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Polymorphic Calcium Carbonate Phases as Adsorbents for Allergens in Natural Rubber Latex

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ABSTRACT: Natural rubber latex contains different allergenic proteins and peptides that restrict its application in consumer products. Small mineral particles have a comparatively high specific surface area and are therefore well suited to adsorb such biomolecules. The adsorption of such biomolecules onto different polymorphic phases of calcium carbonate, i.e., calcite, aragonite, and vaterite, was quantitatively determined, both from solution as well as from cured natural rubber latex. All phases were able to adsorb the allergens, with slight differences between the different allergens. Desorption experiments showed differences between the allergens of natural rubber latex, but only small differences between the polymorphic phases of calcium carbonate. The release of the allergens from latex objects with incorporated calcium carbonate particles showed that a retention of allergens is possible by adding calcium carbonate as a filler material. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41271.

KEYWORDS: adsorption; applications; biocompatibility; properties and characterization; rubber

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INTRODUCTION

Natural rubber latex (NRL) is harvested from *Hevea brasiliensis*, called the rubber tree, and used in many consumer products.[1] So-called dipped products, i.e., products for which molds are immersed into the liquid latex, such as gloves, balloons and condoms, are typical examples. In solid form, latex is also used in many consumer products, e.g., pacifiers, bottle caps and plungers.[2–5] Natural rubber latex contains up to 250 different proteins and polypeptides. These are present in the natural product in small, but allergologically relevant amounts. Approximately 50 (20%) of these proteins and polypeptides have antigenic properties, but only 13 of them were classified as allergenic by the World Health Organization (WHO). The sensitization occurs through skin, mucous membranes, and by inhalation of allergens that will be transferred together with the powder from powdered products. This can lead to edema, urticaria, conjunctivitis, asthma, and even to an anaphylactic shock.[4,6,7] Furthermore, the "latex-fruit syndrome" is of considerable interest for consumer products because cross sensitivities between natural rubber latex on the one hand and banana, avocado, kiwi, and potato on the other hand have been observed.[8,9] After the manufacturing process of different products where a number of biomolecules is removed, four major proteins remain which have an allergenic effect: Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02.

Hev b 1, also known as rubber elongation factor (REF), is a protein with a molecular weight of 14.6 kDa and an isoelectric point (IEP) of 4.9. It is hydrophobic and typically attached to large latex particles with a diameter above 350 nm. Its amino acid sequence shows no homology to known sequences of other proteins.[10] Hev b 3 has a molecular weight of 23 kDa and an IEP of 4.8. It is also bound to latex particles and has similarities to Hev b 1 as 6 of the 14 sequenced peptide strands correspond to Hev b 1.[8] These two allergens have a connection with the neural tube defect *spina bifida*. People with this genetic defect frequently show latex allergies, especially to these two allergens. The relationship between this genetic defect and the latex allergy is not yet understood.[8,11–13]

Hev b 5 is an acidic protein with a molecular weight of 16 kDa and an IEP of 3.5. Its amino acid sequence shows strong similarities to an acidic protein that was found in kiwi fruits. Their sequences overlap by 47% which may be a possible explanation for the observation that many patients who have a latex allergy shows a cross-allergy to kiwi fruits.[9,12]

Hev b 6.02 (hevein) is a protein that is associated with the larger protein prohevein (Hev b 6.01). Prohevein is a 20 kDa protein, consisting of two parts: First, the 4.7 kDa N-terminal unit, the hevein, which consists of 43 amino acids and has an IEP of 4.9. Second, the 14 kDa C-terminal unit, which consists of 144 amino acids, and is denoted Hev b 6.03. Hev b 6.02 is a

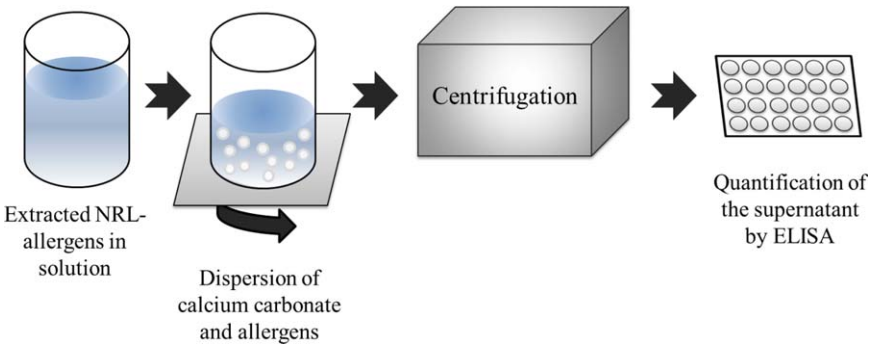

Figure 1. Schematic illustration of the adsorption experiments for allergens on calcium carbonate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

small protein which consists of cysteine and glycine and has a similar structure as lectins (proteins that specifically bind to carbohydrate structures).[12] Studies have shown that about 80% of healthcare workers have developed a sensitisation to Hev b 6.02. Therefore, Hev b 6.02 is the protein with the strongest allergenic potential.[8,11–15]

The latex allergens are included into the polymer matrix after polymerization (vulcanization), but can be slowly released, e.g., in skin contact. The binding of latex allergens to the powder of powdered gloves and the transport of latex allergens by the powder have also been reported.[5] Therefore, the reduction of latex allergens is of great current interest. There are different methods to reduce the allergen concentration in dipped products, especially in latex gloves, e.g., by treating the latex glove either with an aqueous solution of urea in the presence of an anionic surfactant, or with a proteolytic enzyme, or with a polar organic solvent.[16–18] Another way to reduce the allergenic proteins of natural rubber latex is their destruction by gamma irradiation or an electron beam.[19]

The loading of latex with calcium carbonate for the manufacture of latex gloves has also been proposed.[20,21] It is known that up to 35 mass equivalents of calcium carbonate per rubber mass can be added to the latex gloves before the mechanical properties are significantly decreased. [22,23] As calcium carbonate is a nontoxic additive that can be prepared in a variety of polymorphic phases and morphologies, we undertook a systematic study to determine its suitability as adsorbent for latex allergens. Three different crystalline polymorphic phases are known (calcite, aragonite, vaterite) which possess different crystal surfaces and surface energies.[24,25] Therefore, the adsorption capacity should be different and variable with the specific surface area. Finally, calcium carbonate is biodegradable, and there are no environmental concerns about its final disposal together with latex-based consumer products.

EXPERIMENTAL

Synthesis of Calcite, Aragonite, and Vaterite

The synthesis of the calcium carbonate polymorphs was performed as described earlier by Sarkar and Mahapatra.[26] A special feature of this synthetic route is the possibility to synthesize calcite and aragonite by conversion from the initially prepared vaterite so that all three phases have almost the same stoichiometric composition. Briefly, calcium carbonate was precipitated from aqueous solutions of calcium chloride and potassium carbonate and subjected to different thermal treatment, depending on the desired polymorphic phase. All solid products were dried in vacuum at ambient temperature and stored in closed vessels in air until further use.

Extraction of NRL Allergens from NRL Gloves

The release of the four allergens from natural latex gloves was performed by a method proposed by the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (Bochum).[27] The latex gloves were cut in approximately 1 cm^2 sized pieces and immersed in a volume-to-mass ratio (mL g^{-1}) of 6.66 to 1 either into double-distilled water or in PBS (phosphate buffered saline). The extraction of the allergens was carried out for 5 h at 37°C under continuous orbital shaking. In addition, the mixture was vortexed every 15 min. The latex objects were removed with a pair of tweezers, the solution was centrifuged at 3500 rpm for 15 min, and the supernatant was removed. The allergens present in double-distilled water or PBS were stored at −20°C until further analysis. The extracted amount of allergen was found to be independent of the extraction medium used.

Adsorption of Allergens onto Calcium Carbonate

Approximately 50 mg of solid calcium carbonate was added to 1 mL of the allergen-containing solution. To study the influence of the concentration of allergens, serial dilutions were performed. All adsorption experiments were performed either at room temperature (RT; i.e., about 25°C) or at 37°C. The samples were shaken for 24 h at 100 rpm on an orbital shaker.

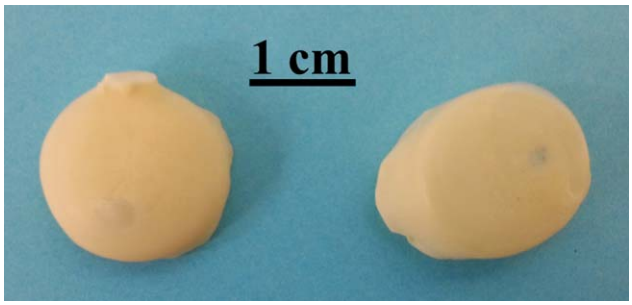

Figure 2. The self-prepared latex objects. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

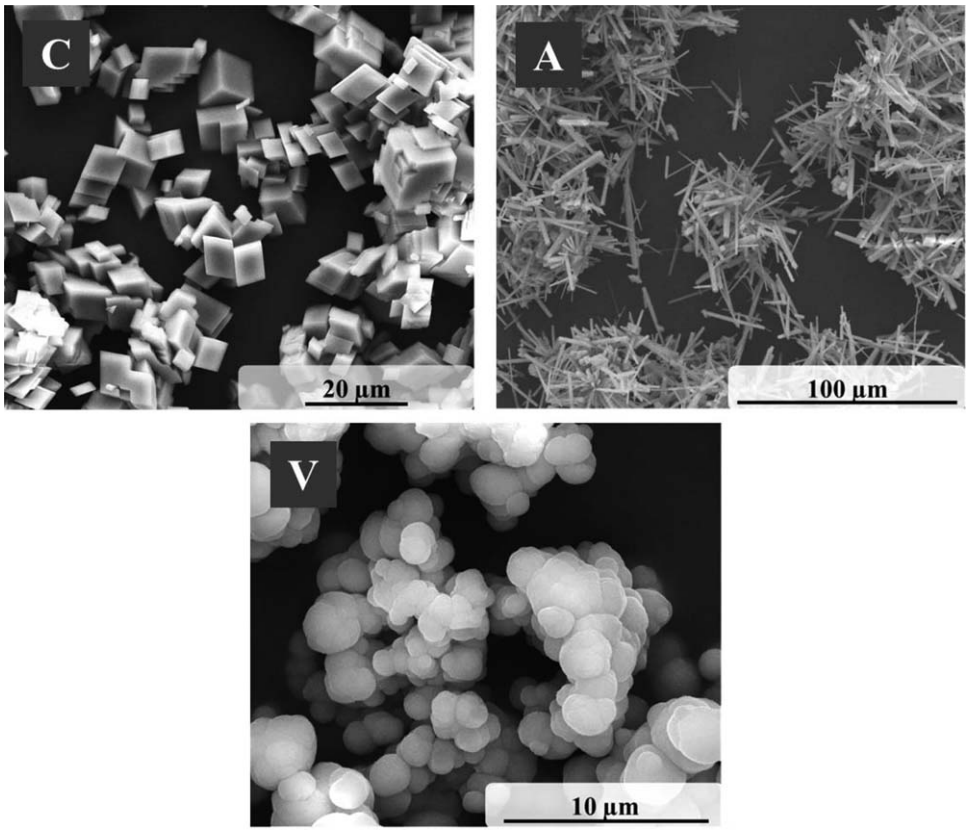

Figure 3. Scanning electron micrographs of calcite (**C**), aragonite (**A**), and vaterite (**V**).

Then, the adsorbent was removed by centrifugation at 3500 rpm for 15 min, and the supernatant was used for the ELISA assay. Figure 1 gives a schematic representation of the experimental procedure. The allergen assay was calibrated by measuring defined concentrations of the pure allergens by ELISA, using the standard samples provided by the manufacturer (see below). All experiments were carried out at least in duplicate.

Desorption of Allergens from the Calcium Carbonate Phases

50 mg of each calcium carbonate with the allergen that was adsorbed at room temperature as described above were used for the desorption experiments, both at room temperature and at 37°C. For this purpose, the solid with adsorbed proteins was mixed with 1 mL of either artificial sweat or double-distilled water. Artificial sweat was prepared according to EN 1811 and consisted of sodium chloride (0.5 wt %), lactic acid (0.1 wt %), and urea (0.1 wt %), dissolved together in double-distilled water. The pH was adjusted with ammonia to 5.9.[28] Desorption experiments were carried out by shaking the samples for 24 h at 100 rpm on an orbital shaker. The solid was quantitatively removed by centrifugation at 3500 rpm for 15 min, and the supernatant that contained the desorbed proteins was quantified by ELISA. All experiments were carried out at least in duplicate.

Preparation of NRL Objects

The components used were produced for the manufacturing of latex objects. As this is a multistep technical procedure with many possible variations, we describe it in detail in the following.

Composite Dispersion. To create the composite dispersion, Dispex® N40 (0.366 g, BASF) was mixed with 21 mL of double-distilled water during vigorous mixing. Subsequently, 7.04 g of zinc oxide (Fluka), 4.68 g zinc diethyldithiocarbamate (Aldrich), and 7.21 g of sulphur (colloidal, 80%, Sigma-Aldrich) were added and the mixture was dispersed for 10 min with an ultrasonic bath. After addition of 0.24 g of bentonite (Sigma-Aldrich), the dispersion was treated in an ultrasonic bath for 5 min. This dispersion was used for the preparation of all latex objects.

Calcium Carbonate Slurry. 0.3 g Dispex® N40 was dissolved in 14.7 mL of double-distilled water by vigorous stirring. 10 g of the corresponding calcium carbonate phase was added in small portions and dispersed by ultrasonication.

Coagulant. A solution of 0.25 g Dispex® N40 and 10.19 g of calcium nitrate tetrahydrate (Merck) in 40 mL double-distilled water was prepared. Subsequently, the calcium carbonate phase (0.51 g) was added and the mixture was ultrasonicated for 15 min.

Dipping Compound. To prepare the dipping compounds, 33.33 g of NRL (45% dispersion in water, kindly provided by CPR, Sarstedt, Germany) were treated with 75 µL of aqueous KOH (2%, VWR) to adjust the pH to 9.5 to 10. After adding of 6 g of the calcium carbonate slurry and 0.75 g of the composite dispersion, the mixture was ultrasonicated for 15 min. After the addition of 210 µL ammonia (30 wt %, Roth) the mixture was strongly stirred. The mixture was stored at room temperature

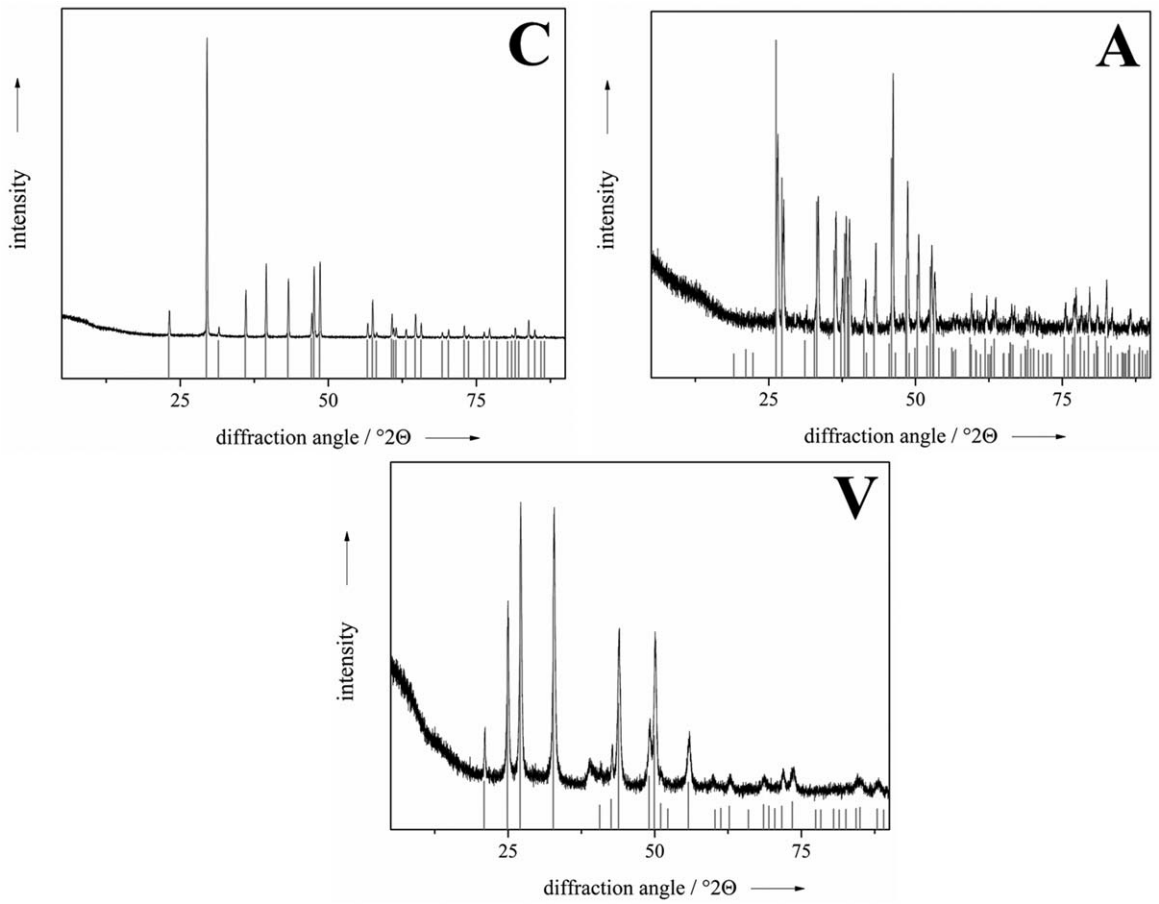

Figure 4. X-ray powder diffractograms of calcite (**C**), aragonite (**A**), and vaterite (**V**). The vertical lines show the reference data of the ICDD database (International Centre for Diffraction Data) for each polymorphic calcium carbonate (calcite, ICDD # 01–083-0577; aragonite, ICDD # 01–076-0606; vaterite, ICDD # 01–072-0506).

for 24 h ("maturation") and then used to prepare the latex objects. For comparison, the same latex objects were prepared without calcium carbonate.

Polymer Dip. The polymer dip was used for all investigated calcium carbonate phases. 60 mL of double-distilled water was mixed with 6.666 g of a 35 wt % solution of polyacrylic acid in water (M_w = 100 kDa, Aldrich) under vigorous vortexing for 5 min.

Latex Objects. To create the latex objects, the mold was washed with water, heated to 75 °C, and dipped into the coagulant dip (55 °C) for 30 s. After drying for 16 s at 110–120 °C, the mold was dipped into the dipping compound (room temperature) for 20 s and dried again at 110–120 °C for 9 s. Then the mold was dipped for 15 s into a water bath (70–80°C) and then dried at 110–120°C for 15 s. Then, the mold was dipped into the polymer dip for 15 s, and then dried at 110–120°C for 30 min. Finally, the mold was dipped for 15 s into a water bath (70–80 °C) and dried for 10 min (70–80 °C). Subsequently, the finished latex objects (see Figure 2 below) were removed from the mold. Their thickness was about 2–3 mm.

Release of the NRL Allergens from the Latex Objects

To study the release of allergens from the finished latex objects, they were cut into pieces of about 1 cm^2 size. The extraction was performed as described above for the NRL gloves.

ELISA Assay for Allergen Concentration Determination

The quantification of the allergen content was performed by ELISA, using FITkits® obtained from Icosagen (Tartu, Estonia). These are monoclonal antibodies which are specific against an epitope of the allergen, based on the ASTM standard D7427-08. For the quantification of the allergen concentrations, the ELISA assay was carried out as specified by the manufacturer at room temperature. Double-distilled water was added to the PBS washing solution up to 500 mL volume. The control was dissolved in 500 μL double-distilled water and allowed to stand for 2 h. First, 100 μL assay buffer was given into each well of a 96-well plate, followed by 25 μL of either the control, or of the calibrant, or of the samples. After incubating the plate for 1 h on a shaker (100–200 rpm), each well was washed four times with 300 μL of PBS washing solution. Then, 100 μL of the enzyme conjugates was given into each well and the plate was shaken again for 30 min. Each well was washed again four times with the PBS washing solution. After 100 μL of the substrate solution was added to each well and shaken again for 15 min, 100 μL of the stopping solution was added and the plate was shaken for 1–2 min. The content of the allergen concentration was measured using a microwell plate reader at a wavelength of 405 nm. The allergen concentration of the samples was determined by a logarithmic calibration plot, using the allergen standards provided by the manufacturer.

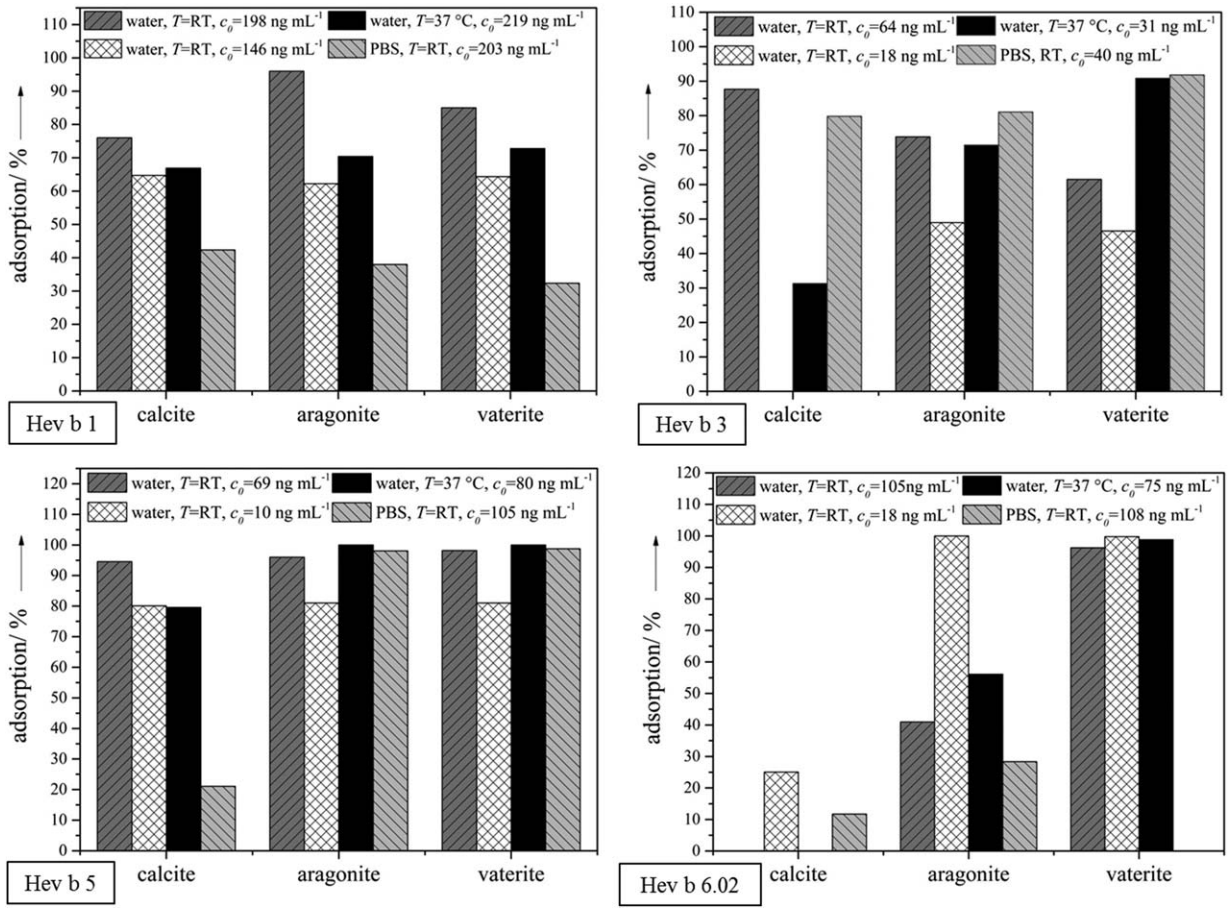

Figure 5. Results of the adsorption experiments for Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02, carried out at room temperature (RT, about 25°C) and 37°C, and with either water or PBS as solvent for the allergens.

Characterization Techniques

The calcium carbonate particles were characterized by thermogravimetric analysis (TGA, STA 409 EP instrument Netzsch; heating from 30 to 1200°C with 2 K min^{-1} in dynamic air atmosphere; 50 mL min^{-1}), scanning electron microscopy (SEM, ESEM Quanta 400 FEG instrument with gold–palladium-sputtered samples), X-ray diffraction (XRD, Bruker D8 Advance Powder Diffractometer with Cu-Kα-radiation; 1.54 Å), elemental analysis (C, H, N; EA 1110, CE instruments), and atomic absorption spectroscopy for calcium analysis (Thermo Electron Corporation M series). The specific surface area of the particles was determined by BET (Micromeritics Gemini 2360 analyzer) on the nitrogen adsorption. The samples used for adsorption were degased overnight at 130°C (FlowPrep 060 degasser).

RESULTS AND DISCUSSION

The calcium carbonate polymorphs were characterized by XRD, TG, EA, and SEM. Figure 3 shows the SEM images of the calcium carbonate polymorphs. The calcite crystals were rhombohedral with an edge length between 3 and 10 μm. The aragonite crystals were needle like with an edge length of 50 μm and a diameter of about 10 μm. The vaterite crystals were large spheres (1–5 μm), consisting of smaller spheres with a diameter of a few hundred nanometres. The phase purity of the calcium carbonate samples was ascertained by XRD. The X-ray diffractograms of the polymorphic calcium carbonate phases are shown in Figure 4.

Thermogravimetry and elemental analysis gave the expected values for the loss of carbon dioxide for all polymorphs; only vaterite contained about 2 wt % of water. To determine the influence of the allergen concentration, the adsorption was performed with the allergens extracted from NRL gloves at two different concentrations at room temperature. Furthermore, the influence of temperature was examined by carrying out experiments at $T = 37$°C with either double-distilled water or PBS as solvent. Figure 5 shows the results for Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 for each calcium carbonate phase. It was possible to adsorb the allergens to most calcium carbonate phases. The adsorption of the allergens Hev b 1 and Hev b 5 was independent of the initial concentration, in contrast to Hev b 3. According to the literature, at high initial concentrations, the adsorption of a protein proceeds in two steps. First, the direct adsorption of the protein occurs without any change of conformation, and second, the slow adsorption of the unfolded protein occurs.[29,30] The adsorption of Hev b 6.02 significantly dependent on the polymorphic calcium carbonate phase. While the allergen was adsorbed only very poorly by calcite, almost 100% of the allergen was adsorbed by vaterite.

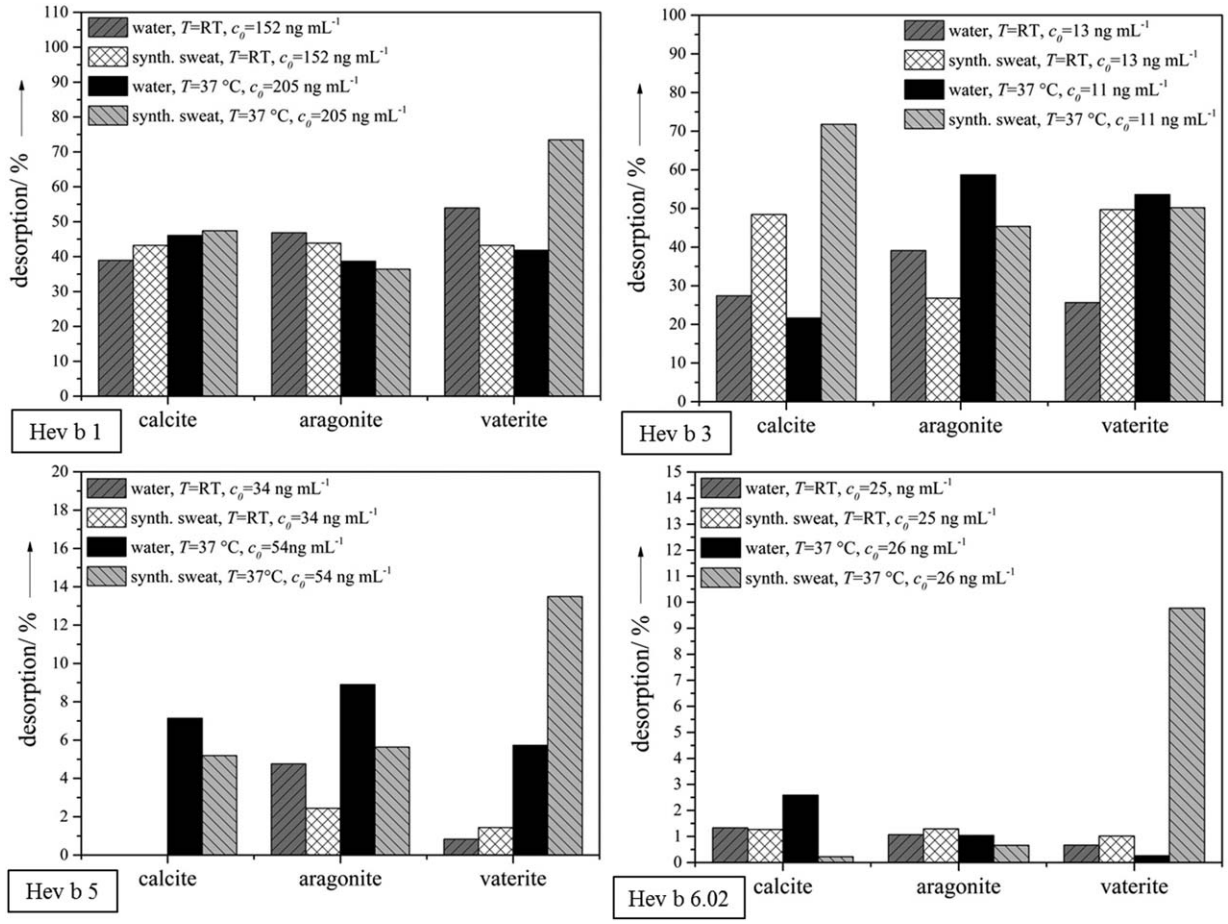

Figure 6. Results of desorption experiments from the calcium carbonate phases for Hev b 1, Hev b 3, Hev b 5, and Hev b 6.02 at room temperature (RT) and 37°C, and with either pure water or synthetic sweat as extraction agent.

All adsorption results were independent on the temperature, but the solvent clearly played a role. With PBS as adsorption medium, the adsorption was greatly reduced or even absent (e.g., for Hev b 6.02 on vaterite). In the literature, the adsorption of proteins was reported to increase significantly with rising temperature due to the denaturation of the protein.[31] In our experiments, no significant influence of the temperature was observed. Overall, we have shown that it is possible to adsorb the allergens onto the surface of calcium carbonate phases with different efficiency.

Subsequent desorption of the adsorbed allergens is of major importance for any allergenic reaction; in fact, it will be decisive for any practical application. From a more fundamental point of view, desorption into water represents the best-defined system. However, in reality, desorption will occur in skin contact. Therefore, we have extended the study by using synthetic sweat as desorption medium. Desorption from the calcium carbonates showed different results for the allergens (Figure 6). Hev b 5 and Hev b 6.02 either did not desorb from the solid at all or only to a very small extent. The allergens Hev b 1 and Hev b 3 desorbed by about 50%. This was independent from the desorption temperature, the initial allergen concentration, and the selected extracting agent. The allergens Hev b 1 and Hev b 3 have structural similarities in their amino acid sequences that may explain the similar desorption of these two allergens. The fact that the adsorption of Hev b1 and Hev b3 showed differences between these two allergens may be due to a different hysteresis with respect to the adsorption/desorption behavior, possibly associated with conformational changes during adsorption.

Adsorption and desorption of complex protein structures are more complicated than the adsorption of small molecules onto solid surfaces. Proteins can adsorb on surfaces in different orientation (e.g., "side-on" or "end-on"), depending on their three-dimensional structure. Proteins can be divided into hard or soft, depending on the conformational stability of the protein during the adsorption. The adsorption depends on many parameters like the protein structure, its unfolding ability, the stability of the protein structure, and whether the protein is hard or soft.[29,30,32–34]

Using the size of the proteins, the concentration, the mass of calcium carbonate and its specific surface area, the number of the protein molecules on the surface of calcium carbonate can be estimated. First, the adsorbed mass of allergens m (adsorbed) on the surface of the calcium carbonate was calculated using the adsorbed concentration c (adsorbed), the mass of calcium carbonate m ($CaCO_3$) used, and the volume ($V = 1$ mL for each experiment) (eq. 1):

Table I. Number of Proteins Molecules per m^2, Adsorbed on or Desorbed from the Calcium Carbonate Surface (T = Room Temperature; RT; Double-Distilled Water)

	Hev b 1		Hev b 3		Hev b 5		Hev b 6.02	
	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
Calcite	2.14×10^{14}	6.82×10^{13}	4.23×10^{13}	2.81×10^{12}	7.33×10^{13}	0	5.6×10^{12}	1.18×10^{12}
Aragonite	5.67×10^{13}	2.72×10^{13}	1.21×10^{13}	5.42×10^{11}	2.43×10^{13}	2.42×10^{11}	5.31×10^{13}	1.38×10^{11}
Vaterite	2.71×10^{13}	1.36×10^{13}	3.99×10^{12}	8.41×10^{11}	1.02×10^{13}	9.83×10^{10}	5.10×10^{13}	2.01×10^{11}

The reference concentrations of the adsorption were $c_{o,ads}$ (Hev b 1) = 203 ng mL^{-1}, $c_{o,ads}$ (Hev b 3) = 64 ng mL^{-1}, $c_{o,ads}$ (Hev b 5) = 70 ng mL^{-1}, $c_{o,ads}$ (Hev b 6.02) = 105 ng mL^{-1}. For the desorption from the solid, we used $c_{o,des}$ (Hev b 1) = 152 ng mL^{-1}, $c_{o,des}$ (Hev b 3) = 13 ng mL^{-1}, $c_{o,des}$ (Hev b 5) = 34 ng mL^{-1}, and $c_{o,des}$ (Hev b 6.02) = 25 ng mL^{-1}. The specific surface areas (BET) were 0.7 $m^2\ g^{-1}$ for calcite, 2.1 $m^2\ g^{-1}$ for aragonite, and 4.9 $m^2\ g^{-1}$ for vaterite.

$$m(\text{adsorbed}) = \frac{c(\text{adsorbed})}{m(CaCO_3)} \cdot V \quad (1)$$

The adsorbed mass of allergen normalized to the specific surface area S of each calcium carbonate was calculated with eq. 2:

$$m(\text{adsorbed})(\text{BET}) = \frac{m(\text{adsorbed})}{S(\text{BET}) \cdot 10^{-3}} \quad (2)$$

The mass of the allergen, m (allergen), necessary to compute the number of allergen molecules on the surface of calcium carbonate, N (adsorbed), was calculated from the molecular weight M_W of the respective allergen and the Avogadro constant N_A, so that the number of molecules follows from eq. 3:

$$N(\text{adsorbed}) = \frac{m(\text{adsorbed})(\text{BET})}{m(\text{allergen})} \quad (3)$$

with

$$m(\text{allergen}) = \frac{M_w}{N_A} \quad (4)$$

The results for the adsorption and desorption experiments are shown in Table I.

The number of protein molecules adsorbed per surface area was similar for all allergens and all calcium carbonate polymorphs. The fraction of the desorbed protein molecules was significant, but typically the majority remained irreversibly adsorbed. As the allergens were extracted from latex gloves, the presence of further proteins and allergens during the adsorption is likely. Thus, there is a competing adsorption of the allergens and other proteins which will influence the total amount of adsorbed protein, but cannot be quantified so far.

Figure 7 shows scanning electron micrographs of the surface of the self-prepared latex objects. The surface of the latex objects containing aragonite (A) or vaterite (V) was comparatively homogenous, while the surface of the latex object prepared with calcite (C) was very rough and showed calcium carbonate particles on the surface.

The release of latex allergens from a latex/calcium carbonate composite is more realistic as it takes into account the competitive adsorption of the allergens on the polymer (latex) and on the mineral (calcium carbonate). As proteins generally adsorb well on surfaces, the release kinetics cannot be predicted but must be experimentally determined. The release of the latex allergens from self-prepared objects was studied under two different conditions. In particular, the damage of the latex surface by cutting and the applied mechanical stress during the extraction of the allergen content are important in the practical application of latex objects. First, as a rather rough method, the release of the allergens was carried out with cut latex objects, shaken at 250 rpm in an orbital shaker for 5 h. As a more realistic and more gentle method, intact (not-cut) latex objects were shaken at 80 rpm in an orbital shaker for 5 h. As a standard for the allergen release, a latex object without added calcium carbonate was tested. Because vaterite is a potentially unstable polymorph of calcium carbonate and because of its easier technical accessibility and availability, calcite was used as a model additive in all latex objects. The reference concentrations of double-distilled water as extracting agent were 221 ng mL^{-1} for

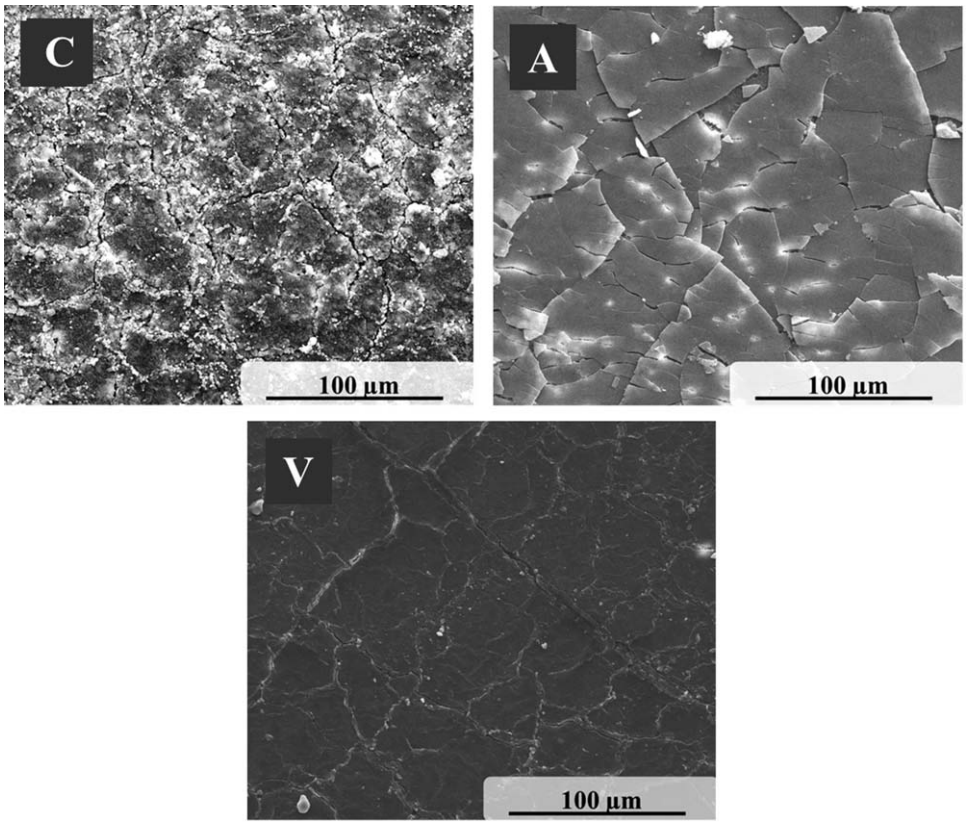

Figure 7. Scanning electron micrographs of the self-prepared latex objects with calcite (**C**), aragonite (**A**), and vaterite (**V**) (weight ratio of $CaCO_3$: latex = 0.12 : 1).

Hev b 1, 62 ng mL^{-1} for Hev b 3, 14 ng mL^{-1} for Hev b 5, and 140 ng mL^{-1} for Hev b 6.02, respectively. For synthetic sweat, the values were 240 ng mL^{-1} for Hev b 1, 78 ng mL^{-1} for Hev b 3, 18 ng mL^{-1} for Hev b 5, and 134 ng mL^{-1} for Hev b 6.02 (extraction method as described before). This shows that the absolute amount of released allergens does not depend on the extraction medium (water or synthetic sweat).

The release of the allergens with the "rough" extraction method amounts to 50 and 100% (Figure 8). This was independent on the extraction medium used. The "gentle", more realistic extraction method, typically gave a smaller release of allergens. Notably, the desorption of the allergens from the latex objects was considerably increased compared to the desorption from the pure calcium carbonate samples. Due to the large number of other compounds which are used in the manufacturing process of the latex objects, there may be different reasons for this effect. One possible explanation is the affinity of the dispersant Dispex® N40, a negatively charged polymer, to the added calcium carbonate. The dispersant is used to disperse the calcium carbonates in water, so that it will probably adsorb onto the surface, thereby preventing the subsequent adsorption of allergens. Dispex® N40 is the sodium salt of polyacrylic acid, and thus carries a negative charge. In general, polyelectrolytes and biomolecules have a high affinity to inorganic particles, as it is exploited by living systems in biomineralization.[35–37] By the adsorption of the dispersant onto the surface of the calcium carbonate phases, the surfaces are no longer fully available for the adsorption of the allergens. There is probably a competitive adsorption of dispersant and allergen on the calcium carbonate surface which clearly constrains the release of allergens, but does not fully prevent it.

The influence of cutting the latex objects and the strong shaking during the release are clearly visible (Figure 8). The release of

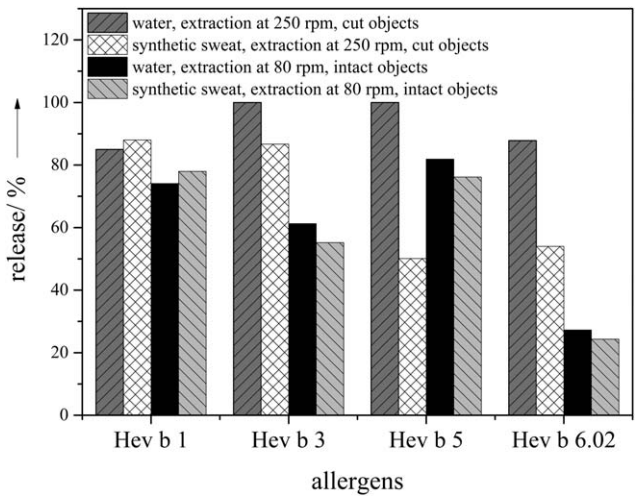

Figure 8. Release of allergens from self-prepared calcite-latex objects at 37°C under different conditions ("rough" with cut objects and "gentle" with intact objects).

the most allergenic protein Hev b 6.02 is most strongly reduced, but all other allergen concentrations are significantly reduced as well. It can be concluded that a significant reduction of allergens by adsorption on the calcium carbonate polymorphs is possible.

CONCLUSIONS

We have shown that the adsorption of NRL allergens to the calcium carbonate polymorphs is possible. However, the adsorption is reversible. Because vaterite shows the best characteristics for the adsorption and desorption, it appears to be a suitable substrate for the adsorption of latex allergens. Furthermore, the production of small latex objects with calcium carbonate in the latex matrix was established, and the release of allergens was examined. The release of the allergens is increased with increasing mechanical stress on the objects, but mostly independent on the nature of the extraction medium, i.e., water or synthetic sweat. This underscores that it is necessary to study not only the inorganic adsorbent, but also the whole product after a typical processing chain. The best way to achieve a good reduction is probably to use a mixture of the different polymorphic phases as they have complementary adsorption capabilities for the four allergens.

ACKNOWLEDGMENTS

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REFERENCES

1. Asrul, M.; Kawahara, S.; Amir-Hashim, M. Y. *J. Rubb. Res.* **2007**, *10*, 89.
2. Peixinho, C.; Tavares-Ratado, P.; Tomás, M. R.; Taborda-Barata, L.; Tomaz, C. T. *Br. J. Dermatol.* **2008**, *159*, 132.
3. Beezhold, D.; Hortin, K.; Hickes, V.; Daddona, J.; Kostyal, D. *J. Long. Term. Eff. Med.* **2003**, *13*, 21.
4. Edlich, R. F.; Woodard, C. R.; Hill, L. G.; Heather, C. L. *J. Long. Term. Eff. Med.* **2003**, *13*, 11.
5. Lim, H. M.; Amir-Hashim, M. Y. *J. Rubb. Res.* **2011**, *14*, 41.
6. Wrangsjö, K.; Boman, A.; Lidén, C.; Meding, B. *Contact Dermatitis* **2012**, *66*, 165.
7. Baur, W. H. *Trans. Am. Crystallogr. Assoc.* **1970**, *6*, 129.
8. Breiteneder, O.; Schneider, O. *Int. Arch. Allergy Immunol.* **1998**, *116*, 83.
9. H. Tamm, In 5th International Rubber Glove Conference and Exhibition, Kuala Lumpur, **2010**, pp 1–12.
10. Chen, Z.; Posch, A.; Raulf-Heimsoth, M.; Rihs, H. P.; Baur, X. *Allergy* **1997**, *52*, 97.
11. Koh, D.; Ng, V.; Leow, Y. H.; Goh, C. L. *Br. J. Dermatol.* **2005**, *153*, 954.
12. Posch, A.; Chen, Z.; Raulf-Heimsoth, M.; Bauer, X. *Clin. Exp. Allergy* **1998**, *28*, 134.
13. Palosuo, T. *Rev. Fr. Allergol. Immunol. Clin.* **1997**, *37*, 1184.
14. Palosuo, T.; Antoniadou, I.; Gottrup, F.; Phillips, P. *Int. Arch. Allergy Immunol.* **2011**, *156*, 234.
15. Dennis, M. S.; Henzel, W. J.; Bell, J.; Kohr, W.; Light, D. R. *J. Biol. Chem.* **1989**, *264*, 18618.
16. Kawahara, S.; Klinklai, W.; Kuroda, H.; Isono, Y. *Polym. Adv. Technol.* **2004**, *15*, 1181.
17. Klinklai, W.; Saito, T.; Kawahara, S.; Tashiro, K.; Suzuki, Y.; Sakdapipanich, J. T.; Isono, Y. *J. Appl. Pol. Sci.* **2004**, *93*, 555.
18. Chaikumpollert, O.; Yamamoto, Y.; Suchiva, K.; Kawahara, S. *Colloid Polym. Sci.* **2012**, *290*, 331.
19. Ratnayake, U.; Makuuchi, K.; Yoshii, F. *J. Rubb. Res.* **2001**, *4*, 153.
20. Deng, C. M.; Chen, M.; Ao, N. J.; Yan, D.; Zheng, Z. Q. *J. Appl. Polym. Sci.* **2006**, *101*, 3442.
21. Poompradub, S.; Luthikaviboon, T.; Linpoo, S.; Rojanathanes, R.; Prasassarakich, P. *Polym. Bull.* **2011**, *66*, 965.
22. Cai, H. H.; Li, S. D.; Tian, G. R.; Wang, H. B.; Wang, J. H. *J. Appl. Polym. Sci.* **2003**, *87*, 982.
23. Khamsook, J.; Magaraphan, R. In International Conference on Latex and Latex Based Products, Rapra Technology, Frankfurt, **2006**, p. Paper 11.
24. Meldrum, F. C. *Int. Mater. Rev.* **2003**, *48*, 187.
25. Sawada, K. *Pure Appl. Chem.* **1997**, *69*, 921.
26. Sarkar, A.; Mahapatra, S. *Cryst. Growth Des.* **2010**, *10*, 2129.
27. IPA. In IPA-Report 4; Institut für Prävention und Arbeitsmedizin der Deutschen Gesetzlichen Unfallversicherung, Ruhr-Universität Bochum, Bochum, **2010**.
28. Marques, M.; Marges, C.; Loebenberg, R.; Almukainzi, M. *Dissolut. Technol.* **2011**, *18*, 15.
29. Miller, R.; Grigoriev, D. O.; Krägel, J.; Makievski, A. V.; Maldonado-Valderrama, J.; Leser, M.; Michel, M.; Fainerman, V. B. *Food Hydrocoll.* **2005**, *19*, 479.
30. Nakanishi, K.; Sakiyama, T.; Imamura, K. *J. Biosci. Bioeng.* **2001**, *91*, 233.
31. Arnebrant, T.; Burton, K.; Nylander, T. *J. Coll. Interface Sci.* **1986**, *119*, 383.
32. Wang, K.; Zhou, C.; Hong, Y.; Zhang, X. *Interface Focus* **2012**, *2*, 259.
33. Rabe, M.; Verdes, D. ; Seeger, S. *Adv. Coll. Interface Sci.* **2011**, *162*, 87.
34. Kojima, C.; Watanabe, K. *J. Drug Deliv.* **2012**, 2012, 4.
35. Gotliv, B. A.; Weiner, S.; Addadi, L. *ChemBioChem* **2003**, *4*, 522.
36. Levi, Y.; Albeck, S.; Brack, A.; Weiner, S.; Addadi, L. *Chem. Eur. J.* **1998**, *4*, 389.
37. Hernández-Hernández, A.; Rodríguez-Navarro, A. B.; Gómez-Morales, J.; Jiménez-Lopez, C.; Nys, Y.; García-Ruiz, J. M. *Cryst. Growth Des.* **2008**, *8*, 1495.