

Adsorption of Triton X-100 on Ultra-Stable Zeolite Y

Daniel Klint* and Jan-Olov Bovin

National Center for HREM, Inorganic Chemistry 2, Center for Chemistry and Chemical Engineering, Lund University, PO Box 124, S-221 00 Lund, Sweden

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Preparation of zeolite particles in the micrometer range was performed by heat treatment of compacted zeolite powder. The fractionated particles were used as a matrix for the adsorption of Triton X-100 in water solution and the rate of adsorption was studied. The rate of adsorption was enhanced either by treatment with a blowing agent prior to compaction into pellets or with metakaolinite, used as a binder in the sintering procedure. For particles in the range 63–125 μm , up to 90% of the Triton X-100 was adsorbed after one minute. The effect of the blowing agent increased with particle size and for particles in the range 250–500 μm , adsorption after one minute increased from 40 to 60%. This implies that shorter contact times can be used in systems working with batch procedures, e.g. in the removal of detergents from protein solutions. In flow systems the residence times may be lowered due to the increased intraparticle diffusion rates. Transmission electron microscopy (TEM) studies confirmed the presence of a secondary pore system in the zeolite, with diameters in the range 5–50 nm. The size of the Triton X-100 micelles was estimated to be 10 nm in diameter by direct imaging with frozen hydrate cryo-TEM.

The rate of adsorption onto porous materials is affected by the microporosity as well as the meso- or macroporosity of the adsorbent. The porosity is defined according to the pore width as micropores, <2 nm, mesopores, 2–50 nm, or macropores, >50 nm.¹ The intra-crystalline microporosity can accommodate the adsorbate provided that it is small enough to enter the pores. Adsorption is also affected by the particle size. In biochemistry, adsorption of, e.g., surfactants and proteins can be performed either in batch procedures or by means of continuous flow systems such as liquid chromatography. Particle sizes of the order of micrometers offer no problems in the former case, because separation of solution and matrix can be achieved by centrifugation. In column chromatography, however, a particle size that is too small is impracticable owing to generation of a high back pressure. Larger particles or smaller flow rates have to be used and the amount of adsorbent material must be adjusted to compensate for the loss of outer surface. One possible way to overcome this would be to increase the macroporosity of the particles in order to facilitate diffusion, thus making a larger portion of the surface available.

Zeolites are microporous aluminosilicates comprising well-defined channels and cavities with aperture diameters ranging from 0.25 to 0.75 nm.² Both chemical and physical properties as well as textural properties of the zeolite can be altered by post-synthetic treatments such as dealu-

mination.³ A dealuminated variety of zeolite Y, abbreviated USY (ultra-stable zeolite Y), has been used to remove detergents from solutions containing proteins.^{4,5} USY has also been used in the purification of proteins.^{6–9} Manufacture of zeolite Y yields crystallites whose particles are a few micrometers or less,¹⁰ which are thus suitable for surfactant and protein adsorption with batch procedures. Larger particles can be produced by compacting the zeolite powder into pellets, either with or without binder, and subsequently heating the pellets at high temperatures. The binder is a matrix in which the zeolite is embedded and clays of the kaolinite type are generally used. During heat treatment kaolinite transforms into metakaolinite. After heat treatment the pellets can be crushed and fractionated into different particle size ranges. Prior to compacting and high temperature treatment, the powder can be mixed with a blowing agent, which is a compound that can be sublimed at moderate temperatures. The blowing agent acts as a generator of pores in the pellets thereby reducing the bulk density.

In order to investigate how different porosities in USY-kaolinite mixtures affect the rate of adsorption of detergents (and proteins), we designed a series of adsorption experiments where Triton X-100 was used as the adsorbate.

Experimental

Dealuminated ultra-stable zeolite Y, USY, with an Si:Al ratio of 230, was manufactured by Tosoh Co., Japan.

* To whom correspondence should be addressed.

Kaolin clay powder and ammonium hydrogen carbonate, analytical grade, were purchased from Kebo, Sweden. The adsorbent matrix was characterized by transmission electron microscopy (TEM). Crushed pellets were embedded in epoxy resin followed by ultrathin sectioning in an ultra microtome (Leica UCT) equipped with a diamond knife (Drukker). Sections of about 20 nm thickness were collected on lacey carbon film, supported on 200 mesh Cu-grids. The samples were examined with a Philips CM120 BioTWIN Cryo ($C_s = 6.0$ mm) equipped with an AutoFilter GIF100 (Gatan Image Filter), used for energy filtering (EFTEM) and elemental mapping. Images were recorded using a cooled multi-scan Gatan CCD camera.

Triton X-100, polyoxyethylene-*p-tert*-octylphenol, was purchased from Sigma, USA. Triton X-100 is a non-ionic surfactant commonly used in biochemical studies. The critical micelle concentration (CMC) is 0.29 mmol l^{-1} .¹¹ The micelles consist of about 140 molecules and in weight they correspond to a protein of 90 kDa. The size of the Triton X-100 micelles was estimated by cryo-TEM performed on frozen hydrates. Samples for cryo-TEM were prepared in a controlled environment vitrification system (CEVS).¹² A droplet of solution containing 10 mg ml^{-1} of Triton X-100 in PBS was mounted on the grid (300 mesh Cu-grids with lacey carbon on Formvar) and the excess volume was blotted off. Vitrification of the aqueous phase was achieved by plunging the sample into liquid ethane. Samples were then stored under liquid nitrogen and prior to microscopy transferred to the cryo holder (Oxford CT3500) in a cryo station cooled with liquid nitrogen.

The following samples were prepared: (1) binderless USY, (2) a mixture of USY and ammonium hydrogen carbonate as a blowing agent, 50% by weight, (3) a mixture of USY and kaolinite, 23% by weight and (4) a mixture of USY and kaolinite, 23% by weight, further mixed with ammonium hydrogen carbonate as a blowing agent, 50% by weight. The acronyms of the samples used in the text and relative amounts of the components are listed in Table 1.

The USY-kaolinite mixture was prepared by grinding the components in water, and the suspension was dried at 50°C . The ammonium hydrogen carbonate was mixed with USY and USY-kaolinite, respectively, by manual grinding in a mortar. The four different preparations were compacted into pellets, 30 mm in diameter and

4–6 mm thick using a pressure of 140 MN m^{-2} . Ammonium hydrogen carbonate was sublimed at about 100°C for 16 h before the high temperature treatment. Y samples were heated for 12 h at 1100°C and the YK samples, 48 h at 800°C . The pellets were crushed in a mortar and fractionated into particle size intervals of 500–250, 250–125 and 125–63 μm .

The fractionated particles were washed and degassed in PBS buffer prior to incubation with a PBS solution (10 mmol l^{-1} phosphate, 150 mmol l^{-1} NaCl, pH 7.4) containing 5 mg ml^{-1} Triton X-100 (8 mmol l^{-1}). After sedimentation of the particles the supernatant was removed, except for the solution in the interparticle volume. The adsorption was stopped at 1, 2 and 5 min by centrifugation at $10\,000 \times g$ for 2 min. The volume of supernatant was determined and the absorbance at 280 nm in each fraction was measured using a spectrophotometer. The concentrations were determined by calibration with Triton X-100 solutions of known concentrations. During the experiment the weight ratio between surfactant and adsorbent matrix in g per g was kept constant at 1/10. The weight ratio was obtained from eqn. (1),

$$q^* = (V_0 C_0 - V_t C_t) / m_M$$

where V_0 is the volume of Triton X-100 solution added ($= 1.0 \text{ ml}$), C_0 is the initial concentration of the Triton X-100 solution ($= 5.0 \text{ mg ml}^{-1}$), V_t is the total volume, i.e. V_0 plus the volume of buffer solution surrounding the solid matrix after the washing solution is decanted off (ml), C_t is the concentration of Triton X-100 at a time t (mg ml^{-1}), m_M is the weight of the matrix (mg).

Adsorption isotherms and adsorption kinetics were obtained by plotting q^* versus C_t and q^* versus adsorption time. In the kinetics experiment, six independent measurements were made for each data point.

Results and discussion

Dealumination, i.e. extraction of aluminium from the zeolite structure, often leads to the formation of a secondary pore system.^{3,13} The size and number of pores are correlated with the extent of dealumination,¹⁴ but generally the diameters are in the mesoporous range, 2–50 nm. Transmission electron microscopy studies on the USY used in this investigation confirmed presence of mesopores. Both small mesopores, with diameters of about 10 nm, and large, 30–50 nm, were detected (Fig. 1). It was also confirmed that metakaolinite retained a lamellar structure after the heat treatment at 800°C , which has been shown earlier.¹⁵ The reason for explicitly pointing out the mesoporosity is that its presence may have a substantial effect on the adsorption of Triton micelles (as well as proteins). The Triton X-100 micelles are about 10 nm in diameter, which was confirmed by direct imaging with cryo-TEM (Fig. 2).

The equilibrium adsorption profiles for Triton X-100 on 63–125 μm particles of Y and YK, and on kaolinite

Table 1. Composition of samples before heat treatment and their acronyms used in the text. The figures within parentheses refer to the composition after heat treatment.

Sample	NH_4HCO_3 wt%	USY wt%	Kaolinite wt%
Y	—	100	—
Y5	50	50 (100)	—
YK	—	77 (80)	23 (20)
YK5	50	38.5 (80)	11.5 (20)

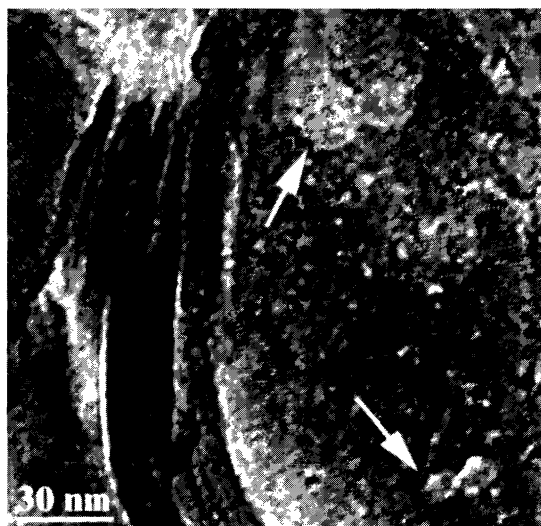


Fig. 1. EFTEM image of USY sintered with metakaolinite. The zeolite crystal to the right is viewed approximately along [110]. The phase with a higher contrast was identified as metakaolinite by elemental mapping (not shown). Mesopores of different sizes are located all over the zeolite particle and two are indicated by arrows. In the upper left-hand corner a cavity is seen between two zeolite crystallites.

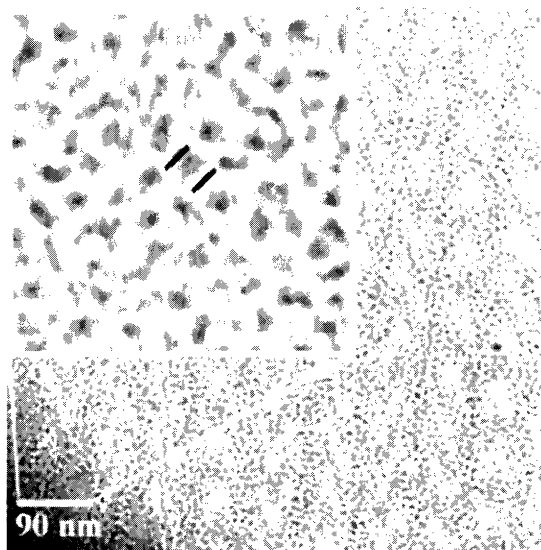


Fig. 2. Triton X-100 micelles, 10 mg ml^{-1} in PBS, imaged by frozen hydrate cryo transmission electron microscopy (cryo-TEM). The inserted image is a magnification of the upper right-hand corner. The edge of the inserted image is 90 nm. The micelles are spheroidal in shape, with a diameter of about 10 nm, which is the distance between the bars in the inserted image.

powder are shown in Fig. 3. It can be deduced that kaolinite exhibits a maximum adsorption capacity which is about 1/30 of that of the YK sample. This implies that the contribution to the adsorption of Triton X-100 from the binder is negligible. Thus, if the binder itself does not adsorb Triton, the difference in maximum capacity between Y and YK must arise for something else. There

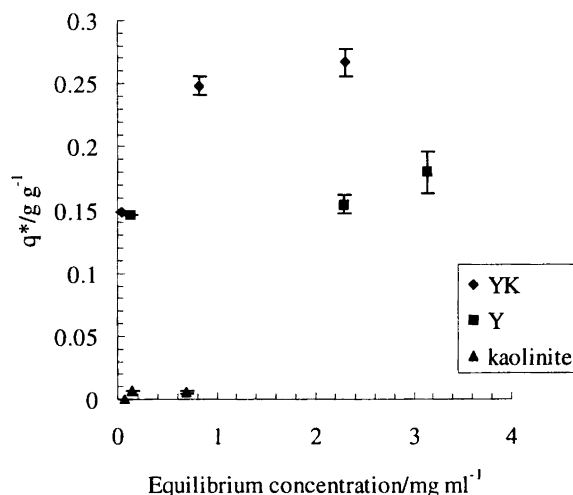


Fig. 3. Adsorption isotherm displaying the weight ratio between Triton X-100 and adsorbent matrix, q^* (g g^{-1}), versus the equilibrium concentration (mg ml^{-1}). The adsorption on binderless sample (Y) and zeolite sintered with metakaolinite (YK) was performed on 63–125 μm particles, but for kaolinite (sub)-micrometer powder was used. The adsorption time was 1 h.

are two differences, both of which may serve as explanations. Firstly, in the sample containing metakaolinite as a binder it is probable that interparticle cavities are formed between agglomerates of zeolite crystallites and metakaolinite wafers, as shown in Fig. 1. These cavities are large enough to accommodate the Triton micelles. Secondly, the sintering temperature differed between the two samples. The binderless Y was sintered at 1100°C and YK was sintered at 800°C . Adsorption sites at grain boundaries are destroyed because of the sintering processes. In the Y sample the packing of crystallites is not disrupted, as kaolin does in the YK sample, thus enabling a higher incidence of sintering reactions. Since the maximum adsorption capacity was 0.24 and $0.14 \text{ g Triton X-100/g matrix}$ for the YK and Y sample, respectively, a weight ratio of 1/10 was chosen in order to study the rate of adsorption on the different preparations.

Taking the binderless sample, Y, as a reference, the rate of adsorption of Triton X-100 was enhanced both with the use of a blowing agent and by using metakaolin as a binder. The one minute adsorption on binderless Y, pretreated with 50% by weight of ammonium hydrogen carbonate (Y5), increased from 0.067 to $0.089 \text{ g Triton X-100/g matrix}$ i.e. approximately 33% (Fig. 4). The relative increase for YK was approximately 20%, 0.067 to 0.080 g g^{-1} . The amount of zeolite in the YK sample is actually 80% of the total weight due to the binder content. Taking only the weight of zeolite into account would yield an even larger increase. Although the binder itself does not contribute to the adsorption, its presence has an effect, therefore comparisons based on the total weight of adsorbent are considered. This increase in the rate of adsorption was valid for all three particle size ranges.

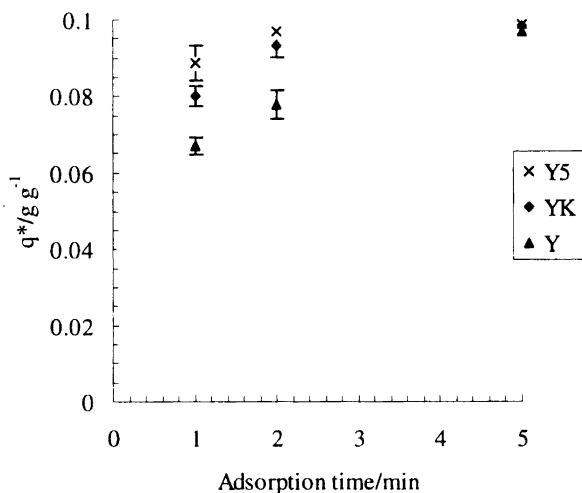


Fig. 4. The rate of adsorption on 63–125 μm particles displayed as the weight ratio between Triton X-100 and adsorbent matrix, q^* , versus the adsorption time. Y represents the binderless sample and YK the one sintered with metakaolinite. Y5 was pretreated with ammonium hydrogen carbonate as a blowing agent as described in the Experimental section. Error bars represent the standard deviation obtained from six measurements for each data point.

It was confirmed that the rate of adsorption was strongly dependent on particle size and Figs. 5 and 6 display clearly the correlation between the increased rate of adsorption with reduced particle size. The (outer) surface area per mass unit is correlated to the square of the radius, approximating the grains to spheres. The decrease in the rate of adsorption with particle size is thus associated with a decrease in surface area, even though the equilibrium capacity, i.e. q^* g Triton X-100/g matrix, may be the same. For particles with diameters of 63–125 μm , up to 90% of the Triton was adsorbed after one minute (Fig. 4). Within five minutes, almost complete removal of Triton X-100 was obtained with Y5, even with 125–250 μm particles [Fig. 5(b)]. The adsorption process could be interpreted as comprising up to three parts, having different rate constants. A possible explanation is as follows. The initial and fastest rate is determined by the adsorption of Triton micelles onto the available sites on the zeolite. The number of these sites per unit mass is directly correlated to the particle size as shown by the differences in q^* after 1 min adsorption (Figs. 5 and 6). The second and approximately constant rate, the slope of q^* versus adsorption time between 1–5 min, is almost the same for particle sizes $>125 \mu\text{m}$, and is determined by some kind of rearrangement of the adsorbed Triton micelles. The diameters of the channel apertures are not large enough for the micelles to enter. However, Triton X-100 monomers are small enough to penetrate the zeolite framework. This intra-particulate or intra-crystalline diffusion is likely to be independent of the particle size. Finally, a third rate of adsorption could be observed, which occurs when the concentration of Triton X-100 falls below the CMC. The rate of

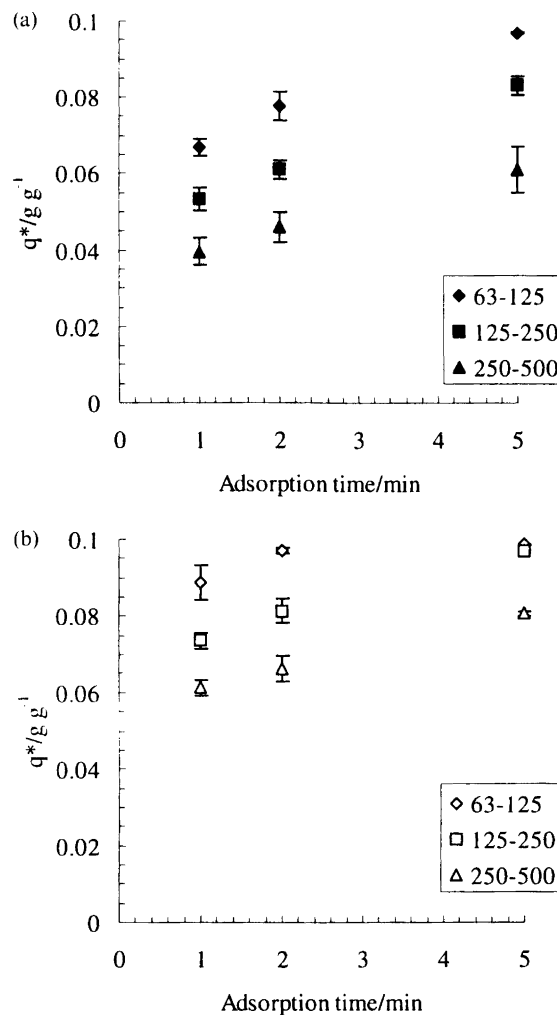


Fig. 5. The rate of adsorption on binderless samples of different particle sizes displayed as the weight ratio between Triton X-100 and adsorbent matrix, q^* , versus the adsorption time. (a) displays the reference sample Y and (b) Y5, pretreated with a blowing agent. Error bars represent the standard deviation obtained from six measurements for each data point.

adsorption is then determined by the bulk diffusion of Triton monomers to the zeolite surface. In the system used, the CMC is reached when $q^* \geq 0.093$, thus is only indicated in the adsorption on 63–125 μm particles [Figs. 5(b) and 6].

Interestingly enough, the effect of the blowing agent differs between the binderless sample, Y, and the one containing binder, YK. For Y, the rate of adsorption was increased in all the particle size ranges, as revealed by comparison of Figs. 5(a) and (b). This means that the porosity obtained by mixing and subsequent sublimation of ammonium hydrogen carbonate was small enough to have an effect on particles less than 125 μm . The effect of the blowing agent was enhanced with increasing particle size. For particles of 250–500 μm , the adsorbed amount of Triton after one minute increased by 50%, from 0.40 to 0.061 g g^{-1} . In the YK sample a statistically

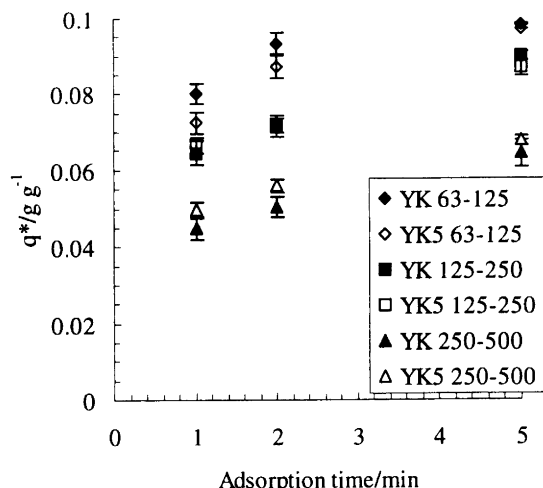


Fig. 6. The rate of adsorption on metakaolinite sintered samples of different sizes displayed as the weight ratio between Triton X-100 and adsorbent matrix, q^* , versus the adsorption time. Empty symbols represent YK samples pre-treated with ammonium hydrogen carbonate. Error bars represent the standard deviation obtained from six measurements for each data point.

significant effect was seen only in the particle range 250–500 μm , as shown in Fig. 6. The lack of effect of the blowing agent in the smaller particle range may once again be due to the binder, forming interparticle cavities, thus keeping a high portion of surface available for adsorption. In the larger particles, however, the pores generated enabled rapid diffusion further into the particles.

N_2 -adsorption/desorption studies have shown that the blowing agent does not affect surface area and pore size distribution attributed to regions below pore diameters of 100 nm.¹⁶ The blowing agent generated macropores, with diameters in the order of micrometers, thereby facilitating transport of micelles further into the particles.

In conclusion, the rate of adsorption of Triton X-100 on heat treated and fractionated zeolite particles was enhanced either by the use of a binder in the sintering procedure or by treatment with a blowing agent prior to compaction into pellets. This implies that shorter contact times can be used in systems working with batch procedures, e.g. in the removal of detergents from protein

solutions. In flow systems the residence times may be lowered due to the increased intraparticle diffusion rates.

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