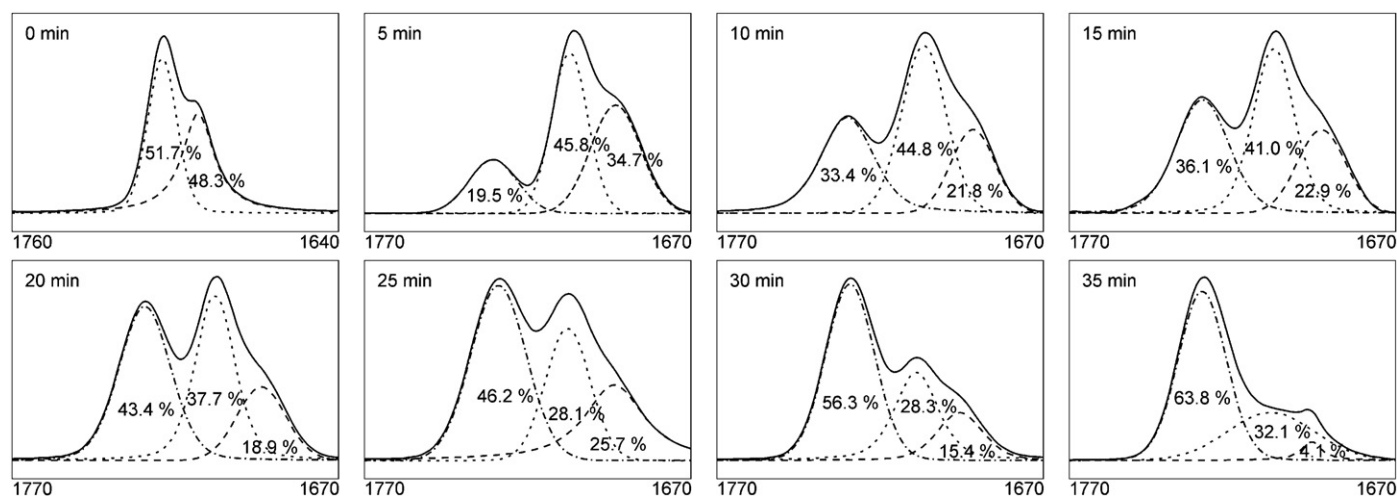


Fig. 8. Inclusion complex formation between GEM and DIMEB during co-grinding.

1690  $\text{cm}^{-1}$ . A new band emerges at 1728  $\text{cm}^{-1}$  as the grinding process advances. Three bands were fitted to all the spectra of the co-ground samples and their areas were compared. The positions of these bands were determined by Fourier deconvolution of the corresponding ranges of the spectra. The same set was used for each spectrum, and the parameters of the fitted bands were liberated for fitting in the sequence of intensities, full widths at half maximum and finally the positions of the bands. Various statistical parameters and the residual spectra were checked to monitor the quality of the curve-fitting. The results are given in Fig. 8 with the percentage contributions of the fitted peaks. The ratios of the areas for the peak at 1708  $\text{cm}^{-1}$  and those of the peak at 1693  $\text{cm}^{-1}$  are plotted against the co-grinding time in Fig. 9 curve (A) and show that their ratios are more or less constant. Curve (B) relates to the corresponding ratios of the peaks at 1730 and 1708  $\text{cm}^{-1}$ , indicating a linear increase in the concentration of the associated species with increasing time of co-grinding.

After co-grinding for 30 min, the peak at 1708  $\text{cm}^{-1}$  broadened, and the ratio of the areas of the peaks at 1708  $\text{cm}^{-1}$  and at 1693  $\text{cm}^{-1}$  therefore changed significantly. The ratio of the areas of the peaks at 1730  $\text{cm}^{-1}$  and 1708  $\text{cm}^{-1}$  remained constant. On

continuation of the co-grinding, this ratio did not change further, indicating that the complex formation process had ended.

#### 4. Conclusion

The complexation of GEM with DIMEB is possible not only with the previously used methods of CD complexation (kneading, co-evaporation, spray-drying, etc.), but also by co-grinding. The process changes can be monitored by DSC, XRPD and FTIR methods. The DSC and XRPD examinations indicated that the endothermic peak corresponding to the melting of GEM continuously decreased with increasing co-grinding time, and the crystallinity of the powder mixture also decreased. After grinding for approximately 30 min, the product no longer contained crystalline GEM and the product was completely non-crystalline. This amorphous product exhibited suitable stability on repeated XRPD investigations. The XRPD and FTIR studies revealed a semiquantitative correlation between the co-grinding time and the GEM complexation. After co-grinding of 35 min, the material was totally amorphous. During this procedure, the GEM was complexed step by step, and the FTIR investigation suggested that the complexation procedure stopped after about 30 min of co-grinding. The process could be described by a linear equation up to 30 min of co-grinding, after which the proportion of complexed GEM did not change with further increase of the grinding time. On the basis of these investigations, the solvent-free co-grinding method can be suggested for production of the GEM + DIMEB complex. The XRPD analysis confirmed the amorphous state of the GEM-DIMEB product. FTIR spectroscopy with curve-fitting analysis may be useful as a semiquantitative analytical method for discriminating the molecular and amorphous states of GEM.

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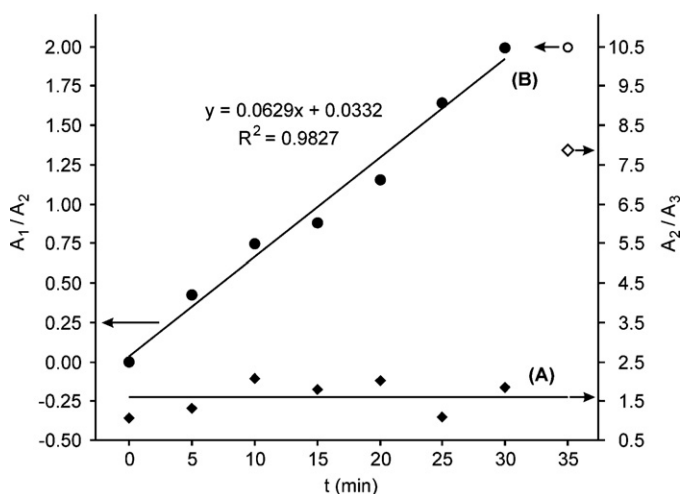


Fig. 9. The ratios of the areas for the fitted peaks ( $A_1$ : 1730  $\text{cm}^{-1}$ ,  $A_2$ : 1708  $\text{cm}^{-1}$  and  $A_3$ : 1693  $\text{cm}^{-1}$ ) are plotted against the grinding time, indicating that the ratio of the intensities of the characteristic bands of pure GEM is nearly constant (A), and the amount of the new species increases with time (B).

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