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# Visualization and quantification of polymer distribution in microcapsules by confocal laser scanning microscopy (CLSM)

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**Abstract**

Confocal laser scanning microscopy (CLSM) was employed in order to characterize microcapsules. Microcapsules were prepared by complex coacervation: gelatin and arabic gum were labelled with fluorescent markers. In the capsule wall a homogeneous distribution for both gelatin and arabic gum throughout the capsule wall was depicted. By the use of CLSM and a computational image analysis the quantification of the labelled polymer in the wall material was possible. Adding fluorescently labelled casein as a macromolecular model compound to the coacervation process, a gradiental distribution in the wall material was observed with highest concentration of casein at the oil–wall interface. The influence of casein concentration on its deposition behaviour in the capsule wall was imaged successfully and thereafter quantified by computational image analysis. 

*Keywords:* Confocal laser scanning microscopy (CLSM); Microcapsules; Microencapsulation; Complex coacervation; Polymer distribution

Microencapsulation is a very popular method for the preparation of controlled release systems. Nowadays, in nearly all cases microparticles and microcapsules for pharmaceutical applications are prepared by using preformed polymers. For many reasons it is of interest to analyze and visualize the deposition/distribution of the involved polymers and to confirm non-ambiguously the encapsulation of the drug. Commonly used visualization techniques for microparticles and microcapsules include the conventional light microscopy and scanning electron microscopy (SEM) (Mathews and Nixon, 1974; Benita et al., 1984; Bodmeier and McGinity, 1987). Light microscopy is always impeded by the scattered or emitted light from structures outside the focal plane. SEM, in contrast, usually requires a relatively complex sample pretreatment (e.g. gold sputtering, etc.) and does not allow to visualize inner structures of objects. Sections of embedded particles allow to inspect the particle wall structure, but the encapsulated phase to be visualized is not possible since it may get lost during the microscopy sample pretreatment.

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Confocal laser scanning microscopy (CLSM) allows to have a non-destructive look through the wall material of microcapsules, while fading out the light from out-of-focus structures. By using different fluorescence labels, marked compounds can be identified unambiguously. A quanitative image analysis allows the inspection of structures not only on the surface but also inside the material.

It was the aim of this study to examine the potential advantages of CLSM as a tool for the characterization of microcapsules. In a previous work, the authors were interested in the localization and identification of the oil phase by CLSM (Lamprecht et al., 1999). Starting from the possibility to depict microcapsules by combining one channel for red fluorescence and another channel for transmission light, we developed a non-destructive method for the calculative determination of the encapsulated oil phase by using an imaging procedure.

In this paper, the visualization of the polymeric components in the capsule wall with regard to their respective distribution within the wall material is described. Furthermore, it is shown that CLSM combined with computational image analysis allows the quantitative determination of polymer ratios in the wall material.

Polymers were fluorescently labelled according to the method of Schreiber and Haimovich (1983). Microcapsules were prepared with the two polymers gelatin and arabic gum containing fish oil as oil phase, following the well established complex coacervation process (Green and Schleicher, 1957; Luzzi and Gerraughty, 1967; Arneodo et al., 1986). A Biorad MRC 1024 Laser Scanning Confocal Imaging System (Hemel Hempstead, UK), was used to investigate the structure and morphology of the oil containing microcapsules. The laser was adjusted in the green/red fluorescence mode which yielded two excitation wavelengths (488 nm/514 nm). Green and red fluorescence images were obtained from these two separate channels, a third picture from the transmitted light detector was optional.

The distribution of the polymers inside the capsule wall was depicted and analyzed afterwards by computational image analysis (Scion Image, 1998). Regarding the polymeric material in previous experiments a homogeneous distribution for both gelatin and arabic gum was found throughout the capsule wall (Fig. 1A,B). In order to coacervate the two polymers the pH is adjusted below the isoelectric point of gelatin which is thereafter consequently positively charged, while the charge of arabic gum is negative (Luzzi and Gerraughty, 1964). The particle wall formation takes place based on electrostatical interaction of both polymers and leads to a statistical distribution of the polymers throughout the wall material because of the necessary charge equivalence of the two counterions for coacervation (Luzzi and Gerraughty, 1964). The homogeneous distribution of all polymers observed by CLSM, are in line with these theoretical considerations. The labelling of gelatin and arabic gum in the same coacervation process was performed with gelatin-FITC and arabic gum-RBITC. A homogeneous distribution of the fluorescence intensity throughout the capsule wall was also observed in this case. By this approach both polymers were visualized in the same coacervate and could be imaged side by side as no influence or hindrance on the distribution behaviour and the coacervation process itself was observed. To characterize the polymer distribution, the fluorescence intensity across the imaged capsule wall was determined quantitatively. Microcapsules were prepared with different ratios of fluorescent labelled to unlabelled gelatin and thereafter, sections of the capsules were depicted by CLSM. Computational image analysis allowed us to quantify the fluorescence signals from the capsule wall, which correlated very well with the percentage of labelled gelatin used for the preparation (Fig. 2A).

In order to quantify the distribution of different polymers within the capsule wall, casein was added as a third component to the complex coacervation process. Casein was fluorescently labelled with FITC. As shown in Fig. 1C, an inhomogeneous distribution of casein in the capsule wall was observed. The highest concentration of casein was found at the oil–wall interface, and its concentration decreased towards the outer wall border. Casein is known to be the dominant component in lowering the surface tension (Mus-

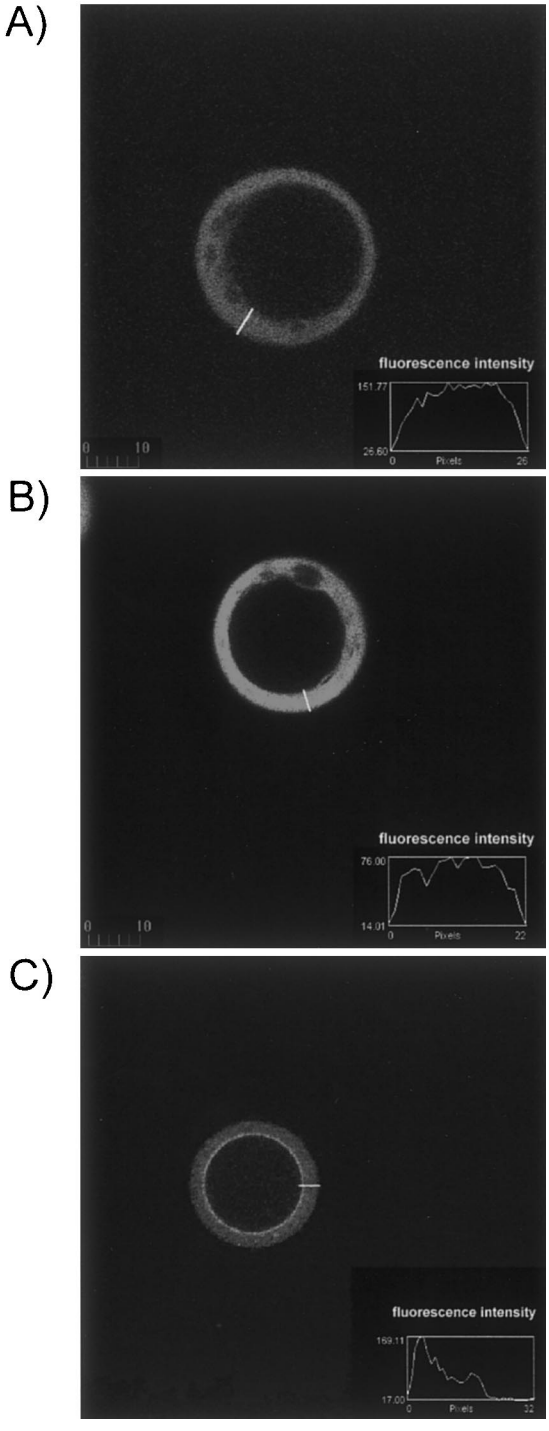


Fig. 1. Microcapsules with fluorescent labelling of gelatin B with FITC (A), of arabic gum with RBITC (B), and with unlabelled gelatin and arabic gum containing additional casein-FITC (C).

sellwhite, 1966) and should accumulate preferentially at the oil–polymer interface. As shown in Fig. 2B the fluorescence signal of casein-FITC decreased with lowering casein-FITC concentrations, but the pronounced gradient in the capsule wall remained.

Another phenomenon was also observed by the use of CLSM. Casein-FITC was added to the oil–water emulsion after lowering the pH where

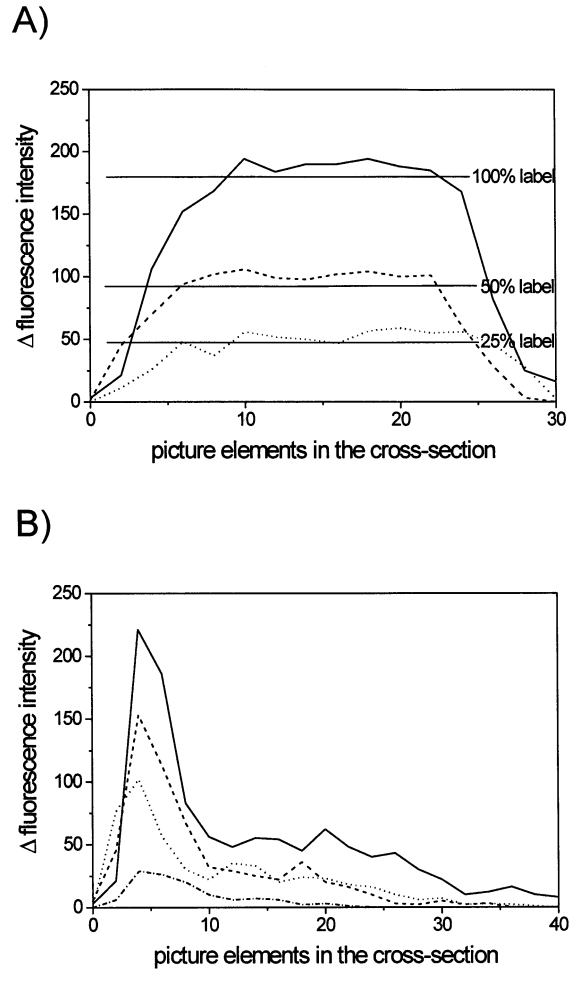


Fig. 2. Fluorescence intensity spectra (A) of cross-sections of particle wall for different ratios labelled/unlabelled gelatin (ratio 1:0, —; ratio 1:1,-- -; ratio 1:3, · · ·), and for different casein concentrations (B) (5% casein, —; 2% casein,-- -; 0.25% casein, · · ·; after coac., 5% casein, ·-·-·-).

gelatin and arabic gum are already present in the coacervated state. In this case an extremely lowered fluorescence signal was obtained from casein-FITC at the oil–water interface compared to prior experiments (Fig. 2B). There, the coacervate seems to hinder casein from penetration through the polymer layer. The immobilization of the two coacervating polymers by their electrostatical interactions seem to reduce the penetration of casein through the gelatin/arabic gum layer. In constrast to the results of gelatin, where the quantification of the fluorescent-labelled polymer was successful, no quantification was possible with casein. A tendency was observed, however, that the fluorescence signal decreased with lower casein concentration. A possible explanation for this phenomenon may be the high amount of casein-FITC which is concentrated at the oil–water interface and therefore is subject to a quenching of the fluorescence signal.

CLSM appeared as a very powerful tool for the characterization of microcapsules. This technique may provide additional structural information, for example the polymer distribution in the wall material. A visualization of the polymer distribution throughout the capsule wall was possible, in particular the detection of unhomogeneities within the capsule wall polymers. By the following computational image analysis the polymer distribution became measurable quantitatively.

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