Fourier Transform-Raman Spectroscopy for the Qualitative and Quantitative Characterization of Sulfasalazine-Containing Polymeric Microspheres

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FT-Raman spectroscopy (FTRS) has been used to characterize microspheres produced from the pharmaceutical polymer Eudragit RS containing a range of concentrations of the drug sulfasalazine. While pure sulfasalazine produced an intense and complex Raman spectrum, the spectrum of drug-free Eudragit RS microspheres was considerably weaker in intensity and contained only a few prominent Raman scattering peaks. In spectra of the drug–polymer microspheres, peaks arising from the individual components could be identified. This enabled a quantitative analysis to be undertaken by calculating the ratio between the area of a sulfasalazine peak and the area of a Eudragit RS peak for each microsphere spectrum. A correlation was shown between the peak area ratio and the microsphere sulfasalazine content. FTRS was then applied to a series of microsphere samples which had been dissoluted into pH 7 buffer for 1, 3, 6, 9, 12, or 24 hr. For each spectrum, the drug–polymer peak area ratio was determined and this in turn enabled calculation of the residual drug content of the microsphere sample. FTRS-calculated data showed good agreement with microsphere drug content values determined spectrophotometrically.

KEY WORDS: Fourier transform (FT)-Raman spectroscopy; Eudragit RS; sulfasalazine; microspheres.

INTRODUCTION

The encapsulation of pharmaceuticals in polymer microspheres may provide a number of potential therapeutic benefits over conventional dosage forms. Such systems can be used for providing sustained or targeted delivery of drug following administration by a variety of routes including oral, parenteral, ophthalmic, and nasal (1,2). As part of a research program investigating aspects of colonic drug delivery using particulate delivery systems, we have evaluated the potential of Fourier transform-Raman spectroscopy (FTRS) as a noninvasive method for qualitative and quantitative characterization of drug within polymer microspheres.

In the past, conventional Raman spectroscopy, an inelastic light-scattering process, suffered from the drawback of laser-induced background fluorescence and photodegradation in molecules sensitive to light of visible wavelength. Consequently, although complementary to infrared spectroscopy, it has not been as widely used. The problem of fluorescence has been overcome by the introduction of near-infrared sources of excitation, but the efficiency of scatter in this region is much lower than for visible light. This loss of signal can be countered by using Fourier transform methods of data analysis (3), and as such, FTRS is becoming established as an important routine technique in vibrational spectroscopy (4,5). FTRS has the particularly attractive features of minimal sample preparation requirements, the ability to analyze systems *in situ*, and, for most materials, a rapid analysis time. In the pharmaceutical field, FTRS has been used to investigate the structure of drugs and polymers and to follow the degradation of biodegradable polymers (6). In this paper, we extend these studies to the analysis of sustained-release microspheres made from the water-insoluble acrylic polymer Eudragit RS and containing sulfasalazine, a drug widely used for the treatment of inflammatory bowel diseases.

MATERIALS AND METHODS

Materials

Sulfasalazine (Sigma, Poole, UK), Tween 20 (Sigma), Eudragit RS (Röhm Pharma, Darmstadt, Germany), methylene chloride (GPR grade; Rhône-Poulenc, Dagenham, UK), sodium hydroxide (BDH, Poole, UK), and potassium dihydrogen phosphate (BDH) were used. All reagents were used as received.

Methods

Microsphere Production

Microspheres were produced by an emulsification-solvent evaporation procedure (7). Eudragit RS was dissolved in 20 ml of methylene chloride and sulfasalazine added to form a suspension. The drug–polymer mixture was then dispersed into 100 ml of 0.1% (w/v) aqueous Tween 20 solution using an overhead stirrer. Stirring was continued for 4 to 5 hr in a fume cupboard until the solvent had evaporated. The completed microspheres were filtered, rinsed with distilled water, and freeze-dried overnight, and a sub-500-μm sieve fraction of the dried product collected. In all cases, the total quantity of drug and polymer in the methylene chloride at the start of manufacture was 2 g. Eleven batches of microspheres were produced containing a range of concentrations of sulfasalazine in order to produce a calibration graph of drug concentration versus Raman peak intensity. The following drug/polymer ratios (by weight) were employed: 6/94, 10/90, 15/85, 20/80, 25/75, 30/70, 35/65, 45/55, 50/50, 55/45, and 60/40. In addition, a drug-free sample of microspheres was produced and a sample fabricated for a dissolution study composed of a 33/67 drug/polymer mixture.

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Microsphere Assay

An accurately weighed quantity of microspheres estimated to contain about 2 mg of sulfasalazine was dissolved in 5 ml of acetone in a 100-ml volumetric flask. The flask was made to volume with a 0.05 *N* aqueous sodium hydroxide solution, resulting in dissolution of the drug and precipitation of the polymer. A sample was filtered (1-μm membrane filter) and the UV absorbance measured (λ_{max} = 458 nm). The drug concentration was calculated by reference to a calibration curve of sulfasalazine in 0.05 *N* aqueous sodium hydroxide containing 5% (v/v) acetone.

Microsphere Drug Release Study

The microspheres made with the 33/67 drug/polymer mixture were dissoluted to provide a comparison of microsphere drug content values obtained from drug release measurements and FTRS analysis. Forty milligrams of the microspheres (250–500 μm) was placed in each of the six flasks in a dissolution apparatus (USP apparatus 2) containing 500 ml of pH 7 phosphate buffer (0.05 *M*) at 37°C. The flask contents were agitated at 100 rpm by paddle stirrer. At 1, 3, 6, 9, 12, and 24 hr, one of the six flasks was removed and the microspheres collected by filtration. The filtrate was assayed spectrophotometrically for sulfasalazine content (λ_{max} = 359 nm) to determine the quantity of drug remaining in the microspheres. The microspheres collected by filtration were dried in preparation for FTRS analysis.

FT-Raman Analysis

FT-Raman spectra were obtained from a Bruker FRA 106 FT-Raman system with an IFS 88 FT-IR optics bench (Bruker Spectrospin, Coventry, UK). All samples were lightly packed into an aluminium sample cup (10-mm overall diameter containing a 2-mm-diameter indentation to hold sample) and irradiated by a diode pumped Nd:YAG laser (Adlas, Lubeck, Germany) of 1.064-μm wavelength. The laser beam was focused to a spot size of 100-μm diameter onto the sample and the resultant Raman spectrum collected after 50 scans. For each spectrum, the signal/noise ratio was optimized by altering the parameters governing spectral acquisition, i.e., resolution and laser power. All of the calibration samples, apart from the four highest drug concentrations, were analyzed at a laser power of 180 mW and an instrumental resolution of 4 cm^{-1}. The remaining four samples were analyzed at 140 mW and 4-cm^{-1} resolution. To achieve a comparable signal/noise ratio, the dissolution samples were analyzed at 300 mW and 4-cm^{-1} resolution.

Spectral reproducibility was examined using a microsphere sample containing 17.4% (w/w) sulfasalazine. To test within-sample variability, 10 individual spectra were obtained for a single microsphere sample (140 mW/4 cm^{-1}/50 scans). To test between-sample variability, single spectra were obtained of 10 individual microsphere samples weighing between 1.2 and 71.1 mg using the same acquisition parameters.

RESULTS AND DISCUSSION

Microsphere Characterization

The results of the microsphere spectrophotometric assay are listed in Table I. In general, drug encapsulation efficiency was in excess of 85% and increased with drug concentration, reaching a plateau above 24% (w/w) sulfasalazine.

Table I. Details of Eudragit RS/Sulfasalazine Microsphere Formulations

Drug/polymer ratio (w/w)	Assayed drug content % (w/w)	Encapsulation efficiency (%)
6/94	5.1	85
10/90	8.9	89
15/85	11.7	78
20/80	17.3	86
25/75	24.0	96
30/70	28.5	95
35/65	33.8	97
45/55	42.9	95
50/50	47.3	95
55/45	51.2	93
60/40	54.6	91
33/67	32.0	96

Drug Release Study

The results of the microsphere dissolution study are presented in Table II. The percentage of the encapsulated drug released into the dissoluting buffer is recorded. From the percentage of drug released, the residual drug content of the microspheres at the various time intervals has been calculated. The sustained-release properties of these microspheres are clearly demonstrated, with 62% of the encapsulated drug released after 12 hr.

FT-Raman Spectra

Qualitative Interpretation

Sulfasalazine produced an intense and complex Raman spectrum (Fig. 1) because of the centrosymmetric vibrational nature of the three aromatic ring structures within the molecule. In Raman spectroscopy, each form of ring substitution produces a specific band pattern distribution. This pattern is evident in the spectrum of sulfasalazine; the peaks at 1395, 1148, 1077, and 708 cm^{-1} probably originate from the ring breathing pattern of the 1,2,4-trisubstituted benzene

Table II. *In Vitro* Release of Sulfasalazine from Eudragit RS Microspheres over a 24-hr Period

Time after dissolution (hr)	% (w/w) of encapsulated drug released	Microsphere drug content (%, w/w)
0	0.0	32.0
1	12.4	28.0
3	29.6	22.5
6	39.8	19.3
9	54.7	14.5
12	62.3	12.1
24	73.2	8.6

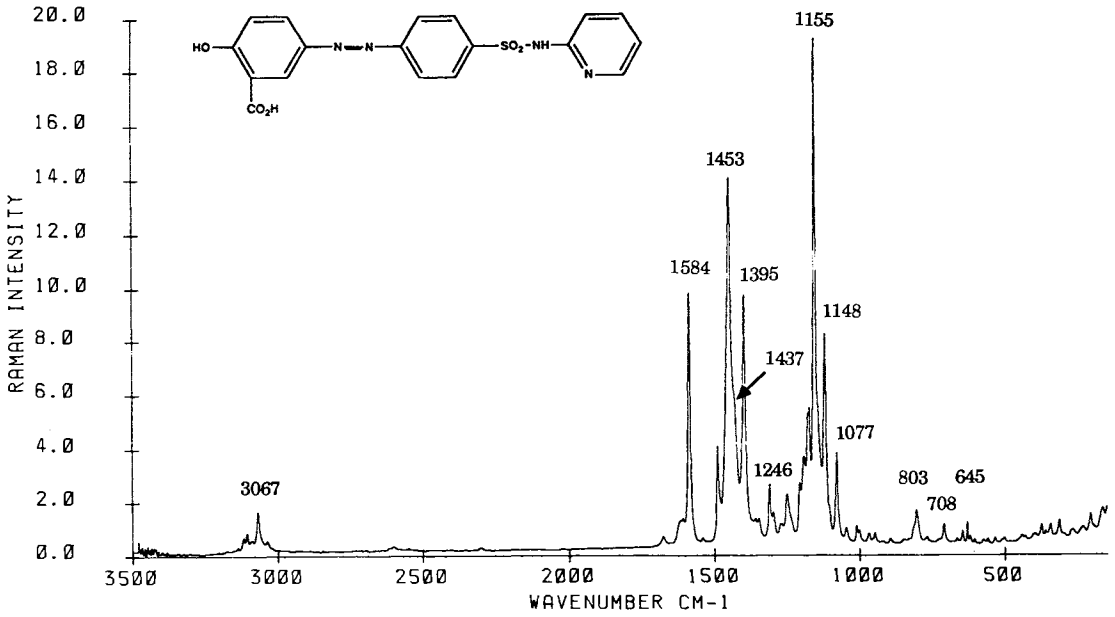

Fig. 1. FT-Raman spectrum of sulfasalazine.

ring, whereas the peaks at 1246, 803, and 645 cm^{-1} may arise from the 1,4-substituted benzene function. The peak at 1584 cm^{-1} could originate from any of the three aromatic groups. Other peaks in the spectrum arise from specific functions within the molecule such as the sulfonamide group ($-SO_2NH-$) at 1155 cm^{-1}. The 2-substituted pyridine function produces a peak at 1453 cm^{-1} and a broad shoulder at 1437 cm^{-1}. There is a relatively weak band at 3067 cm^{-1} arising from C–H stretching vibrations within one of the molecule's benzene rings.

Eudragit RS is a commercially available copolymer of methyl methacrylate, ethyl acrylate, and trimethylammonium methacrylate chloride. Approximately 1 in 40 of the polymer backbone units contains a trimethylammonium methacrylate chloride monomer which provides the polymer with a degree of water permeability. Compared to sulfasalazine, the spectrum of drug-free Eudragit RS microspheres (Fig. 2) appeared considerably weaker in intensity and less complex. Three major bands dominate the spectrum, at 1728, 1449, and 2949 cm^{-1}. The 1728-cm^{-1} band arises from the carbonyl bond of the polymer ester function. The 1449-cm^{-1} band originates from deformation of the methylene groups, while the 2949-cm^{-1} band is a composite originating in the symmetric and asymmetric C–H stretching of the large number of methyl and methylene groups in the polymer structure.

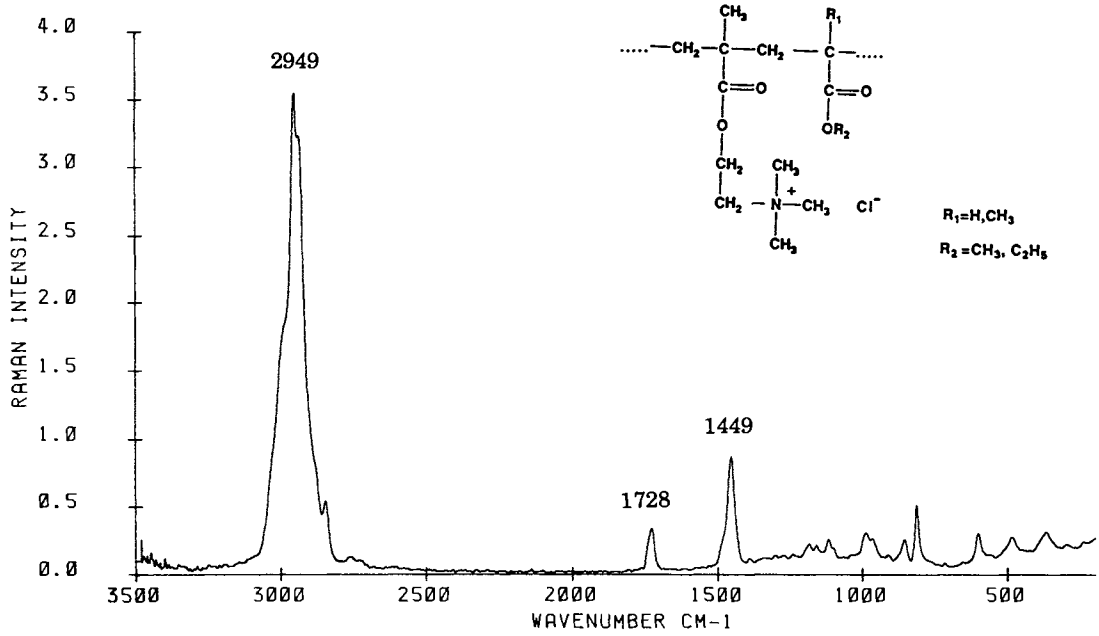

Fig. 2. FT-Raman spectrum of drug-free Eudragit RS microspheres.

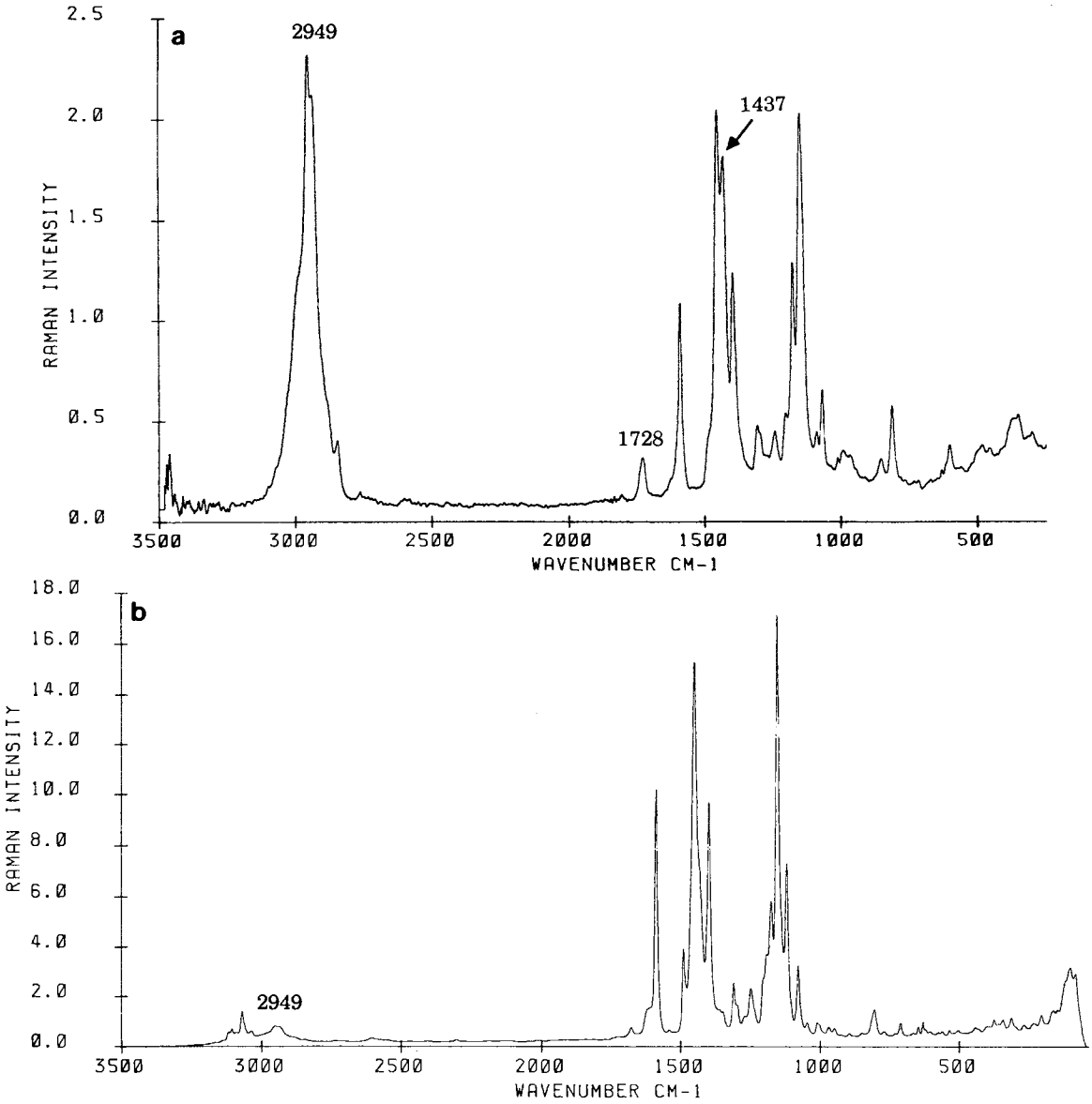

Fig. 3. FT-Raman spectra of Eudragit RS:sulfasalazine microspheres containing (a) 5.1% (w/w) sulfasalazine and (b) 54.6% (w/w) sulfasalazine.

In Raman spectroscopy certain functional groups are good or bad scatterers. Randomized molecular systems, particularly if aliphatic, produce very weak spectra. Their random nature results in a complex vibrational fingerprint, and as a consequence, the numerous bands overlap, producing a broad, diffuse, weak spectrum. In comparison, heterocyclic and aromatic compounds and crystalline materials usually give intense, sharp spectra. Sulfasalazine and Eudragit RS are good examples of a good and a poor Raman scatterer, respectively, and illustrate this principle well.

Figure 3a shows the FT-Raman spectrum of microspheres containing 5.1% (w/w) sulfasalazine. The Eudragit RS peaks at 2949 and 1728 cm^{-1} can be clearly seen, but the 1449-cm^{-1} peak has been masked by sulfasalazine peaks. In contrast, the characteristic sulfasalazine scattering peaks are clearly evident, e.g., 1584, 1453, 1395, 1148, and 1077 cm^{-1}. These peaks are of equivalent intensity to those from the polymer and reflect the high scattering intensity of the sulfasalazine despite its low bulk concentration. In this spectrum, the 1437-cm^{-1} shoulder, barely discernible in the sulfasalazine spectrum (Fig. 1) now appears resolved and relatively intense. Figure 3b shows the spectrum of microspheres containing 54.6% (w/w) sulfasalazine. It is dominated by peaks arising from the drug and contains few visible polymer peaks. For example, the 1728-cm^{-1} peak, diagnostic of Eudragit RS, is no longer visible and the intensity of the 2949-cm^{-1} peak has become very weak. Figures 3a and b demonstrate that sulfasalazine can clearly be distinguished from Eudragit RS in this mixed system, even at low concentrations. In Fig. 3b, it is interesting that the sulfasalazine 1437-cm^{-1} peak has virtually disappeared again. In fact it is apparent from the spectra of the other samples that, as the sulfasalazine content of the microspheres decreases, the 1437-cm^{-1} peak increases in intensity. This may

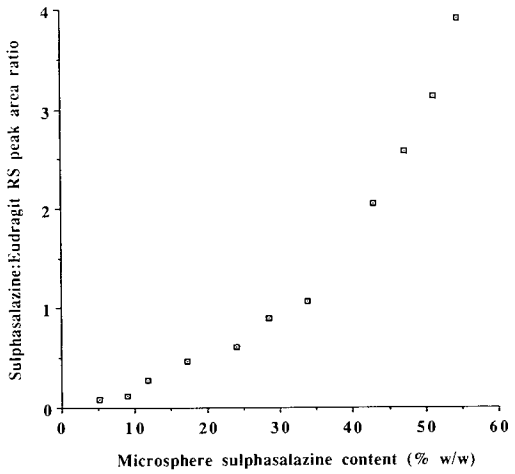

Fig. 4. Relationship between sulfasalazine:Eudragit RS peak area ratio (using sulfasalazine 1584-cm^{-1} peak and Eudragit RS 2949-cm^{-1} peak) and microsphere sulfasalazine content.

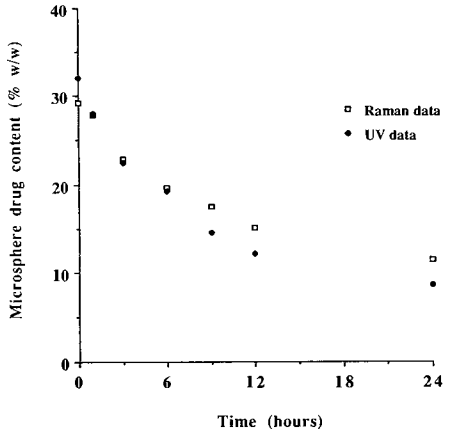

Fig. 6. Depletion of sulfasalazine from microspheres during *in vitro* dissolution measured by UV spectrophotometry and FTRS.

be indicative of a degree of drug–polymer interaction, although there appear to be no shifts in the position of the major peaks in the sulfasalazine spectrum (e.g., 3067, 1584, and 1440 cm^{-1}).

Quantitative Interpretation

A quantification of drug content based on sulfasalazine peak intensities was made for each of the microsphere samples. The intensity of Raman scattering arising from the sulfasalazine will depend upon the total quantity present within the sampling area, but due to variations in sample density and packing, the absolute intensity of the drug peaks within the Raman spectrum cannot be directly related to the concentration present. In order to eliminate this problem, a peak area ratio was taken between sulfasalazine (1584 cm^{-1}) and Eudragit RS (2949 cm^{-1}) for each spectrum. For sulfasalazine, the peak area was determined between 1600 and 1557 cm^{-1}, and for Eudragit RS, between 3047 and 2857 cm^{-1}, thereby avoiding the small sulfasalazine peak at 3067 cm^{-1}.

For each spectrum, the peak area ratio (sulfasalazine:Eudragit RS) was calculated and plotted against the sulfasalazine content assayed spectrophotometrically (from Table I). This relationship, shown in Fig. 4, is not a simple one, since as the concentration of drug increases, the concentration of polymer decreases. Thus, even if the intensity of scattering from polymer and drug were linear with concentration, the relationship in Fig. 4 would not be linear. To simplify data interpretation, a plot of log[peak area ratio] vs log[%sulfasalazine/(100 − %sulfasalazine)] has been produced (Fig. 5). A linear relationship was shown between the two parameters (r = 0.995), with the y axis intercept corresponding to the relative difference in scattering intensity between the chosen drug and the polymer peaks. Theoretically, if the area of the polymer and drug peaks were linear with concentration, the gradient of the line would be 1.0. The gradient of 1.26 in Fig. 5 might indicate that this is not so for this system.

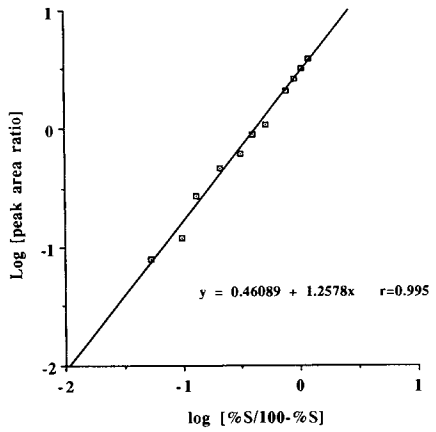

Fig. 5. Graph of log[sulfasalazine:Eudragit RS peak area ratio] versus log[%sulfasalazine/(100 − %sulfasalazine)].

For each of the spectra from the dissolution samples, the sulfasalazine:Eudragit RS peak area ratio was also calculated. The peak area ratios were substituted into the Fig. 5 calibration equation and the concentration of sulfasalazine was calculated. In Fig. 6, the microsphere drug content values calculated from the spectrophotometric data (Table II) and the Raman data are plotted versus time. For the 1-, 3-, and 6-hr samples the Raman and UV-calculated values were in excellent agreement. For the 9-, 12-, and 24-hr samples, however, the Raman-calculated drug content values were all higher than the UV values, perhaps indicating deviations from linearity in the calibration curve at low sulfasalazine values.

The same drug:polymer peak area ratio was employed to assess spectral reproducibility. For the within-sample test, the standard deviation was ±3.0% (n = 10) of the mean peak area ratio, whereas for the between-sample test, the standard deviation was ±4.4% (n = 10) of the mean peak area ratio. The shape of Fig. 4 implies that errors in peak area ratio will have the greatest effect at low concentration values. Thus, using the calibration equation in Fig. 5, a 3.0% variation in peak area ratio would produce a ±0.6% (w/w) error in concentration for a sample containing 50% (w/w) sulfasalazine, whereas the error in concentration at 5% (w/w) sulfasalazine would be ±0.1% (w/w). The between-sample variation of ±4.4% would result in a ±0.9% (w/w)

error in concentration at 50% (w/w) sulfasalazine and a ±0.15% (w/w) error at 5% (w/w) sulfasalazine.

CONCLUSIONS

In this study we have demonstrated that FTRS can simultaneously provide qualitative and quantitative information on sulfasalazine-containing Eudragit RS microspheres. The successful application of the technique to this system depended on the ability to distinguish drug and polymer peaks in spectra of the drug–polymer microspheres. For assay of drug levels in the microspheres, FTRS was simpler and faster compared to UV spectrophotometry and showed a good reproducibility. No sample preparation was required other than loading the material into the sample holder, and acquisition of each spectrum and determination of its peak area ratio took no more than 5 min. While problems may arise in the analysis of samples containing water (water absorbs in the near-infrared region), FTRS has a significant role to play in the characterization of pharmaceuticals [e.g., polymorphism (8)] and drug-delivery systems.

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