CONCENTRATION MAPPING OF COMPLEX MICROSTRUCTURES BY CONFOCAL RAMAN SPECTROSCOPY AND MULTIVARIANT ANALYSIS

Paul D.A. Pudney, Tom M. Hancewicz, Dale Cunningham

Unilever R&D, Colworth Laboratory UK, Paul.Pudney@unilever.com Unilever R&D US Edgewater Laboratory

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Abstract: This paper shows how fully quantitative concentration images of complex microstructures can be obtained using confocal Raman spectroscopy. A number of measurement and data analysis obstacles needed to be examined and overcome. This included the spatial resolution limitation in the z-direction and spectrometer stability. An extension to the multivariate curve resolution (MCR) method is described that allows concentration maps to be produced.

Complex microstructures are present in many areas of research and technology, however characterizing their structures can be very challenging [1]. Confocal Raman has shown much promise in being able to make a significant impact by probing these structures non-invasively. However to be able to this consistently a number of issues have to be addressed, both experimentally and in data analysis. In this paper these problems are examined and it is shown how by using a careful experimental set-up and procedures, along with developments in the data analysis method MCR [2] these can be overcome. For instance, as highlighted by Everall [3], depth resolution can be a problem due to changes in refractive index. It is shown in figure 1 how this effect can be negated, by using the correct index matching objectives. Also knowledge of instrumental stability is needed so robust long-term calibrations can be produced.

Once a spectral image has been collected, highly overlapping spectra often cause great problems in extracting the pertinent information inherent in the data especially when concentrations are required. It has been shown previously that pure spectral factors can be obtained by statistical methods like self-modelling curve resolution (e.g. MCR) to produce individual component images [2]. Here we show an extension of this method that allows these component images to be made quantitative. The idea involved in producing quantitative data from the modified MCR method is analogous to that used in quantitative absorption spectroscopy. In addition to the data matrix of unknown component spectra, in this case the Raman image data, (which is unknown in the sense that the concentration of each component in each sample is unknown), a matrix of calibration spectra for pure components and/or mixtures of components are added to the data matrix. This data then causes the resultant scores (i.e. intensity component of each spectra) to be scaled relative to the calibration data. A calibration equation is then generated from the scores of the calibration spectra, which is subsequently used to scale the scores from the Raman image data. These calibrated scaled scores can then be plotted to produce concentration images of the components within a microstructure. A full account of this methodology is given elsewhere [4].
Soft solids are technologically important in many areas e.g. foods and pharmaceuticals. These often consist of many biopolymers in complex mixtures, which then often phase separate to form very complex microstructures. Here we show how such a microstructures can be examined by using the methods described above. The system examined is two carbohydrates, Gellan and κ-carrageenan in water. These will phase separate under certain concentration regimes and form gelled soft solid microstructures. The spectra from Gellan and κ-carrageenan are highly overlapping, see Fig. 2, so represent a great challenge for the MCR method and cannot be analysed using single peak intensities. Calibration spectra were obtained from pure gels and a number of miscible mixtures. Confocal Raman maps were then obtained from the phase separated microstructures. The spectra from these images where then augmented with the calibration spectra and analysed using the MCR method. The MCR factors were obtained and compared with the spectra from the pure gels, see Fig. 2. A good separation into two pure component factors was achieved. A calibration equation was then generated and used to scale the image data as described above and subsequently concentration maps produced e.g. Fig. 3. This paper thus demonstrates that using the correct experimental methods and MCR data analysis methodology allows fully quantitative images of complex microstructures to be produced.

![MCR factors (i) and Raman spectra (ii) of a) gellan and b) κ-carrageenan. Rescaled and offset for comparison](image1)

![Concentration images of a) κ-carrageenan b) gellan, produced from Raman mapping the phase-separated area shown in the optical microscope picture c), which is an xy optical slice of the microstructure at a constant depth just below the coverslip. The microstructure was produced from 2% κ-carrageenan / 2% gellan. 480 spectra (24 by 20) were collected each scanned for 100s. 1 µm stepsize was used.](image2)

**Figure 2** MCR factors (i) and Raman spectra (ii) of a) gellan and b) κ-carrageenan. Rescaled and offset for comparison

**Figure 3** Concentration images of a) κ-carrageenan b) gellan, produced from Raman mapping the phase-separated area shown in the optical microscope picture c), which is an xy optical slice of the microstructure at a constant depth just below the coverslip. The microstructure was produced from 2% κ-carrageenan / 2% gellan. 480 spectra (24 by 20) were collected each scanned for 100s. 1 µm stepsize was used.

**References:**