

NMR based Diffusion and Interaction Studies of Silver Nanoparticles and Triblock Copolymers

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BS-MS dual degree in Science*



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Certificate of Examination

This is to certify that the dissertation titled “NMR based diffusion and interaction studies of silver nanoparticles and triblock copolymers” submitted by Mr. Abhishek Mishra (Reg. No. MS09005) for the partial fulfillment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Kavita Dorai at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

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In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Kavita Dorai
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Abbreviations

rf	R adio F requency
AgNP	S ilver N anoparticles
PEG	P oly E thylene G lycol
PPG	P oly P ropylene G lycol
PFD	P er F luoro D ecalin
NMR	N uclear M agnetic R esonance
CPMG	C arr P urcell M eiboom G ill
1H	1 H ydrogen
^{13}C	13 C arbon
M	M agnetization
1D	1 D imensional
2D	2 D imensional
COSY	C ORrelations S pectroscop Y

Contents

Bibliography	ix
1 Basics of Nuclear Magnetic Resonance	1
1.1 NMR	1
1.2 Spin	1
1.2.1 Spin as Magnetic Moment	2
1.2.2 Larmor frequency and Magnetization	2
1.2.3 Spin packets	3
1.3 Relaxation	3
1.3.1 Longitudinal Relaxation	3
1.3.2 Transverse Relaxation	4
2 Diffusion and Pulsed Field Gradient NMR	6
2.1 Diffusion	6
2.2 Stokes Law	7
2.2.1 Derivation of Stokes Law	7
2.3 Einstein's Law	9
2.3.1 Derivation of Einstein's Law	10
2.4 Stokes Einstein equation	11
2.5 Pulsed Field Gradient	11
2.6 Stejskal Tanner Equation	12
2.6.1 Derivation of Stejskal-Tanner equation	12
2.7 Measurement of Relaxation Times	15
2.7.1 Determination of T_1	15
2.7.2 Determination of T_2	15
3 Nanoparticles and Biomolecules	18
3.1 Nanoparticles	18

3.2	Triblock copolymers	18
3.3	Dextran	19
3.4	Ficoll	19
3.5	Blood substitute	20
4	Experiments and Results	21
4.1	AgNP and triblock copolymer	21
4.2	Effect of Dextran	24
4.2.1	Experiments	24
4.3	Effect of Ficoll	26
4.3.1	Experiments	26
4.4	Effect of Perfluorodecalin	29
4.4.1	Experiments	29
5	Conclusion	33
	Bibliography	34

List of Figures

1.1	Spin Lattice relaxation.On the application of a 180 degree pulse,net magnetization get transferred in the negative z-axis.After the removal of rf pulse,the spins try to relax and get back to the equilibrium condition. ⁶	4
1.2	Spin-Spin relaxation.On the application of a 90 degree rf pulse,the net magnetization get transferred in the x-y plane.After removing the rf pulse,the spins dephase owing to different environments thus decreasing the magnitude of magnetization.	5
2.1	Larmor frequency of different spins	11
2.2	Inversion recovery experiment	16
2.3	Spinecho	17
3.1	Structure of Dextran ²⁴	19
3.2	Structure of Ficoll ²⁵	19
3.3	Structure of Perfluorodecalin ²⁶	20
4.1	1H spectra of AgNP+Triblock copolymer+D-Benzene	21
4.2	Graph of M_z vs t	22
4.3	Graph of M_{xy} vs t	22
4.4	Graph of $\ln(S/S_0)$ vs g^2	23
4.5	2D COSY of AgNP+Triblock+D-Benzene	23
4.6	1H of Dextran+AgNP+Triblock copolymer+D-Benzene	24
4.7	Graph of M_z vs t	24
4.8	Graph of M_{xy} vs t	25
4.9	Graph of $\ln(S/S_0)$ vs $-g^2$	25
4.10	2D COSY of Dextran+AgNP+Triblock+D-Benzene	26
4.11	1H spectra of Ficoll+AgNP+Triblock copolymer+D-Benzene	27
4.12	Graph of M_z vs t	27
4.13	Graph of M_{xy} vs t	28

4.14	Graph of $\ln(S/S_0)$ vs $-g^2$	28
4.15	^1H - ^1H 2D COSY of Ficoll+AgNP+Triblock copolymer+D-Benzene . .	29
4.16	^1H spectra of Perfluorodecalin+AgNP+Triblock copolymer+D-Benzene .	30
4.17	Graph of M_z vs t	30
4.18	Graph of M_{xy} vs t	31
4.19	Graph of $\ln(S/S_0)$ vs $-g^2$	31
4.20	^1H - ^1H 2D COSY spectra of Perfluorodecalin+AgNP+Triblock copolymer+D-Benzene	32

Abstract

Nanoparticles are proving to be immensely helpful in the field of medicinal research. Being smaller in size and having a higher reactivity, they are being used in various biological systems and these studies can be further used in targeted drug deliveries. Triblock copolymers are very powerful tools for biological studies as they form meshes which are quite similar in structure to the membranes present inside our body. And so diffusion and interaction of nanoparticles with these triblock copolymers can further aid to the medicinal purposes. Here we have studied the relaxation, diffusion and interaction studies of the system containing triblock copolymer mesh and silver nanoparticles.

Various chemical compounds when added to a system can change the diffusion and interactions occurring in the system. If we want to make a system more biologically favorable, we can add chemical compounds that can make it more similar to the fluids present in the body. Then studies conducted in these type of systems will yield results which are of better use. We added Dextran and Ficoll to our existent system and then did the relaxation, diffusion and interaction studies of the system.

To make the system more blood like, we added a blood substitute perfluorodecalin and then did the relaxation, diffusion and interaction studies of the system.

Chapter 1 deals with a short introduction to NMR and its basic concepts of relaxation. Chapter 2 deals with the theory regarding diffusion and the concept of pulsed field gradient. It also contains a brief overview of the methods used in the calculations of relaxation times T_1 and T_2 . Chapter 3 deals with a description of silver nanoparticles and the biomolecules used in the studies of this project. Chapter 4 deals with the experiments performed on various systems. It also contains the results of these experiments and an analysis of these results. Chapter 5 deals with the conclusions drawn from these experiments.

Chapter 1

Basics of Nuclear Magnetic Resonance

1.1 NMR

The phenomenon of NMR was first studied by Isidor Rabi in 1938. Rabi used the Stern-Gerlach experiment and modified it to study NMR.¹ Later significant developments in this field were made by Bloch and Purcell who extended it to study liquid systems.² NMR can be understood as a phenomenon which occurs when the nuclei of certain atoms are immersed in a static magnetic field and are exposed to a second oscillating magnetic field. Some nuclei show this phenomena while the others doesn't. Thus the nuclei which exhibit it must possess some property which is responsible for NMR. This property is known as spin.

1.2 Spin

Spin is a fundamental property just like electric charge and can be understood as an intrinsic form of angular momentum. This spin is not the spin that signifies the rotational motion in Classical Physics but is purely a quantum mechanical phenomena. It can have half integral or integral values. Protons, electrons and neutrons all possess a spin whose value is $1/2$.

1.2.1 Spin as Magnetic Moment

If we consider a proton, then its spin can be understood in terms of a magnetic moment vector. The magnitude of this magnetic moment vector depends on the value of the spin angular momentum which is given by the relation

$$\mu = \gamma I \quad (1.1)$$

where μ is the magnetic moment, γ is the gyromagnetic ratio and I is the spin angular momentum.

1.2.2 Larmor frequency and Magnetization

When a proton is placed in a magnetic field, it will try to align itself in the direction of the applied magnetic field. This will lead to two configurations; one in which all spins are aligned in the direction of the magnetic field and the other in which they are aligned opposite to the magnetic field. The angular momentum of the nucleus will lead to a precessional motion of magnetic moment vector around the z-axis (if the external magnetic field is applied in z-axis). The angular frequency of this precessional motion is known as Larmor frequency and is given by the formula

$$\omega = -\gamma B_0 \quad (1.2)$$

where B_0 is the magnitude of the external magnetic field.

In the presence of this magnetic field, the energy of the spin states depend on the magnetic moment and the strength of the magnetic field and is given by

$$E = -\mu \cdot B = -\gamma I \cdot B \quad (1.3)$$

At room temperature, the number of spins in the lower energy state are more than that in the higher energy state in accordance with the Boltzmann distribution which is given by

$$\frac{N_\beta}{N_\alpha} = \exp\left(-\frac{(E_\beta - E_\alpha)}{k_B T}\right) \quad (1.4)$$

where α and β are the energy levels, E_α and E_β are their energies, N_α and N_β are the number of spins in the energy levels respectively, k_B is the Boltzmann constant and T is the thermodynamic temperature of the spin system.⁴

The difference in the number of the spins in the two energy states leads to the devel-

opment of a net magnetization in the sample. The signal in NMR spectroscopy results when transition between these states occur. So Nuclear Magnetic Resonance (NMR) can be defined as a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. The redistribution of the net magnetization can be done by applying a radio frequency pulse whose frequency matches with the Larmor Precession Frequency of the nuclei.⁴

1.2.3 Spin packets

It is quite cumbersome to describe NMR on the basis of a single spin when we talk about complex systems. So if we can think of a group of spins that experience the same magnetic field, then this problem becomes easy. These groups are called **Spin Packets**.³ Each spin packet has a net magnetization which is represented by a magnetization vector. The vector sum of these magnetization vectors gives us the net magnetization of the system.

1.3 Relaxation

Relaxation can be understood as a process or number of processes by which nuclear magnetization in a system in its state of disturbance return to that of the equilibrium distribution.⁴ To get a NMR signal, a strong radio frequency radiation is applied to the sample at the appropriate frequency so that flipping of spins can occur. From quantum mechanical point of view, the RF photons are absorbed by some of the protons to flip them from parallel to the magnetic field to anti-parallel which is a higher energy state.⁵

When the exciting RF field is switched off, the protons return to their lower energy state. So they relax back to the state where their spins are parallel to the static magnetic field. This transition produces a small amount of RF radiation which is detected as the NMR signal.⁵

1.3.1 Longitudinal Relaxation

At equilibrium, the net magnetization vector M points in the direction of the applied magnetic field B_0 and the value of this magnetization is called the equilibrium magnetization M_0 . On applying frequencies equal to the difference in the energy states, the

value of M starts decreasing and soon reaches zero. This decrease in the value of net magnetization is exponential in nature and is given by the relation

$$M_z = M_0(1 - e^{-\frac{t}{T_2}}) \quad (1.5)$$

where T_2 is a constant and is known as spin-lattice relaxation time (initially solids were used in the study of NMR and hence the term spin lattice). If we apply a 180 degree pulse, then the net magnetization will get transferred in the $-z$ direction. Thus, its characteristic equation will be given by

$$M_z = M_0(1 - 2e^{-\frac{t}{T_2}}) \quad (1.6)$$

The following figure shows spin-lattice relaxation.

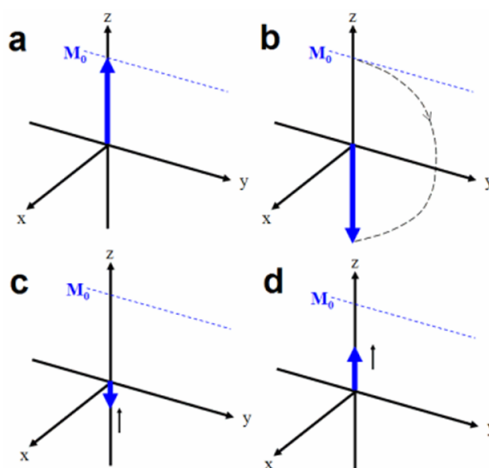


Figure 1.1: Spin Lattice relaxation. On the application of a 180 degree pulse, net magnetization gets transferred in the negative z -axis. After the removal of rf pulse, the spins try to relax and get back to the equilibrium condition.⁶

1.3.2 Transverse Relaxation

Each spin packet experiences a slightly different magnetic field due to its environment. Thus the net magnetization starts to dephase and each spin packet rotates at its own Larmor frequency.³ So when we apply a 90 degree pulse to the nuclei, the net magnetization gets transferred in the x - y plane. The value of the net magnetization then starts decreasing because of different fields. This decrease is also exponential but

is different from that in the case of spin lattice relaxation and is given by

$$M_{xy} = M_0 e^{-\frac{t}{T_2}} \quad (1.7)$$

where T_2 is constant and is known as spin spin relaxation time.

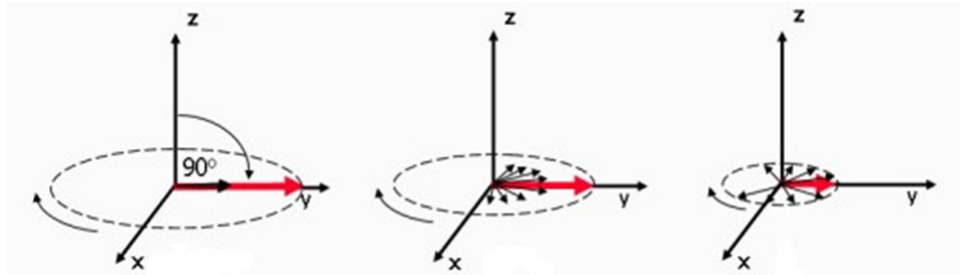


Figure 1.2: Spin-Spin relaxation. On the application of a 90 degree rf pulse, the net magnetization gets transferred in the x-y plane. After removing the rf pulse, the spins dephase owing to different environments thus decreasing the magnitude of magnetization.

Chapter 2

Diffusion and Pulsed Field Gradient NMR

2.1 Diffusion

Diffusion is the random translational motion of particles that happens because of thermal energy of the particles.⁹ Diffusion is a very important physical phenomenon as it leads to collisions between the particles in a system which results in chemical reactions. Diffusion is governed by two laws known as Fick's Laws.⁹

Fick's first law states that particles in a medium move from regions of higher concentration to regions of lower concentration and the magnitude of the flux is proportional to rate of change of concentration gradient.⁹ Thus,

$$\frac{\partial J}{\partial x} = -D \frac{\partial C(x, t)}{\partial x} \quad (2.1)$$

where D is a constant and is known as diffusion coefficient. It is measured in the units of cm^2s^{-1} or m^2s^{-1} . Fick's second law states that change in the concentration of particles over time is equal to the change in local diffusion flux.⁹ Thus,

$$\frac{\partial C(x, t)}{\partial t} = -\frac{\partial J}{\partial x} \quad (2.2)$$

Combining the two laws, we get;

$$\frac{\partial C(x, t)}{\partial t} = D \frac{\partial^2 C(x, t)}{\partial x^2} \quad (2.3)$$

In an isotropic system, without thermal or concentration gradients, the average molecule displacement in all three directions is zero. But the mean square displacement is non-zero and can be calculated by treating diffusion as random walk. The mean square displacement by that notion comes out to be

$$\langle r^2 \rangle = 6Dt \quad (2.4)$$

If we assume the diffusion is taking place only in a single direction (1D), then

$$\langle r^2 \rangle = 2Dt \quad (2.5)$$

2.2 Stokes Law

This law was given by George Gabriel Stokes in 1851 and talks about the drag experienced by a sphere (particles are generally assumed to be perfect spheres). Mathematically it is given by

$$F = 6\pi\eta rv \quad (2.6)$$

where F is the force experienced by the particle (sphere), r is the radius of the sphere, η is the viscosity of the medium and v is the velocity of the sphere in the medium. This formula applies to only those flows where Reynold's number ($Re = \frac{\rho r v}{\eta}$) is less than unity. So this law is applicable to spheres with smaller radii and present in media with lower densities.

2.2.1 Derivation of Stokes Law

Consider a spherical coordinate system where a sphere of radius $r=a$ is placed at the origin. Since the divergence of the velocity field is zero, we introduce a function $\psi(r, \theta)$ such that

$$v_r = \frac{1}{r^2 \sin \theta} \frac{\partial \psi}{\partial \theta} \quad (2.7)$$

$$v_\theta = -\frac{1}{r \sin \theta} \frac{\partial \psi}{\partial r} \quad (2.8)$$

Since we are considering the case of an incompressible fluid, $\nabla^2 v$ can be replaced with $-\text{curl}(\text{curl} v)$.¹² Therefore, the momentum equations can be written as

$$\frac{\partial p}{\partial r} = \frac{\eta}{r^2 \sin \theta} \frac{\partial(Q\psi)}{\partial \theta} \quad (2.9)$$

$$\frac{\partial p}{\partial \theta} = -\frac{\eta}{r \sin \theta} \frac{\partial(Q\psi)}{\partial r} \quad (2.10)$$

where Q is an operator and is defined as

$$Q = \frac{\partial^2}{\partial r^2} + \frac{\sin \theta}{r^2} \frac{\partial}{\partial \theta} \left[\frac{1}{\sin \theta} \frac{\partial}{\partial \theta} \right] \quad (2.11)$$

From the two momentum equations, we can eliminate p and thus can get a fourth order partial differential equation-

$$Q^2 \psi = 0 \quad (2.12)$$

To solve further we make the following substitution-

$$\psi = f(r) \sin^2 \theta \quad (2.13)$$

This allows us to reduce the equation to a fourth order ODE of the standard Euler type

$$r^4 f'''' - 4r^2 f'' + 8r f' - 8f = 0 \quad (2.14)$$

Next, by using a simple substitution $f = r^k$ we can get a fourth order polynomial in k whose roots are $k=-1, 1, 2,$ and 4 .¹² And thus the general solution for f becomes

$$f(r) = \frac{A}{r} + Br + Cr^2 + Dr^4 \quad (2.15)$$

Now we have four constants $A, B, C,$ and D whose value we can calculate from the fact that both velocity components vanish at the sphere surface and that v_r goes to $v_0 \cos \theta$ as r starts attaining larger values.¹² Thus taking these things into account, we get-

$$\psi(r, \theta) = v_0 \left[\frac{a^3}{4r} - \frac{3ar}{4} + \frac{r^2}{2} \right] \sin^2 \theta \quad (2.16)$$

Using the last result and the definitions for the velocity components and radial pressure gradient given above, we get

$$v_r = v_0 \left[\frac{a^3}{2r^3} - \frac{3a}{2r} + 1 \right] \cos \theta \quad (2.17)$$

$$v_\theta = v_0 \left[\frac{a^3}{4r^3} + \frac{3a}{4r} - 1 \right] \sin \theta \quad (2.18)$$

$$p = - \left[\frac{3a\eta v_0}{2r^2} \right] \cos \theta \quad (2.19)$$

If we now integrate the shear stress and normal stress over the entire surface of the sphere, then we get the drag force.¹² The shear stress has the form

$$\tau_{r\theta} = -\eta \left[r \frac{\partial v_\theta}{\partial r} \frac{1}{r} + \frac{1}{r} \frac{\partial v_r}{\partial \theta} \right] \quad (2.20)$$

Now we do the integration and get the force associated with the pressure field in the z direction

$$F_{pressure} = 2\pi a^2 \int_0^\pi p \sin \theta \cos \theta d\theta = 2\pi a\eta v_0 \quad (2.21)$$

The force in the z direction due to viscous shear stress is

$$F_{shear} = 2\pi a^2 \int_0^\pi \tau_{r\theta} \sin^2 \theta d\theta = 4\pi a\eta v_0 \quad (2.22)$$

Adding the two forces we get

$$F_{Total} = 6\pi a\eta v_0 \quad (2.23)$$

which is the Stokes Law.¹²

2.3 Einstein's Law

Einstein from his study of Brownian motion of particles discussed in his 1905 paper that

$$D = \mu k_B T \quad (2.24)$$

where D is the diffusion coefficient, μ is the mobility and is equal to $\frac{v}{F}$, k_B is Boltzmann constant and T is the temperature.¹¹

2.3.1 Derivation of Einstein's Law

Here diffusion is discussed for motion in 1D but same analogy can be worked out for 2D and 3D also. Suppose there is potential energy U which creates a force $F = -\frac{dU}{dx}$ on a particle. But there are many particles in the system. So two things can happen. First, the particles can pile up around the region of low U (drift current) and second, particles can move out due to diffusion (diffusion current). At equilibrium, these currents balance each other.¹⁰ The net flow of particles due to drift current is

$$J_{drift}(x) = \mu F(x)\rho(x) = -\rho(x)\mu \frac{dU}{dx} \quad (2.25)$$

The net flow of particles due to diffusion current is

$$J_{diff}(x) = -D \frac{d\rho}{dx} \quad (2.26)$$

But sum of these two current at equilibrium must be zero. Thus,

$$0 = J_{drift} + J_{diff} = -\rho(x)\mu \frac{dU}{dx} - D \frac{d\rho}{dx} \quad (2.27)$$

From Boltzmann statistics, we can say that

$$\rho(x) = Ae^{\frac{U}{k_B T}} \quad (2.28)$$

Applying chain rule, we get;

$$\frac{d\rho}{dx} = -\frac{1}{k_B T} \frac{dU}{dx} \rho(x) \quad (2.29)$$

Putting this in the equilibrium equation, we get

$$0 = J_{drift} + J_{diff} = -\rho(x)\mu \frac{dU}{dx} + \frac{D}{k_B T} \frac{dU}{dx} \rho(x) = -\rho(x) \frac{dU}{dx} \left(\mu - \frac{D}{k_B T} \right) \quad (2.30)$$

Since the above equation must hold everywhere, we have;

$$D = \mu k_B T \quad (2.31)$$

which is the Einstein's equation.¹⁰

2.4 Stokes Einstein equation

Combining the Stokes Law and Einstein equation, we get the Stokes Einstein equation which is given by the formula

$$D = \frac{k_B T}{f} \quad (2.32)$$

$$f = 6\pi\eta r v \quad (2.33)$$

2.5 Pulsed Field Gradient

In normal experiments we only use a constant external field B_0 . This magnetic field makes the spins precess at Larmor frequency which is given by

$$\omega_0 = \gamma B_0 \quad (2.34)$$

If we apply an another magnetic field B_z , then the effective Larmor frequency is given by

$$\omega_{eff} = \omega_0 + \gamma B_z \quad (2.35)$$

Now if we keep the magnitude of the magnetic field gradient to be constant ($g = \frac{\partial B_z}{\partial z}$), then the effective frequency can be written as

$$\omega_{eff} = \omega_0 + \gamma g z \quad (2.36)$$

In Figure 2.1, the effective Larmor frequency of different spins in a sample is shown.

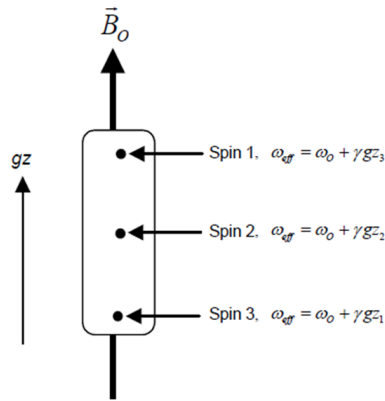


Figure 2.1: Larmor frequency of different spins

2.6 Stejskal Tanner Equation

The PFG-NMR method was first used by Stejskal and Tanner to study diffusion.²⁰ They varied the gradient strength of the magnetic field and observed the attenuation of the signals and related the value of gradient strength to the intensity of the signals. They derived the Stejskal Tanner equation which is given by

$$\log_e \left(\frac{A(2\tau)}{A(0)} \right) = -D\gamma^2\delta^2g^2 \left(\Delta - \frac{\delta}{3} \right) \quad (2.37)$$

where $A(2\tau)$ is the intensity of the signal in the presence of the magnetic field gradient, $A(0)$ is the intensity of the signal in the absence of the magnetic field gradient, D is the diffusion coefficient, γ is the gyromagnetic ratio, δ is the gradient duration, g is the gradient strength and Δ is the diffusion time.

2.6.1 Derivation of Stejskal-Tanner equation

The magnetizations in the xy plane can be written as

$$M_{x,y} = M_x + iM_y \quad (2.38)$$

But the spins precess with a frequency ω around the direction of the magnetic field and thus the above relation can be written as

$$M_{x,y} = M_0(\cos \omega t + i \sin \omega t) = M_0 e^{i\omega t} \quad (2.39)$$

But since the transverse magnetization decays with T_2 , we can write the transverse magnetization as

$$M_{x,y} = M_0 e^{(i\omega - \frac{1}{T_2})t} \quad (2.40)$$

If we have a magnetic field gradient of magnitude G in the z direction, then the total magnetic field in the z-direction is

$$B_z = B_0 + G.r \quad (2.41)$$

where r is the position vector for a given nucleus. The Larmor frequency in the rotating frame can be written as

$$\omega_R = -\gamma G.r \quad (2.42)$$

Therefore, in the rotating frame the magnetization in the xy plane can be written as

$$M_{x,y} = M_0 e^{(-i\gamma G.r)t} \quad (2.43)$$

In the above equation, we have removed the terms containing T_2 and have considered the system to not undergo transverse spin-spin relaxation as we here are only concerned with the signal attenuation that occurs due to diffusion with and without the magnetic field gradient and so the spin-spin relaxation will be normalized out in our data analysis. Replacing $M_{x,y}$ by ψ for further calculations, we have

$$\psi = M_0 e^{(-i\gamma G.r)t} \quad (2.44)$$

Differentiating ψ with respect to time we have

$$\frac{\partial \psi}{\partial t} = -i\gamma G.r M_0 e^{(-i\gamma G.r)t} = -i\gamma G.r \psi \quad (2.45)$$

Integrating the above equation we get;

$$\begin{aligned} \int_{\psi_0}^{\psi} \frac{\partial \psi'}{\partial t'} &= \int_0^t -i\gamma G.r \psi \, dt' \\ \Rightarrow \psi &= \psi_0 e^{-i\gamma r.F(t)} \end{aligned} \quad (2.46)$$

where

$$F(t) = \int_0^t G(t') dt' \quad (2.47)$$

Now if we consider the diffusion of the spins, then by Fick's second law of diffusion

$$\frac{\partial \psi}{\partial t} = D \nabla^2 \psi \quad (2.48)$$

Thus, the total rate of change of transverse magnetization can be written as

$$\frac{\partial \psi}{\partial t} = -i\gamma G.r \psi + D \nabla^2 \psi \quad (2.49)$$

Since diffusion is taking place, we need to replace ψ with a function that is dependent on t . Let it be $A(t)$. Then, ψ can be written as

$$\psi = A(t) e^{-i\gamma r.F(t)} \quad (2.50)$$

At a later time in the pulse sequence the π RF pulse rotates the position of the magnetization vector about the axis of the pulse (or it can be said to have been advanced by twice the angle it had previously precessed in time τ).²¹ So, we replace the exponent in the expression:

$$\psi = A(t)e^{-i\gamma r(F(t)-2f)} \quad (2.51)$$

where f is a constant defined by $f \equiv F(\tau)$, and it corresponds to the phase angle that has accumulated between the $\pi/2$ and π RF pulses, due to the gradients. But the above equation does not correctly describe the magnetization before the π pulse. Hence, a new parameter, $\xi = 1$ for $t < \tau$ and $\xi = -1$ for $t > \tau$ is introduced to set up a single expression for the magnetization before and after the π pulse:

$$\psi = A(t)e^{-i\gamma r(F(t)+(\xi-1)f)} \quad (2.52)$$

Differentiating the above equation, we get:

$$\frac{\partial \psi}{\partial t} = \frac{dA(t)}{dt} e^{-i\gamma r(F(t)+(\xi-1)f)} - A(t)i\gamma r \cdot \left(\frac{dF(t)}{dt} + (\xi-1)\frac{df}{dt} \right) e^{-i\gamma r(F(t)+(\xi-1)f)} \quad (2.53)$$

where

$$\frac{dF(t)}{dt} = G(t)$$

and

$$\frac{df}{dt} = 0$$

as f is a constant. From equations 2.49, 2.50 and taking Laplacian double derivative wrt r , we have;

$$D\nabla^2 A(t)e^{-i\gamma r(F(t)+(\xi-1)f)} = -DA(t)(\gamma(F(t) + (\xi-1)f))^2 e^{-i\gamma r(F(t)+(\xi-1)f)} \quad (2.54)$$

Substituting 2.53 in 2.50, we have;

$$\frac{\partial \psi}{\partial t} = -i\gamma G.r\psi - DA(t)(\gamma(F(t) + (\xi-1)f))^2 e^{-i\gamma r(F(t)+(\xi-1)f)} \quad (2.55)$$

From equations 2.52, 2.53 and 2.55 and putting $\psi = -i\gamma(F(t) + (\xi-1)f)$ we have;

$$\frac{dA}{dt} e^\phi - A(t)(i\gamma r.G)e^\phi = -A(t)(i\gamma r.G)e^\phi - DA(t)(\gamma(F(t) + (\xi-1)f))^2 e^\phi \quad (2.56)$$

Dividing both sides by e^ϕ and doing some rearrangements, we have;

$$\frac{dA}{dt} = -A(t)D(\gamma(F(t) + (\xi - 1)f))^2 \quad (2.57)$$

Integrating the above equation using separation of variables we have;

$$\int_{A(0)}^{A(2\tau)} \frac{dA(t)}{A(t)} = -D\gamma^2 \int_0^{2\tau} (F(t) + (\xi - 1)f)^2 dt \quad (2.58)$$

This results in

$$\log_e \left(\frac{A(2\tau)}{A(0)} \right) = -D\gamma^2 \delta^2 g^2 \left(\Delta - \frac{\delta}{3} \right) \quad (2.59)$$

which is the Stejskal Tanner equation as mentioned earlier.²¹

2.7 Measurement of Relaxation Times

Methods used for T_1 and T_2 determination are described in the following subsections.

2.7.1 Determination of T_1

The prominent method used to calculate T_1 is the inversion recovery method.¹³ Non selective rf pulses are used and then the value of delay is changed. So the size of the peak in different spectra change. The required peak is selected and its area is integrated and the signal intensity of the various other peaks are calculated with respect to this peak. And so we get varying values of signal intensities. Then a graph between signal intensities and delay are plotted and then fitted using the characteristic T_1 equation. As mentioned above, inversion recovery involves the usage of two rf pulses separated by τ . The experiment is done and the pulse sequence is repeated with keeping a long interval τ_{wait} thus giving the spins time to come back to the equilibrium state. For each value of τ , we repeat the pulse sequence and acquire the data number of times. Each repetition of the pulse sequence is separated by a long interval τ_{wait} , so that spins can return to thermal equilibrium state.¹³

2.7.2 Determination of T_2

The prominent method used for the calculation of T_2 is the CPMG spin echo method.

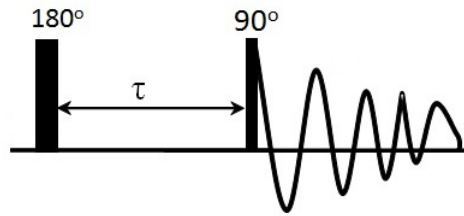


Figure 2.2: Inversion recovery experiment

Spin Echo

It was proposed by Hahn who pointed out that intensity of the magnetization signals will be affected if diffusion is taking place in a system.¹⁵ Spin echo tells us about the self diffusion which in turn can provide us with detailed information about molecular organizations and phase structures.

The spin echo sequence is given in the following figure. Initially, a magnetic field is applied and thus magnetization is pointing in the z-direction. Then, a 90 pulse is applied along the x direction. This shifts the magnetization in the xy-plane. After that the RF pulse is removed.

During the period of time following the removal the RF pulse, each spin experience a slight variation in magnetic field and thus starts to scatter in the xy-plane. These variations in the magnetic field come due to transverse relaxation and inhomogeneities in the external field. Now after a time τ , a 180 degree pulse is applied which reverses the effect of these homogeneities. The spins which were traveling faster gets behind and those traveling slower gets ahead. And because of this, the spins all gather up in the end after time τ resulting in the signal and hence forming a echo.¹⁵

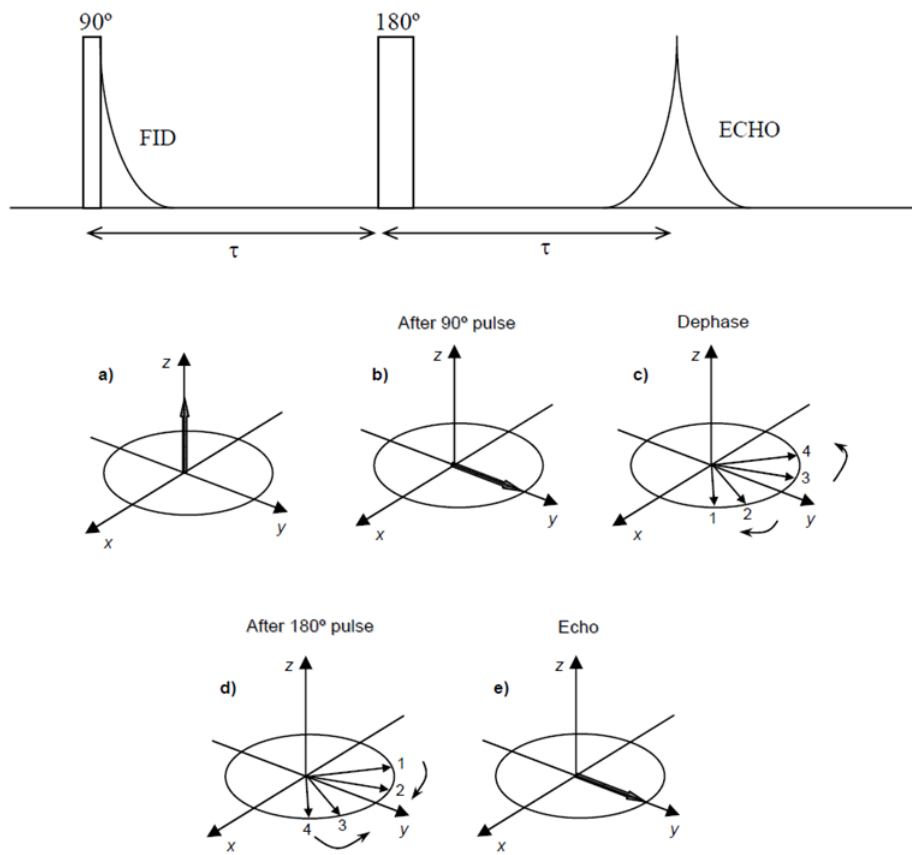


Figure 2.3: Spinecho

Chapter 3

Nanoparticles and Biomolecules

3.1 Nanoparticles

As the name suggests, nanoparticles are particles with radii in nanometers. They can be classified into three categories on the basis of their size. Coarse particles whose size varies between 2500 and 10000 nm, fine particles whose size varies between 100 and 2500 nm and ultrafine nanoparticles whose size varies between 1 and 100 nm.¹⁶ Here it is important to note that size of various molecules and other units come near to the size of nanoparticles but they are not classified as nanoparticles as behaving as a single entity is important which they might not show. The reactivity of a material is more if it is made up of nanoparticles as they have a greater surface area per weight than their counterparts.

Their size make them important to be used as probes in various biological environments. They find great usage in the fields of medicine. Nanoparticles made from the oxides of Cerium are very useful in absorbing oxygen and thus are helpful in oxygen transport. They are also being used to deliver vaccines and in chemotherapy to treat brain tumour. They are also being heavily used in the manufacturing and energy sectors owing to their properties.

3.2 Triblock copolymers

Copolymers are polymers that consists of atleast two types of constituent or structural units. They can be classified into various categories and block copolymer is one of them.¹⁷ These block copolymers have two or more homopolymer subunits which are

linked through covalent bonds. A block copolymer with three distinct blocks is known as a Triblock copolymer. I used Polyethylene Glycol(PEG)-Polypropylene Glycol(PPG)-Polyethylene Glycol(PEG) as the triblock copolymer in my experiments and studies. They vary their configurations and can form meshes depending on their concentrations in the solutions. Thus they are very helpful in the studies of target drug deliveries where they can be used as vesicles for nanoparticles .

3.3 Dextran

Dextran is a complex organic compound made up of polysacchrides of glucose. It is widely used to reduce the viscosity of human blood and as a volume expander in the case of anemia.¹⁸

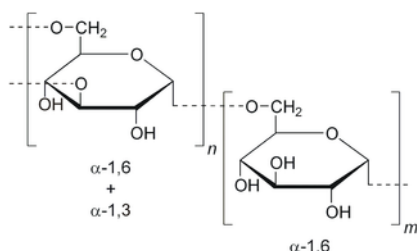


Figure 3.1: Structure of Dextran²⁴

3.4 Ficoll

Ficoll is complex organic compound consisting of polysaccharides. The compound on a whole is neutral and is highly branched. It is often used in the laboratories to separate the various constituents of human blood.²³

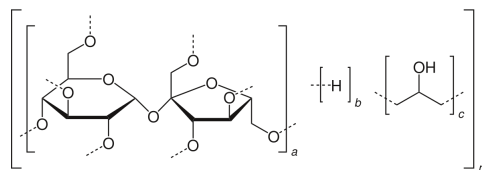


Figure 3.2: Structure of Ficoll²⁵

3.5 Blood substitute

A blood substitute can be something which can more or less mimic the properties of human blood. They are helpful in the cases of blood transfusion and can also be used as volume expanders. There are mainly two types of blood substitutes on which biological research is going on. One of them is hemoglobin based whereas the other one is perfluorocarbon based. The perfluorocarbon based blood substitutes are immiscible in water and therefore are used as emulsions by dispersing small drops in water. One of the important perfluorocarbon that is used for blood substitute is perfluorodecalin. Perfluorodecalin has been used to dissolve high amount of Oxygen (100 ml of perfluorodecalin at 25°C will dissolve 49 ml of oxygen at STP). It was also used as a component in the manufacture of Flusol which was once used for the treatment of heart attacks and in the cases of carbon monoxide poisoning.²²

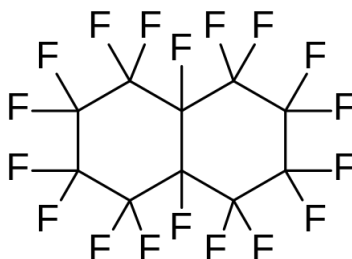


Figure 3.3: Structure of Perfluorodecalin²⁶

Chapter 4

Experiments and Results

4.1 AgNP and triblock copolymer

A system containing AgNP(3-7nm in size and suspended in Decanethiol),Triblock copolymer(PEG-PPG-PEG) and D-Benzene was taken.The 1H spectra of the solution is given below.

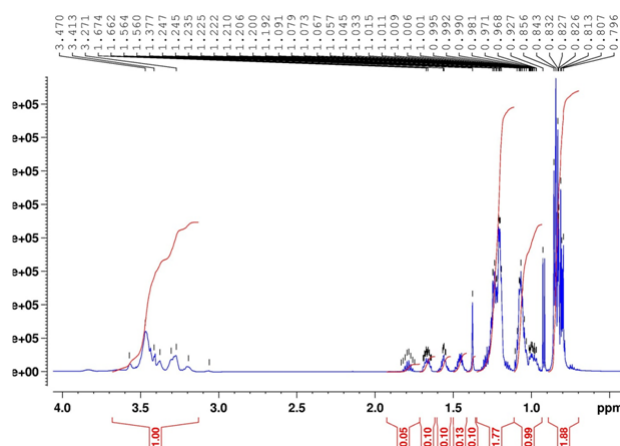


Figure 4.1: 1H spectra of AgNP+Triblock copolymer+D-Benzene

Silver Nanoparticles signals come in the range (0.5-1.8 ppm).¹⁹The region around of (0.80-1.30) and (3.2 - 3.6) corresponds to the signals of triblock copolymers.¹⁹The addition of silver nanoparticles to triblock copolymer leads to some overlapping of the spectra of the two constituents. The t1ir1d sequence was run to calculate T_1 of triblock copolymers.A set of readings were taken by varying the time,then the graph of M vs t was plotted and the value of T_1 was calculated which came to be 0.938 seconds.

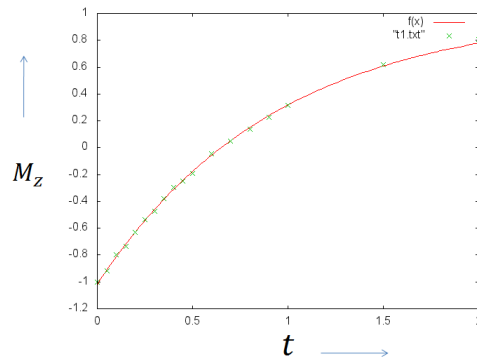


Figure 4.2: Graph of M_z vs t

Then the cpmgd1d sequence was run and T_2 was calculated by varying the time 2τ . The value of T_2 was found to be 0.85203 seconds.

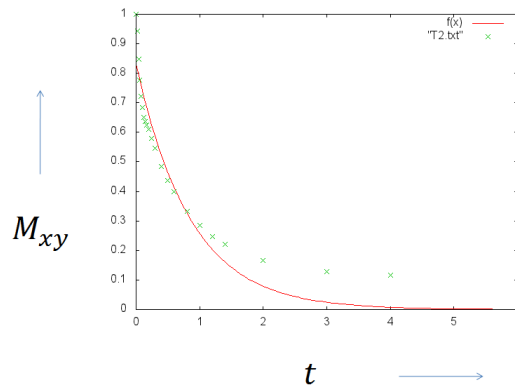


Figure 4.3: Graph of M_{xy} vs t

The ledbpgp2s sequence was used and the readings of intensity of the signals were taken while varying the gradients from 5 percent to 95 percent. The graph between log of the intensity and -ive of the square of the gradient was plotted and the diffusion coefficient was calculated using the Stejskal-Tanner equation. The value of D was found to be $1.6 \times 10^{-6} m^2 s^{-1}$.

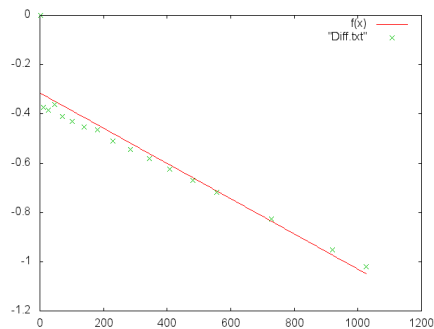


Figure 4.4: Graph of $\ln(S/S_0)$ vs g^2

If only the COSY spectra of AgNP is observed, there is no correlation peaks around (3.90 and 3.95 ppm).⁷ 2D COSY spectra of triblock copolymers alone reveal the existence of peaks around (3.90 and 3.95 ppm).⁷ But the 2D COSY spectra of AgNP+Triblock shows some shifting in these peaks suggesting the fact silver nanoparticles are binding to triblock copolymers at some sites.

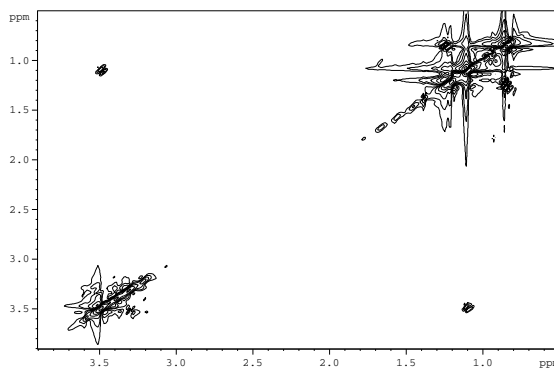


Figure 4.5: 2D COSY of AgNP+Triblock+D-Benzene

4.2 Effect of Dextran

4.2.1 Experiments

Small amounts of Dextran was added to the previous system of AgNP and triblock copolymer.

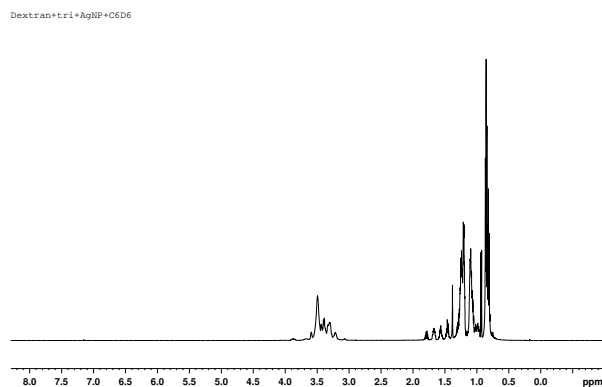


Figure 4.6: ^1H of Dextran+AgNP+Triblock copolymer+D-Benzene

Then the t1r1d sequence was run to calculate T_1 of this system which came to be 0.923 seconds.

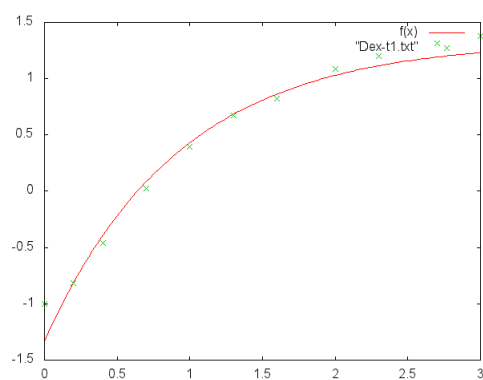


Figure 4.7: Graph of M_z vs t

Then the cpmg1d sequence was run and T_2 was calculated by varying the time 2τ . The value of T_2 was found to be 0.352 seconds.

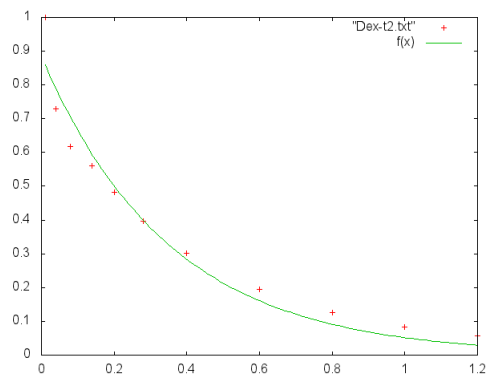


Figure 4.8: Graph of M_{xy} vs t

The ledbpgp2s sequence was used to calculate the diffusion coefficient whose value came to be $1.917 \times 10^{-6} m^2 s^{-1}$. Dextran is often used to reduce the viscosity of a medium. Using 2.32 and 2.33, we can assume that it must increase the diffusion coefficients. This is in accordance with the value of the diffusion coefficient mentioned above.

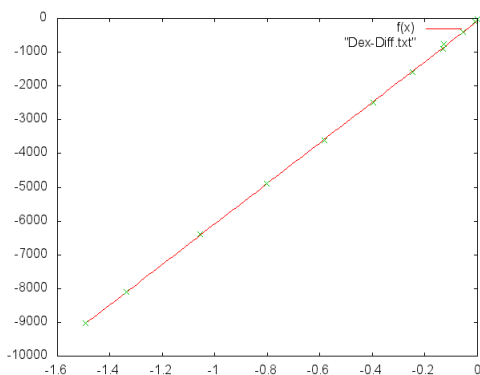


Figure 4.9: Graph of $\ln(S/S_0)$ vs $-g^2$

The 2D COSY spectra was taken to study the correlation. There are no new peaks which suggest that the addition of dextran has not affected in the interactions happening between AgNP and triblock copolymer mesh.

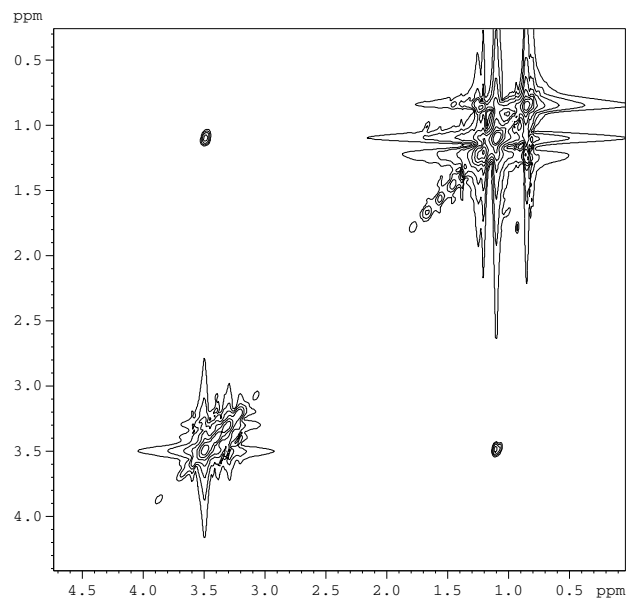


Figure 4.10: 2D COSY of Dextran+AgNP+Triblock+D-Benzene

4.3 Effect of Ficoll

4.3.1 Experiments

Small amounts of Ficoll was added to the previous system of AgNP and triblock copolymer.

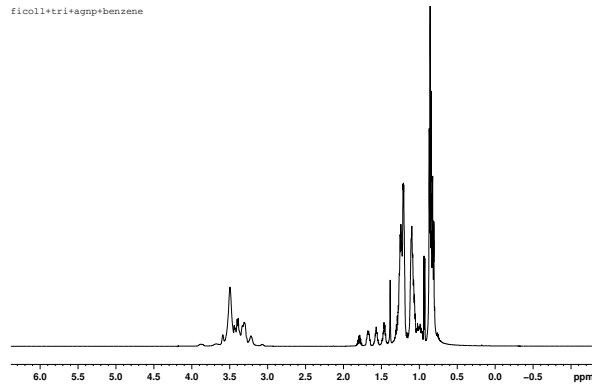


Figure 4.11: ^1H spectra of Ficoll+AgNP+Triblock copolymer+D-Benzene

Then the `t1r1d` sequence was run to calculate T_1 of this system which came to be 0.949 seconds.

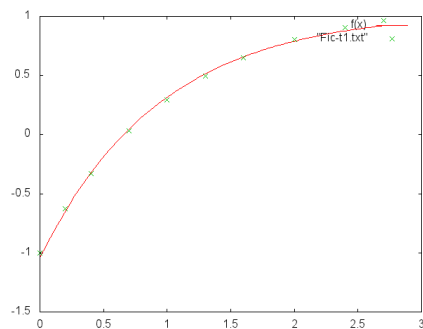


Figure 4.12: Graph of M_z vs t

Then the `cpmg1d` sequence was run and T_2 was calculated by varying the time 2τ . The value of T_2 was found to be 0.352 seconds.

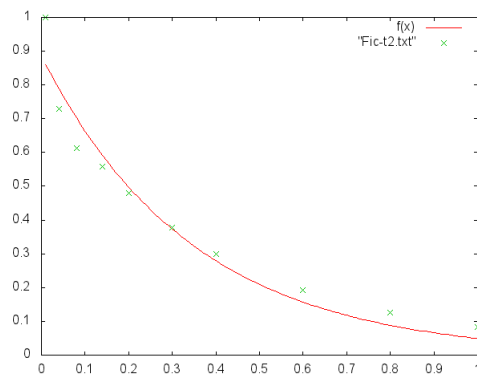


Figure 4.13: Graph of M_{xy} vs t

The ledbpgp2s sequence was used to calculate the diffusion coefficient whose value came to be $1.198 \times 10^{-6} m^2 s^{-1}$. The ficoll added to the system may have decreased the free motion of the various constituents of the system thus decreasing the diffusion coefficient.

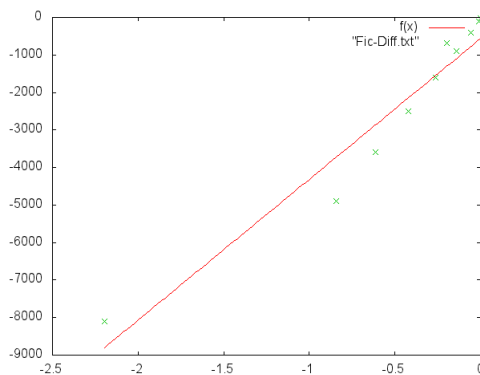


Figure 4.14: Graph of $\ln(S/S_0)$ vs $-g^2$

The 2D COSY spectra was taken to study the correlation. Appearance of some small peaks near the diagonals suggest that the addition of ficoll might have affected the binding of AgNP to triblock copolymers.

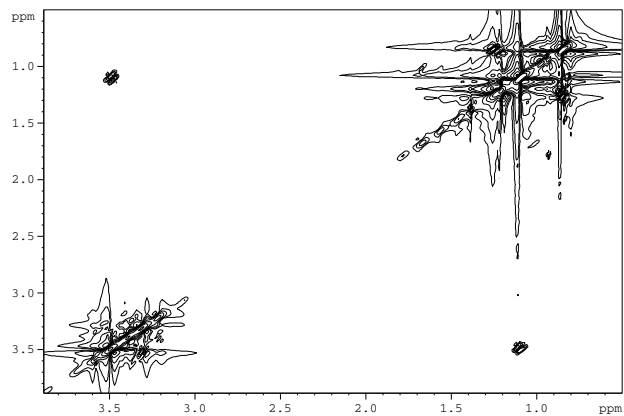


Figure 4.15: ^1H - ^1H 2D COSY of Ficoll+AgNP+Triblock copolymer+D-Benzene

4.4 Effect of Perfluorodecalin

4.4.1 Experiments

Small amounts of perfluorodecalin was added to the previous system of AgNP and triblock copolymer.

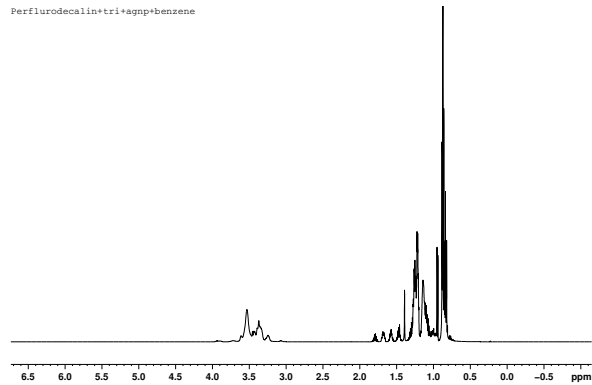


Figure 4.16: ^1H spectra of Perfluorodecalin+AgNP+Triblock copolymer+D-Benzene

Then the t1r1d sequence was run to calculate T_1 of this system which came to be 0.969 seconds.

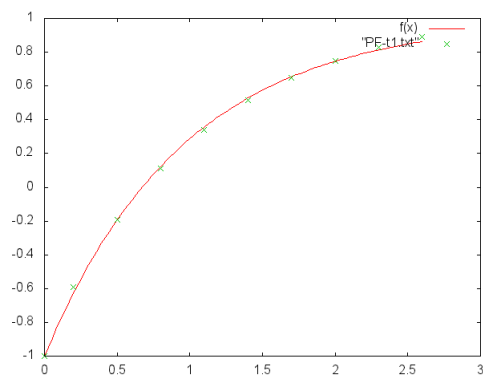


Figure 4.17: Graph of M_z vs t

Then the cpmgd1d sequence was run and T_2 was calculated by varying the time 2τ . The value of T_2 was found to be 0.568 seconds.

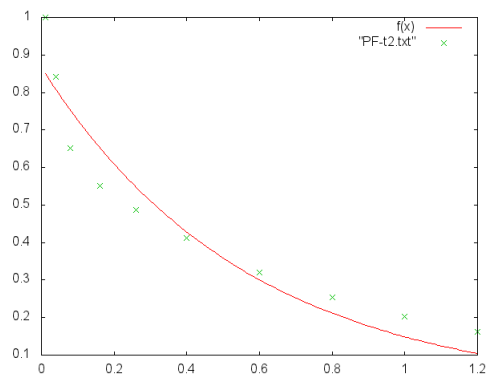


Figure 4.18: Graph of M_{xy} vs t

The ledbpgp2s sequence was used to calculate the diffusion coefficient whose value came to be $1.609 \times 10^{-6} m^2 s^{-1}$. There is a slight decrease in the value of the diffusion coefficient which can be due to the addition of perfluorodecalin to AgNP and triblock copolymers.

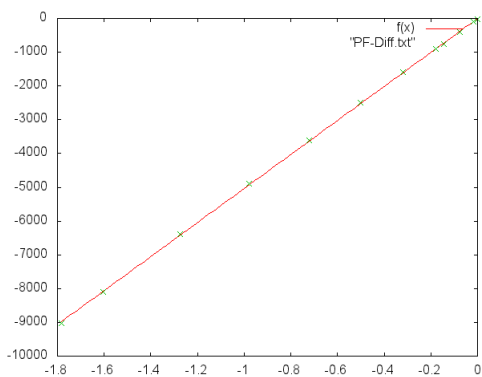


Figure 4.19: Graph of $\ln(S/S_0)$ vs $-g^2$

The 2D COSY spectra was taken to study the correlation. The 2D COSY spectra shows no significant different peaks suggesting the presence of perfluorodecalin has not affected the interaction and binding between AgNP and triblock copolymers.

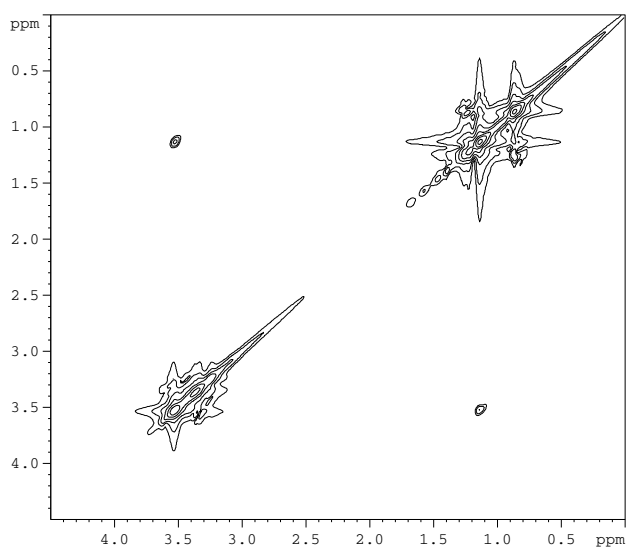


Figure 4.20: ¹H-¹H 2D COSY spectra of Perfluorodecalin+AgNP+Triblock copolymer+D-Benzene

Chapter 5

Conclusion

2D COSY and diffusion studies of AgNP in triblock copolymer and D-Benzene solution suggest that the nanoparticles are binding to some of the sites in the triblock copolymer mesh. Addition of Dextran to the system of AgNP and triblock copolymer lead to an increase in diffusion but did not not affected the interactions between AgNP and Triblock Copolymer mesh.

Addition of Ficoll to this system led to a decrease in diffusion which shows that Ficoll may have posed some hindrance to the movement of the components of this system. Appearance of some new peaks in the 2D COSY spectra suggest that it might have affected the interactions between AgNP and the triblock polymer mesh. Addition of Perfluorodecalin to this system didn't lead to any significant change in the diffusion. The 2D COSY spectra also suggest that there was almost no change in the interaction profile of AgNP and triblock copolymer mesh on adding Perfluorodecalin.

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