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Temporal evolution of self-organization of gelatin molecules and clusters on quartz surface

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Aqueous gelatin solutions when spread on hydrophilic substrates form self-organized structures where the gelatin molecules and clusters are arranged as self-similar objects giving a mass fractal dimension $d_f = 1.67$ and 1.72 for solutions made with KCl and NaCl salts as estimated from atomic force microscopic studies. The dehydration driven self-organization of particles changed the d_f values to 1.78 and 1.81, respectively, after 24 h. This further changed to 1.83 and 1.85 after a time lapse of 10 days. The dynamics of formation of these structures are modeled through spin-exchange kinetics in the nonequilibrium steady state regime in order to understand their complex behavior. Kawasaki spin exchange dynamics has been applied to a diffusion limited aggregation type fractal object, and the growth of the domains was observed by minimizing the free energy. The fractal dimension of such a system changed from 1.70 to 1.82 which inferred the loss of fractal behavior and the generation of a more compact object. The experimentally observed temporal evolution of these complex structures could be adequately described through the results obtained from the computer simulation data.

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INTRODUCTION

Free-energy driven self-organization of molecules and clusters is observed in a variety of systems of interest in biology, immunology, polymer science, colloidal chemistry, metallurgy, etc. For example, the self-assembly dynamics underlying the protein shell formation in spherical viruses is an interesting biological problem. The dynamics of evolution of icosahedral symmetry observed in these supramolecular polyhedral structures could be modeled through molecular dynamics studies [1]. Dynamics of nanoscale self-assembly of ternary epilayers formed on elastic substrates was examined in excellent detail by Lu and Kim [2]. In this approach, the continuum phase field model of spinodal decomposition and the surface stress was suitably combined to account for the concentration and stress fields in the substrate. Dynamics of formation of vesicle self-assemblies was studied by Noguchi and Gompper [3] who showed that these self-assemblies are formed via three distinguishable steps: particle assembly into discoidal clusters, cluster aggregation into larger membrane patches, and finally, the vesicle formation. The time-dependent Landau-Ginzburg formulation was successfully applied to explore the phase separation in the presence of surfactants by Patzold and Dawson [4]. Coarse-grained simulation studies of amphiphilic self-assembly structures was reported by Michel and Cleaver [5] who showed that micellar, bilayer, and reverse micellar arrangements could be achieved for a single model parametrization. These studies reinforce the importance of numerical simulation techniques in solving problems related to self-assembly of soft matter.

Normally, self-organization and aggregation processes have been described through diffusion limited cluster aggregation (DLA) [6], reaction limited cluster aggregation (RLA) [7], random sequential adsorption (RSA) [8,9], etc. Adhesion of macromolecules onto surfaces, polymer chain reactions, association of noncrystalline granular materials and arrangement of nanostructures, quantum memory devices, biosensors on substrates, car parking, etc., fall into the category of the random sequential adsorption process (RSA). These processes are generated with sequential addition of particles at a constant rate to a substrate where overlapping and leaving the cluster once apart is forbidden. Brilliantov *et al.* [10] have shown that geometric and kinetic features of the RSA clusters for a system showing polydispersity in size are governed by the smallest size particles.

Such characteristics often lead to interesting pattern formations in two-dimensional (2D) and 3D Euclidean space, and at the microscopic level the growth of self-similar domains is governed by a range of physical parameters. The quantification of these structures is done through Hausdorff mass fractal dimension d_f, which is much smaller than the corresponding Euclidean dimension (D). In this picture, the density-density correlation function, $g(r) = \langle \rho(r)\rho(0)\rangle \sim r^{-A}$, where $A = D - d_f$. Diffusion driven processes rapidly coagulate to yield DLA clusters of fractal dimension, $d_f \cong 1.7$ for $D=2$ and 2.6 for $D=3$ which is strongly supported by simulations and experimental results [9]. On the other hand, RLA clusters grow rather slowly because of reduced collision probability between particles and the growth is through interparticle reactivity involving equilibrium constant. The corresponding fractal dimensions are much higher than that of DLA clusters. Though the crossover from DLA to RSA has been observed, the intermediate region remains relatively unexplored [10]. Determination and understanding of self-organized behavior in biopolymers such as proteins, polypeptides, nucleic acids, and polysaccharides are even more intriguing, because these carry charged side groups and the net charge carried is a function of solution pH, among other things [11,12].

In this paper, we address the problem of dehydration driven self-organization on a substrate pertaining to a biopolymer, gelatin, a low charge density polyampholyte from both the experimental and theoretical standpoints. The experimental observations comprise of recording the evolution of self-organized patterns created by a thin film of gela-

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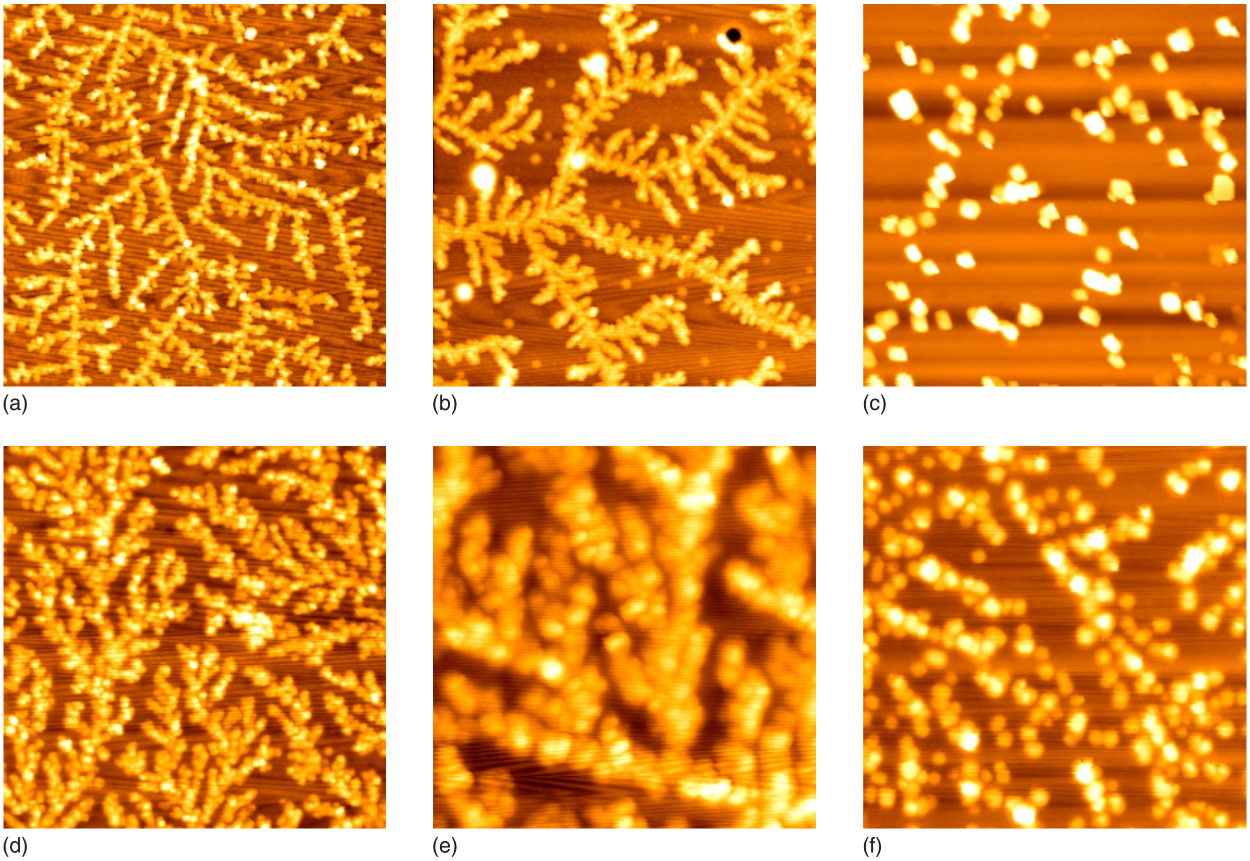

FIG. 1. (Color online) Temporal evolution of self-organized structures. AFM pictures (5 × 5 micron) of gelatin nanostructures spread on quartz substrate. The 1% w/v aqueous gelatin solution was prepared with monovalent salt concentration fixed at 0.1 M and measurements taken at (20±2) °C. The mass fractal dimensions of (a) and (d) are 1.67 and 1.72, respectively. This increases to 1.78 and 1.81 after 24 h of time lapse. This further increased to 1.83 and 1.85 after a lapse of 10 days.

tin solution on a hydrophilic surface as a function of time. We model this dehydration driven morphological change through Kawasaki spin exchange dynamics to generate similar patterns via computer simulation. Gelatin has been chosen as the target molecule due to the following reason. This biopolymer contains acid and base residues in almost a 1:1 ratio at neutral *p*H. The molecular charge distribution is symmetric at isoelectric *p*H, but gets skewed as one moves away from *p*I [13]. The properties of aqueous solutions, gels, and coacervates of gelatin have been well studied and characterized in the past [14].

MATERIALS AND METHODS

Gelatin (type-B, 300 Bloom, microbiology grade devoid of *E. coli*, *p*I=4.9) was obtained from E. Merck (India). The solvent used was Millipore deionized water. The gelatin solutions (1% w/v) were prepared by dispersing gelatin in 0.1-M sodium chloride (a second set used 0.1-M KCl) solution at 60 °C. The macromolecules were allowed to hydrate completely; this took 1–1.5 h. The gelation concentration of gelatin in water is 2% (w/v); the gelatin concentration chosen in these experiments was deliberately kept lower than this to avoid formation of gels. A twin sample stored in a test tube at room temperature did not exhibit gelation. All other chemicals used were bought from Thomas Baker, India. All the chemicals were of analytical grade. During the experiment, a drop of the solution was removed from the beaker and allowed to spread out uniformly on a quartz plate over a period of 30 min. Atomic force microscope (AFM) pictures were taken using an Autoprobe CP Research AFM system, model AP-2001 (Thermo-microscopes, USA) using a 90-mm scanner and in tapping mode. All experiments were carried out at (22±2) °C. The evolution of AFM micrographs was recorded for 10 days which is displayed in Fig. 1 (available online in color) for samples made with both KCl and NaCl salts. These self-assemblies are made of gelatin nanoparticles and clusters that are generated through intra- and intermolecular interactions [15]. Such interactions produce partial charge neutralization of gelatin molecules, and release counterions previously bound to the charged segments into the solution thereby increasing the solution entropy which facilitates more interactions [16]. The phenomenology of this has been discussed earlier [16]. The gelatin particles and aggregates could be visualized in the solvent environment because the hydration solvent (bound water molecules) has a refractive index (and dielectric constant) different from that of the solvent background (free water molecules), which is responsible for providing the necessary optical contrast.

The self-similarity and scaling in the fractal structures observed in Fig. 1 (available online in color) was ascertained from the fractal dimension d_f. This is defined as [17]

$$d_f(s) = \lim_{\varepsilon \to 0} \frac{\ln M(\varepsilon)}{\ln(1/\varepsilon)}, \tag{1}$$

where s is a subset of N-dimensional space occupied by the fractal object and $M(\varepsilon)$ is the number of N-dimensional cubes required to cover the subset s. Our analysis yielded $d_f = 1.67 \pm 0.05$ and 1.72 ± 0.05 for Figs. 1(a) and 1(d), respectively. This increased to 1.83 and 1.85 after 10 days of time lapse making the structures more compact. The corresponding values after 24 h of drying were 1.78 and 1.81, respectively. These clearly indicated that the dehydration driven self-organization was driving the system towards the formation of more compact structures.

NUMERICAL SIMULATION STUDIES

The AFM pictures obtained distinctively showed time-dependent morphological changes that called for a clear physical understanding of the dehydration driven self-organization phenomenon. In such problems several parameters become important like the rate of solvent evaporation, interfacial tension and viscosity, Donnan equilibrium, interparticle interactions, etc. Much of this information is not available which required the phenomenon to be probed through computer simulation studies. The following presents the details of the simulation work performed in two dimensions which demonstrates that correct phenomenology observed could be captured rather extensively.

We have taken a square lattice size (128×128) in which we fixed the origin as the initial site for inorganic low molecular weight salt which acts as a nucleation center for a local fractal structure to grow via the DLA model. We have taken 5000 particles that perform random walk (diffusion) within the lattice until a hit is registered with the nucleation site or a growing cluster. Following this the particle sticks to the site or cluster leading to the growth of a microscopic fractal structure (defined as $t=0$). We allow the local spin variable to interchange which is appropriate for kinetics of phase separation studies in binary mixtures. The Kawasaki spin exchange model adequately mimics phase segregation via diffusion. There are systems where the bulk mobility diminishes drastically due to physical processes, where one component may undergo gelation transition.

We mapped the water and ampholytic molecules (gelatin) by Ising spin variables with spin $S = \pm 1/2$. Monte Carlo simulation was performed on a fractal employing Kawasaki spin exchange dynamics. In order to incorporate the dehydration mechanism into the model the following protocol was used. The rate of creation of new vacancies was considered as directly proportional to evaporation of solvent molecules. As the solvent evaporates it causes cooling to the system which develops a temperature gradient in the system. The probability of evaporation of solvent from the outer surface of the film is more. We select a gelatin molecule at random and flip the same with the nearest solvent molecule, and then calculate the total energy for the new system, if the new energy is lower than the previous then accept the change, otherwise discard that change. This process gives rise to a domain type of growth mechanism. Thus clearly the system prefers to be in a phase-separated state at the lower temperature.

The standard model for binary mixtures is the Ising model, and the Hamiltonian is given as [18–23]

$$H = -J \sum_{i \to 1, j \to 1}^{N, N_1} \sigma_i \sigma_j, \tag{2}$$

where the spins $\{\sigma_{\mathrm{i}}\}$ with $i = 1 \to N$ are located on a discrete lattice and J is the strength of the exchange interaction between spins. The states $\sigma_{\mathrm{i}} = +1/2$ or $-1/2$ denote the presence of gelatin particles or solvent molecules at site i, respectively. The concentration of gelatin particle is less in comparison to the solvent. In a Monte Carlo simulation of this model, a pair of nearest-neighbor sites i and j is randomly selected, and the spins σ_{i} and σ_{j} are exchanged. The probability that this exchange is accepted is

$$P = \min[1, \exp(-\beta \Delta H)], \tag{3}$$

$$\Delta H = J(\sigma_i - \sigma_j)\left(\sum_{Li \neq j} \sigma_{Li} - \sum_{Lj \neq i} \sigma_{Lj} \right). \tag{4}$$

Here, ΔH is the energy change due to the proposed spin exchange, and $\beta = 1/k_{\mathrm{B}}T$ is the inverse temperature, with k_{B} denoting the Boltzmann constant. L_i denotes the nearest neighbors of "i" on the lattice. We have performed the simulations at 22 °C. A single Monte Carlo step (MCS) corresponds to N such attempted exchanges.

The phase separation kinetics follows a simple diffusion equation [24],

$$\frac{\partial u(r,t)}{\partial t} = \eta \nabla^2 u(r,t), \tag{5}$$

where $u(r,t)$ is the density and η is the diffusion constant, so the future profile of $u(r,t)$ can be predicted by solving the ordinary differential equation. The evaporation of solvent induces an outward flow, which brings the solute towards the perimeter of the polymer film. We have taken snaps in the interval of 10×10^6 Monte Carlo steps. The first snap matches with the initial stage of grown fractal [Fig. 2(a)], and then we allow the system to evolve with Monte Carlo steps. Finally, the snap (6th) taken after 50×10^6 MCSs can be compared with the corresponding AFM data [Fig. 2(f)]. We are comparing the Monte Carlo steps with the real time of experiments only at the end points. It is not possible to establish one to one correspondence in the intermediate steps.

RESULTS AND DISCUSSION

The process described in the present study pertains to the retraction pattern of a viscous liquid phase at the liquid-vapor interface. The highest rate of evaporation occurs at the edges whereas the bulk of the solvent is concentrated closer

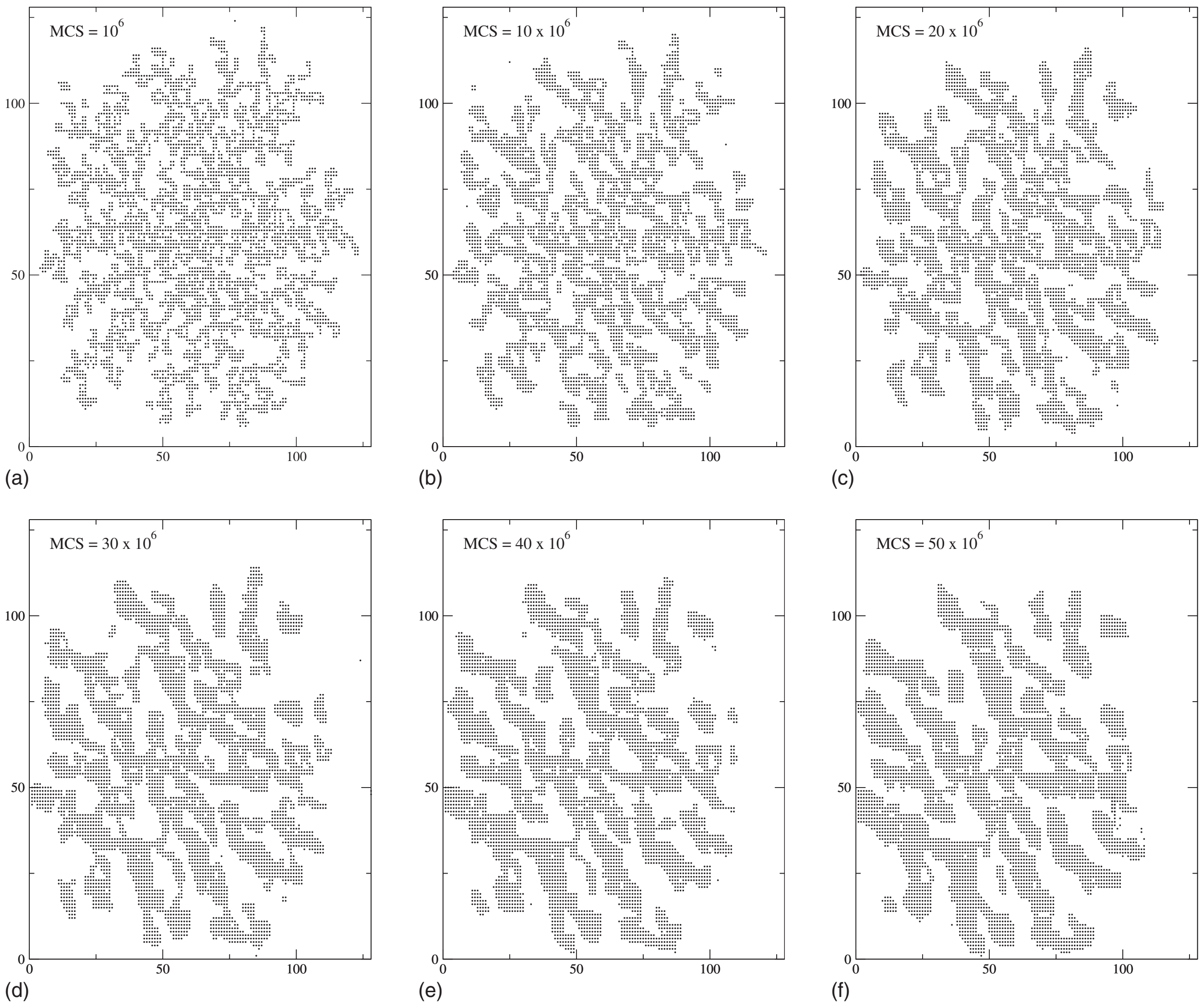

FIG. 2. Simulated evolution of the initial DLA fractal structure (a) $d_f = 1.7$ shown in various Monte Carlo time steps. The fractal dimension undergoes continuous change. Notice that the final states (e) and (f) are not too different from the AFM pictures shown in panels Fig. 1(c) and 1(f), available online in color. The final structure shown in panel (f) has $d_f = 1.82$.

to the center of the film. In order to replenish the liquid removed by evaporation at the edge, a solvent flux from the inner to the outer regions must exist inside the film. Such a flux will drive the solute molecules towards the center in order to minimize the free energy of hydration thus leading to more compact structures and the concomitant loss to the fractal features. The effective concentration of the gelatin molecules is increasing during the evaporation of solvent which will exceed the gelation concentration (≈2% w/v) when 50% of the solvent is lost due to evaporation. Thus there is a definite possibility of formation of thin gelatin gel films on the substrate which will hinder further solute movement. Notice that the experimental temperature is less than the gelation temperature of gelatin which is ≈28 °C [25].

In a real system, the gelatin particles are charged and strong screened electrostatic interactions prevail between these entities. When we make a thin film on quartz plate the particles diffuse and adhere to each other to form fractal structures through diffusion-limited aggregation process. The dehydration driven growth process owes its origin to the presence of hydrodynamic instability caused due to the asymmetric drying of the film. There is a positive osmotic pressure gradient at the tip of the fractal structures with respect to that of the backbone which sets up a solvent flux. Recall that free energy of hydration is negative which forces the structural changes. The diffusion between microscopic zones is possible only if a percolating solvent channel connects these regions. The death of branches as they are screened by their neighbors is balanced by the creation of new branches via microscopic tip splitting process. Temperature influences a number of parameters which play a key role in the drying process: the interfacial viscosity and surface tension of liquid are of paramount interest here. But, these are not amenable to experimental measurements. As the water evaporates the salt (NaCl/KCl) present in the system crystallizes and the crystal generates a stress on the fractal structure leading to its disintegration. In our case, the gelatin molecules are highly miscible in water so these form finger-like structure initially, but as the water molecules evaporate the local concentration of the polymer gets affected which

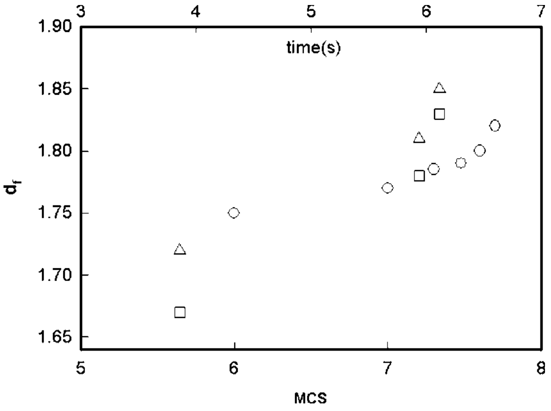

FIG. 3. Evolution of fractal dimension d_f as a function of real time (top scale) [from AFM data: □ (KCl) and △ (NaCl)] and Monte Carlo steps (bottom scale) [simulation data: ◦]. The two time scales are qualitatively same and a clear correspondence is observed implying the validity of the simulation model used.

results in the loss of the specific structure. Ultimately, the disintegrated structure appears as a polydisperse assembly of clusters scattered over the quartz plate randomly. This is clearly seen from Figs. 1(c) and 1(f), and Fig. 2(f). Both NaCl and KCl samples produced identical self-similar nanostructures. However, the fractal dimensions for KCl aggregates was observed to be lower than those of NaCl samples according to the AFM pictures shown in Fig. 1 (available online in color). We believe that the cation size may be playing a role in this process since it is driven by nucleation. As these structures evolve with time their fractal dimensions (a loose measure of compactness) alter considerably as shown in Fig. 3.

An alternative way of looking at the problem is through random sequential adsorption (RSA) where particles are added sequentially to a surface until a jamming limit is reached. Brilliantov *et al.* [10] have shown that geometric and kinetic features of the RSA clusters for a system showing polydispersity in size are governed by the smallest size particles but prior to this an intermediate asymptotic regime prevails. Brilliantov *et al.* [10] assumed the particle size distribution to be continuous and represented it as a power-law distribution function $P(R)$ given by

$$P(R) = \begin{cases} \alpha R^{\alpha-1}; & R \le R_{\max} \\ 0; & R > R_{\max} \end{cases}. \qquad (6)$$

The system is assumed to have very large polydispersity in size with the maximum size given by R_{max}. The geometrical structure of the resultant RSA cluster evolving from a system with characteristics defined by particle size distribution as in Eq. (6) is fully governed by the spatial correlations inside the system, which is accounted for by the parameter α ($\alpha > 0$). As α increases from 0 to ∞, d_f reduces from 2 to 1.305. For smaller α fractal clusters are formed while the Apollonian packing limit is reached as $\alpha \to \infty$. In an earlier work, the self-assembly in two dimensions was discussed within this framework while the same in three dimensions could be explained through Smoluchowski aggregation kinetics [15]. The role played by dehydration driven instability was not adequately discussed earlier which constitutes the main objective of the present work. We have modeled our physical situation in accordance with the DLA framework mainly because the d_f values measured were consistent with such a prescription, and second, in our case the particles were not added sequentially as required by the RSA model. It is possible that a proper description of the dehydration driven self-organization necessitates the involvement of a combination of these two models.

CONCLUSION

The structural evolution of self-assembled microstructures comprising gelatin nanoparticles and clusters, prepared on quartz substrates, was recorded by AFM as a function of time. The evaporation of solvent produces dehydration driven temperature and osmotic pressure gradient in these assemblies that result in the setting up of a solvent flux. Such a flux redistributes the solute molecules in a bid to minimize the free energy of hydration which generates an assortment of assemblies. During this process the self-assembled structure is under continuous asymmetric stress due to continuous evaporation of solvent. The dynamics of this has been modeled through Monte Carlo simulations within the mechanism of Kawasaki spin exchange dynamics. The simulation results agree with the experimentally observed features to a very good extent. However, the exact role played by interfacial surface tension and viscosity among other parameters like rate of cooling, concentration of low molecular weight salt and protein, etc., could not be accounted for in the model. Regardless, an attempt has been made to understand the intricacies of dehydration driven self-organization of nanoassemblies. The dynamics governing the various aspects of self-assembled nanostructures and condensates manifests itself through a variety of observable phenomenon like syneresis which could be modeled through nonequilibrium statistical mechanics [26].

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