Modelling of Aggregation of Nanoparticles and its effect on their Structural and Biological Functions

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Abstract: Nanoparticles have applications such as drug delivery and cancer treatments, reinforcement of the polymer or metal matrix, consumer products and environment. This work concentrates on how aggregated nanoparticles might realistically effect performance of the intended structural or biological function. As a conceptual basis, primary aggregation is assumed to produce the backbone of micro-structures which then cluster, covering a large portion of the material. This process is assumed to be chaotic and to occur rapidly. Molecular dynamic analysis of this aggregated model is difficult because the problem is not clearly bound and regions not spatially defined. Moreover the modulus of the micron-sized aggregate within the cluster is also difficult to measure directly. Instead an indirect method is developed of the polymer/particle interface in the aggregate which can be verified by bulk modulus experiments on nano-composite samples produced specifically for this work. A computer program equates minimum free-energy of the absorbed polymer molecule to dipolar interaction energies having a Boltzmann’s Distribution. Fractal numbers are used to characterise the molecular/particle interface and configuration of the aggregate backbone. After the principle has been established it is extended to other applications for example how aggregation might effect the probability of release of artificial DNA from silica nano-particles within the body.

1 Introduction
In this work two different applications of nanoparticles are examined for the effect of agglomeration. One application is reinforcement filler in a polymer nanocomposite and the other is as means of delivering artificial DNA (CpG) to stimulate the immune system in diseased target cells. It may be conceptually easier to consider the nanoparticles as separate entities however this condition does not normally exist during processes such as inoculation or injection in the body. Once any electrostatic repulsion between the particles, if it exists, has been overcome then van der Waal’s forces dominate often irreversibly. In both examples experimental data are compared to results from a theoretical analysis. The purpose of the analysis is to determine the effect of agglomeration on performance. Molecular dynamic modeling is a powerful tool in simulations of material interaction [1, 2, 3], however, full scale molecular dynamic modelling of the aggregated model is difficult because the problem is not clearly bounded, and regions are not spatially defined. Also such simulation would also be computationally expensive [4]. The method of analysis in this work depends on the application.

In the case of the nanocomposite filler, a Cluster-Cluster Aggregation (CAA) model is assumed, in which primary clustering produces short fibre-like groupings. These have a backbone of nanoparticles connected by a flexible nanoscopic bridge of glassy polymer between the nanoparticles. A computer simulation based on fractal properties of the particle estimates the modulus of this primary grouping. Then, because nanoparticle content is high (40% by weight) the composite consist of secondary clustering. Linear approximations are used to estimate the
secondary modulus and hence elastic modulus of the entire nanocomposite. This value is compared to elastic modulus obtained from particulate (separated) and fibre filler models.

For the nanoparticle application where they are required to deliver artificial DNA (CpG), the measure of performance is as probability of release for theoretical separated particles, compared to experimentally derived agglomerated particles. The rate of release must be sufficient to ensure that CpG is released in the vicinity of the target cell but not so great that all the CpG load is released before it reaches the target.

2 Agglomeration of the Nano-filler in the Polymer Matrix
This section describes the preparation of a nanocomposite with agglomerated filler, experimental estimates of its composite strength and comparison of values with those from a theoretical analysis.

2.1 Preparation of Nanocomposite Samples
It was important to devise a method for producing the nanocomposite that ensured agglomeration of the nano-filler after inculcation in the polymer matrix. Basically this was done by directly mixing a colloid suspension of nanoparticles with a polymer solution. The method is fully described elsewhere [5] but is now explained briefly: Powdered PVC was added to DMF in a mass ratio of 25:75. The mixture was manually stirred, at 2 hourly intervals, over a period of 10 hours until there was no discernible solid in the mixture. A known weight of the polymeric mixture was placed in a glass-dish. The colloid suspension with approximately 30% mass-ratio of silica nanoparticles was then poured into the container to immerse the sample. The sample remained immersed for 11 hours during which time the DMF solvent diffused out of the polymeric mixture and the aqueous suspension of Silica Nano-Particles diffused in. The sample was then removed dried in a warm air-flow and weighed.

Fig. 1 Scanning Electron Microscope (SEM) image of nano-silica dispersion in a PVC Polymer Matrix. The image shows an area of approximately 10x10μm. Red-dots indicate the presence of nano-silica. The porous microstructure of the matrix is indicated by variation in background tone; grey and dark areas represent polymer and voids/pores respectively.
The micrograph in Fig 1 shows a porous microstructure. This porosity is also described in the literature [6, 7]. The large internal surface area due to porosity is likely to increase uptake of nanoparticles, which in these experiments reached a maximum of 40% by weight. For compression testing purposes the sample porosity was later removed by re-dissolving in DMF and allowing it to solidify by evaporation. Table 1 shows the measured weights of nanoparticles in three samples produced with different concentrations (8%, 18% and 30%) in the precipitation bath.

| Concentration of Silica Nanoparticles (% volume) | Measured amount of Nanoparticles in PVC Matrix (% mass) |
|-----------------------------------------------|------------------------------------------------------|
| 30                                            | 40                                                   |
| 18                                            | 20                                                   |
| 8                                             | 11                                                   |

Table 1 Measured amounts of nanoparticles in the three nanocomposite samples produced with three different Silica nanoparticle concentrations

| Measured Nanoparticles in PVC Matrix (% weight) | Measured Nanoparticles in PVC Matrix (% volume) | Measured Composite Modulus (MPa) |
|------------------------------------------------|-----------------------------------------------|---------------------------------|
| 0                                              | 0                                             | 61                              |
| 11                                             | 5.4                                           | 95                              |
| 20                                             | 10                                            | 105.5                           |
| 40                                             | 20                                            | 130.7                           |

Table 2 Comparison between measured composite modulus for four samples with different concentrations of nanoparticles in the precipitation bath and theoretical values

2.2 Compression Tests and Results

The samples in Table 1 were subjected to compression testing which was repeated until Young’s Modulus had the same value following two consecutive tests. The Young’s Modulus was estimated from the linear part of the stress/strain curve. Composite moduli of the samples are shown in Table 2 together with percentage weight and volume of nanoparticles. The volumetric percentage of nanoparticles in the sample was calculated using a Silica density of 2.65 g/cm$^3$ and a PVC density of 1.3 g/cm$^3$.

Fig. 2 shows composite modulus (Ec) plotted against filler fraction (by volume). Also plotted are the theoretically derived values for spherical particulate and fibrous composites based on the Halpin-Tsai equations [8]. Trial values of filler strengths (Ef) were substituted into the formulae; 1000 MPa and 700 MPa respectively for spherical and fibrous filler. Fig. 4 shows that the experimental sample modulus is greater than that of the spherical particulate composite over the
entire range of filler fraction (Ef); only a small increase in composite modulus is made by further increasing tri axial filler strength. The fibrous filler model provides a result slightly closer to experimental data. Similar comparisons are made in the literature [9, 10] for dry mixed silica nanoparticles with polyimide and nylon matrix; composite strengths were much greater than that obtained with dispersed spherical-particulate filler.

Using the Halpin-Tsai equations, neither the spherical model nor the fibrous models can predict the effects of the filler volume fraction on composite modulus. A new approach based on a Cluster-Cluster Aggregation (CCA) model is presented.

![Graph showing comparisons between measured composite modulus for four samples with different concentrations of nanoparticles, and theoretical values for fibrous and dispersed particulate filler based on the Halpin-Tsai equations.](image)

Fig. 2 Comparisons between measured composite modulus for four samples with different concentrations of nanoparticles, and theoretical values for fibrous and dispersed particulate filler based on the Halpin-Tsai equations.

2.3 Analysis of the elastic properties based on a Cluster-Cluster Aggregation (CAA) model

Nanofiller aggregation has been proposed as a reinforcing mechanism in nanocomposites [11]. The notion is that aggregated particles form fillers that are geometrically similar to a micro-fibre; hence greater reinforcement. A Cluster-Cluster Aggregation (CCA) model of the filler microstructure [12] is used as a conceptual basis and is illustrated in Fig. 3. According to the CCA model, clusters are formed from strongly bonded primary aggregates of nanoparticles as shown in Fig. 3a. In this work the modulus of the primary aggregate bond is estimated using a computer-based model. The predicted Filler-Filler Bond Modulus is then used in a theoretical
analysis of composite modulus for filler fractions above the gel point ($\varphi > \varphi_G$) as shown in Fig. 3b.

Fig. 3 Schematic view of aggregated cluster in the polymer matrix. (a) The local structure of the cluster is built from primary particles and strongly bonded primary aggregate. (b) shows connected clusters in a composite where filler fraction is above the gel point (c) illustrates a composite where filler fraction is below the gel point. Every disk in (b) and (c) represents a primary aggregate [12,13]

2.3.1 Primary Aggregation of Filler Reinforcement

The basic concept for primary bonding (shown in Fig. 3a) is illustrated in Fig. 4 [14]. The strength of a polymer/nanoparticle bond depends on its location within the cluster. The bond strength within a confined region between the nanoparticles is greater than that at the cluster/matrix boundary. A flexible nano-sopic bridge of glassy polymer exists between the nanoparticles. This bridge effectively provides a backbone to the primary aggregate structure.

Fig. 4 Schematic representation of nanoparticle aggregation in a polymer matrix. The concept provides a method for estimating the filler-filler modulus which is an essential part of calculating the composite modulus.
A MatLab computer model was used to compute the filler-filler bond modulus between nanoparticles i.e. modulus of the nanoscopic bridge described above. The model equates dipole interaction energy using two equations, each having a different theoretical basis. One of the equations is derived from minimization of free-energy of the absorbed polymer molecule which is treated as a Gaussian Chain [15]:

\[ G_p = R_v^{(1-ds)} b^{(ds-3)} (Nb/N) \] (1)

Where:
- \( G_p \) is the modulus of the filler-filler bonds (immobilised polymer) (GPa)
- \( R_v \) is the mean height of the bound polymer molecule above the particle surface (nm)
- \( ds \) is surface fractal dimension of nanosilica
- \( b \) is the effective length of the Carbon-Carbon bond in the PVC backbone ≈ 0.126 (nm)
- \( Nb/N \) is the ratio of bound monomers to unbound monomers

The other equation is the so-called Keeson Equation for the interaction energy between dipoles having configurations with a Boltzmann’s Distribution [16]. The computer algorithm, iteratively calculates the interaction energy using the Keeson Equation for each dipole interaction between the absorbed polymer-molecule and nanoparticle. The simulation stops when the iterative total equals the interaction energy obtained from the free-energy equation. Convergence is achieved when the number of iterations equals the guessed number of bound to unbound monomers \( Nb/N \).

The value of surface fractal dimension \( ds \) has a profound effect on bond modulus due to the exponential function in Equation 1. For a flat surface \( ds = 2 \) and for a Brownian surface \( ds = 2.5 \). The glassy-polymer bond between particles is assumed to be a space-filling surface where both particle surfaces are in contact with the same polymer molecule. This is represented by a limiting value of \( ds \rightarrow 3 \); for simulation purposes \( ds=2.95 \). The thickness of the immobilised polymer layer \( \Delta \) (bond gap size) is most commonly taken to be \( \approx 2 \)nm [17]. At this thickness and a value of \( ds =2.95 \) the calculated filler-filler modulus is 11.5 GPa. This modulus is near the upper limit of moduli for crystalline polymers [18]. Results from the computer simulation for filler-filler bond modulus are shown in Table 3.

| Surface Fractal Dimension ds | Thickness of Polymer Layer between Primary Aggregate Particles \( \Delta \) (nm) | Ratio of Bound to Unbound Polymers \( Nb \) | Modulus of Polymer Layer between Primary Aggregate Particle \( G_p \) (GPa) |
|-----------------------------|---------------------------------|----------------|----------------------------|
| 2.95                        | 0.212                           | 0.65           | 11.5                       |
| 2.9                         | 0.245                           | 0.49           | 2.93                       |
| 2.8                         | 0.285                           | 0.44           | 0.92                       |
| 2.7                         | 0.305                           | 0.43           | 0.54                       |
| 2.6                         | 0.32                            | 0.42           | 0.38                       |

Table 3 Simulation output values of the filler-filler bond moduli (\( G_p \)) for different input values of the particle surface fractal dimension (\( ds \))
2.3.2 Composite Modulus for Filler Fractions greater than the Mechanical Gel-Point (φ > φG)

For composites with filler fractions greater than the gel-point, it is assumed that the clusters are connected and start to form the network as shown in Fig. 3(b). The cluster backbone then becomes an essential factor in the estimation of cluster modulus. The formula used to calculate composite modulus (Gc) takes the form of filler-filler bond modulus (Gp) multiplied by filler fraction (φ) with an exponent that involves the size and geometrical structure of the cluster and cluster backbone [19,20,21]:

\[
G_c = G_p \left(\frac{(d+2\Delta)^3 - 6d\Delta^2}{d^3}\right) \phi^{(3+df_b)/(3-dfc)}
\]

(2)

Where:
Gp is the modulus of the filler-filler bond
d is the particle diameter
Δ is the thickness of the immobilised polymer layer shown in Fig. 4
φ is filler fraction
dfc is the fractal dimension of the cluster
dfb is the fractal dimension of the cluster backbone

Trial values of dfb and dfc were substituted into Equation (2). For example a straight backbone would have a theoretical (dfb) ≈ 1 corresponding to a cluster value (dfc) < 2 [22].

2.3.3 Model for Filler Fractions less than the Mechanical Gel-Point (φ < φG)

For composites with filler fractions less than the gel-point it is assumed that the clusters are unconnected as shown in Fig. 3(c) and are considered simply as rigid inclusions. The formula used to calculate composite modulus (Gc) takes the form of matrix modulus (Gm) multiplied by a factor which depends on the filler fraction of the clusters:

\[
G_c = G_m X \quad X = (1 + 2.5 \times \phi_{eff} + 14.1 \times \phi_{eff} + ...)
\]

(3)

Where:
Gc is the Composite Modulus
Gm is Matrix Modulus
\(\phi_{eff} = \phi / \phi_A\) i.e. filler-fraction ratio of the composite to the cluster

\[
\phi_A = K_0 \left(\frac{R_g}{a}\right)^{df-3}
\]

(4)

Where:
\(\phi_A\) is the filler fraction of the cluster
Rg is the radius of gyration (nm)
\(K_0\) is a constant ≈ 1.01
a is radius of the nanoparticle = 15 (nm)

Radius of gyration $R_g$ for the cluster [23] is scaled from Small Angle Neutron Scattering (SANS) data for 9.8 nm radius silica particles using the fractal dimension for the data set corresponding to the filler fractions $\phi$ used in the experimentation.

2.4 Results from theoretical calculations and comparisons with experimental data

Table 4 lists the results from calculations using Equations (2) and (3). Results from calculations using Equation (2) are in good agreement with experimental data for the highest filler fraction $\phi = 0.2$. To obtain this agreement, it was necessary to assume a value for the exponent $= 2.97$ which is possible with $df_b = 1$ and $df_c = 1.65$. These are minimal values that imply that the material has a very simple cluster structure with a straight backbone. Results from calculations using Equation (3) are in good agreement with experimental data at most filler fractions. There is a discrepancy at the lowest filler-fraction (=0.054) with a +10% difference.

| Filler Fraction | Measured Composite Modulus Gc (MPa) | Calculated Value of Composite Modulus (MPa) |
|-----------------|------------------------------------|------------------------------------------|
|                 |                                    | Using Equation 2: $G_c = G_p (\phi_A)^{(3+df_b)/(3-df_c)}$ | Using Equation 3: $G_c = G_m X_0$ |
| 0               | 61                                 | -----                                    | -----                                    |
| 0.054           | 95                                 | -----                                    | 103                                      |
| 0.1             | 105.5                              | -----                                    | 105                                      |
| 0.2             | 130.7                              | 130.7                                    | 131                                      |

Table 4 Comparisons between predicted composite modulus (Gp) using equations 2-3 and experimental data.

The results in Table 4 and Fig. 2 suggest that lower filler fractions (0.054 and 0.1) are likely to be below the mechanical gel-point. The highest filler fraction ($\phi = 0.2$) is likely to be a transition point; at or near the gel-point. This is inferred from the fact that both Equation (2) (for $\phi > \phi_G$) and Equation (3) (for $\phi < \phi_G$) provide results in good agreement with the experimental data. The fractal dimensions associated with this transition suggests that the clusters have a simple structure and straight backbone.

3.0 Delivering of synthetic DNA (CpG) to stimulate immune response of target cells using Mesoporous Silica Nanoparticles (MSN)

Synthetic DNA cytosine-phosphate-guanine oligodeoxynucleotides (CpG ODN) has proved to be effective for treating cancer, infectious diseases and allergies. The presence of the alien DNA is detected on the surface of the target cell by pattern recognition Toll-like receptors (TLR9), and the cell immune system is stimulated. However CpG ODN tends to be easily degraded by the
body’s immune mechanisms. It has been shown that when CpG ODN is attached to the surface of porous nanoparticles MSN, they are protected from this attack and can thus be delivered to targets cells. A CpG ODN delivery system has been developed [24] by binding CpG ODN non-covalently onto the modified surface of the MSNs. However, to optimise the induction rate the CpG ODN/MSN system must be designed with binding energy which is sufficient to carry the CpG load to the target cell, yet not so strongly bound that it cannot release the CpG in the vicinity of the TLR9 receptor. In this case it is assumed that Silica Nanoparticles are initially agglomerated based on micrographic evidence in Fig. 5. The Scanning Electron Microscope (SEM) image shows MSN agglomeration, while in addition the Transmission Electron Microscope (TEM) shows the internal MSN structure in which CpG accumulates. Thus there are two mechanisms by which release is restrained. The question then is how does this effect the release rate of DNA from the nanoparticle.

![Fig. 5 (A) SEM and (B) TEM images of mesoporous silica nanoparticles](image)

The experimental technique used for estimating release rate is fully described elsewhere [X]. But briefly, a set of experiments were conducted to determine the rate of free dissociation of CpG ODN from the MSN. The experiments were ex-Vivo (out-of-body) to determine the amount of CpG ODN dissociated during time intervals of 2, 5, 8 and 24 hours. No external force was applied i.e. separation of CpG from the MSN was by free dissociation. Quantitative analysis of the released CpG ODN was determined by Ultraviolet visible spectroscopy (UV-vis).

### 3.1 Analysis of Experimental Data for agglomerated CpG separation from the MSN

The data was expressed as a probability density distribution. Basically, this involved obtaining a mathematical function by curve fitting for each set of data. This mathematical function was then differentiated to determine the release rate over each time interval. Multiplying the release rate by time interval gave the mass released. The probability density of free dissociation was then obtained from the ratio of the mass released and the mass remaining on the aminated MSNs. This
was then expressed as a cumulative probability versus time. The results are shown as blue-diamonds on the graph in Fig. 6.

3.2 Theoretical Simulation of the Non-agglomerated CpG separation from the MSN

In order to compare the agglomerated experimental data with a non-agglomerated state, the CpG is assumed to be connected to the MSN by uninhibited single bonds with MSNs non-agglomerated. The probability density of release is then equal to the failure probability of a single bond with no force acting. Bell’s Reliability Theory [25] provides Equation 5:

$$\Pr(t) = \lambda e^{-\lambda t}$$  \hspace{1cm} (5)

Where: $\Pr(t)$ is the failure probability density based on Bell’s Reliability Theory

$\lambda$ is the constant probability density at time = 0 obtained from experimental data

$t$ is time corresponding to the time at which experimental data was collected

Results of from Equation 5 were expressed as a cumulative probability versus time and shown as orange-squares on the graph in Fig. 6.

3.3 Theoretical Simulation of the Non-agglomerated CpG separation from the MSN compared to Experimental data obtained for the agglomerated state

Fig 6 compares experimental data of dissociation of CpG from the MSN for agglomerated MSNs to hypothetically single-bonded CpG with non-agglomerated MSNs.

Fig. 6 Comparison of experimentally derived probability of dissociation (blue-diamonds) with calculated dissociation due to failure probability of CpG/MSN bond (Orange-squares)
The graph shows that the hypothetical non-agglomerated bonding has a greater probability of dissociation. This is to be expected since the release would be less inhibited. However, despite the large conceptual difference between the two release mechanisms the variation is relatively small; 5% after 5 hours, 15% after 15 hours and 17% after 24 hours.

4.0 Conclusions

Two applications of silica nanoparticles were considered: a nanocomposite and a delivery system for artificial DNA (CpG). In both applications experimental data was available for functioning of the agglomerated nanoparticle state. In this work a theoretical analysis is made of each case in order to gain insight into how agglomeration effects function.

For the nanocomposite, experimental results are not explained by assuming a separated nanofiller. Instead an analytical methodology based on a cluster-cluster model together with a model of the polymer chain-nanoparticle interface is successfully used to estimate composite modulus when compared to experimental data. The nanocomposite with agglomerated filler has a greater modulus than that of the separated filler. A method is presented to achieve agglomeration with very high particulate content (= 40% by weight); without aggregation.

In the case of the agglomerated CpG/MSN delivery system, a relatively simple analysis compares probability of dissociation of CpG from MSN derived from experimental results to failure probability of an non-agglomerated bond. As expected the non-agglomerate bond has a higher probability of dissociation (failure) but the difference is not great, reaching a maximum of 17% after 24 hours. This would suggest that even if it was possible a non-agglomerated system would have marginal advantages.

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