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Formation of Two-dimensional Structures from Colloidal Particles on Fluorinated Oil Substrate

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We propose a new type of liquid substrate, perfluorinated oil (F-oil), for the formation of two-dimensional arrays from colloidal particles. The appropriate conditions for particle ordering (experimental cell, type and concentration of surfactants, *etc.*) are reported. Large and well ordered structures from μm-sized latex particles are obtained. Ordered clusters of globular protein (ferritin) macromolecules are also observed. The structures formed are directly transferred (after the F-oil evaporation) onto a solid substrate for subsequent study by means of optical and electron microscopy. The mechanism of the ordering process is studied and the advantages and disadvantages of the liquid substrates (in comparison with the solid ones) are discussed. Some possible ways for control of the ordering process and for improvement of the quality of the arrays are pointed out.

The interest in ordered two-dimensional (2D) colloid structures fixed on solid substrates[1–11] is stimulated by their possible applications in optical devices[4–6,9] and some other techniques like data storage, microelectronics and synthetic membrane production. 2D arrays from μm[5–8,11] and sub-μm[5,9,10] latex particles, from a number of proteins and protein complexes[12–25] and even from viruses,[18,20] were obtained and analysed with respect to their structure and orientation. Since 3D crystals are obtained from a very limited number of integral membrane proteins,[26] 2D crystallization provides a unique possibility for investigating their structure.[18,27–30] The experience gained during the experiments on formation of ordered two-dimensional structures from colloid particles[1–11,31,32] and proteins[12–25,33,34] shows that the choice of an appropriate substrate is of crucial importance for the ordering process.

As a rule, the 2D arrays obtained at a single fluid interface[3,18,31–34] are built up by small domains comprising no more than several hundred particles. Most probably the reasons are related to the specific mechanism of the ordering process controlled by diffusion, and to the small magnitude of the forces acting between the adsorbed particles. Some recent experimental studies[31,32] report the formation of 2D structures of high quality from μm-sized latex particles in a Langmuir trough. It is not yet clear to what extent this method will be applicable for obtaining 2D arrays of high quality from sub-μm latex particles and biocolloids.

Solid substrates[1–20] (mica, glass, graphite, metals) can also be used for colloid particle array formation. In the case of latex particles it is proven[7,8] that the ordering process starts when the thickness of the water layer containing the particles becomes approximately equal to the particle diameter. It is shown[7,8] that the mechanism of the ordering process consists of two stages: (i) nucleus formation and (ii) array growth. The first stage is governed mainly by lateral capillary forces[35–38] between the particles that appear when the liquid layer thickness becomes equal to the particle diameter. As shown theoretically[35–37] the corresponding attractive energy can be many orders of magnitude larger than the thermal energy, kT, even for nm-sized particles. The second stage (array growth) is connected with the evaporation of water from the already ordered regions.[7,8] Water evaporation causes the appearance of particle flux directed from the disordered toward the already ordered regions, see ref. 7 for details. Thus new particles are continuously reaching and sticking to the boundary of the ordered array. The control of the water evaporation rate turned out to be a convenient tool for improvement of the array quality and for overall control of the ordering process. It is possible that similar factors play an important role in the experiments with proteins.[25]

The investigations of the mechanism of 2D array formation on solid substrates[7,8] also revealed the main shortcomings of these substrates. The two most important ones are the roughness of the solid surface and the irreversible sticking of the particles against the substrate before their incorporation inside the array. The first problem becomes very important for small particles (in particular, biomolecules). In the works of Harris *et al.*[12–14] it was overcome by using a cleaved mica for substrate (the so-called 'mica spreading technique'). The sticking of the particles causes the appearance of defects in the 2D structure and makes impossible the application of any 'annealing' procedure for improvement of the quality of the obtained array.

The method developed by Nagayama and co-workers[21,22,25] for array formation in a thin aqueous layer on a mercury substrate combines the advantages of both aforementioned types of substrate. On the one hand the mercury surface is molecularly smooth and tangentially mobile. On the other hand, the immersion capillary forces and the hydrodynamic flux are most probably the important factors for the process of ordering. The 2D structure obtained is then successfully transferred to a solid support for fixation and investigation by electron microscopy and image reconstruction. Ordered arrays from a large number of proteins, latex particles and viruses were obtained by this method.[25] The application of mercury as a substrate needs a special experimental procedure.[22,25] The high surface tension of mercury demands ultra-clean experimental conditions because the mercury surface is easily contaminated. The necessity of a low-humidity oxygen atmosphere and the cleaning procedure require complicated equipment. This suggests that one might search for some alternative liquid as a substrate which can combine the advantages of mercury with simpler experimental conditions.

The aim of this study is to investigate the possibility of the application of perfluorinated oil (F-oil) as a liquid substrate for 2D array formation. Experiments with μm-sized latex particles, which allow direct observation of the ordering process,

and with the globular protein ferritin are performed. We use perfluoromethyldecalin (PFMD) which possesses some of the appropriate features of the mercury substrate (molecularly smooth and tangentially mobile surface) and some additional advantages: (i) it is chemically inert and hazardless,[39–41] (ii) it allows the merging and rearranging of already ordered domains into larger ones, (iii) the 2D structure formed can be gently deposited onto another surface after evaporation of all F-oil, and (iv) it is difficult to contaminate the fluorocarbon surface (the common surfactants adsorb poorly at the fluorocarbon/water interface[42]). As a final result, large and well ordered domains from latex particles and clusters from ferritin molecules are obtained under appropriate conditions and after that are transferred onto the solid substrate.

Experimental

Materials

We use perfluoromethyldecalin, PFMD (commercial name Flutec PP-7, Rhone Poulenc S.A.) as a substrate. PFMD has a higher density than water. It has high vapour pressure at room temperature and can be easily evaporated after the formation of a 2D array. Thus the obtained array can be transferred to a solid substrate for fixation and investigation, see Fig. 1. Some of the relevant physico-chemical properties of PFMD are: density, $\rho_{25°C} = 1.94$ g cm^{-3}; boiling temperature, $T_b = 141$ °C; melting temperature, $T_m = -10$ °C; insoluble in water or liquid hydrocarbons; molecular mass, $M_w = 512.1$; refractive index, $n_d^{20} = 1.315$; relative permittivity, $\varepsilon_r = 1.99$; surface tension, $\sigma = 19.2$ mN m^{-1}; interfacial tension against pure water, $\gamma = 53.4$ mN m^{-1}.

To ensure the spreading of drops from the latex suspension or ferritin solution on the fluorinated oil one should use appropriate surfactants (see, *e.g.*, ref. 42 and references therein). We chose the surfactant perfluorononyl-polyoxyethylene, $C_9F_{19}CH_2CH(OH)CH_2(OCH_2CH_2)_9OCH_3$, abbreviated hereafter as PFPE ($M_w = 995$). This substance was laboratory synthesised and used without additional purification. As a cosurfactant we used the fluorinated alcohol $C_8F_{17}(CH_2)_2OH$ with $M_w = 464$ (Daikin Kougyou Ltd., Japan).

Since saturated perfluorocarbons and some fluorinated surfactants have found biomedical applications,[39,41] one can conclude that these substances do not denature the proteins in the organism. Nevertheless, we cannot be sure that the fluorinated surfactant and cosurfactant used by us do not affect the structure of the ferritin molecules. A more detailed investigation on the protein structure after 2D array formation requires special techniques like electron diffraction and image reconstruction and can be the subject of a separate study.

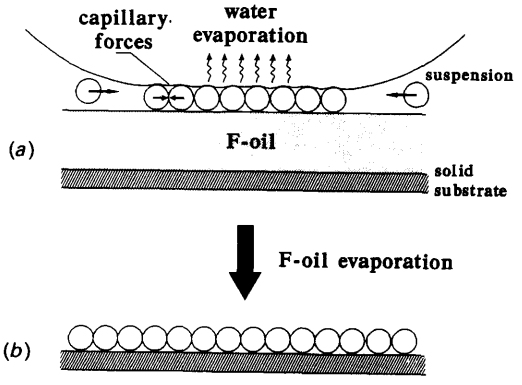

Fig. 1 Experimental system: (*a*) 2D ordering of particles in an aqueous layer over F-oil substrate; (*b*) the formed 2D array is deposited on a solid substrate after the evaporation of water and F-oil

The latex suspension used (JSR, Japan) contained particles of diameter 1.70 μm with concentration 1 wt.%. In some experiments glucose (Chameleon, Japan) was used without additional purification.

The experiments on 2D protein array formation were performed with aqueous solutions containing 10^{-4} g ml^{-1} ferritin (Sigma) and 0.15 mol l^{-1} NaCl. The ferritin was purified by gel chromatography (gel Sephacryl S300, Pharmacia) and filtered through a 0.22 μm filter unit (Millipore) just before use. The purification quality (in particular, the absence of aggregates) was controlled by measuring the size of the particles by means of a dynamic light scattering method (Malvern 4700C, Malvern Instruments Ltd.). The main fraction used in the experiments consisted of single molecules of diameter 12 ± 0.5 nm. The acidity of the solutions corresponded to pH 5.4 ± 0.1 as measured after the purification procedure.

Methods

Wetting of PFMD by an Aqueous Phase

To find appropriate conditions for the formation of a stable aqueous layer on the PFMD substrate we carried out preliminary experiments on the spreading of water drops on the surface of PFMD. Certain quantities of the surfactant and cosurfactant were dissolved in PFMD. The PFMD phase was placed in a cylindrical glass vessel of diameter 3.5 cm and volume 35 ml. Then 20 μl drops of pure water were gently deposited in the central part of the fluorocarbon surface. These drops spread over a certain area and the radii of the formed aqueous lenses were used as a measure of the wettability of the PFMD phase by water. The average diameter of pure water drops spread over pure PFMD was about 3 mm. The drop diameter increased up to 17 mm with the increase of the surfactant (PFPE) concentration. At PFPE concentrations above 10^{-3} mol l^{-1} the fluorocarbon phase became turbid and the drop diameter did not increase any more. This means that the solubility of PFPE in PFMD is about 10^{-3} mol l^{-1} and it is meaningless to use higher surfactant concentrations. We improved the wettability further by adding cosurfactant (the fluorinated alcohol) to the system. Fluorinated alcohol alone did not change the wettability even at concentrations as high as 10^{-1} mol l^{-1}. However, the mixture of PFPE and alcohol ensured better spreading of the water droplet, *i.e.* the fluorinated alcohol plays the role of a cosurfactant. For the experiments with latex and ferritin we used 5×10^{-2} mol l^{-1} alcohol and 10^{-3} mol l^{-1} PFPE which corresponded to a spread water drop of 22 mm diameter. As discussed below, this composition provided spreading of aqueous layers which were very stable and did not rupture during the experiments with latex and ferritin.

Note that the system is very sensitive to the nature and concentration of the surfactants. A deviation from the optimal composition quoted above changes substantially (for the worse) the spreading and 2D array formation behaviour.

Experimental Cell and Method for 2D Array Formation

The experimental cell used for obtaining ordered structures is a modification of the one described previously.[7] It consists of a Teflon ring which is pressed against a glass plate. To design an appropriate cross-section of the Teflon ring for the present study we performed experiments on the wetting of Teflon by water and F-oil. These experiments showed that the pure water formed a three-phase contact angle air/water/Teflon close to 90°, which substantially decreased when the water phase is pre-equilibrated with F-oil containing fluorinated

surfactants. The independently measured oil/water/Teflon contact angle was very close to 90°.

These experiments suggest the use of a Teflon ring of particular cross-section, as shown in Fig. 2. The inner wall of the Teflon ring is cut in a way which allows the three-phase contact line air/water/Teflon to be 'attached' to the concave corner of the wall. Thus, by loading the cell with appropriate volumes of F-oil and suspension one can ensure the formation of a thin, almost flat, aqueous layer with slightly concave upper surface (Fig. 2). As shown previously[7] such a configuration ensures better ordering of the latex particles.

For control of the water evaporation rate the cell was covered with a thermostatted glass container. We were able to vary the evaporation rate and the array formation rate by controlling the temperature of the water circulating through the glass container.[7]

To form sufficiently thin aqueous films over the F-oil substrate we controlled the oil level *via* ejection and injection of oil by means of a microsyringe. Taking into account that the three-phase contact line ferritin solution (latex suspension)/Teflon/air is fixed at the concave corner of the wall, the variation of the oil level results in different meniscus shapes which, in turn, is important for controlling the process of array formation. By sucking out some oil we succeeded in promoting the thinning of the aqueous film.

In the experiments with latex particles the ordering process was observed from below by a metallographic type optical microscope in transmitted or reflected light. The final 2D array, deposited on the glass plate after the water and oil evaporation, was studied and photographed by an optical microscope (Optiphot 2, Nikon) and by a scanning electron microscope (SEM). The samples for SEM (JEOL, Superprobe 733) were prepared by vacuum-coating with thin layers of carbon and gold.

In the experiments with ferritin the following procedure was used. We loaded the cell with the necessary amounts of F-oil and ferritin solution and waited for *ca.* 1 h to allow the ferritin molecules to be adsorbed at the water/air surface. Then we sucked out some F-oil which gave rise to a fast film thinning. At a given moment (when the water layer thickness became smaller than *ca.* 1 μm) Newton rings appeared, owing to interference of reflected monochromatic light from the lower and upper surfaces of the aqueous film. If we did not change the oil level in the initial stage, it would take a long time (>7 h) for water to evaporate before observation of the first Newton rings. The reason for this sluggish thinning of the aqueous layer is the decreased evaporation rate due to the adsorbed monolayer of ferritin molecules at the air/water interface.

The obtained ferritin-containing aqueous films were stable and did not rupture during the thinning process. After film formation the system was left for a period of a few days to allow total evaporation of the water and oil. In order to prepare samples for electron microscopy we put a specimen grid at the bottom of the cell before loading it with F-oil and ferritin solution. The specimen grid was coated with Formvar plastic film. After drying, the ferritin structures formed in the aqueous film were deposited directly on the specimen grid and were studied by transmission electron microscopy (Hitachi H-500).

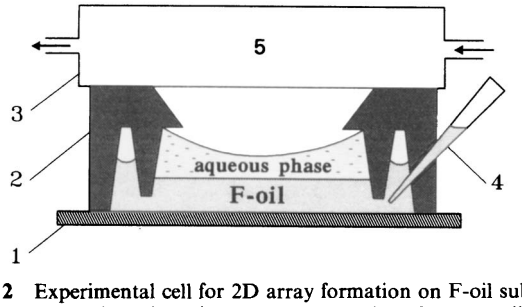

Fig. 2 Experimental cell for 2D array formation on F-oil substrate: 1, glass plate; 2, Teflon ring; 3, glass container for controlling the water evaporation rate; 4, micro-syringe for varying the oil level and the meniscus shape; 5, jacket through which thermostatting liquid flows

Results and Discussion

Description of the Main Stages of the 2D Array Formation Process

After loading the cell with a latex suspension one observes the formation of a slightly concave aqueous layer (Fig. 2) similar to the one in the earlier experiments on glass substrates.[7,10] The water evaporates and the layer thins with time. At the moment when the layer thickness becomes equal to the particle diameter many small 'islands' of ordered particles form in the centre of the substrate. If two such islands approach each other closely, so that the aqueous menisci formed around them overlap, they attract each other due to lateral capillary forces,[35–38] merge and form one large island. The latter often represents a single, well ordered domain. Later the water in the central zone of the substrate is totally evaporated and the islands become dry. After that moment they no longer coalesce upon collision. This can be explained with the irreversible coagulation of the neighbouring particles within an island when the water is evaporated. It is known that the polystyrene latex particles immersed in water are strongly charged owing to dissociation of ionic surface groups. This surface electrical charge gives rise to a strong electrostatic repulsion between the particles as long as they are immersed in the aqueous phase. The electrostatic repulsion disappears after drying, and the particles irreversibly adhere to each other owing to the van der Waals attraction.

At the same time a circular meniscus is formed around the central zone of the substrate where the layer thickness is still larger than the particle diameter. At the boundary of this meniscus small groups of particles are formed (Fig. 3) which slowly move towards the centre of the substrate. During this motion the water is evaporated from between and around the particles and they are pressed against each other by the lateral capillary forces acting in the thin aqueous layer. The shape of the liquid meniscus is visualized in reflected monochromatic light by the Newton interference fringes around each group of particles (Fig. 3). When two such groups come

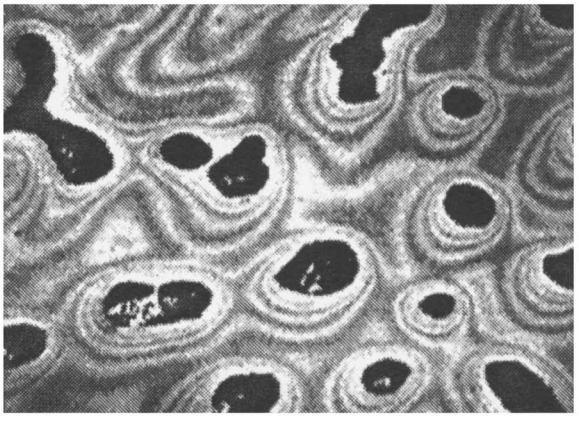

Fig. 3 Groups of ordered particles (formed when the layer thickness becomes equal to the particle diameter) are seen as dark spots. When two such groups approach each other closely they coalesce owing to the lateral capillary forces. The meniscus shape is visualised in reflected monochromatic light by the interference fringes.

closer together, they merge into a larger group (this process is also driven by lateral capillary forces), which can rearrange in a single domain (but only if there is water around the particles). With time the central zone is filled with such more or less well ordered groups of particles.

After a certain period of time one observes the formation of a '2D foam' of particles which encircles the region of the already formed islands (Fig. 4). The observations with a high magnification objective show that this foam consists of circular fields free of particles surrounded by narrow ordered domains. In reflected light, the presence of a concave aqueous surface is observed in the regions free of particles. A similar 2D foam was observed previously[7] with latex particles on a glass substrate in the presence of water-soluble surfactant (sodium dodecyl sulfate). Note that similar structures are observed also with proteins on mercury, see Fig. 4 in ref. 25. This type of structure can be formed due to the action of capillary forces.

During the later stages of the ordering process one can observe the formation of a ring-shaped homogeneous particle monolayer. This process resembles very much the corresponding stage of array formation on solid substrates. The particles carried by the convective water flux are moving in a radial direction towards the centre of the substrate and upon reaching the ordered zone are incorporated in it. Large and well ordered monolayers are formed during this process, see Fig. 5. Transitions from a monolayer to a bilayer and *vice versa* are observed.

During the final stage of water evaporation, multilayers are formed in the close vicinity of the cell wall. After the evaporation of the water from the F-oil surface, the oil itself starts to evaporate. Thus, the latex structure formed gradually approaches the upper surface of the glass plate and after complete evaporation of the oil, the latex array is transferred to the solid substrate.

In summary, the experiments described show that a 2D latex array can be formed over the F-oil substrate. They also prove that if the groups of particles are not completely dry, they can merge upon collision to form larger domains. The results imply that one must strive for precise control of the process to obtain very large (mm size) ordered domains of high quality.

Control of the Process

Control of the Evaporation Rate

The experiments described in the preceding section show that 2D array formation is a complex dynamic process. We should be able to control the experimental conditions during every stage of the process. Our experiments showed that it is preferable to decrease gradually the temperature at the top of the cell during the ordering process. Thus by varying in a vertical direction the temperature gradient inside the cell we were able to regulate the particle flux from the periphery towards the ordered regions in the central zone (see ref. 7 for details). One of the main reasons for obtaining the 2D foam described above was the low intensity of the convective particle influx. By gradually decreasing the temperature of the water circulating in the glass container above the cell (see Fig. 2), condensation of water on the glass cover is initiated. Thus the water vapour pressure in the cell is decreased, the evaporation from the aqueous film is increased and the influx of water from the thicker periphery towards the ordered particle structures is accelerated. In this manner we were able to 'compress' the 2D foam into the central zone, and to enlarge the well ordered regions. Fig. 5 shows an ordered monolayer of particles obtained in this way. Photographs are taken by using optical microscopy and SEM.

A more rapid decrease of the temperature at the top of the cell results in the formation of multilayers. On the other hand, an increase of the temperature (*i.e.* suppression of the evaporation) leads to a transition from dense monolayer formation back to 2D foam formation and even to a cessation of the ordering process. In conclusion, control of the evaporation rate can be a useful tool for improvement of array quality.

Impact of Glucose upon the 2D Array Quality

The addition of a small amount of glucose (0.1 wt.%) does not change significantly the process and the final result. A high concentration (10 wt.%) completely stops the evaporation and no ordering is observed even for a period of up to 24 h. A medium concentration (0.25 wt.%), however, turns out to improve the array formation process. As expected, the evaporation from very thin aqueous layers containing glucose was slower. Because of the decreased drying of the water

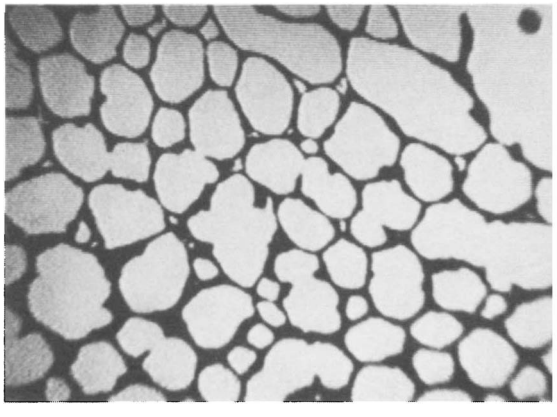

Fig. 4 Formation of a '2D foam' in the region where the thickness of the water layer is equal to or smaller than the particle diameter. The bright spots are areas free of particles.

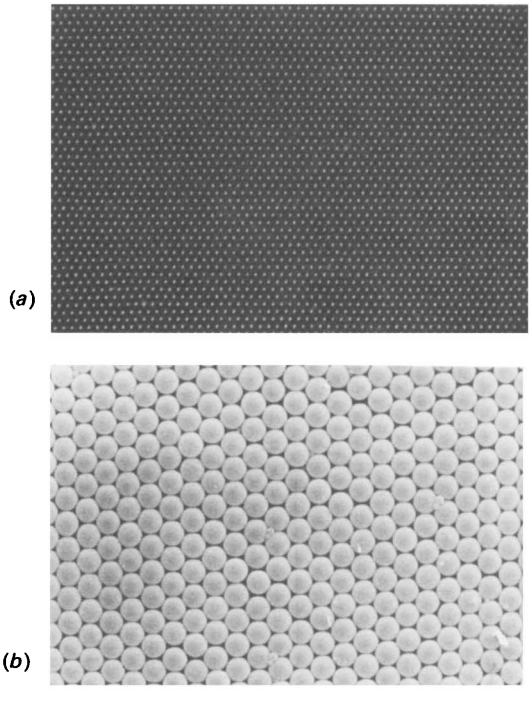

Fig. 5 Large, well ordered domains of 1.7 μm latex particles obtained in the presence of glucose (0.25 wt.%) by regulation of the evaporation rate: (*a*) optical microscope view; (*b*) SEM micrograph. A slight deformation or shrinking of the latex particles may take place in the vacuum chamber of the electron microscope.

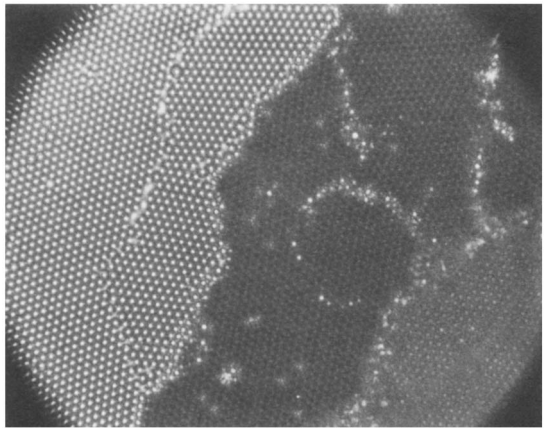

Fig. 6 View of the transition zones monolayer–bilayer–triple layer, 1△–2△–3△, obtained on the F-oil substrate. No tetragonal lattices are seen at the transition boundaries.

layer between the smaller groups of particles, their coalescence into larger domains was more pronounced. As a result, very large, well ordered domains, comprising more than 15000 particles, were obtained.

Effect of the 'Softness' of the Substrate Surface

The ordering process on F-oil shows many similarities with that on a glass substrate.[7] However, there are several differences between the two cases. They are connected mainly with the 'softness' (vertical flexibility) of the surface of the F-oil substrate and are briefly described and discussed below.

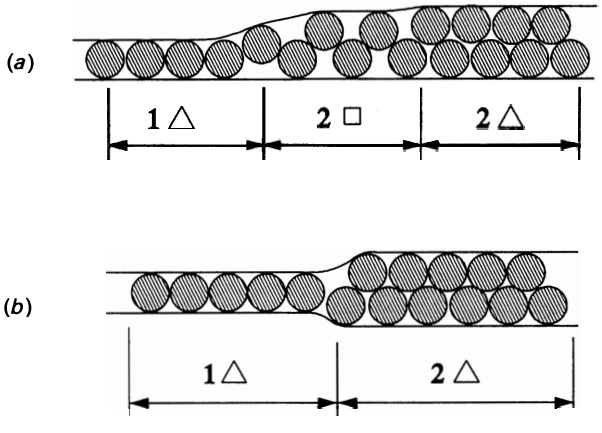

Fig. 7 Schematic presentation of the transition zone between monolayer and bilayer: (*a*) on a solid substrate and (*b*) on an F-oil substrate

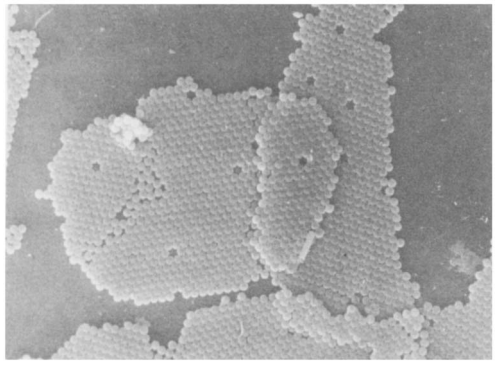

Fig. 8 Subduction of two approaching ordered domains takes place upon collision (instead of merging) when the evaporation rate is rather high; scanning electron micrograph

Almost Complete Absence of the Square Lattice

It has been demonstrated[7,9] that when using glass plate as a substrate, multilayers can be obtained with the following sequence of layers: 1△–2□–2△–3□–3△–···. Here the ciphers correspond to the number of layers and the symbols mean hexagonal (△) or tetragonal (□) packing of the particles. This order exactly coincides with the phase diagram calculated and observed by Pieranski *et al.*[43] for an equilibrium system of charged particles confined inside a thin liquid layer between two solid plates.

In the present experiments we have not observed tetragonal lattices between the hexagonally packed domains, see Fig. 6. Inspection of the transition regions between monolayers and bilayers of hexagonally packed particles by SEM confirms the absence of a developed tetragonal lattice in between them. The lack of a tetragonal lattice is most probably a result of the mobile and 'soft' oil/water interface which can bend at the transition zone between the multilayers [*cf.* Fig. 7(*a*) and (*b*)].

Subduction of Ordered Domains One under the Other: 'Superlattices'

As described above, two ordered groups of particles can merge when they approach each other closely (unless they have already dried). In such a way, larger and well ordered regions were formed. However, if the evaporation rate is rather high, the approaching domains (which are almost dry) can tuck one underneath another upon collision instead of merging and rearranging (see Fig. 8). If two hexagonally packed monolayers (placed one over the other) have lateral axes of symmetry, rotated with respect to each other at an angle not divisible by 60°, they form a peculiar structure which we term the 'superlattice'. Superlattices exhibit an interesting optical property, known in the literature as the 'moire effect'.[44] For instance, in Fig. 9(*a*) one can see regions of hexagonal structure with different unit-cell constants (but which are always larger than the constant of the particle monolayer). The presence of superlattices with different unit-cell constants (Fig. 10) is due to the different relative orientations of the two layers in the different domains.[44] To our knowledge, this is the first observation of such a phenomenon in experiments on 2D array formation from latex particles.

'Black Holes'

We sometimes observe the spontaneous formation of large 3D agglomerates of latex particles which we term 'black holes'. They are seen as dark spots in transmitted light and, like their cosmic namesakes, draw in all particles nearby and bring about intensive hydrodynamic fluxes.

We hypothesise that the 'black holes' form due to the presence of some larger particle (a result of the polymerisation procedure during latex production) or particle aggregate in the water layer, which then bends the two film surfaces. Since the oil/water interfacial tension is rather low, the oil/water interface gives way and becomes concave around the larger particle (or aggregate). The neighbouring latex particles (which are slightly heavier than water) tend to fill this concavity, which, in turn, spontaneously grows and sinks into the oil phase. The larger the number of particles gathered, the greater the sagging of the oil/water interface. We tried to avoid the formation of such features because they disturbed the formation of good 2D arrays.

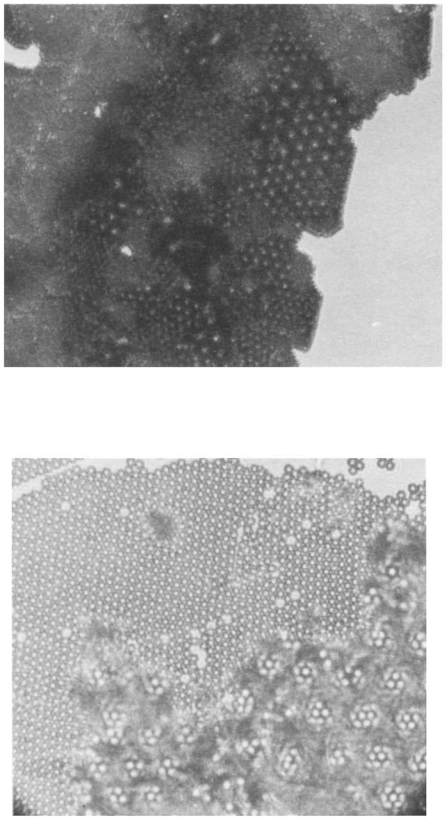

Fig. 9 Photograph of 'superlattices' with different unit-cell constants (due to the different relative orientation of the two overlapping layers) in transmitted light: (*a*) overview; (*b*) superlattice at higher magnification

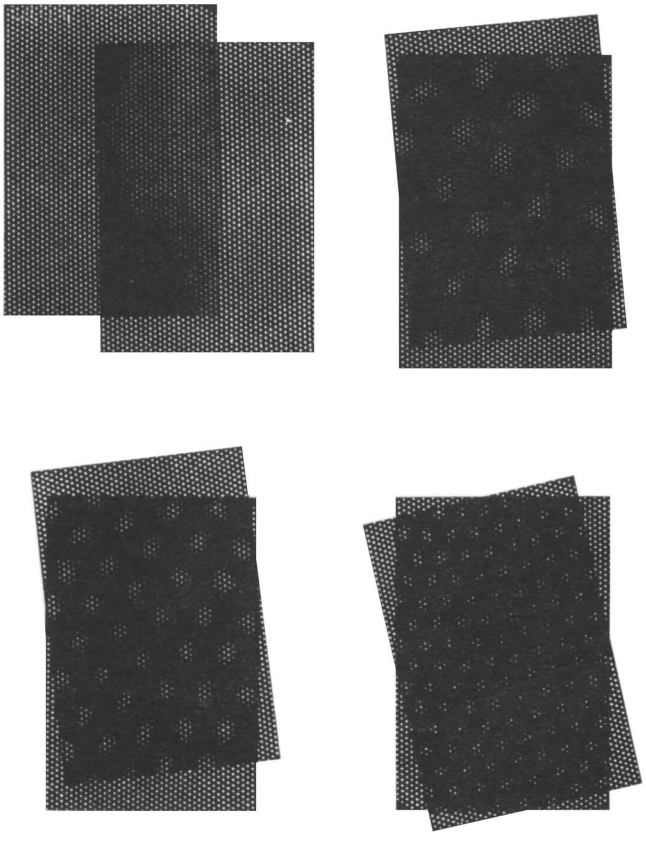

Fig. 10 Illustration of the superlattice constant dependence on the relative orientation of two overlapping layers

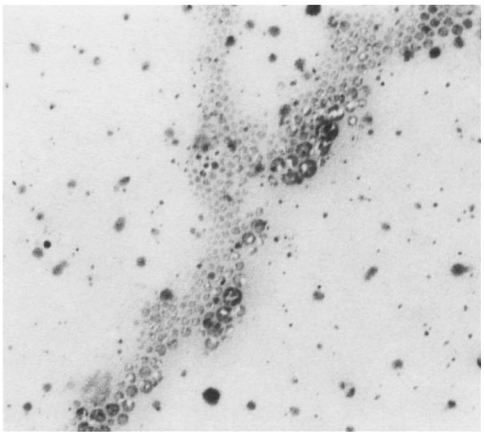

Fig. 11 Transmission electron micrograph of ferritin structures containing ordered regions

2D Ferritin Structures

We studied by transmission electron microscopy (TEM) specimen grids with deposited ferritin structures. In some cases we observed groups of hexagonally ordered ferritin molecules (see Fig. 11). The lattice constant measured from the photographs was *ca.* 13 nm, which is close to the diameter of the ferritin molecules. Note that a partial destruction of the Formvar film took place during the observation of the sample by TEM. Deposition of the ordered structures directly onto the microscope specimen grid (after F-oil evaporation) was also employed as a method of TEM sample preparation. The deposition process could be additionally promoted by gentle ejection of the oil after 2D array formation. We hope that further experiments, with more precise control of the conditions, will allow us to obtain a 2D protein array of higher quality.

Conclusions

The experiments performed demonstrate that F-oil substrates can be applied to the formation of 2D ordered structures from latex particles or ferritin molecules. Under appropriate conditions, larger and well ordered 2D arrays from latex particles can be obtained, see Fig. 5. The arrays can be transferred onto solid substrates without damage after complete evaporation of the F-oil, Fig. 1 and 5. The results confirm that the capillary forces and the convective particle flux are the main factors governing the 2D array formation process. The quality of the arrays was increased by controlling the evaporation rate and the meniscus shape. Unforeseen phenomena like domain subduction, the moire effect and 'black holes', which are connected with the specific properties of the oil/water interface, were observed by optical microscopy or SEM. Ordered 2D clusters from ferritin molecules were obtained in a similar way. They were then deposited directly onto the specimen grids and studied by TEM. A further development of this experimental technique can lead to obtaining larger ordered domains from colloidal particles, *i.e.* latex microspheres, globular or membrane proteins.

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