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THE DETERMINATION OF RELATIVE AMOUNTS OF PHASES IN BINARY MIXTURES WITH QUANTITATIVE X-RAY DIFFRACTION

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ABSTRACT

The quantitative x-ray diffraction analysis offers often an exact and practical technique to determine the relative amounts of the phases of the solid in question. The sample may contain only one amorphous phase.

The purpose of this paper is to estimate the suitability of this technique to quantify the phase fractions of pharmaceutical substances. Three different method were used to determine the relative amounts of phases. Methods were compared and the major sources of error were estimated. The measurements and calculations are carried out using selegilin hydrochloride, mannitol and modified starch as an example. The results show that the amount of about 2 % of selegilin hydrochloride can be observed reliably from among the mannitol (crystalline substance) or modified starch (amorphous substance). During the work it became obvious that the accuracy of these calculations can essentially be improved planning the measurements carefully.

INTRODUCTION

The knowledge of the percentual amounts of different phases in a solid pharmaceutical mixtures might be necessary for several reasons. The manufacturing of drugs is composed of many processes where the phase transition may exist. Some excipients also may retard or increase this transition. Some phases might be unstable affecting the quality of the product adversely. For that reason the determination of the phase composition of both the raw material and the product is essential.

X-ray diffraction analysis is a fast and reliable method for the determination of the crystalline phase fractions of the metal alloy. The following text considers binary pharmaceutical systems where at least one component is crystalline. The extension of the method to the multi-component systems is however straightforward.

The integrated intensity I of one reflection can be expressed in the form

$$I \propto \left[|F|^2 p \left(\frac{1 + \cos^2 2\theta}{\sin^2\theta \cos\theta} \right) \right] \left(\frac{e^{-2M}}{2\mu} \right), \qquad (1)$$

where F = structure factor, p = multiplicity, θ = Bragg angle, e^{-2M} = temperature factor and μ = linear absorption coefficient.

The integrated intensity of the specific reflection (fixed θ) of substance is proportional to the amount of that substance in the sample. Therefore in the mixture of two phases the integrated intensity of one reflection depends on the amount of corresponding phase in the sample. This relation can be expressed with the aid of equation (1) in the form

$$\frac{I_\alpha}{I_{\alpha p}} = \frac{w_\alpha \frac{\mu_\alpha}{\rho_\alpha}}{w_\alpha \left(\frac{\mu_\alpha}{\rho_\alpha} - \frac{\mu_\beta}{\rho_\beta} \right) + \frac{\mu_\beta}{\rho_\beta}} = \frac{w_\alpha}{w_\alpha (1 - A_{\beta\alpha}) + A_{\beta\alpha}}, \qquad (2)$$

where I_α and $I_{\alpha p}$ are the integrated intensities of the diffraction line of the phase α corresponding the samples made of mixture of phases α and β and of pure phase α, respectively. The factor w_α is weight fraction of the α-phase, ρ_α, ρ_β are the densities and μ_α, μ_β are the linear absorption coefficients. The quantity μ_α/ρ_α is mass absorption coefficient. The factor $A_{\beta\alpha}$ is an abbreviation for the ratio of mass absorption coefficients of phase α and phase β, i.e.

$$A_{\beta\alpha} = \frac{\mu_\beta/\rho_\beta}{\mu_\alpha/\rho_\alpha} \quad . \tag{3}$$

Equation (2) states that the weight fractions can be determined from the intensity measurements if the ratio of mass absorption coefficients $A_{\beta\alpha}$ is known. For farmaceuticals it is not usually known and the method is not so straightforward. Instead of direct analysis the calibration must be done by determining the quantity $A_{\beta\alpha}$.

If the two phases mentioned above comes from the different forms of same substance the mass absorption coefficients μ/ρ are same for both phases. Equation (2) simplifies to the form

$$\frac{I_\alpha}{I_{\alpha p}} = w_\alpha \tag{4}$$

and no calibration is needed. This equation can be used as an approximation if $\mu_\alpha/\rho_\alpha \approx \mu_\beta/\rho_\beta$.

This paper treats of the possibilities of quantitative x-ray diffraction in the study of drug substances. The investigations are carried out with different compositions of selegilin hydrochloride and the excipient (mannitol or modified starch) for which the corresponding integrated intensities of specified reflections are measured. The intensity ratios are calculated and the calibration curves are made using different diffraction lines and different techniques for the measurements. The results of analysis are compared with equation (2).

MATERIALS AND METHODS

Materials

The samples were made mixing different amounts of selegilin hydrochloride (Orion, Finland) and mannitol (Merck, Germany) or selegilin hydrochloride and modified starch (Starch 1500®, Colorcon, England). The samples were prepared weighing the components and mixing them carefully. The amounts of component were chosen such that the total volume of mixture was same as the volume of the specimen holder.

Methods

The measurements were carried out using Philips PW1820 diffractometer. The measuring conditions were the following: Ni filtered CuK_α radiation (λ = 0.15418 nm), voltage 50 kV, current 40 mA, automatic divergence slit (irradiated sample length 12.5 mm), receiving slit 0.1 mm, scatter slit 4° and proportional detector.

Data was collected and analyzed using Philips APD1700 program. This program makes possible to determinate the integrated intensities with three different methods.

First method is to measure ordinary x-ray diffractogram of a sample and find integrated intensities of the reflections with PW 1869 profile fitting program. The program asks values of positions, intensities and widths of the reflections and two positions where the background will be determined. The program makes the best fit using these values as a starting-point. The fitting is based on Marquardt non-linear least squares algorithm[1]. If the fitting succeeds the measured diffractogram and the profile fit match and the residue curve, which describes the difference between them, is thus straight and match the background. If the fitting is not satisfactory the procedure is repeated using the results of previous fitting as a starting-point. The profile fit curve is a

superposition of the individual peak profiles and the integrated intensity is the same as the area of peak profile corresponding that peak.

Although the residue curve represents the difference between measured diffractogram and the profile fit the method is somewhat subjective. Good fit might be achieved by different background determination that affects the integrated intensities of reflections. For that reason the background should be determined same way every time. For overlapping peak profiles or complicated background the method is powerful. The integrated intensity of the reflection can be obtained with reasonable accuracy from the superposition of the overlapped components. In spite of that the analysis of overlapped peaks should be avoided, when possible, even though the intensity of reflection in question is high.

Second method is to measure only the integrated intensities. This can be done giving the exact 2θ–range where the integrated intensity and background will be measured. The method uses linear background correction and thus the goodness of the results depends on the linearity of the background. Slight difference in linearity might be corrected by assuming that the intensity of the non–linear background caused by matrix is decreasing linearly when the proportion of phase in interest is increasing. If the background is very complicated the first method is to be recommended. Because the vertical sample displacement moves peaks in the diffractogram the preparation of the sample must be made with care.

Third method is to measure the intensity maxima of the reflection and subtract the background from it. The method is useful in the case of slight overlap when the minor line do not effect on the intensity maxima of analyzed reflection. The shape of diffraction line depends on the quality of the sample surface and therefore this method is poor accurate especially at low 2θ–angles. The results are usually only qualitative.

The main errors in all three method was caused by the preparation of the sample. If the sample is non–homogenous the relative amounts of phases in the

surface of the sample, which is in preferential role in the x-ray diffraction, might differ critically from the corresponding values in the whole sample. The error due to preferred orientation produced in sample preparation was tried to avoid tapping powder gently as possible. Also the roughness and the level of the surface of the sample causes the change in the profile of the diffractogram.

The errors due to the change of the temperature, air pressure and the variation of the tube voltage and the current are usually small if the measurements are carried out in a short period. The effect of these errors might be reduced by using external standard when the time between measurements is long or as high accuracy as possible is wanted. The effects of extinction and microabsorption were minor because of small particle size and low degree of crystalline perfection and were same in all samples and therefore negligible.

The densities of all three materials were determined with AccuPyc 1330 helium pycnometer. The measurements were repeated until the standard deviation of last five results was under 0.0005 g/cm^3.

RESULTS

The diffractograms of selegilin hydrochloride, mannitol and modified starch are presented in Fig. 1. Selegilin hydrochloride and mannitol are clearly crystalline whereas modified starch is amorphous. Although the correction due to the overlapping of peaks from different materials is possible in quantitative analysis the best result is obtained if the chosen peak does not overlap with any other. For selegilin hydrochloride and mannitol the quantitative analysis is carried out using two reflections characteristic for selegilin hydrochloride at $2\theta \approx 13.1°$ and $2\theta \approx 26.3°$ and one reflection characteristic for mannitol at $2\theta \approx 9.7°$ whereas for selegilin hydrochloride and modified starch reflections at $2\theta \approx 10.2°$ and double reflection at $2\theta \approx 20.5° - 21°$ are selected. The measured densities of materials are presented in the Table 1.

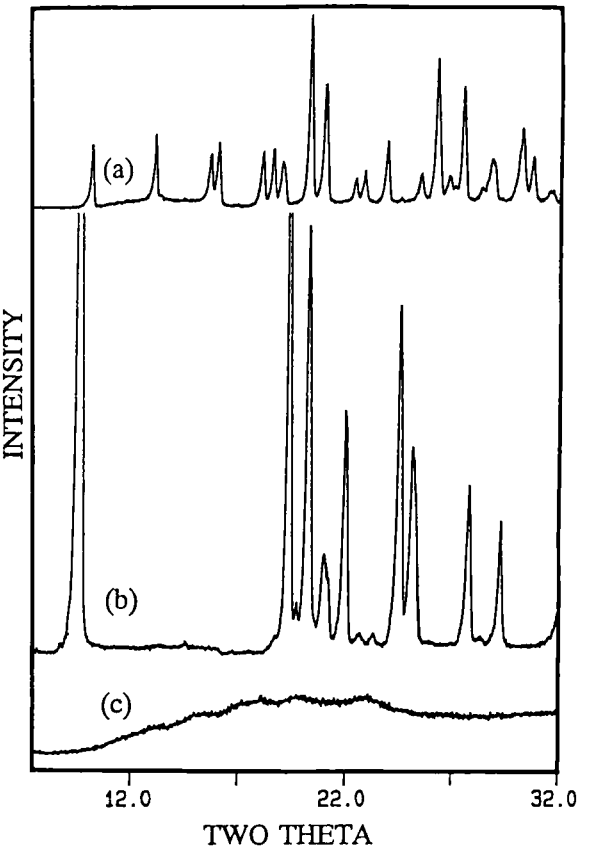

FIGURE 1

Diffractograms of selegilin hydrochloride (a), mannitol (b) and modified starch (c) between $8° \leq 2\theta \leq 32°$. Intensity scales are same for all diffractograms.

The Mixture of Selegilin Hydrochloride and Mannitol

The quantitative analysis of mixtures of selegilin hydrochloride and mannitol were determined using all three before-mentioned methods. The profile fitting were done to the peak at $2\theta \approx 13.1°$. The integrated intensities of reflections at $2\theta \approx 13.1°$ and $2\theta \approx 26.3°$ and the intensity maxima of reflection $2\theta \approx 9.7°$ were measured directly.

TABLE 1

The Densities of Studied Materials

material	density (g/cm^3)	standard deviation (g/cm^3)
selegilin hydrochloride	1.1338	0.0003
mannitol	1.4999	0.0004
modified starch	1.5011	0.0003

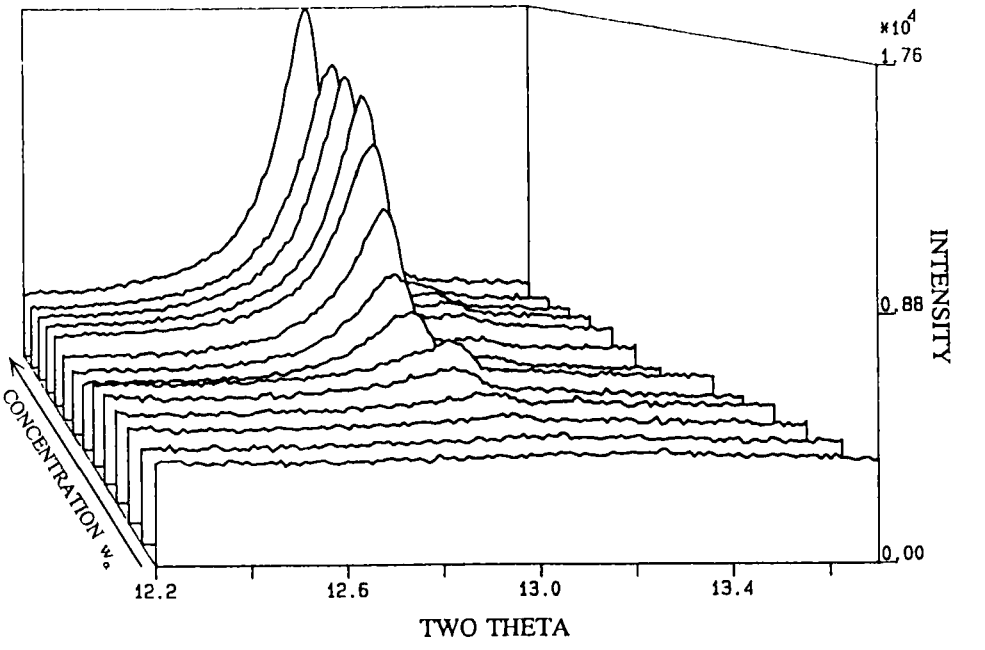

FIGURE 2

Diffractograms of the mixtures of selegilin hydrochloride and mannitol between 12.2° – 13.7°. The percentual amounts of selegilin hydrochloride are since the front 0 %, 0.5 %, 1.1 %, 2.4 %, 4.6 %, 9.8 %, 18.3 %, 26.3 %, 36.7 %, 49.7 %, 64.6 %, 80.3 %, 94.1 % and 100 %.

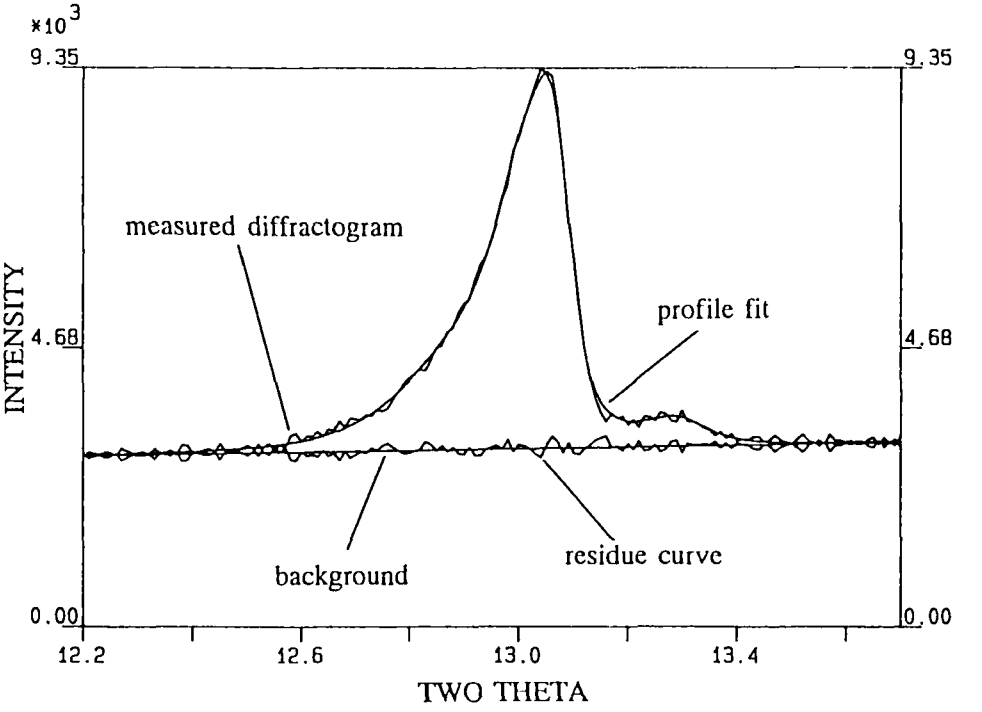

FIGURE 3

The result of the profile fitting. If the profile fitting succeeds the measured diffractogram and the profile fitting match. The residue curve is almost straight and joins with the background.

The measured diffractograms of mixtures between $12.2° \leq 2\theta \leq 13.7°$ are presented in the Fig. 2. The Fig. 3. is an example of profile fitting that is done to the mixture consisting of 35.67 % of selegilin hydrochloride. Here the profile fit yields the value of 119178 cts for integrated intensity.

The results of profile fit and the regression curve are stated in the Fig. 4. The best correlation with the measurements and equation 2 is obtained when $A_{\beta\alpha} = 0.69$, where α refers selegilin hydrochloride and β mannitol. Using the direct integrated intensity measurement the results lead to the values $A_{\beta\alpha} = 0.54$

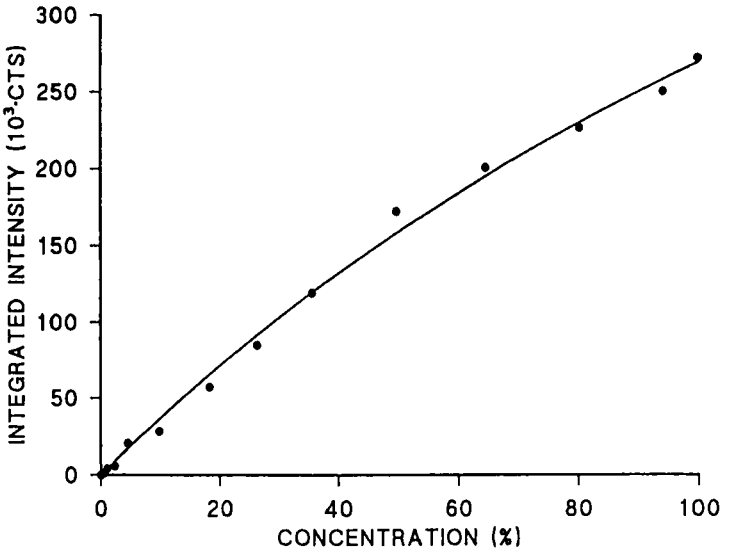

FIGURE 4

The results of quantitative analysis of the mixtures on selegilin hydrochloride and mannitol. The determination is made with the profile fitting using reflection characteristic for selegilin hydrochloride at $2\theta \approx 13.1°$.

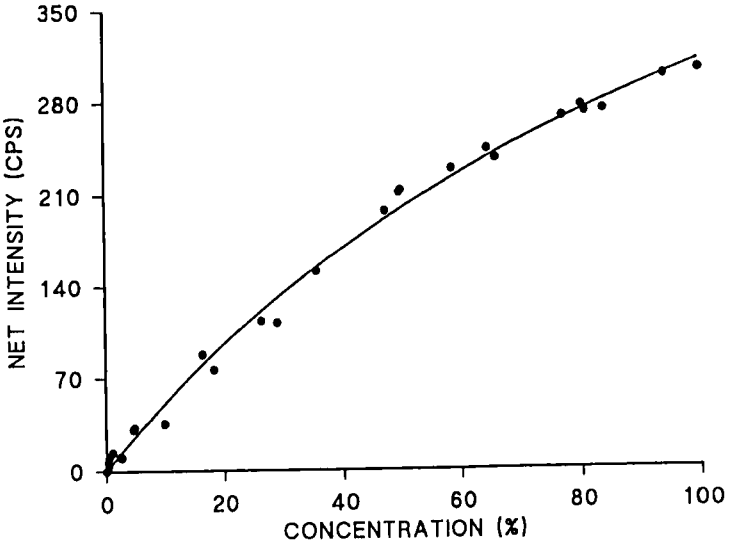

FIGURE 5

The results of quantitative analysis based on direct integrated intensity measurements of reflection at $2\theta \approx 13.1°$. The reflection is characteristic for selegilin hydrochloride.

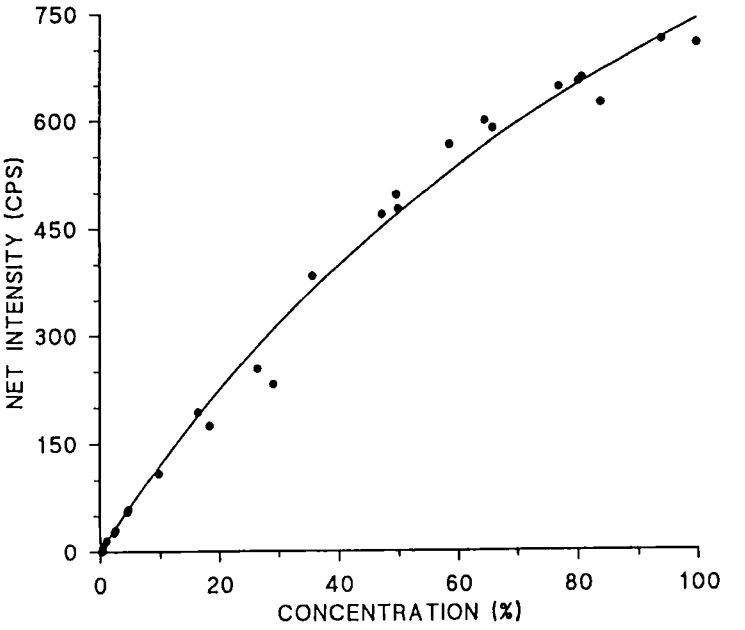

FIGURE 6

The results of quantitative analysis based on direct integrated intensity measurements of reflection at $2\theta \approx 26.3°$. The reflection is characteristic for selegilin hydrochloride.

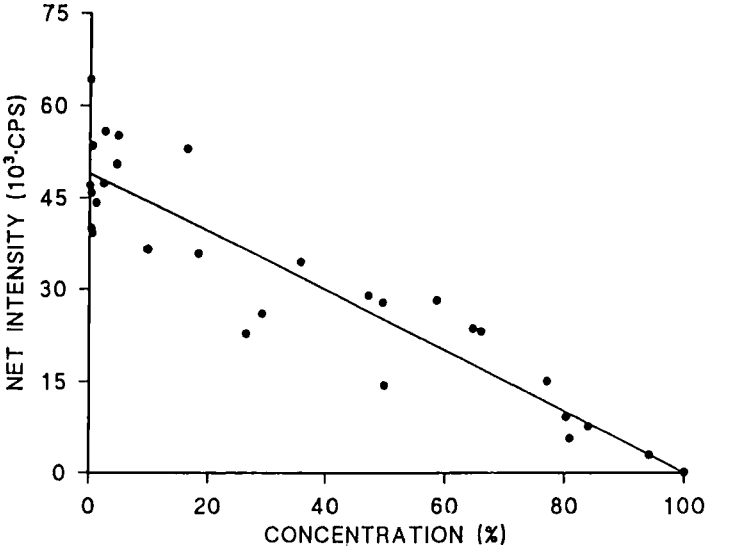

FIGURE 7

The results of quantitative analysis based on intensity maxima measurements of reflection at $2\theta \approx 9.7°$. The reflection is characteristic for mannitol.

TABLE 2

The Results of Quantitative Analysis of the Mixtures of Selegilin Hydrochloride and Mannitol

method	measuring parameters	$A_{\beta\alpha}$ [a]	μ_β/μ_α [b]	r [c]
profile fit (13.1°)	step size 0.01 ° sample time 9 s	0.69 ± 0.09	0.92 ± 0.12	0.9982
integrated intensity measurement (13.1°)	speed 0.005 °/s	0.54 ± 0.06	0.71 ± 0.09	0.9965
integrated intensity measurement (26.3°)	speed 0.005 °/s	0.52 ± 0.06	0.69 ± 0.08	0.9965
maximum intensity measurement (9.7°)	measuring time 300 s	1.05 ± 0.43	1.39 ± 0.57	0.9434

[a] The ratio of mass absorption coefficients
[b] The ratio of linear absorption coefficients
[c] The correlation factor of the regression curve

(13.1°) and $A_{\beta\alpha}$ = 0.52 (26.3°). The results of quantitative analysis are expressed in Fig. 5 and Fig. 6. When using the intensity maxima measurement to the peak at 9.7° the best fit is obtained with $A_{\beta\alpha}$ = 0.95 (Fig. 7). The results and measuring parameters are summarized in the Table 2. The results show that the maximum intensity measurement is not satisfactory here.

The Mixture of Selegilin Hydrochloride and Starch

The quantitative analysis of mixtures of selegilin hydrochloride and modified starch were determined using profile fit and direct integrated intensity

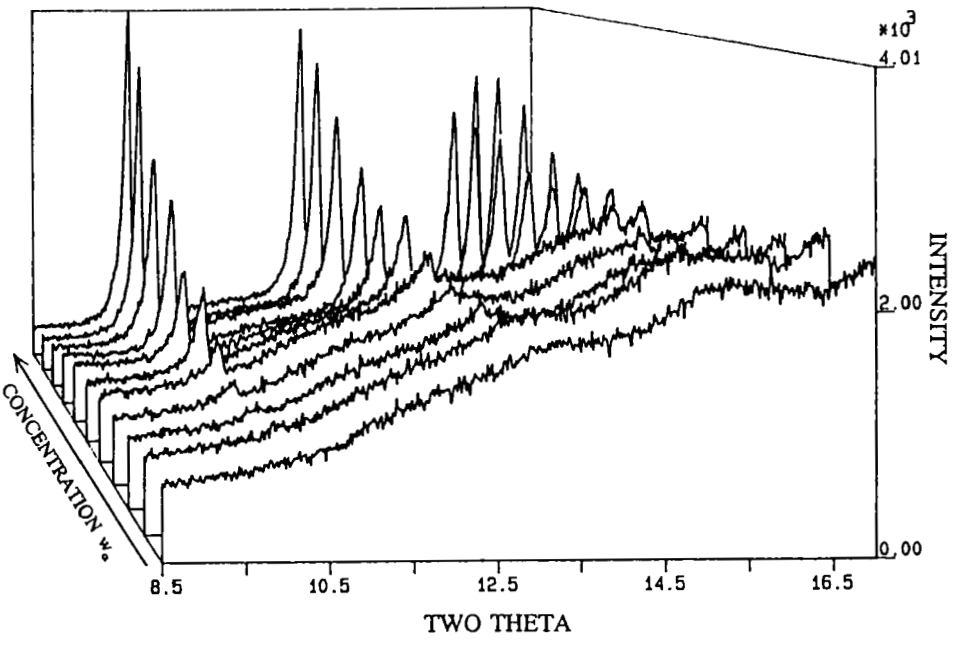

FIGURE 8

Diffractograms of the mixtures of selegilin hydrochloride and modified starch between 8.5° −17.0°. The percentual amounts of selegilin hydrochloride are since the front 0 %, 0.6 %, 2.5 %, 5.1 %, 8.1 %, 19.9 %, 26.1 %, 40.8 %, 54.1 %, 84.1 % and 100 %.

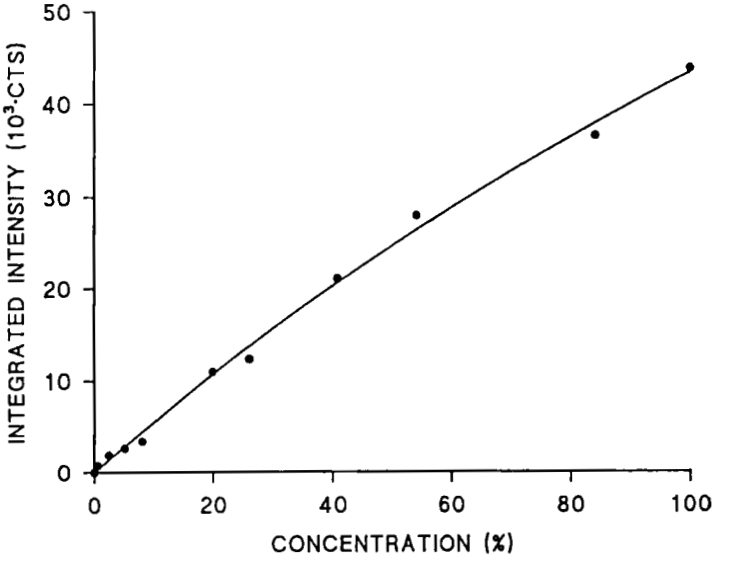

FIGURE 9

The results of quantitative analysis of the mixtures on selegilin hydrochloride and mannitol. The determination is made with the profile fitting using reflection characteristic for selegilin hydrochloride at $2\theta \approx 10.2°$.

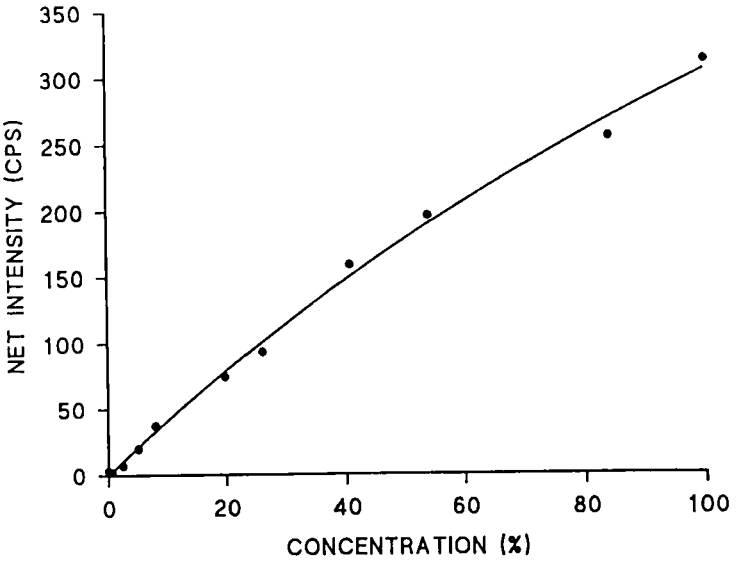

FIGURE 10

The results of quantitative analysis based on direct integrated intensity measurements of reflection at $2\theta \approx 10.2°$. The reflection is characteristic for selegilin hydrochloride.

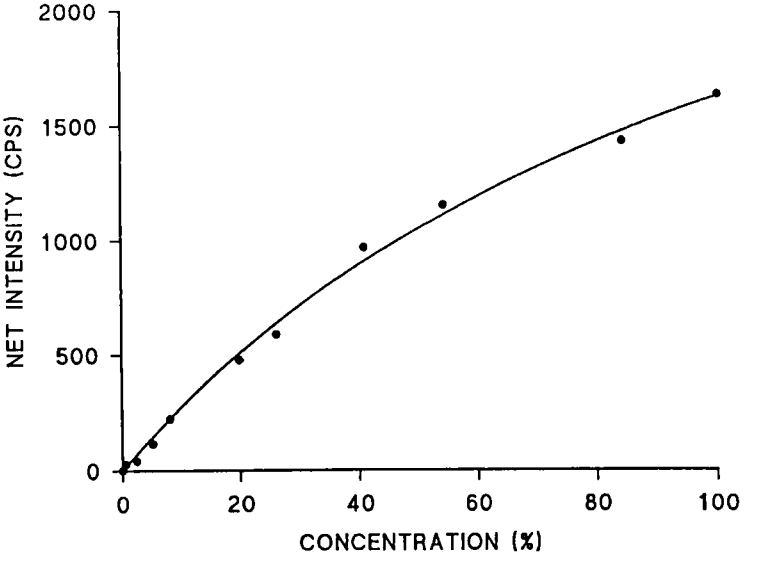

FIGURE 11

The results of quantitative analysis based on direct integrated intensity measurements of reflections at $2\theta \approx 20.5° - 21.0°$. The reflections are characteristic for selegilin hydrochloride.

TABLE 3

The Results of Quantitative Analysis of the Mixtures of Selegilin Hydrochloride and Starch

method	measuring parameters	$A_{\beta\alpha}$[a]	μ_β/μ_α[b]	r[c]
profile fit (10.2°)	step size 0.015 ° sample time 2 s	0.76 ± 0.10	1.01 ± 0.14	0.998
integrated intensity measurement (10.2°)	speed 0.003 °/s	0.74 ± 0.10	0.98 ± 0.13	0.998
integrated intensity measurement (20.5 – 21.0°)	speed 0.003 °/s	0.55 ± 0.08	0.73 ± 0.10	0.998

[a] The ratio of mass absorption coefficients
[b] The ratio of linear absorption coefficients
[c] The correlation factor of the regression curve

measurements. The maximum intensity measurements were omitted because of poor results in previous case. The profile fitting was performed on the peak at $2\theta \approx 10.2°$ and the integrated intensities of the reflections at $2\theta \approx 10.2°$ and $2\theta \approx 20.5° - 21°$ were determined.

The measured diffractograms of mixtures between $8.5° \leq 2\theta \leq 17.0°$ are presented in the Fig. 8 and the results of quantitative analysis and the regression curves are presented in the Fig. 9, Fig. 10 and Fig. 11. The results are summarized in the Table 3.

TABLE 4

The Accuracy of the Phase Composition Measurements as a Function of the Concentration of Selegilin Hydrochloride

sample	method and reflection	the accuracy of the quantitative analysis with different phase concentrations of selegilin hydrochloride		
		2 %	5 %	50 %
selegilin hydrochloride and mannitol	profile fit (13.1°)	(2.0 ± 0.4) %	(5.0 ± 0.9) %	(50 ± 6) %
	integrated intensity measurement (13.1°)	(2.0 ± 0.3) %	(5.0 ± 0.7) %	(50 ± 5) %
	integrated intensity measurement (26.3°)	(2.0 ± 0.4) %	(5.0 ± 0.9) %	(50 ± 6) %
selegilin hydrochloride and modified starch	profile fit (10.2°)	(2.0 ± 0.4) %	(5.0 ± 0.9) %	(50 ± 6) %
	integrated intensity measurement (10.2°)	(2.0 ± 0.4) %	(5 ± 1) %	(50 ± 7) %
	integrated intensity measurement (20.5 – 21.0°)	(2.0 ± 0.4) %	(5 ± 1) %	(50 ± 7) %

The Comparison of the Results

The determination of the factor $A_{\beta\alpha}$ makes possible to find out the relative amounts of phases in the unknown sample. By substituting the measured value of integrated intensity of the unknown sample to the equation (2) the weight fraction w can be determined. The accuracy of the results is also essential and can be estimated from the deviations of the parameters I_α and $A_{\beta\alpha}$. The accuracies of the measurements in both previous stated cases are expressed in the Table 4 as a function of the concentration of selegilin hydrochloride.

CONCLUSIONS

The results show that the concentration of 2 % of selegilin hydrochloride can yet acceptable be detected. Both methods based on the integrated intensity give almost same accuracies. On the other hand the intensity maxima measurements gives only qualitative results. The accuracy is slightly better if both phases are crystalline. In conclusion, quantitative x–ray analysis can be successfully used e.g. to control the variation of the concentrations in solid drug dosage from tablet to tablet.

ACKNOWLEDGMENTS

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