Confocal Raman-Spectroscopy: Analytical Approach to Solid Dispersions and Mapping of Drugs

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Purpose. To compare the physical state of a drug in a liquid with a polymeric matrix.

Methods. Solid solutions of ibuprofen in polyvinylpyrrolidone were obtained from the hot melt extrusion technique. In order to investigate the physicochemical stability, content, and homogeneity of the formulation, the tablets produced by a subsequent calendering step were examined using confocal Raman spectroscopy. In addition, a dimeric vinylpyrrolidone was synthesized and used to compare the physical state of embedding in a polymeric matrix with a physical solution of the active in a solvent, i.e. the dimeric vinylpyrrolidone. The spatial resolution of confocal Raman spectroscopy was used to image the drug distribution in the final form.

Results. Confocal Raman spectroscopy has been successfully used to determine the state of ibuprofen in a solid matrix showing equivalence to a physical solution. Moreover, the physicochemical stability of the formulation under stress conditions and content, as well as homogeneity of drug distribution in the formulation matrix, has been examined with the same method, proving the efficiency of the approach.

Conclusions. Confocal Raman spectroscopy offers a new approach for the analytical assessment of solid dispersions both covering the physical state as well as the distribution of the drug via its spatial resolution. Moreover, it is a promising tool for observing changes in a formulation due to physicochemical processes, e.g. recrystallisation and at the same time for locating the area where changes occur. Therefore, it may contribute to standard analytical methods to evaluate content and homogeneity.

KEY WORDS: solid dispersions; polyvinylpyrrolidone; confocal Raman spectroscopy; ibuprofen; solid oral dosage forms; melt extrusion.

INTRODUCTION

The improvement of the solubility and absorption properties of sparingly water-soluble drugs is a very important problem with respect to the development of pharmaceuticals. Solid dispersions of drugs with poor solubility revealed remarkably higher bioavailability (1). Less drug material may be applied and the possibility of reduced inter-patient variability are obvious advantages.

Among the suitable excipients most commonly used have been polyvinylpyrrolidone or its co-polymers, polyether diols, sugar, or dextrin and fat matrices. Reviews give a detailed overview on the matrices and methods applicable (2).

Solid dispersions have been obtained conventionally as co-precipitates, e.g., by solvent methods, by freeze and spray-drying, by simple fusion methods, and by means of co-grinding and as extrusion or injection moulding processes (3).

Only recently, we have introduced polymer/drug melt extrusion to prepare solid dispersion therapeutic systems. The breakthrough is brought about by having made feasible a great variety of therapeutic systems using a comparatively simple integrated technological system (4). The essential advantage of polymer/drug melt extrusion in this domain is its solvent free formation process.

Nevertheless, solid dispersions, in which the drug is in an amorphous state, suspended in the polymer carrier, exhibit the intrinsic problem of recrystallization due to physicochemical changes in the formulation matrix especially under stress storage conditions. The presence of crystals of the active could always influence the therapeutic performance, e.g., by changing the dissolution kinetics and has to be closely investigated (5).

The NSAID ibuprofen belongs to the group of drug compounds with poor solubility in acidic media. Therefore, subtherapeutic plasma levels may result. Ibuprofen has been studied in solid solutions with urea, polyvinylpyrrolidone, and polyethylenglycols (6).

Methods to monitor and prove the stability of the amorphous state of the drug dispersed in the polymer have been explored for a long time (7). The crystallization process and other physicochemical characteristics were determined by powder X-ray diffractometry, differential scanning calorimetry (DSC), differential thermo analysis, solution calorimetry, infrared spectroscopy, and FT-Raman spectroscopy (8).

Also, Raman spectroscopy has been used to investigate drug-polymer conjugates from sulfathiazole-polyvinylpyrrolidone co-precipitates (9).

In this article we demonstrate that confocal Raman spectroscopy (10) has great potential in evaluating such questions. Compared to standard Raman technology, confocal Raman technology offers the capability to monitor drug distribution in a matrix. This gives additional information about the homogeneity in a final dosage form and is suitable for answering major questions related to solid dispersions and, their manufacturing process as: (a) physical state of the active, (b) physicochemical stability of the formulation, and (c) content and homogeneity—via an imaging process—of the active in the matrix.

It was the aim of our study to investigate all these relevant issues using simply one analytical tool.

MATERIALS AND METHODS

Confocal Raman Spectroscopy

Figure 1 gives a schematic representation of the confocal Raman spectrometer. A laser (He-Ne or krypton) is focused onto the sample by the objective of a fluorescence microscope. The Raman backscattered light is collected by the high numerical aperture objective (100x, numerical aperture N.A. 0.8) and passed through the confocal aperture into the detection system. This confocal set-up leads to a 3-dimensional confinement of the measuring volume down to 1 μm^3 depending on the width of the aperture and the magnification of the objective. The weak Raman signal is first separated from the laser Rayleigh

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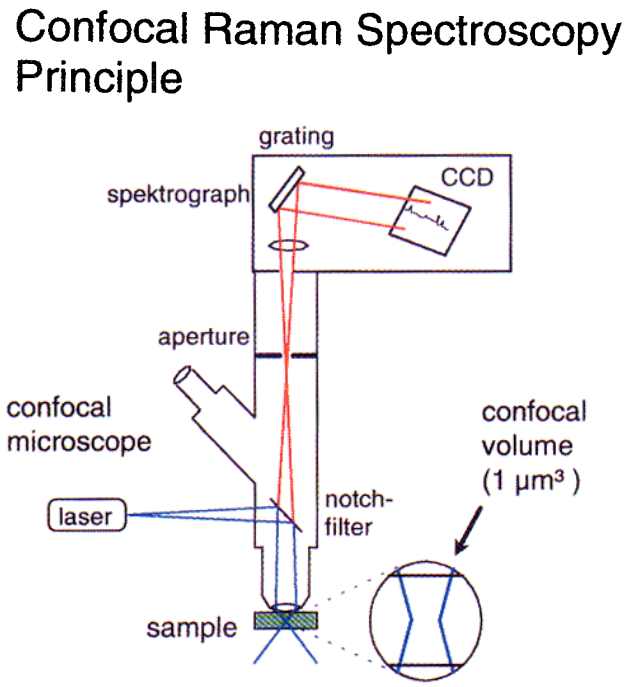

Fig. 1. Schematic layout of confocal Raman spectrometer.

background by a notch filter and afterwards spectrally analyzed by a single grating stage followed by a cooled CCD-detector. The Raman microscope used in this work (Labram®, Dilor GmbH, 64625 Bensheim, Germany) allowed short measuring times of less than a minute with a spatial resolution of 2 μm^3 and a spectral resolution of 7 cm^{-1} for the extrudate samples. Changing the grating from 600 to 1800 grooves per mm improves the spectral resolution to 2 cm^{-1}.

In order to investigate the spatial distribution of the active compound in a tablet, the sample is moved by a computer controlled translational stage while Raman spectra are continuously taken. Thus, chemical composition can be mapped by rationing characteristic band intensities. Depth profiles are recorded by moving the microscope focus into the sample. A diamond cutter generates plane tablet surfaces suitable for Raman mapping. The penetration depth into the tablets was 30

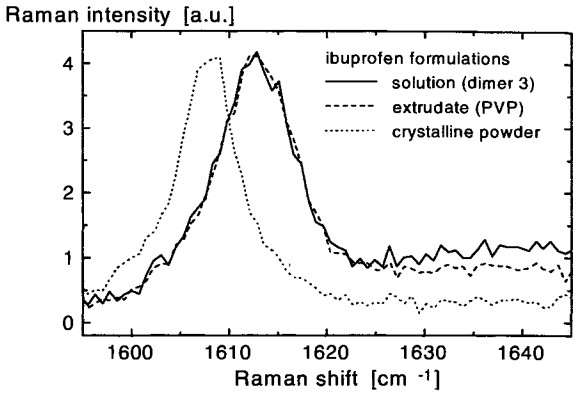

Fig. 3. Part of the Raman spectra (1595–1645 cm^{-1}) monitoring the characteristic Raman band of ibuprofen: VP dimer solution (solid line), extrudate (dashed line) and crystalline powder (dotted line). Crystalline ibuprofen shifts to lower vibrational energies.

µm. The measurements of the results presented were performed at a depth of 10 µm. The measurements took advantage of the clear, glassy appearance of the matrix containing the ibuprofen.

A microscope oven is used to apply temperature stress to the formulation. Raman spectra are taken at temperatures ranging from −90°C to 90°C.

Solid Oral Dosage Forms—Extrudates

An extruder with a conrotating twin-screw configuration (Werner & Pfleiderer, ZSK 30) gives the opportunity to vary and combine different types of e.g. screw, kneading, and housing elements. Feed screws and a kneading paddle are incorporated as screw segments. The two screws rotate equiaxially, while the screws accurately engage within the barrels. The temperatures of all the barrels were independent and were accurately controlled.

All solid dispersions were prepared using the hot-melt extrusion process thus without the use of organic solvents. Drug and polymer were intensively mixed before extrusion using a mixer (Bohle mixer, type: Bohle PM 4000). The mixture contained 20–40% weight of ibuprofen. During the passage through

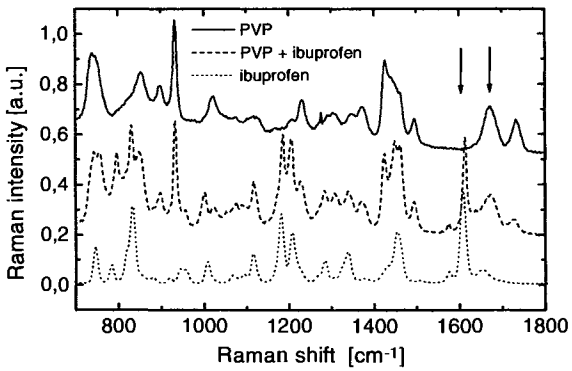

Fig. 2. Raman spectra (fingerprint region 700–1800 cm^{-1}) of polyvinylpyrrolidone (PVP) (top), crystalline ibuprofen powder (bottom) and ibuprofen-PVP-extrudate (center). The arrows mark the bands used for evaluation of the relative contents.

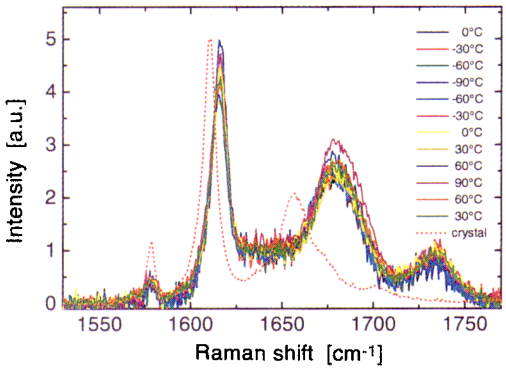

Fig. 4. Temperature stressed ibuprofen extrudate: for comparison the Raman spectrum of crystalline ibuprofen is given. No evidence for crystalline aggregates formed during the temperature stress cycle procedure was found.

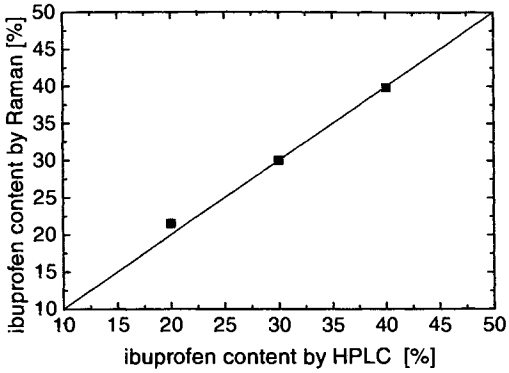

Fig. 5. Determination of ibuprofen content by HPLC and Raman spectroscopy, the solid line is given as a guideline for the theoretical optimum, e.g., linear correlation.

the extruder the mass is heated and the matrix polymer thereby plastified to incorporate the molten drug material. The temperatures of five barrels were fixed at 40–100°C towards the extruder die.

The resulting preparations represented glass like solid dispersions of the drug in the embedding matrix.

In order to obtain samples for analytical assessment, the thermoplastic strand leaving the extruder was forced on-line between two metal plates cut and cooled to room temperature or molded between two calendar rollers.

HPLC Method

The HPLC method was performed essentially according to DAB 96. Mobile Phase: A mixture of 0,5 ml phosphoric acid (85%), 340 ml acetonitrile and 659,5 ml water was prepared. Chromatographic system: The liquid chromatograph was equipped with a 214-nm detector, a 4-mm × 12,5-cm column that contains 5-µm octadecyl silane chemically bonded to porous silica (e.g., Merck Lichrospher 100 RP18), a guard column 4-mm × 4-mm of the same material, and was maintained at 40°C. The flow rate was about 2 ml per minute. 20 µl-Injections of test preparations and of standard solutions were performed.

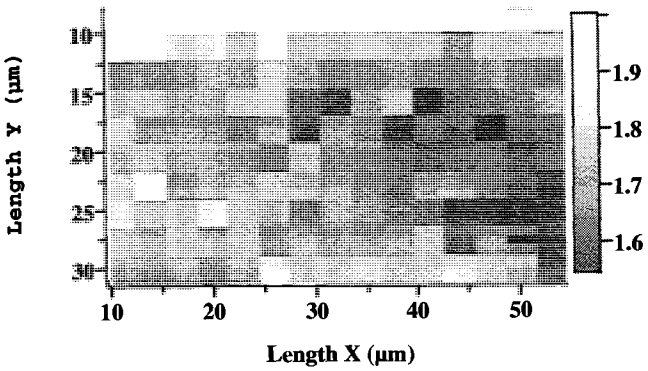

Fig. 6. Homogeneity of ibuprofen extrudate sample: the ratio of the Raman bands of ibuprofen (1613 cm^{-1}) and PVP (1673 cm^{-1}) is mapped for an area of 45 × 25 μm^2 (200 measurements in total), the standard deviation is less than 10%.

Drug Compound/Polymer

Ibuprofen: melting point 78°C, (Knoll AG, Ludwigshafen).

Polyvinylpyrrolidone, softening point 120°C (Kollidon K 30®, BASF AG, Ludwigshafen, Germany).

N-Vinylpyrrolidone, BASF AG, Ludwigshafen, Germany.

Synthesis of 1,3-bis (pyrrolidonyl) butene(11) (2):

To 25 g of N-vinylyprroldidone (1), which was purified by crystallisation before drying, HCl gas was added under nitrogen for 10 sec.. The flask was stored at 5°C over night. The dimeric 1,3-bis(pyrrolidonyl)butene crystallised in thin, colourless needles which were removed by filtering from the mother liquor. The dimer was recrystallized from an ether-acetone mixture once.

m.p.: 72°C; yield: 80 %; ^{1}H-NMR (MHz, $CDCl_3$):δ = 7.0 (d, ^3J = 14Hz, trans, 2H), 4.9, 3.4 (dt), 2.25 (m), 1.3 (d) ppm. ^{13}C-NMR (MHz, $CDCl_3$): δ = 174.01, 173.17, 125.14, 110.58, 46.08, 45.17, 42.31, 31.44, 31.15, 17.92, 17.39, 17.29 ppm. MS (Cl): m/z = 223 (M^+,18), 222 (95), 207 (95), 138 (100), 137 (85), 112 (35), 81 (90).

Synthesis of 1,3-bis (pyrrolidonyl) butane (3):

60 g of 1,3-Bis-(1-pyrrolidonyl)-butene-(1) (2) 1000 ml acetic acid and 10 g Of Pd/C (10%) were stirred at room temperature for 5 min. Hydrogen was added for 30 min. and an exothermic reaction was observed. Stirring was continued for another 90 min. The catalyst was filtered off and the reaction mixture distilled under vacuum to yield the dimeric butane in 85% yield.

b.p.: 205–215 °C (0.2 mbar), yield: 85%; ^{13}C-NMR (MHz, $CDCl_3$): δ = 174.58, 174.42, 47.16, 44.29, 41.87, 39.59, 31.45, 31.32, 30.91, 18.08, 17.94, 17.92 ppm. MS (Cl): m/z = 224 (M^+,70), 196 (60), 126 (100), 113 (95), 98 (50). IR (Film) ϑ [cm^{-1}]: 2995 (CH, s), 1680 (CO, s), 1425, 1286.

RESULTS

Physical State of the Drug

Figure 2 shows the Raman spectra of crystalline ibuprofen, polyvinylpyrrolidone (PVP), and the hot melt extrudate. In the fingerprint region the Raman band (1613 cm^{-1}) of the ibuprofen extrudate is shifted compared to the crystalline ibuprofen. The shift reveals a different chemical environment of the active possibly due to the physical state of ibuprofen in the extrudate matrix.

To prove this observation we synthesized the dimeric 1,3-bis (pyrrolidonyl) butane (3) (Fig. 7) and recorded Raman spectra of the dissolved ibuprofen in the viscous solvent 3. The fingerprint region of the Raman spectrum of this solution is shown in Fig. 3 in comparison to the spectrum of the solid dispersion. As the spectra are almost identical one may deduce from these spectra that ibuprofen is obviously present in a non crystalline form in the extrudate, i.e., as 'solution' in the polymer matrix dispersed at a molecular level in a solid matrix. By means of powder X-ray diffractometry and differential scanning calorimetry of the extrudate the same result was observed: ibuprofen was present in an amorphous state.

Physicochemical Stability of the Formulation Under Stress Conditions

We applied a triangle like temperature stress profile for the extrudate to verify the physicochemical stability of the

hot melt extruded formulation, i.e. the physical state of the ibuprofen dissolved. The temperature was changed by 30 degrees every 30 minutes yielding the following series of measuring temperatures: 0°C, −30°C, −60°C, −90°C, −60°C, −30°C, 0°C, 30°, 60°C, 90°C, 60°C, 30°C. No changes of the sensitive ibuprofen band positions were detected (Fig. 4). The spectrum of the crystalline ibuprofen powder is shown for comparison.

Content and Homogeneity of Drug Distribution in the Formulation Matrix

Using confocal Raman spectroscopy the signal intensity ibuprofen (1613 cm^{-1}) was determined at 14 different locations of three extrudate tablets with different ibuprofen content. In parallel, the drug content was measured using HPLC. Figure 5 shows the calibration curve.

To evaluate the homogeneity of the formulation the ratio of the Raman bands of ibuprofen (1613 cm^{-1}) and PVP (1673 cm^{-1}) were mapped for an area of 45 × 25 μm^2 (200 measurements in total). Each measurement was performed with a resolution of 2 μm^3. As a result a homogeneous distribution of the drug in the mot/melt extruded matrix was found within the range of the standard deviation over the three samples examined (Fig. 6).

DISCUSSION

Of the analytical techniques available, Near Infrared and Raman spectroscopy can be applied without any sample preparation, thus avoiding mechanical influence on the preparation which may alter the physicochemical properties of the formulation. Complementary to Infrared spectrometry Raman techniques offer an interesting non-invasive method. A major problem for Raman measurements can be caused by fluorescence (intrinsic or caused by impurities) overlaying the Raman bands. This can be avoided to some extent by shifting the laser wavelength to the NIR spectral region. In this investigation a 633 nm He-Ne laser was used without fluorescence problems.

In this paper we demonstrate confocal Raman spectroscopy is a suitable tool to answer the important questions related to the development of solid dispersions systems. Confocal Raman spectroscopy combines the chemical and structural information of modern microscopic methods extending the use of Raman spectroscopy. The capability to image final dosage forms may lead to new on-line analytical tools to ensure product quality.

Also, our aim was to prove the physical state of the drug in the solid dispersion as well as the homogeneous distribution of the drug in the matrix and to study storage stability of the formulation using a fast, reliable, and convenient single analytical method. The imaging studies verify the quality of mixing and dissolution while the solid dispersion is manufactured.

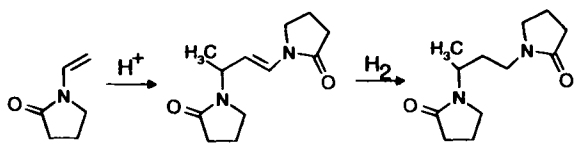

Fig. 7. Schematic of synthesis of 1,3-bis (pyrrolidonyl) butane.

Moreover, 1,3-bis (pyrrolidonyl)butane (3) represents an interesting tailored solvent mimicking the solid, high viscous polymer polyvinylpyrrolidone, but still being a liquid solvent. We believe the properties of this solvent can be used as a convenient test system to predict the capability of drugs to form solid dispersions in PVP.

Combining the results from Raman spectroscopy in the polymeric matrix and the solvent test system we believe there is strong evidence the drug is distributed on a molecular level throughout the polymer PVP.

State of the Drug

By means of Raman spectroscopy the morphological state of ibuprofen in the extrudate was examined. This method has the advantage that, in contrast to DSC measurements and powder X-ray diffractometry measurements, no sample preparation is required. The dimeric vinylpyrrolidone 3 mimics the chemical environment of ibuprofen molecules embedded in the polyvinylpyrrolidone matrix but represents a liquid physical solution. As both the spectrum of this solution in the solvent and the Raman spectrum of the ibuprofen in the extrudate show no significant deviation we came to the conclusion that the active is present in a dissolved form, dispersed at a molecular level.

This result was cross-examined by DSC measurements. No endothermic peak could be observed at the melting temperature of crystalline ibuprofen. DSC measurements were performed using a Mettler-Toledo TA 8000 differential scanning calorimeter. The weight of the samples was about 10 mg in closed aluminium pans. The heating rate was 10°C/min from −10°C to 220°C. Nevertheless, DSC only verifies the non-crystalline character but does not reveal the break down to a molecularly dispersed form.

Physicochemical Stability of the Formulation Under Stress Conditions

The physical character, be it an amorphous or molecularly dispersed form of the active in any given formulation, has to be closely monitored as it exhibits the risk of recrystallization. Product quality and in vivo performance might be influenced if changes of the state of the drug occur.

The triangle like temperature profile was chosen to favor crystallization processes of the dissolved active in the melt extrudate. The ibuprofen bands did not show any shift, thus revealing no detectable amount of crystalline ibuprofen arising from the stress model chosen.

Content and Homogeneity of Drug Distribution in the Formulation Matrix

Quantification of pharmaceutical products thus excipients or actives in a formulation is of high interest to specify the formulation. The homogeneous distribution of the drug in the formulation matrix is favorable to achieve high stability of the formulation in respect to the dissolved state of the drug. Aggregates of active or phase separation will lead to changes in the matrix. In addition, data on the dissolution capabilities and mixing quality of the process can be obtained.

We were able to demonstrate the determination of the amount of active ingredient in a pharmaceutical preparation in good correlation between HPLC and Raman spectroscopy. Moreover, it was possible to map the ibuprofen distribution for

an area of 45 × 25 μm^2. We believe this area is large enough to demonstrate the homogeneously distributed content of ibuprofen over the molten preparation. In addition to Raman spectroscopy confocal Raman spectroscopy allows determination of drug content as well as determination of drug distribution at the same time. This attempt may be seen as a first approach to document imaging processes for application to drug distribution in pharmaceutical formulations. Confocal Raman spectroscopy thus represents a new application of Raman spectroscopy offering the potential to investigate different layers, e.g. coatings on a tablet, areas, e.g. phase separation or simply quality of mixing in a manufacturing process.

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