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ORIGINAL ARTICLE

# Distribution and accessibility of cyclodextrins covalently bound onto silica gel

**Ronnie Nielsen · Jeppe L. Nielsen · Kim L. Larsen**

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**Abstract** Silica gel coated with a layer of β-cyclodextrins is a well investigated system. Earlier literature studies suggest that the coating changes the mesoporous structure of the silica gel and that the coating obtained might be heterogeneous. From surface area measurements it was found that the average pore diameter and accessible pore volume was reduced after grafting with 3-glycidyloxypropyltrimethoxysilane and β-cyclodextrin, but the change was reversible by simple pyrolysis of the organic compounds. To investigate the amount of accessible β-cyclodextrin on the silica particles, a Langmuir adsorption study was used. From the Langmuir binding isotherm it was found that the 8 Anilinonaphtalene-1-sulfonic acid ammonium (1,8 ANS) guest had a much larger stability constant with surface bound β-cyclodextrin compared to that found for free β-cyclodextrin. The accessible amount of β-cyclodextrin was found to be approximately 12% lower than the total amount of β-cyclodextrin indicating that some β-cyclodextrin is inaccessible for complex formation on the silica particles. The distribution of the cyclodextrin on the particle was investigated using the enhanced fluorescence of 1,8 ANS when complexed to β-cyclodextrin using confocal laser scanning microscopy. The confocal images of the particle show that the β-cyclodextrin was positioned on the outer layer of the particle, explained by the difficulty of diffusion of the β-cyclodextrin into the small pores. It was observed that smaller particles (>20 μm) has a more homogeneous distribution of β-CD compared to larger particles where a heterogeneous distribution was observed.

**Keywords** β-Cyclodextrin · Silica gel · Distribution · CLSM · BET

R. Nielsen (✉) · J. L. Nielsen · K. L. Larsen (✉)
Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, 9000 Aalborg, Denmark
e-mail: Ronnie01@gmail.com

K. L. Larsen
e-mail: kll@bio.aau.dk

## Introduction

The unique ability of cyclodextrins (CDs) to form complexes with guest molecules at a molecular level has been utilized in a broad range of applications [1] e.g. for improving the bioavailability of pharmaceuticals, as odour or taste masker, for separation and purification of compounds etc. For the latter type of applications the CDs are normally bound to a solid matrix in order to facilitate an efficient separation. One method of covalently attaching CDs onto a solid matrix was patented by Armstrong who utilizes a grafting arm to covalently graft the CDs onto a surface [2–4]. This is predominantly done on a matrix of $SiO_2$ in the form of silica gel [2]. The coating of cyclodextrins onto silica gel has been applied by several research groups and the products used e.g. as stationary phases in chromatography in order to access the efficacy of the CDs for separation of enantiomeric compounds [5–7]. Earlier research on the synthesis and the products obtained by use of the Armstrong synthesis route suggests that the CDs are not evenly distributed throughout the silica particles [2], and coating of silica gel with organic grafting arms and β-CD changes the mesoporous structure of the silica gel [7–9]. The change is a consequence of the organic components being grafted inside the pores, thereby reducing the accessible pore area and total accessible surface area. Silica gel is often used because of its mesoporous structure with a large accessible surface area, and thus maintaining this trait is an important, but less investigated

feature. Besides the reported changes in surface and pore characteristic found from BET analysis, little has been reported on the change in mesoporous structure and the possible impact it has on the final product or the impact it has on the distribution of β-CD throughout the particle. Results obtained by this research group also suggest that the concentration of β-CD at the surface of the silica particles is much higher than the bulk concentration which can only be explained by a heterogeneous distribution. If the β-CD blocks the pores it will reduce the accessible surface area, and if some β-CD are trapped in the pores it may result in a fraction of β-CD being inaccessible and thus unable to form complexes. Therefore there might be a discrepancy between the accessible β-CD (e.g. the amount of CD available for complex formation) and the total amount of β-CD. This property is an important factor for determining the effectiveness of β-CD immobilized and should be included when determining the effective β-CD content. In earlier studies thermogravimetric analysis (TGA) and elemental analysis has been the preferred approaches for the determination of the β-CD content [7, 9–12]. However, these methods reveal the total amount of β-CD and not the amount of accessible β-CD. To better understand the relation between the total amount of β-CD and the accessible amount of β-CD both fractions need to be determined. While quantification of the total amount of β-CD can be obtained with methods such as TGA or elemental analysis, quantification of accessible amount of β-CD can be determined by evaluating the complex formation. By choosing a guest with a distinct and detectable change in its physico-chemical behaviour upon complex formation it is possible to separate the complex from the non-complexed guest and thus distribution of accessible β-CD on the particle. 8 Anilinonaphtalene-1-sulfonic acid ammonium (1,8 ANS) is a fluorescent molecule with two important properties; the quantum yield of the molecule increases in polar environments and 1,8 ANS is able to form an inclusion complex with CD [13–20]. The use of 1,8 ANS as a probe for accessible β-CD cavities holds two advantages. Firstly, the adsorption of 1,8 ANS by the β-CD bound to the silica particles can be determined enabling the quantification of the accessible β-CD. Secondly, the particles with 1,8 ANS complex bound in β-CD can be analyzed in a Confocal Laser Scanning Microscope (CLSM) allowing for a visual determination of the distribution of the inclusion complex. Using this combination of methods it should be possible to investigate the distribution and accessibility of β-CD on the silica particles.

The Langmuir isotherm given in Eq. 1 can be used for the description of the complexation between surface bound β-CD and a guest molecule, here 1,8 ANS.

$$\theta = \frac{\alpha * P}{1 + \alpha * P} \quad (1)$$

where $\theta$ is the fraction of cyclodextrins that has formed complex with the guest molecule, $\alpha$ is the stability constant between β-CD and 1,8 ANS and P is the concentration of free CD. The isotherm is based on the adsorption of a guest molecule onto a surface with a finite number of adsorption sites, each having the same binding affinity unaffected by the degree of binding on the surface. A guest molecule is expected to exhibit intermolecular interactions if they are charged and in proximity [21]. ANS is charged, however it is assumed that the intermolecular interaction will be negligible due to the small size when compared to the size of the binding sites (β-CD). The association constant found in the Langmuir equation is equal to the stability constant between the β-CD and the guest molecule. From $\theta$ the number of absorptions sites on the surface, corresponding to the number of β-CD cavities accessible for formation of complex with 1,8 ANS, can be determined.

Here we report the investigation of silica particles coated with β-CD with respect to the amount of accessible CD as determined by titration and fitting to a Langmuir isotherm. The mesoporous structure of the particles are investigated by use of BET before and after coating to understand the changes in porosity caused by the chemical modification. The particles are further investigated by use of CLSM to determine the distribution of the CD on the silica particles.

## Materials and methods

### Materials

β-CD was purchased from Wacker Chemie AG (Munich, Germany). Silica gel was supplied by Merck (Darmstadt, Germany) with a size distribution of 0.063–0.200 mm and a surface area of 480–540 $m^2/g$. Reference silica gel, 3-Glycidyloxypropyl silica gel (Gly-Si) and β-cyclodextrin 3-Glycidyloxypropyl silica gel (β-CD-Gly-Si) was produced as described elsewhere [22]. The amount of organic compounds on the β-CD-Gly-Si determined by use of TGA was 532 μmol Gly/g Si and 33 μmol β-CD/g Si. By use of elemental analysis a yield of 699 μmol Gly/g Si and 38 μmol β-CD/g Si were found for the same material. 8 Anilinonaphtalene-1-sulfonic acid ammonium salt (1,8 ANS) was purchased from Sigma–Aldrich (Saint Louis, USA) and used as received.

### Methods

#### *Titration of silica gels*

For titration of the surface binding sites varying amounts of reference Si, Gly-Si or β-CD-Gly-Si were mixed with

10 mL of $3 \times 10^{-5}$ M 1,8 ANS in MilliQ water. The solution was left on a shaking table overnight (1.5 Hz and 4 cm oscillation) and following centrifuged at 1,922*g* (Sigma 6k10 centrifuge). The top fraction (3 mL) liquid phase was removed and measured in a fluorescence spectrophotometer (Varian Cary Eclipse). The solid fraction was recovered and resuspended in 3 mL of MilliQ water. The suspension containing the solid particles was used for CLSM measurements.

### *Confocal laser scanning microscopy*

Transmitted light microscopy combined with CLSM was used for the analysis of silica particles. A Zeiss LSM 510 META scanning confocal microscope equipped with a 80 mW UV laser was used. Excitation at a wavelength of 364 nm and emission collected at all wavelengths between 385 and 470 nm with standard Zeiss software (LSM 510, version 2.01) were used for the recording of images. The images were further analysed by use of ImageJ (ver. 1.40 g) where the area of the particles were found by use of "Analyze particles" function and the mean grey value was found by use of the "Measure" function. The mean grey value was divided by the particle area to obtain a value corresponding to the amount of fluorescent light per particle area. Some images were analysed using the function "surface plot" to give a 3D representation of the fluorescence intensity of each pixel in the picture. "Plot profile" was used to mark a certain area of the image and get the fluorescence intensity as function of position on the particle.

### *Surface area analysis*

Specific surface area was calculated using the Brunauer, Emmet and Teller (BET) Isotherm to fit nitrogen adsorption onto the surface of the silica gel [23] and the pore volume was calculated using the Barrett-Joiner-Halenda method [24]. BET measurements were done on a Quantachrome Autosorb 3. For analysis 0.3 g of sample was degassed at 70 °C under vacuum for 20 h. After degassing nitrogen was introduced to the chamber and the pressure was monitored and used in the BET isotherm.

### *Fluorescence spectroscopy*

Fluorescence spectroscopy was measured on a Varian Cary Eclipse system. For the Langmuir adsorption isotherm experiment the excitation wavelength was 388 nm and the emission was recorded at 590 nm. The excitation and emission slit were set at 10 nm.

## Results and discussion

### Surface area

After synthesis the reference Si gel, Gly-Si and $\beta$-CD-Gly-Si was characterized using TGA, elemental analysis, X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, direct pyrolysis mass spectroscopy, solid state nuclear magnetic resonance and wide angle X-ray diffraction [22]. All used methods confirm that the products contain the desired organic components; Gly and $\beta$-CD. The data from the XPS analysis clearly show that the concentration of the organic components is higher in the outer 10 nm layer compared to the bulk concentration, found by elemental analysis and TGA. This clearly shows that the surface coating is heterogeneous and not homogeneous. Earlier studies on an identical system also show that the mesoporous structure of the silica gel is changed after synthesis [7, 8] and it is expected that these two phenomena might be linked. To investigate this observation and link it to earlier literature observations, BET analysis of the reference Si-gel and $\beta$-CD-Gly-Si were done and the results are shown in Table 1.

The BET data clearly shows a changed mesoporous structure of the silica gel as a result of the coating with Gly and $\beta$-CD onto the silica gel. The surface area, pore volume and average pore diameter is significantly reduced as a result of the coating. An explanation for the reduced pore volume is that the pores in the silica are very small and coating the inside of the pores with organic molecules thereby renders the pores inaccessible for nitrogen adsorption. At the same time the pores will be coated with a layer of grafting arm and $\beta$-CD which reduces the average pore diameter. This observation is in agreement with earlier studies [8] where it is suggested that the $\beta$-CD is capable of coating the larger pores and thereby reduce the average pore diameter and surface area. To confirm the change in mesoporous structure can be ascribed to the organic compounds, the $\beta$-CD-Gly-Si was pyrolysed in order to remove the organic compounds from the silica gel.

**Table 1** BET data obtained from measurement of $\beta$-CD-Gly-Si and reference Si gel

| | Surface area ($m^2$/g) | Pore volume ($cm^3$/g) | Average pore diameter (nm) |
|---|---|---|---|
| Si-gel | 517 | 0.752 | 5.8 |
| $\beta$-CD-Gly-Si | 320 | 0.427 | 5.3 |
| $\beta$-CD-Gly-Si, pyrolyzed | 521 | 0.762 | 5.8 |

One gram of $\beta$-CD-Gly-Si is heated to 350 °C under atmospheric air for 5 h and measured after pyrolysis

If the mesoporous structure change is due to the organic content it is expected that the change in mesoporous structure is reversible by use of pyrolysis. After pyrolysis it is seen that the pore volume and average pore volume is reverted to the same values as prior to coating with the organic compounds. From the BET data it can be concluded that the coating of the particle with the organic molecules are responsible for the change in the mesoporous structure and that the change is reversible by simple pyrolysis.

## Langmuir adsorption isotherm

To investigate the accessibility of the $\beta$-CD coated on the silica particles titration of the particles with 1,8 ANS was fitted to the Langmuir adsorption isotherm. For the Langmuir experiment the silica particles with $\beta$-CD were equilibrated with the 1,8 ANS for 20 h. The large timescale insures that the data is based on equilibrium and not kinetic observations. As similar silica particles are used for separation in high performance liquid chromatography (HPLC) it is known that smaller molecules are able to interact with the silica gel rapidly and therefore the silica gel can be used in experiments with a much shorter time span such as HPLC.

The total amount of both Gly and $\beta$-CD was found by TGA and elemental analysis. However, the total and the accessible amounts are expected to differ as the changed mesoporous structure might result in some $\beta$-CD getting "trapped" inside pores, thereby rendering some $\beta$-CD inaccessible for complex formation. The results from the Langmuir fitting can be seen in Fig. 1, the same experiment was repeated with silica gel and Gly-Si where none of them showed any adsorption of ANS (data not shown).

The fit made by use of the Langmuir isotherm is close to the experimental data ($R^2 = 0.92$) and therefore the values obtained are a good fit to the Langmuir adsorption isotherm used to model the interaction between 1,8 ANS and $\beta$-CD. From the fit of the Langmuir isotherm to the experimental data, the concentration of accessible $\beta$-CD is found to be 26.5 μmol $\beta$-CD/g Si and $K_{\beta\text{-CD:1,8 ANS}}$ is found to be 35,300 $M^{-1}$. The accessible amount of $\beta$-CD is close to the total amount of $\beta$-CD found by TGA and elemental analysis and only a fraction corresponding to approximately 12% of the total amount of $\beta$-CD is inaccessible. This result indicates that the mesoporous structure of the silica gel or the modification itself renders some cavities of the $\beta$-CD inaccessible due to sterical hindrance. If the $\beta$-CD is grafted inside a pore and the pore subsequently is blocked by additional grafted $\beta$-CD, then the $\beta$-CD grafted deepest in the pore may become inaccessible. Another possibility is that the $\beta$-CD grafted in the smallest pores may not be accessible for complex formation as the distance between

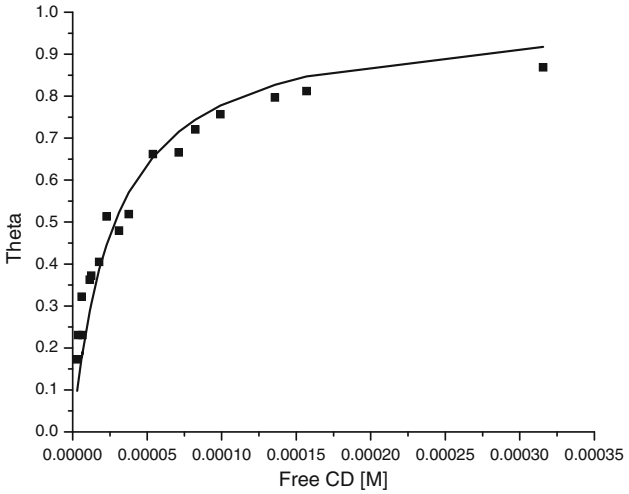


**Fig. 1** The value of Theta as a function of the concentration of uncomplexed $\beta$-CD, denoted free CD. The black dots are the experimental data where the black line is the optimal fit from which the amount of accessible $\beta$-CD and binding constant is calculated. The free $\beta$-CD is calculated from the theoretical amount found from the Langmuir isotherm

the silica wall and the cavity may be too small to allow diffusion of 1,8 ANS into the cavity. Both phenomena could explain the fraction of inaccessible $\beta$-CD. The stability constant found is two orders higher than the stability constant between $\beta$-CD and 1,8 ANS in liquid which has been reported to be around 300 $M^{-1}$ [13, 16–18, 20]. Despite this large difference it is not unexpected as literature values for a similar system have found K values between $\beta$-CD and 1,8 ANS up to $3.9 \times 10^5$ $M^{-1}$[25]. As $\beta$-CD is already covalently bound to a surface the entropy loss due to the complexation will be smaller as compared to the complexation in solution and this gives rise to a higher stability constant [12]. Another reason for the large stability constant is due to the confinement effect from the combined structure of the grafting arms and the $\beta$-CD [26]. Using the Langmuir isotherm it is possible to both estimate the stability constant as well as the amount of accessible $\beta$-CD on the silica gel, which makes it a very powerful tool for characterizing the surface bound $\beta$-CD with a very simple technique.

The values obtained from the titration experiment clearly illustrate the amount of accessible $\beta$-CD under the assumptions used but give little information on the distribution of the $\beta$-CD on the particle. CLSM is used for the investigation of the $\beta$-CD-Gly-Si with 1,8 ANS complex bound in the $\beta$-CD cavity.

## Confocal scanning laser microscopy images

To better analyze the images it is assumed that particles without $\beta$-CD does not emit fluorescence and therefore all

fluorescence is due to complex formation. This assumption is valid as the reference silica gel and Gly-Si (data not shown) does not fluoresce in the CLSM experiment as demonstrated in Fig. 2.

From the fluorescence images it is possible to measure the area fraction covered by particles of each image. The mean grey (proportional to the intensity of fluorescence) value of the fluorescent images can be measured and when dividing the mean grey value with the area of each particle that emit light, a value of the average emission intensity from each light emitting pixel can be obtained. Assuming that the intensity of the fluorescence is related to the amount of complexes, the particles with the most complexes will emit the most light and if quantified this tool can be used to measure concentration on an unknown surface. The measured fluorescence emission per surface area for different samples and the theoretical amount of complex in each sample is shown in Fig. 3. From the figure it is apparent that a higher concentration of complex leads to higher emission intensity per surface area as the curve generated from the experimental data is very similar to the theoretical curve, which is calculated from the 1,8 ANS concentration, added amount of $\beta$-CD-Gly-Si, $K_{\beta\text{-CD-Gly-Si:1,8 ANS}}$ and accessible Wt% $\beta$-CD on the particles.

The relationship between the theoretical amount of complex in the particles and their fluorescence intensity/area relates well and show the viability of using CLSM as a tool for semi quantitative analysis of a surface. If a surface contains inclusion complexes heterogeneously distributed, this technique can also be used to give a semi quantitative estimate of inclusion complex as function of light intensity throughout the surface, thus quantifying the heterogeneous distribution. This method is however very dependent on the instrumental setup and a curve as shown in Fig. 3 must be measured for a new instrument setup. However, the generation of a standard curve allows for an approximate estimation of the amount of complex in an unknown sample under the assumption that only the 1,8 ANS/$\beta$-CD complex emit fluorescence. The CLSM images used for the data analysis described above can be obtained by use of simple fluorescence microscopy. The advantage of using a CLSM is the ability to image focal planes in the particles and determine the fluorescence through the particle and these images can be used for further data treatment [27]. CLSM images of a representative particle are shown in Fig. 4.

From Fig. 4 it is clear that each confocal plane contains different concentrations of inclusion complex throughout the particle. As the confocal planes are closer to the centre of the particle it is seen that the outer layer of the particle emits more fluorescence light. This indicates that the distribution of the inclusion complex is heterogeneous throughout the particle with a higher concentration at in the surface layer. This fits with the theory that the grafting of CD onto the surface of the silica gel is diffusion limited and the mesoporous structure might make it harder for the CD to diffuse to the inside of the particle and graft it. The heterogeneous distribution observed is believed to be depended on the pore size of the silica gel used and synthesis time. For smaller particles (>20 μm) no heterogeneous distribution was observed (images not shown) but a

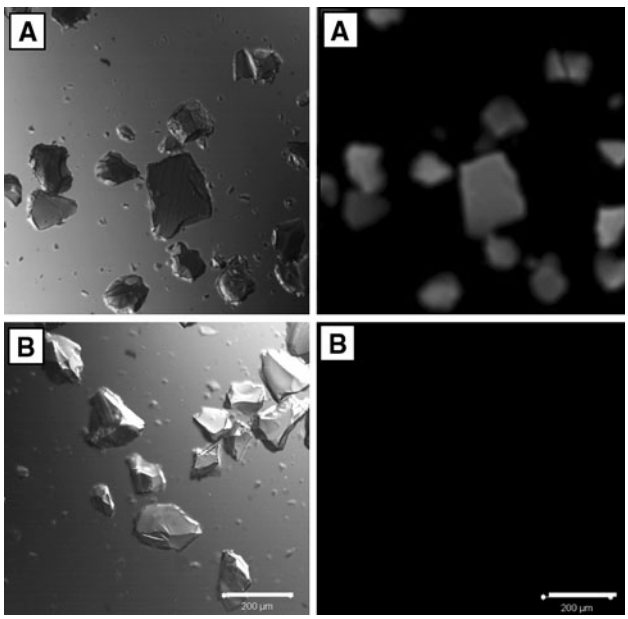


**Fig. 2** Reference $\beta$-CD-Gly-Si (**a**) and reference silica gel (**b**) particles in transmission mode (*left*) as well as fluorescence excited with 364 nm UV laser with emission recorded at all wavelengths between 385 and 470 nm (*right*). Both reference Si gel and $\beta$-CD-Gly-Si have been treated with ANS prior to analysis. All images are recorded at the same magnification with the scale bar being 200 μm

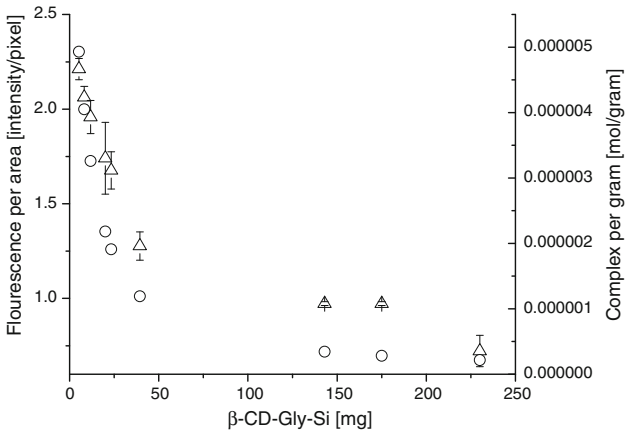


**Fig. 3** The figure shows the fluorescence intensity per area found from the CLSM images (minimum 5 images per data point) with standard deviation (Δ). The secondary *Y*-axis shows the theoretical amount of complex bound ANS per gram of $\beta$-CD-Gly-Si (o)

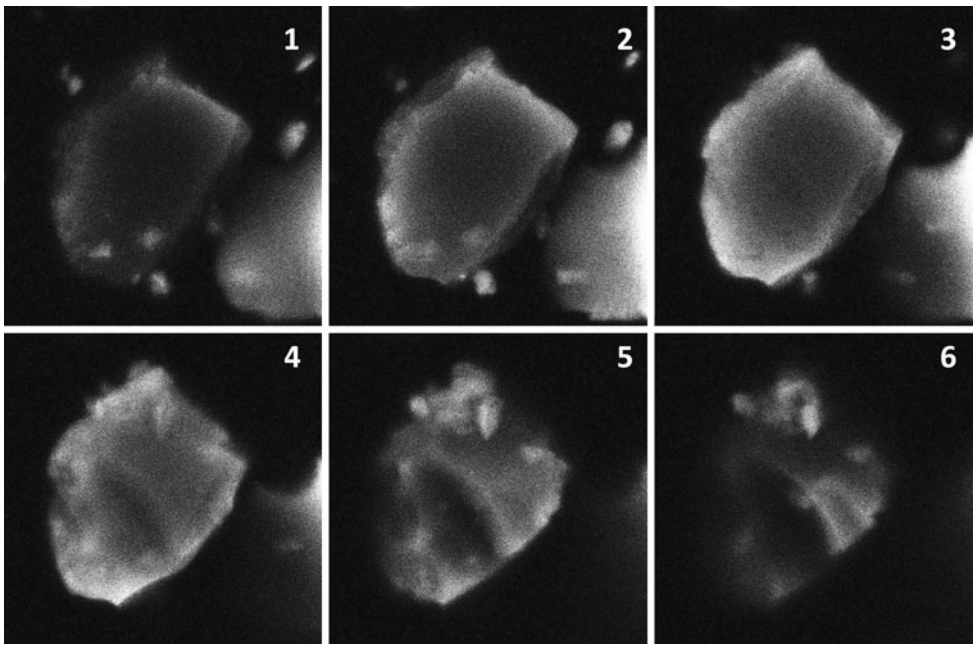


**Fig. 4** The images are from the top of the particle and down through the bulk of the particle. The particle is excited with a UV laser at 364 nm and emission collected at all wavelengths between 385 and 470 nm. Each image is 104 × 104 μm. The thickness of each confocal image is 10.4 μm

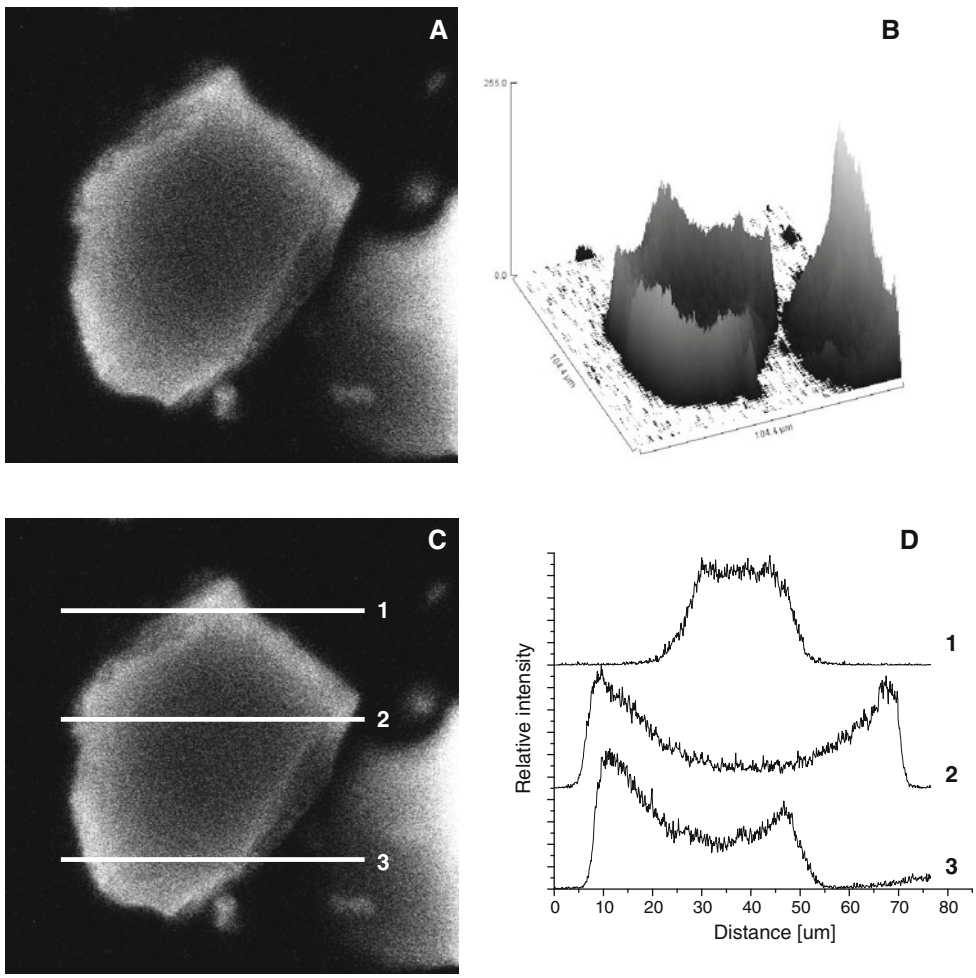


**Fig. 5** The images show the original image (**a**) with the intensity of each pixel shown as in a 3D representation (**b**). It should be noted that another particle is causing the high intensity in the right part of the figure. The fluorescence intensity measured at three different locations as function of distance over particle (**c**) with the corresponding intensity versus distance shown (**d**). The confocal images are 104 μm × 104 μm

rather homogeneous distribution was obtained. It is believed that give enough time the diffusion of the β-CD into the particle is possible. From previous experiment it is known that the light intensity of the fluorescence is related to the concentration of inclusion complex present. It is possible to analyze the distribution of the inclusion complex in the particle by use of ImageJ. The distribution of the inclusion complex can be shown by measuring the distribution of the fluorescence intensity on the particle by use of a surface plot as shown in Fig. 5 where the original confocal image is also shown.

From the image analysis it is clear that the concentration of inclusion complex at the surface it much higher than the concentration of inclusion complex in the centre of the particle. For each particle additional information can be extracted from the confocal scan, where the concentration of inclusion complex can be determined throughout the particle by use of the relationship between intensity and inclusion complex concentration shown in Fig. 3. From the image analysis it is also apparent that the background gives no fluorescence emission, seen as an intensity of nearly zero in Fig. 5. The intensity of the fluorescence through the particle as shown in Fig. 5, line 1, shows that a near equal coating is present when the CD only has to diffuse through 20 μm silica gel or below. Overall the CLSM images gives a lot of information that help understand the distribution of the accessible β-CD, but one has to take care on how to do the image analysis as there are no set standard for the method when analysing these materials.

## Conclusion

The accessibility of β-CD was investigated for a silica gel containing covalently surface bound β-CD. The accessibility was investigated using the Langmuir adsorption isotherm by use of the fluorescent guest 1,8 ANS. From the isotherm the concentration of accessible β-CD and stability constant were determined. The accessible amount of β-CD was found to be 12% lower than the total amount of β-CD. The reduced accessible amount of β-CD could be explained by the changed mesoporous structure as found by BET, which suggests that some pores are blocked by grafting and this might trap some β-CD in inaccessible pores. The fluorescence intensity from the silica gel containing β-CD/1,8 ANS complex was quantified and it was shown than the light emission of the particles could be used as a semi quantitative tool for investigating the amount of formed complex. The distribution of the β-CD on the silica particles were investigated using confocal scanning laser microscopy. The CLSM clearly showed that the β-CD was heterogeneously distributed with the highest concentration of accessible β-CD located in the outer layer of the particle.

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