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Automatic particle analysis by Raman spectroscopy – evaluation of sample preparation and optimum background material

Abstract: The particle generation of medical implants and drug coated balloons (DCB) is evaluated by simulating the implantation process and collecting the released particle material. In addition to size and particle counts their material composition is of interest. Raman spectroscopy and microscopy are established methods for chemical identification. For the manual analysis of the particles different filter or rather background materials are suitable because different requirements in height and intensity of the spectra can be compensated by the user. The aim of this study was to find suitable background materials for automatic particle identification.

Raman analysis of background and spherical polystyrene standard particles on filters and plain surfaces was performed manually to receive evaluable spectra for automation. Automatic analysis was done by a) single-point spectrum measurement using the coordinate list of all particles, and b) scanning a large sample area pixel by pixel measuring background and particles.

For automatic analysis with method a) the polycarbonate membrane provided best results. With method b) the polystyrene spectrum of the particles could be found on both plain surfaces and the polycarbonate membrane. Influences from the background spectrum could be kept small by thoroughly defining the focal plane with a wavelength specific autofocus mode for method a) and manually for method b).

Keywords: Particle analysis, Raman spectroscopy, sample preparation

1 Introduction

The test of medical implants such as drug coated balloons (DCB) contains the simulation of use under environmental conditions. To evaluate the amount of particle generation during the procedure particles are collected and counted. The additional analysis of these particles is important to identify the materials that may cause a higher amount of particles or to find sources of contamination during the production process. Raman microscopy and spectroscopy enable differentiation of polymeric materials. For the evaluation of Raman spectra under dry conditions the particles have to be filtered or applied on a surface and dried. Four different background materials were analysed and the focus was to evaluate the quality of the particle spectra that were recorded with automatic methods provided by the Raman microscope.

2 Materials and methods

2.1 Sample preparation

Instead of particles that were released by medical implants spherical standard particles made of polystyrene were used for development of the test method (Count-Cal, Thermo Scientific, diameter: 10 µm, 3000 particles per ml, aqueous

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suspension). The particles were applied on four different materials shown in **Table 1**.

Table 1: Background materials for the particle analysis

Sample	Material	Properties/ manufacturer
1. Silver mirror	Silver coated glass, silicium oxide covering	Plain surface, Thorlabs
2. Microscope slides	Glass	Plain surface, Menzel
3. Silver membrane	Pure Silver	Filter, Pore Size 0.4 µm, Merck
4. PC membrane	Polycarbonate (PC)	Filter, Pore size 0.4 µm, Sartorius

All samples were prepared under clean room conditions to avoid contamination. The samples 1 and 2 are plain surfaces and the particle suspension (300 µl) was applied in several steps in drops directly on the surface and air dried. The samples 3 and 4 are filters so the particle suspension (500 µl) was applied in one step. The filters were fixed on a microscope slide for analysis.

2.2 Overview image and single spectrum

The particle analysis was done with the Raman microscope Alpha300R (WiTec). All samples were placed under the lens (10 fold magnification, NA 0.25). To generate the Raman spectra a laser with a wavelength of λ=532 nm was used. An overview microscope image of 1000 x 1000 µm² to 5000 x 5000 µm² was taken via image stitching. On the basis of these images particles can be localised by the user.

For the single Raman spectra localisation and parameters as laser power, integration time, height of focal plane and the number of accumulation scans were optimized by the user. The results were used to choose the parameters for automatic analysis. One spectrum of the background material was taken to evaluate its influence on the measurement.

2.3 Automatic analysis

For the analysis of particles by recording multiple spectra automatically we tried two options: the single point spectrum measurement and the large area scan.

2.3.1 Single point spectrum measurement

For the single point spectrum measurement several points were defined by the user on the basis of the overview image. 3 to 20 points were defined for the different samples depending on how many particles were identifiable. A good visibility of the particles on the background is assumed for this method. Parameters such as laser power, integration time and number of accumulation scans had to be set in advance and were kept for all spectra. They were set according to the results of the manual adjustment under point 2.2 (results see point 3.1). To set the focal height the autofocus mode was used. For this method the spectrum intensity is determined in a defined z range of 200 µm and on ten steps (1 second per step). The spectrum was taken at the focal height with the maximum intensity in a defined wavenumber range.

2.3.2 Large area scan

The spectra were taken pixel by pixel for the large area scan. We defined an area with a side length between 30 µm and 200 µm and a resolution between 0.5 and 1 line per µm. The parameters were set in advance. The focal height was defined in advance by the user observing an example particle and was kept for all points.

3 Results and discussion

3.1 Overview image and single spectrum

The overview images were taken for all samples to locate the particles. They can be seen in **Figure 2** with the points that mark the location of the single point spectrum measurement. The visibility of the particles differs for the several background materials. Particles can be recognized well on the two plain surfaces and on the PC membrane. The localisation was more difficult for the particles on the silver membrane. Its surface is rough and reflecting. Particle identification might be confused with shining spots that appear bright as well. The parameters that were determined for the record of the spectra are shown in **Table 2**.

The laser power that was needed to take the particle spectra is higher for the microscope slides and the PC membrane. For the microscope slides even a higher integration time and more scans were needed to see the shape of the polystyrene spectrum and to improve the signal to noise ratio. This may be caused by surface enhancement of silver background

materials. This effect is described in literature and it is the reason to expect the sample backgrounds that contain silver to be advantageous for the particle analysis with Raman spectroscopy [1].

Table 2: Parameters for the single point spectrum measurement

	Silver mirror	Microscope slide	Silver membrane	PC membrane
Laser power/ mW	0.5	3.6	0.5	3.6
Integration time/ s	0.5	1.0	0.5	0.5
Accumulation scans	10	20	10	10

Figure 1 shows the single spectra of one particle on each sample. The fifth spectrum shows a reference of a polystyrene spectrum that was taken on a cluster of larger polystyrene particles.

All spectra contain characteristic peaks of the reference polystyrene spectrum ($k=1005, 1605, 3050$ rel. cm-1). The peaks of the spectrum recorded on the silver membrane are weaker because of a poor signal to noise ratio. Here the particle position was probably not clearly identified. That shows that all sample materials are theoretically usable for the automatic particle analysis with Raman spectroscopy.
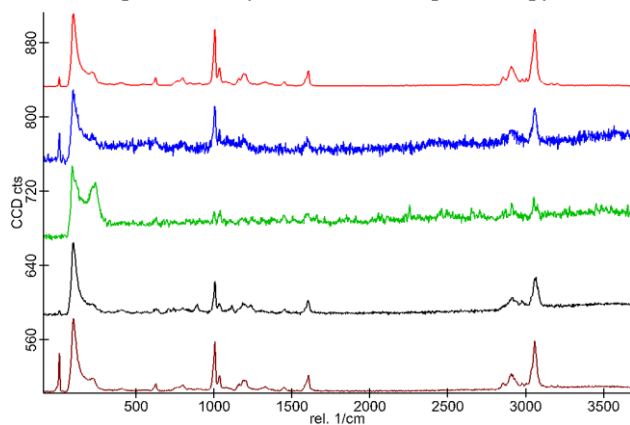

Figure 1: particle spectra on the different samples (from top): 1. silver mirror, 2. microscope slide, 3. silver membrane, 4. PC membrane 5. reference spectrum of polystyrene

3.2 Automatic analysis

The results of the automatic analysis show the single point spectra and examples of the spectra that were taken with the large area scan.

3.2.1 Single point spectrum measurement

The points for the measurement of the spectra are shown in **Figure 2**. One example of the particle spectra on each background is shown in **Figure 3**.
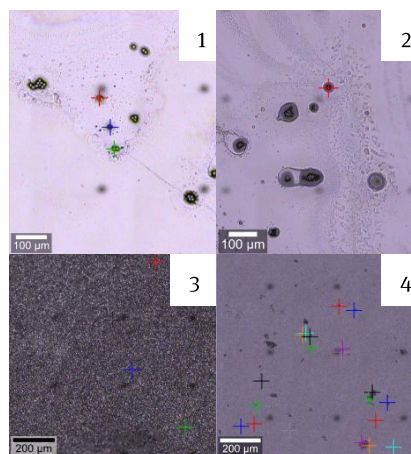

Figure 2: Overview about the localization of the points for the single point spectrum measurement 1. silver mirror, 2. microscope slide, 3. silver membrane, 4. PC membrane
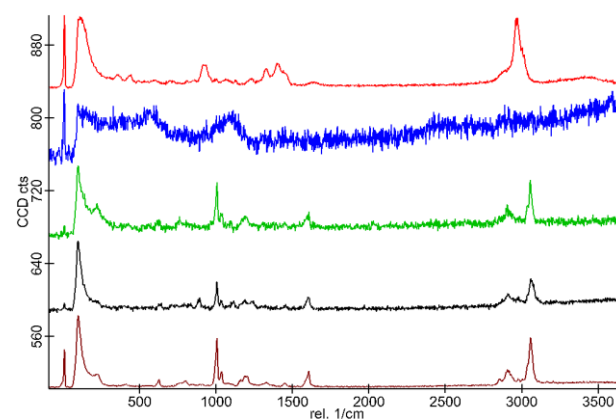

Figure 3: particle spectra recorded via single point list (from top): 1. silver mirror, 2. microscope slide, 3. silver membrane, 4. PC membrane 5. reference spectrum of polystyrene

No spectrum is comparable to the reference polystyrene spectrum on the silver mirror. The reason might be that the focal height was not determined exactly on the particle but on the mirror surface. The wavenumber range for the autofocus was probably defined too broadband in this case. Another reason might be a small shift on the XY-plane so that the spectra were taken directly on the background material. For the microscope slide only one spectrum was recorded and it does not show any of the characteristic peaks of polystyrene either probably because of the same reasons. Additionally the signal strength compared to the noise is lower which makes it even harder for the focus automatic to find the best focal plane. Two of four spectra were defined as polystyrene for the particle spectra on the silver membrane. Here it was harder to localise the particles on the XY

plane because of the poor contrast between surface and particles. The autofocus works better because of the narrow-banded background spectrum. The best result we got was for the PC membrane. The characteristic peaks of the polystyrene spectrum are included by 18 of 20 spectra.

3.2.2 Large area scan

The results of the large area scans are shown as overlay images in **Figure 4**. The peak height on one selected wavenumber is represented as a colour gradient. In our case high values are yellow and low values are red. We chose one of the characteristic peaks of polystyrene. Particles are easy to identify on all samples in spite of the silver membrane. Additionally we selected one spectrum located on a particle to show the shape of the spectrum in **Figure 5**.The shape of the polystyrene spectrum can be recognized for the silver mirror, the microscope slide and the PC membrane. The signal is lower on the silver mirror because the integration time was abbreviated to 0.2 s instead of 0.5 s to scan a larger area. The characteristic peaks cannot be found in the particle spectra on the silver membrane. The uneven surface is a big disadvantage for this preparation method because the focal plane is not adapted and all particles that do not lay in the chosen height cannot be measured.
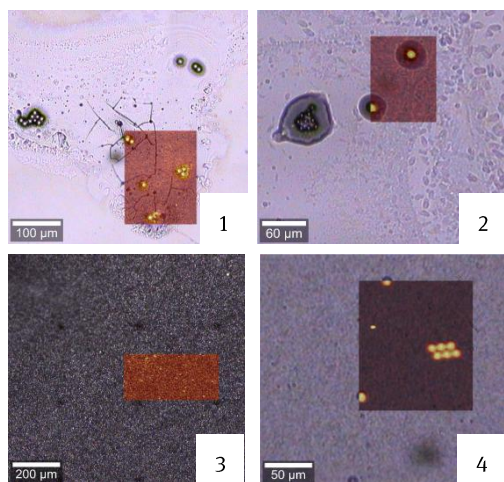

Figure 4: overlay images of the samples: 1. silver mirror, 2. microscope slide, 3. silver membrane, 4. PC membrane

4 Conclusion

All sample surfaces are usable for the measurement of polymeric particle spectra. For the manual record polystyrene could be found on all samples. However localization of parti-

cles on a silver membrane might fail because of poor visibility. The automatic analysis with the single point spectrum
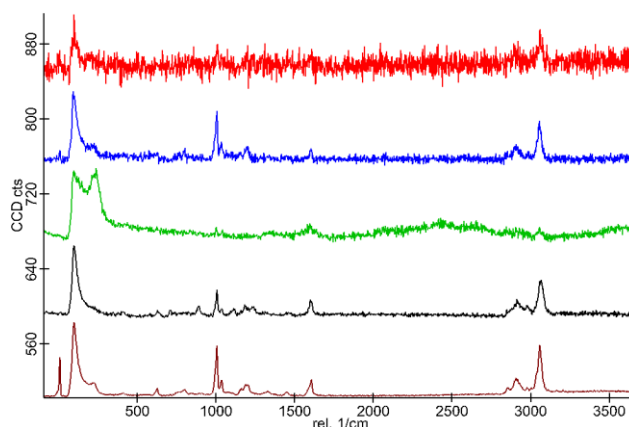

Figure 5: particle spectra of the yellow marked regions (from top): 1. silver mirror, 2. microscope slide, 3. silver membrane, 4. PC membrane 5. reference spectrum of polystyrene

measurement is influenced by the background spectra of the surface when using the autofocus. Even though the PC membrane showed a background spectrum this method worked best on this sample. Selecting the wavelength range for the autofocus to distinguish the background spectra from particle spectra is an important additional option to focus on for further measurements. Main requirement for both automatic particle analysis methods is a smooth surface. This applies as well for large area scans. Polystyrene spectra could be identified on both plane surfaces and on the PC membrane but not on the rough silver membrane.

Further measurements will be done with real particles released during the simulated use of DCB.

References

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