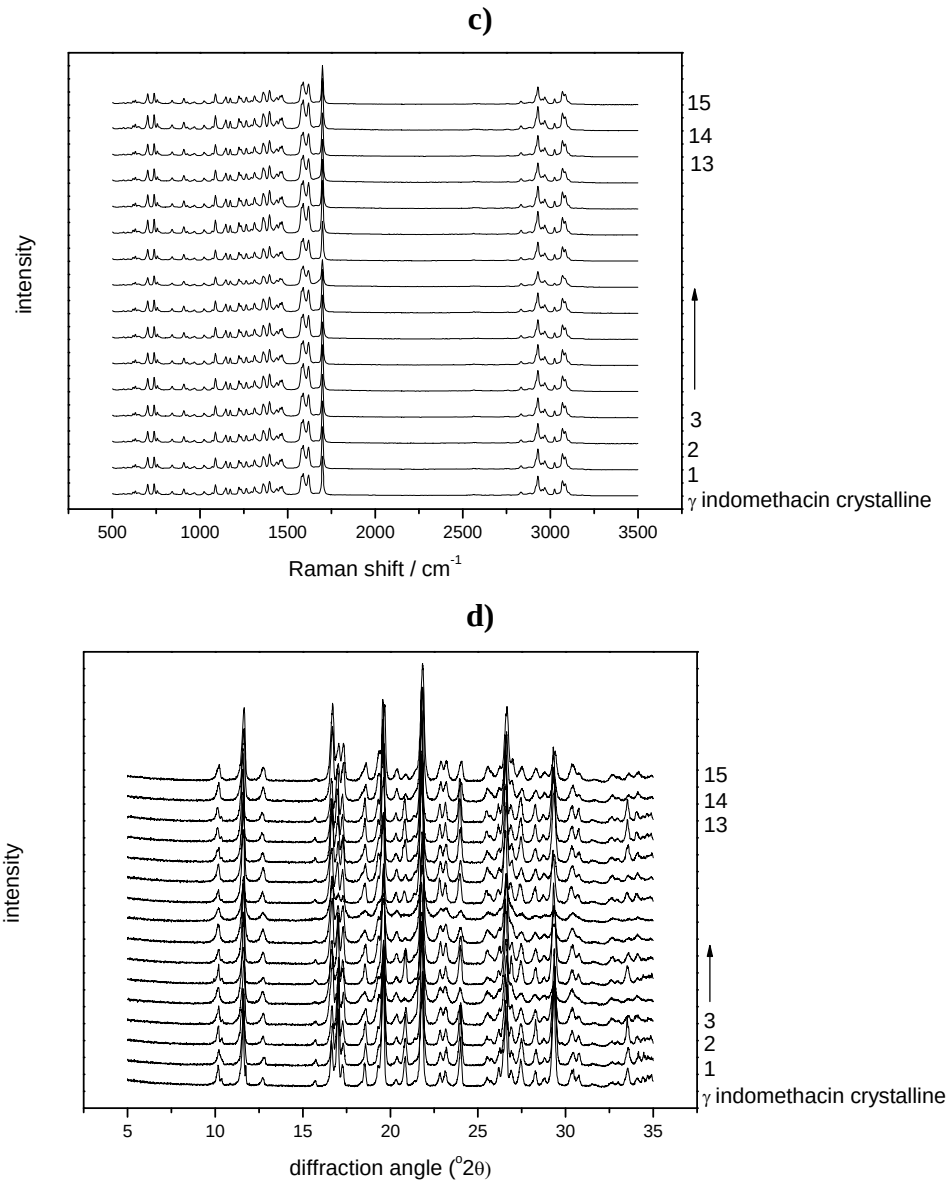
Figure 7. *Cont.*



3.3.3. DSC

For DSC analysis the change in heat capacity at the glass transition was used as a measure for amorphousness. The average change in heat capacity (Δc_p) for the quench cooled, X-ray amorphous indomethacin samples was 0.45 J/g^{o}C at the glass transition temperature (T_g) of 43.2 ^{o}C. Only in the indomethacin sample milled under the harshest milling conditions (600 mg of compound milled for 60 min with sixty 4 mm balls at 25 Hz) a glass transition, immediately followed by an exothermic event, *i.e.*, recrystallisation could be detected (Figure 9). The change in heat capacity for this sample was 0.08 J/g^{o}C at T_g (42.4 ^{o}C), corresponding to an amorphous content of 17.8%.

Figure 8. a) PLS model for XRPD data of indomethacin according to cross validation; **b)** Loading weights of the PLS factor (XRPD, indomethacin); **c)** PLS model for XRPD data of simvastatin according to cross validation; **d)** Loading weights of the PLS factor (XRPD, simvastatin).

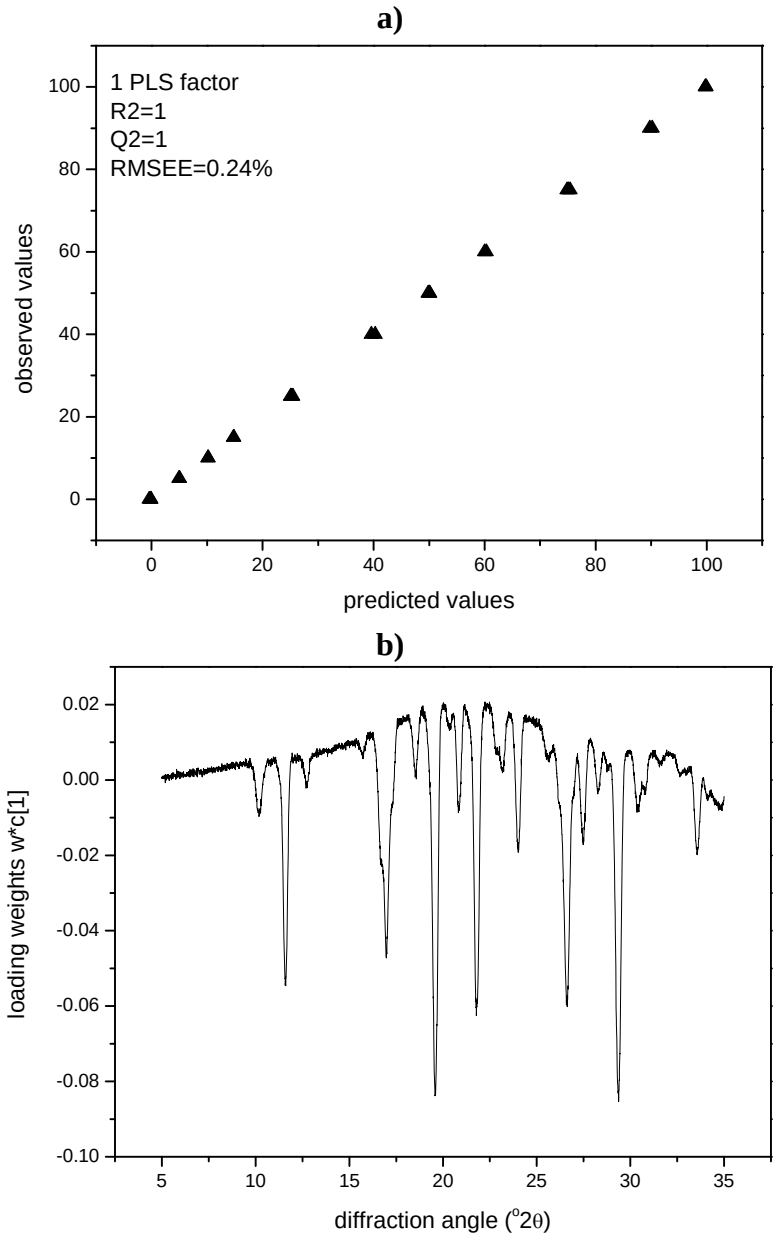
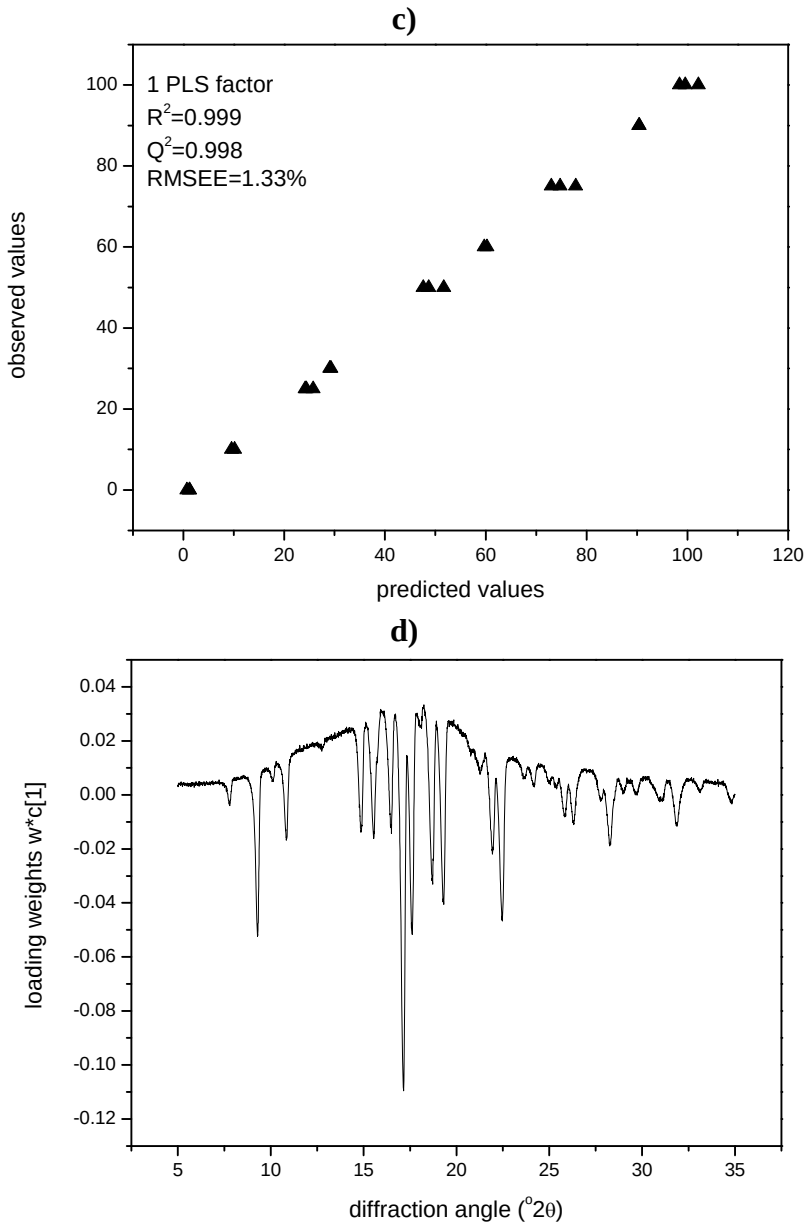
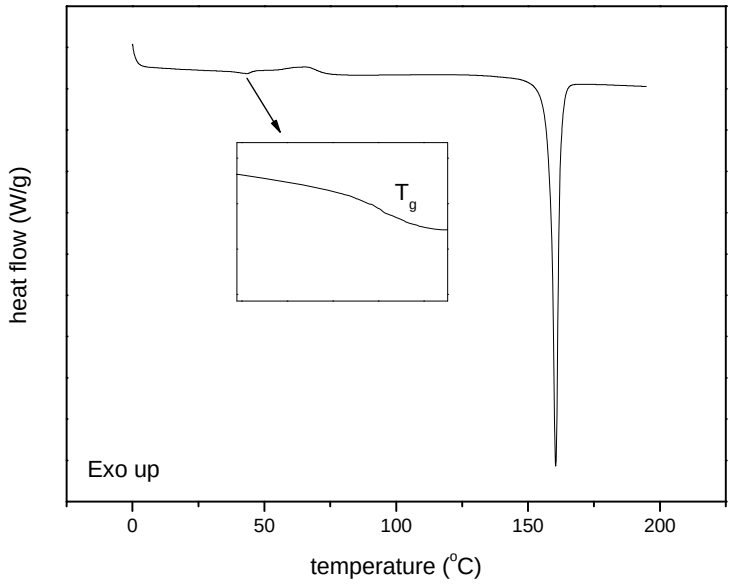


Figure 8. *Cont.*



For the quench cooled, X-ray amorphous simvastatin Δc_p was 0.49 J/g°C (±0.01) at a T_g of 30.3 °C (±0.9). For the entire set of milled simvastatin samples no glass transition could be detected, indicating that process induced changes in the ball milled samples did not lead to amorphisation. Only for the sample milled under the harshest milling conditions (milled for 60 min with 60 balls at 25 Hz) an exothermic recrystallisation event could be observed at 49.5 °C. Although no glass transition could be detected for the milled simvastatin samples, the one recrystallisation event in the sample with the harshest milling conditions can be interpreted as recrystallisation of a disordered form and therefore points to some kind of crystalline disorder. This finding was confirmed by Raman spectroscopy and XRPD (Table 1).

Figure 9. Thermogram of the indomethacin sample milled under the harshest milling conditions applied (milled for 60 min with 60 balls at 25 Hz).



For other simvastatin samples as well as for indomethacin samples without a glass transition, process induced disorder was detected by Raman spectroscopy and XRPD.

The comparison of the results for process induced disorder detected by Raman spectroscopy, XRPD and DSC revealed that the respective outcome regarding the content of process induced disorder in the ball milled drugs strongly depends on the analytical method used. Of all methods investigated, XRPD is the one that tends to give a lower percentage of crystallinity than Raman spectroscopy and DSC.

We understand these discrepancies as being method inherent. Thermal analysis detects the existence of the truly amorphous state, defined as a second order phase transition in form of the glass transition. In contrast, XRPD predominately detects crystallinity, the absence of which may be due to several forms of process induced disorder ("total process induced disorder"). Also, the decrease of crystalline peaks may be due to particle size effects, as very small particles may lead to weaker diffraction peaks and bigger particles may facilitate preferred orientation, which again may influence peak intensities [8]. Thus, PLS modeling of XRPD data in order to quantify the amount of process induced disorder could be problematic as the method might not differentiate between changes in the diffractograms induced by crystalline disorder and diffractogram changes that are induced by changes in particle size. Consequently, quantification of the solid state of milled drugs by XRPD may be biased.

3.4. Influence of the drug itself on the quantification outcome for process induced disorder

For simvastatin the differences between crystalline disorder detected by Raman spectroscopy and XRPD were not as distinct as for indomethacin. This phenomenon could be attributed to the drugs itselves. Quench cooled amorphous indomethacin exists predominantly as dimer as does the γ crystalline form [28]. Hydrogen bonding in simvastatin facilitates the formation of molecule chains along the *a* axis in the crystal [29]. The remaining near order of quench cooled simvastatin has not been published to the best of our knowledge, but it is suspected that the differences between crystalline and amorphous near order are more distinct for simvastatin than for indomethacin. This could explain

the more consistent Raman and XRPD results for simvastatin, as Raman spectroscopy being a near order analytical method might not easily pick up on disordered states, where the near order of amorphous and crystalline form are very similar.

Taken together, our findings suggest the presence of different disordered states in the milling process. On the basis of the different results obtained by the different analytical methods, existence of pre-amorphous (without exhibition of a glass transition) and amorphous (with glass transition) disordered states are assumed.

4. Conclusions

This study has compared three different techniques, namely XRPD, DSC and Raman spectroscopy, with respect to the quantification of the content of processed induced crystalline disorder in a milling process. Care must be taken when interpreting the results obtained with these three methods. Within XRPD the assignment of changes in the diffractogram due to "total process induced disorder" (*i.e.*, amorphous content and other forms of crystalline disorder) and particle size effects appears to be problematic. However, XRPD is the method of choice for the detection of remaining crystallinity. For the detection of the truly amorphous state, defined by a glass transition, DSC is the method of choice. Raman spectroscopy combined with multivariate analysis detects and quantifies crystalline disorder before the compound can be described as DSC amorphous as well as thereafter. However, Raman spectroscopy as a molecular level technique is sensitive to the near order of solid materials and therefore could 'underestimate' the degree of disorder if the material remains near range ordered to a certain extent (*i.e.*, exists as a dimer) in the amorphous state. Thus, for the quantification purpose of milled drugs Raman spectroscopy combined with multivariate analysis appears to be advantageous for drugs with significant differences in the near order of the crystalline and amorphous form as particle size effects are not as apparent and the whole range of crystalline disorder is covered. However, to understand the investigated system fully, all methods need to be used complementary.

Acknowledgements

We like to thank Damian Walls (Department of Geology, University of Otago) for help with the XRPD measurements and Katharina Paulsen for help in data acquisition. The University of Otago is acknowledged for providing a scholarship for U.Z. The Academy of Finland, Finnish Cultural Foundation and Finnish Pharmaceutical Society are also acknowledged for funding (J.A.).

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