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Application of attenuated total reflectance FTIR spectroscopy to the analysis of mixtures of pharmaceutical polymorphs

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Abstract

Full characterization of the polymorphic content of bulk drug chemicals has become increasingly important in the pharmaceutical industry. A multitude of analytical techniques are commonly employed in this process. In this work the feasibility of the Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) to the qualitative and quantitative analysis of mixtures of polymorphs is investigated using three known polymorphs of ganciclovir as model compounds. Definitive identification of all three polymorphs is achieved from their ATR-FTIR spectra obtained from the sample in their native state (no sample preparation). Quantitation of polymorphic mixtures is carried out using a partial least-squares procedure. Quantitative results obtained with validation samples for both binary and ternary crystal form mixtures clearly demonstrate the strong potential of ATR-FTIR technique for use in quantitative analysis of polymorphic content of bulk pharmaceutical materials.

Keywords: Polymorphism; Fourier transform infra-red spectroscopy; Attenuated total reflectance; Quantitative analysis; Partial least-squares; Ganciclovir

1. Introduction

Characterization of polymorphism in solid pharmaceuticals is a very important aspect of drug development and manufacturing. It is well known (Haleblian and McCrone, 1969; Haleblian, 1975; Wall, 1986) that changes in the polymorphic behavior of a drug substance can adversely impact its bulk chemical properties (e.g. mixing, milling, processibility, filling) as well as its pharmaceutical performance (e.g. bioavailibility, dissolution, stability, suspendibility). Therefore, it is of critical importance that accurate assessment of the polymorphic behavior and correct identification

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of the proper polymorph of the drug substance be made in the early phases of drug development. Moreover, in situations where the most stable and efficacious crystal form is not easy to manufacture as a pure form, it is often necessary to characterize the polymorphic content of the bulk material. This places a high demand for an analytical technique capable of providing rapid qualitative and quantitative monitoring of the crystal form composition of the drug substance. Analytical techniques commonly used to characterize and/or follow polymorphic changes in pharmaceutical solids include powder X-ray diffraction (XRD), thermal analysis (DSC, DTA, TGA, etc.), microscopy, and dissolution study. Among these, powder X-ray diffraction provides the most definitive data to aid crystal form identification. Infrared spectroscopy and solid state NMR have also been used (Fletton et al., 1987; Brittain et al., 1993) in the study of polymorphism, but mainly as ancillary techniques. However, since differences in spatial relations of functional groups and of nuclei in alternate crystal polymorphs give rise to different vibration modes in IR and different resonance frequencies in NMR, both techniques can be used to study polymorphism at the molecular level and gain localized information.

In recent years (Brittain, 1997), FTIR has found wider applications in the study of pharmaceutical solids. This has been, in part, due to the availibility of cost-effective FTIR instruments with high resolution and signal-to-noise ratio, equipped with powerful multivariate quantitative techniques such as partial least-squares (PLS) modeling software. Another equally important development has been the emergence of some new reflectance sampling techniques which allow for direct measurement of the IR spectrum of solids in their native state. That is sample pretreatment, such as KBr pellet preparation, that subjects the sample to extreme high pressures ($\sim 20\,000$ psi), or grinding, as is sometimes done for XRD are not necessary. This eliminates an important concern with the use of FTIR transmission spectrometry for studying crystal polymorphism; namely, the potential for polymorphic changes induced by mechanical treatment (Ketolainen et al., 1995), such as grinding or KBr pellet preparation. One such new technique, Diffuse Reflectance FTIR spectroscopy (DRIFTS), has been successfully applied to the quantitative analysis of binary mixtures of sulfamethoxazole polymorphs (Hartauer et al., 1992). However, the authors caution about the influence of particle size on the DRIFTS spectral quality and correctly point out the need for careful and uniformly sized sample preparation. In addition to the well demonstrated effect of particle size on the diffuse reflectance spectra of solid samples (Fuller and Griffiths, 1978; Christy et al., 1993), quantitative analysis using DRIFT technique require a certain level of expertise on the part of the user as has been pointed out by Christy and coworkers (Christy et al., 1995). A somewhat less serious concern with the use of the DRIFT technique (and the KBr pellet) is the possibility of solid state interaction between KBr and crystal polymorphs.

Another FTIR sampling technique, which has become quite popular in recent years is Attenuated Total Reflectance (ATR). The technique was originally developed by Fahrenfort (Fahrenfort, 1961) and was suggested as a technique for analyzing polymorphs of pharmaceuticals as early as 1969 (Higuchi et al., 1969). However, in that study only qualitative spectral data were obtained from compressed KBr pellet rather than directly from native solid samples. Other workers (Kang et al., 1974) have also reported on the use of ATR for identification of pharmaceutical solids. Very limited quantitative applications of ATR-FTIR to the analysis of pharmaceutical solids have been reported. For example, circle cell liquid ATR has been used to quantitate mixture of trimethoprim and sulfamethoxazole in pharmaceutical formulation (Hartauer and Guillory, 1989). To date however, no application of ATR-FTIR to the quantitation of polymorphs has been reported in the literature. In this paper, the feasibility of ATR-FTIR to the qualitative and quantitative analysis of mixtures of pharmaceutical polymorphs is described using three known polymorphs of ganciclovir as a model compound. Ganciclovir is the active ingredient of Cytovene, an antiviral drug marketed by Roche Group, Ltd. for cytomegalovirus infection. The three known polymorphs of ganciclovir used in this work will be referred to as phase I, phase II, and phase III.

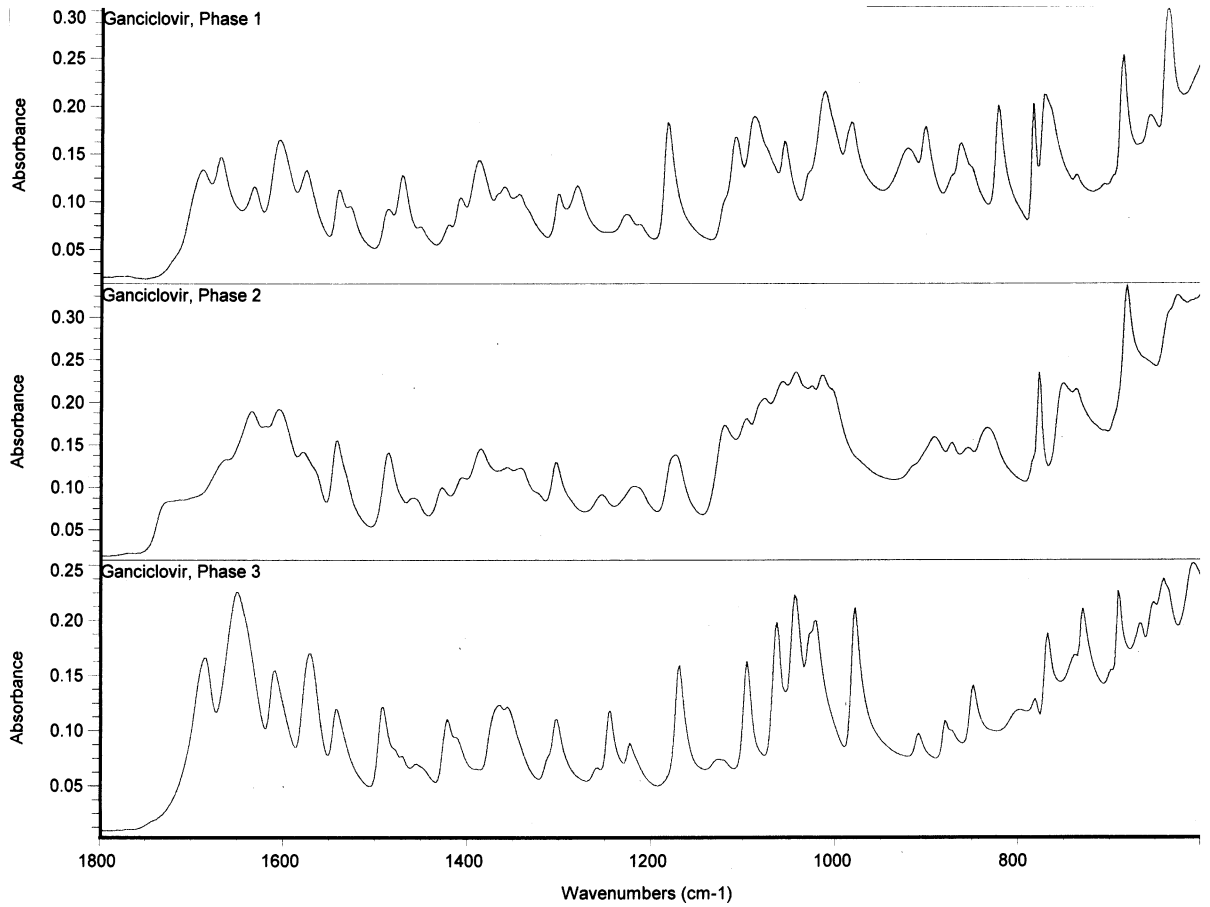

Fig. 1. ATR-FTIR spectra of three pure polymorphs of ganciclovir.

2. Materials and methods

2.1. Instrumentation and software

A Nicolet MAGNA-550 FTIR (Nicolet Instrument Co., Madison, WI) equipped with DTGS detector and a 9-bounce DuraSamplIR™ diamond horizontal ATR sampling accessory (ASI Applied Systems, Norwalk, CT) was used for all of the experiments. Each spectrum represents 200 co-added scans measured at a spectral resolution of 4 cm^{-1} in the 4000–600 cm^{-1} range with an aperture of 130, and Happ–Genzel apodization. Dry air purge was maintained during all data acquisition.

Instrument control and data analysis was done using a Compaq 486-DX/66 MHz computer. Data was acquired using OMNIC (ver. 3.1, Nicolet Instrument Co.). Calibration and quantitation was performed using Partial Least Squares (PLS) as implemented in TurboQuant (ver. 1.0, Nicolet Instrument Co.). All Software were executed under Windows™ (ver. 3.1, Microsoft Corp.) with MS–DOS® (ver. 6.2, Microsoft Corp.).

2.2. Materials

Standards were prepared from three pure polymorphs of ganciclovir. Mixtures of the polymorphs were weighed in 2-ml GC borosilicate autosampler vials using an analytical balance accurate to 0.00001 g. The standards were then mixed by vibration using a Burgess Vibrograver pressed against the outer wall of the vial. Three

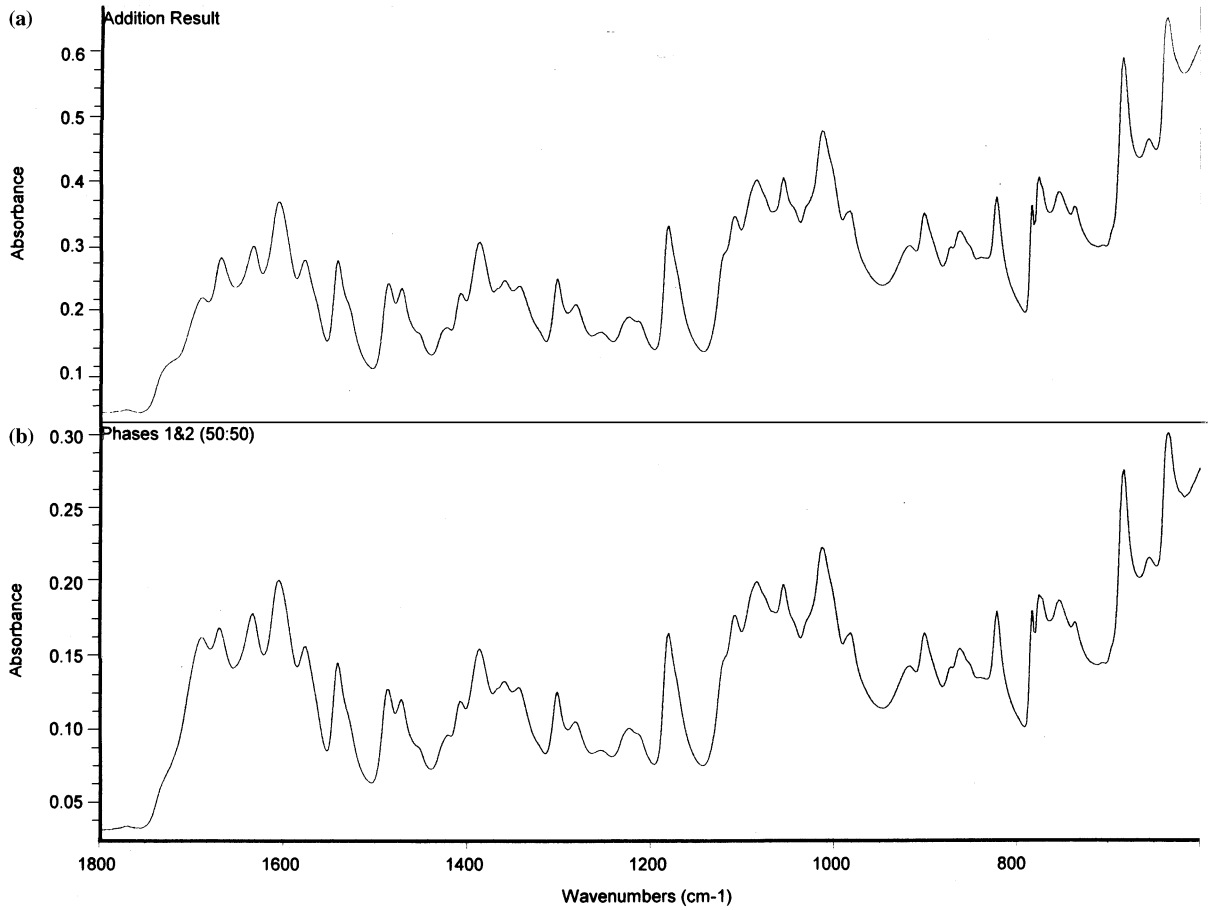

Fig. 2. (a) Result spectrum of mathematically added spectra of ganciclovir pure polymorphs I and II. (b) Spectrum of the actual physical 1:1 mixture of ginciclovir polymorphs I and II.

binary-phase calibration standard sets were created consisting of phases I and II, phases I and III, and phases II and III. Each of these sets consisted of seven binary-phase mixture standards with wt% concentrations of 5, 10, 25, 50, 75, 90, and 95%. A set of ternary-phase calibration standards were also prepared in the same manner as the binary-phase mixture standards. Six ternary mixture standards were made with each phase at 10, 30, and 60%. Additionally, a three-phase mixture standard was prepared that consisted of 33.3% of each phase. Another set of ternary standard mixtures was also prepared having varying ratios of each of the three phases (see Table 3 for the values of each phase). All of the pure component and mixture standards were sealed with Teflon-lined caps and kept refrigerated at 4°C.

2.3. Analytical procedure

Before the acquisition of experimental data, the DurasamplIR™ was aligned in accordance to the manufacturer's recommendation. A few mg of a standard was placed on the surface of the diamond (Internal Reflection Element, IRE) and held in place by the Calibrated Pressure Applicator (ASI Applied Systems) to ensure even and reproducible contact between the sample and the IRE (diamond) surface.

Each set of standards (binary-phase and the ternary-phase sets), along with samples of the pure phases represented in that particular set were

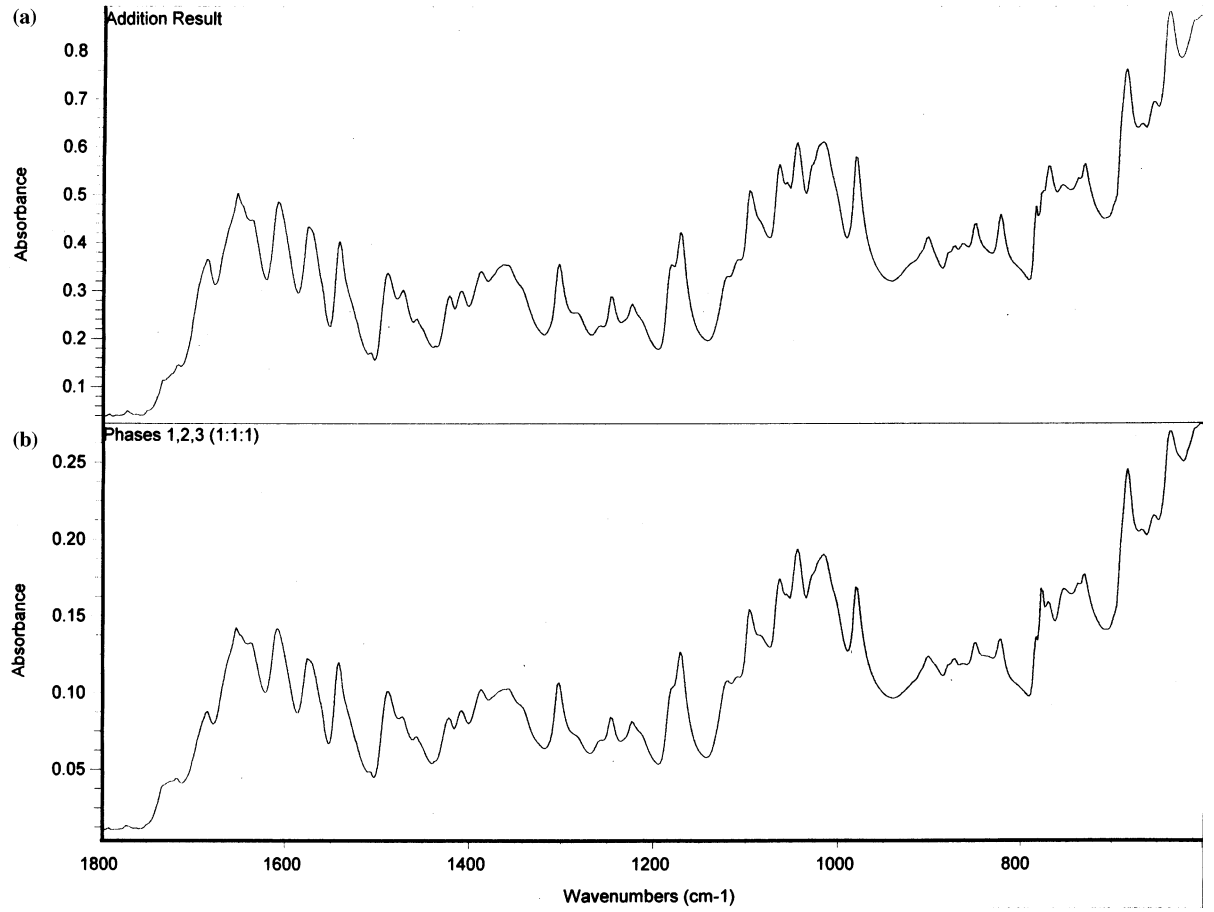

Fig. 3. (a) Result spectrum of mathematically added spectra of pure ganciclovir phases I, II, and III. (b) Spectrum of the actual physical ternary 1:1:1 mixture of phases I, II, and III.

analyzed in triplicate with each replicate acquired on a different day. The additional ternary-phase standards, represented in Table 3, were analyzed with single determinations on a day separate from the analysis of any other standard set.

3. Results and discussion

The ATR-FTIR spectra of the three pure phases of ganciclovir are shown in Fig. 1. It is evident from comparison of the three spectra in Fig. 1 that each polymorph exhibits a characteristic spectrum in the IR fingerprint region (1800–600 cm^{-1}), with significant differences whereby each polymorph can be readily identified. Furthermore, various mixture combinations of pure phases produced very distinct and predictable spectra. Spectra obtained by mathematical addition of various pure polymorph spectra closely matched the spectra measured for the corresponding actual physical mixtures. For example, Fig. 2a and Fig. 2b show the ATR-FTIR result spectrum for the mathematically added spectra of pure phases I and II, and the spectrum of the actual physical 1:1 mixture of the two phases, respectively. Similarly, Fig. 3a and Fig. 3b show the same type of comparison between the added ATR-FTIR result spectrum and that of the physical ternary 1:1:1 mixture of phases I, II and III. These comparisons also illustrate that the physical mixtures were properly prepared.

Table 1
PLS parameters used for calibration and validation

Parameter	Phase I	Phase II	Phase III
Number of factors	6	4	6
% Concentration explained*	98.1	98.2	98.6
Regions (cm^{-1})	1115–1101	1115–1101	1115–1101
	870–791	870–791	870–791
	781–773	781–773	781–773
	762–712	762–712	762–712
	687–662	687–662	687–662

*Variance of a phase's concentration explained by the cummulative effect of factors.

Calibration and quantitation was done using the Partial Least Squares (PLS) model as provided in Nicolet TurboQuant (ver. 1.0) quantitative software. The first replicate measurements of three data sets consisting of both binary and ternary mixtures were used as calibration while the second and third replicates were used as validation. The forth ternary mixture set was used for validation. Prior to using PLS, all spectral data were normalized by mean centering and the proper spectral regions as well as optimal number of factors were determined. Table 1 lists the parameters, which were used by PLS model for calibration and validation. For all three polymorphs a good linear relationship was observed between the actual and predicted concentrations for calibration standards. Fig. 4 shows the calibration plots for the three polymorphs with each having correlation coefficient of 0.981, 0.982 and 0.986, respectively. Table 2 and Table 3 show the actual and predicted percent concentrations, and the absolute errors obtained for the different sets of validation standards. Table 2 consists of the

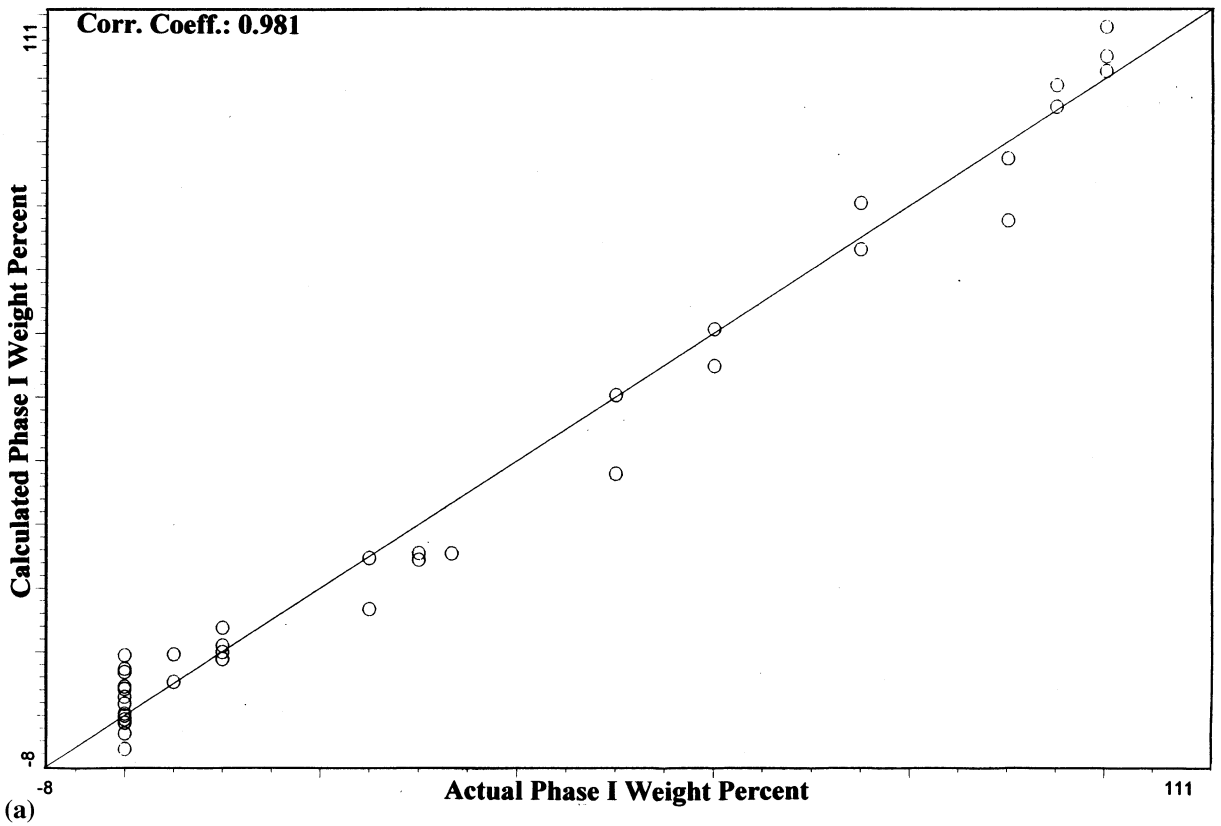

Fig. 4. Calibration plots of the three phases of ganciclovir: a) phase I, b) phase II, c) phase III.

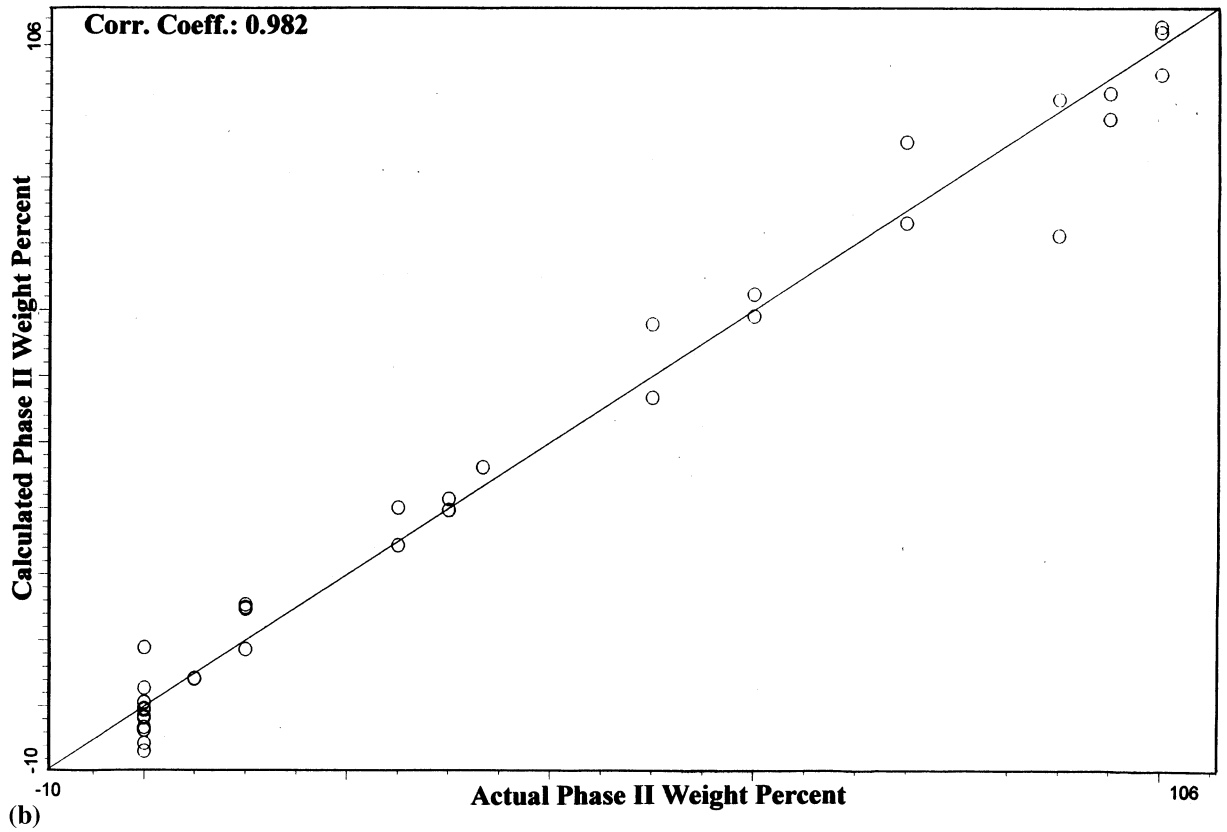

Fig. 4. (*Continued*)

second and third replicate measurements of the same physical standards that were used in calibration. Table 3 consists of the independent set of ternary mixtures prepared for further validation and testing of the predictive power of the model. As can be seen from the data in the Table 2 and Table 3, each polymorph can be quantitated in either binary or ternary mixture standards with a mean absolute error of better than 4.0% with slightly better performance for phase III compared to phases I and II. The data in Table 3 demonstrate that for the independently prepared standards (mixtures of three phases), the model is successful in predicting the phase purity (100% concentration) with a mean absolute errors of less than 3% for phase I and II, and about 6% for phase III. The absence of each of the three phases (0.0% concentration) is also predicted with a mean absolute errors of 3%, 0.3%, and 3.7% for phases I, II and III, respectively. The overall mean absolute errors for the three phases are 3.4, 4.6, and 5.8% as shown in Table 3.

In conclusion, the results obtained in this work clearly demonstrate that ATR-FTIR spectroscopy in conjunction with PLS modeling can be successfully applied to the qualitative and quantitative analysis of mixtures of pharmaceutical polymorphs. The technique is fast and nondestructive, relatively easy to use, and most importantly requires no sample pretreatment whereby offering great advantage when normal transmission IR could not be used due to concerns for phase transformation. Finally, it should also be pointed out that although this research was undertaken merely to demonstrate the feasibility of the ATR-FTIR technique to the characterization of polymorphism in solid pharmaceuticals, the data presented here show that the technique can poten-

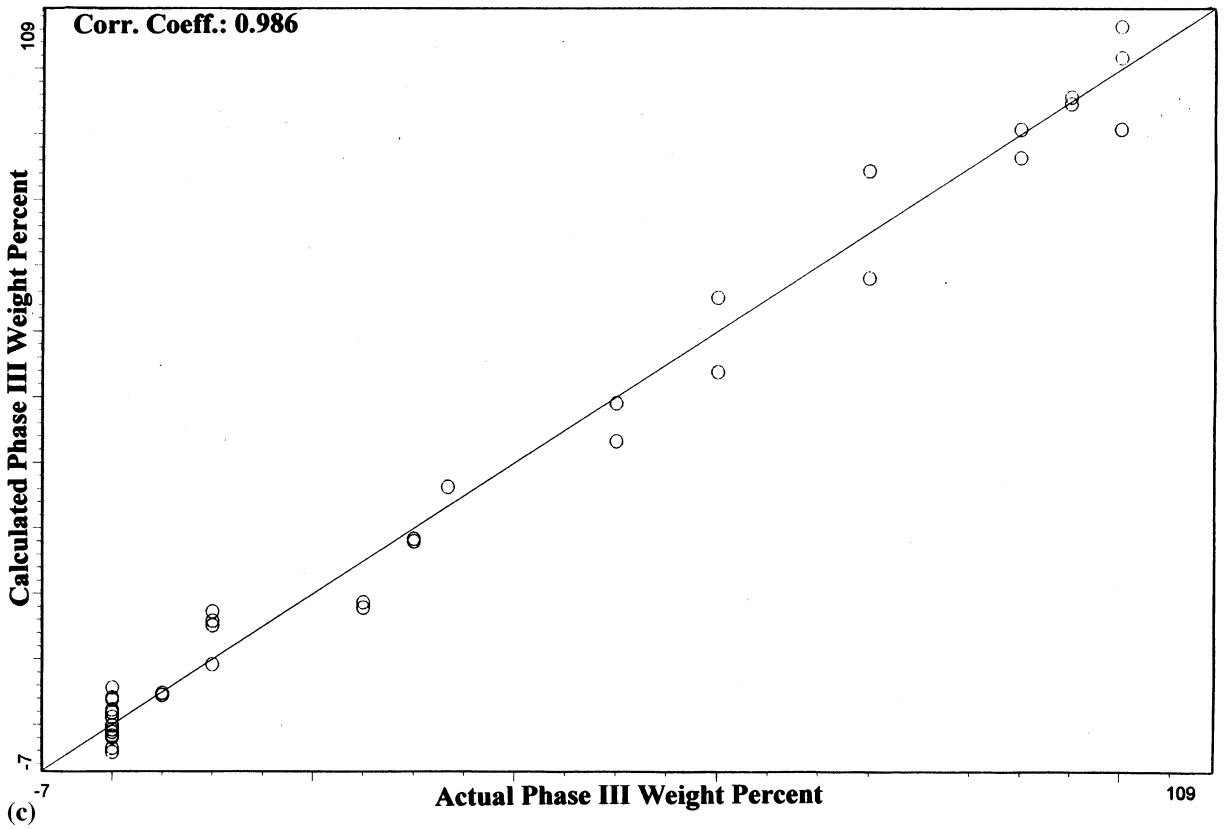

Fig. 4. (*Continued*)

Table 2
Actual vs. mean predicted values and absolute errors for the first validation set

Actual wt%	Mean predicted wt%								
	Phase 1			Phase 2			Phase 3		
	Mean	S.D.	Difference	Mean	S.D.	Difference	Mean	S.D.	Difference
0.0	2.8	4.0	−2.8	−0.9	4.3	0.9	2.9	5.6	−2.9
5.0	7.6	5.7	−2.6	4.0	1.3	1.0	4.8	1.3	0.2
10.0	8.3	1.8	1.7	12.5	2.8	−2.5	12.6	5.8	−2.6
25.0	21.8	3.5	3.2	29.2	3.0	−4.2	22.2	6.9	2.8
30.0	19.7	1.8	10.3	32.6	3.8	−2.6	27.3	0.6	2.7
33.3	25.2	0.8	8.1	39.8	3.0	−6.5	33.9	3.2	−0.6
50.0	50.9	5.2	−0.9	47.0	5.4	3.0	48.1	6.6	1.9
60.0	56.3	3.2	3.7	66.8	2.7	−6.8	62.8	7.3	−2.8
75.0	71.6	9.2	3.4	73.0	8.8	2.0	75.4	6.4	−0.4
90.0	92.4	8.8	−2.4	87.1	8.0	2.9	89.1	7.6	0.9
95.0	95.0	1.2	0.0	89.1	10.5	5.9	95.9	2.0	−0.9
100	106	3.2	−6.0	89.5	13.5	10.5	102.0	7.7	−2.0
			3.8*			3.7*			1.8*

*Mean of the absolute values of the differences.

Table 3
Actual vs. predicted values and absolute errors for the second validation set

Validation standard ID	Phase 1			Phase 2			Phase 3		
	Actual wt%	Predicted wt%	Difference	Actual wt%	Predicted wt%	Difference	Actual wt%	Predicted wt%	Difference
Phase 1	100.0	102.0	−2.0	0.0	−2.1	2.1	0.0	−1.1	1.1
Phase 2	0.0	3.2	−3.2	100.0	104.0	−4.0	0.0	−6.7	6.7
Phase 3	0.0	5.6	−5.6	0.0	1.2	−1.2	100.0	94.1	5.9
Mix 1	5.6	9.0	−3.4	21.2	22.0	−0.8	73.2	68.5	4.7
Mix 2	7.3	9.0	−1.7	9.8	11.1	−1.3	82.8	80.3	2.5
Mix 3	14.2	21.4	−7.2	43.1	46.0	−2.9	42.7	32.2	10.5
Mix 4	14.1	12.5	1.7	78.6	86.8	−8.2	7.4	0.7	6.7
Mix 5	26.8	23.6	3.2	56.0	65.9	−9.9	17.2	10.2	7.0
Mix 6	33.6	39.5	−5.9	11.4	7.2	4.2	55.0	54.7	0.3
Mix 7	40.8	45.6	−4.8	18.2	22.7	−4.5	41.0	31.6	9.4
Mix 8	46.4	47.7	−1.3	32.9	37.5	−4.6	20.7	14.3	6.4
Mix 9	46.5	47.2	−0.7	28.2	35.4	−7.2	25.4	17.0	8.4
Mix 10	58.2	61.5	−3.3	18.2	21.1	−52.9	23.5	17.6	5.9
			3.4*			4.1*			5.8*

*Mean of the absolute values of the differences.

tially provide highly tailored, rapid and reliable information in situations where assessments of batch polymorphic purity, consistency or control of relative composition are required.

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