# Experimental Report



**Proposal:** **9-11-1611** **Council:** 4/2012

**Title:** Structure of PLGA nanoparticles for drug delivery to the brain

**This proposal is resubmission of: 9-11-1567**

**Researh Area:** Chemistry

**Main proposer:** **EDLER Karen**

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**Samples:** PLGA ([C3H4O2]x[C2H2O2]y), PEO (CH3(C2H4O)n), TFMU (4-(Trifluoromethyl)umbelliferone, C10H5F3O3), D2O/H2O
PLGA ([C3H4O2]x[C2H2O2]y), TFMU (4-(Trifluoromethyl)umbelliferone, C10H5F3O3), or Nile Red (C20H18N2O2) D2O/H2O
PLGA ([C3H4O2]x[C2H2O2]y), mPEG-DSPE (1,2-Distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000), Nile Red (C20H18N2O2) D2O/H2O

| Instrument | Req. Days | All. Days | From | To |
|---|---|---|---|---|
| D11 | 3 | 2 | 30/10/2012 | 01/11/2012 |

**Abstract:**

Biodegradable polymer nanoparticles are a commonly used method for controlled drug release. Recently in collaboration with medics in Bristol, we have studied the feasibility of using PLGA nanoparticles for controlled release of drugs to the brain where the nanoparticles are delivered by injection via convection-enhanced drug delivery through microcatheters. Incorporation of small molecules into the nanoparticles can be hindered by the relatively hydrophobic nature of PLGA so PEG and PEG-lipid conjugates have been introduced into the particles to assist drug/tracer molecule incorporation however the effect on particle nanostructure is not known. Also spreading of the particles into the brain tissue is governed by particle size and charge but recent work has suggested that the particle mass is also important for distribution and persistance of nanoparticles in the brain. Here we propose to use SANS to determine the structure & density of PLGA nanoparticles prepared with or without PEG or lipid via contrast variation. Understanding the particle structure will help to rationalise the location of more hydrophilic species in the particles, to assist understanding the release profiles.

## Introduction

Polylactide-co-glycolide (PLGA), a US FDA-approved polymer, is widely used due to its desirable characteristics, including good biodegradability and biocompatibility. Preparation of biodegradable PLGA particles and their application in the delivery of a number of active agents have been extensively studied in the past decades.[1] Being assisted with convection enhanced drug delivery (CED) platform technology provided by a team of medics at Bristol, nanoparticles formulated with desired size, charge and drug loading can be delivered to brain tissue and homogeneously spread over a desired anatomical target with extremely high accuracy, sustained drug release and prolong the tissue half-life of the chemotherapeutic.

In this method, the main parameters to be controlled to achieve predictive and clinically relevant volumes of drug distribution are the size, surface charge and structure of the nanoparticles.[2] Therefore understanding the internal structures forming within the particles will help to explain the difference in drug loading, variations in release rate, leading to a optimized design of the drug delivery nanoparticles. In this experiment, we aimed to determine the structure and density of 1) PLGA only particles prepared with two different methods, and several solvents; and 2) similarly prepared particles made from PLGA-PEG polymer conjugates. Since we requested 3 days but were awarded only 2 we did not have time to study the PLGA-DSPE conjugate particles and decided to concentrate our studies on the particles which have been shown by our medical collaborators to be most relevant to their clinical studies (the lipid-containing samples proved to be toxic to cells when exposed to them for several days during release experiments).

### Experiment

Before the experiment, PLGA-PEG conjugates were synthesized from an acid terminated PLGA with an amine terminated PEG,[3] and this polymer was used to prepare blank nanoparticles via two methods (w/o/w emulsification vapor evaporation method and nanoprecipitation method) in the present of different solvents (acetone, acetonitrile, dichloromethane, ethyl acetate and tetrahydrofuran). Similar methods were used to prepare PLGA-only particles. Carboplatin was also incorporated into some particles to determine whether incorporation of small amount of drugs affects the particle structure. All the particles were collected by centrifugation for 15min, the supernatant solution decanted and replaced with clean water. Particles were washed three times using this method to remove unencapsulated species and any free polymer. The samples were freeze dried and stored at -10$^{o}$C. Mean particle diameter, size distribution and zeta potential were measured using DLS prior to the neutron experiment. Particle diameters ranged from 200nm-500nm.

During the SANS experiment, 1mg of prepared nanoparticles were re-suspended in 1ml $D_2O$ or $70D_2O/30H_2O$ solution using a bath ultrasonicator for 30 minutes before injecting the suspension into the scattering cells. Samples were held in double stopper quartz Hellma cells with a path length of 1mm. In order to measure both the particle scattering and internal nanostructure within the particles, two difference beam wavelengths (6Å and 13Å) and four sample-detector distances (38.998m, 8.003m, 5.503m and 1.204m) were combined to cover a wide range of scattering vectors, Q, from 0.0009Å$^{-1}$ to 0.5Å$^{-1}$.

### Results

Figure 1 shows plots of intensity against Q for three PLGA-PEG samples prepared with THF, acetone and acetonitrile via nanoprecipitation method. All the scattering profiles could be well fitted with a model for a fractal structure with primary building blocks composed of polydisperse spheres, which describes a PLGA matrix with an internal fractal structure composed of polydisperse PEG spheres. The obtained fitting parameters are summarized in Table 1. Models indicate that PEG spheres of 12-19nm packed in a fractal geometry were included inside PLGA nanoparticles, and

thus the PEG chains are not situated solely on the exterior surfaces of the particles. The aggregation number and gyration radius can be determined from correlation length, dimension, and block radius. The calculated radius of gyration is consistent with the PLGA-PEG particle sizes measured by DLS.

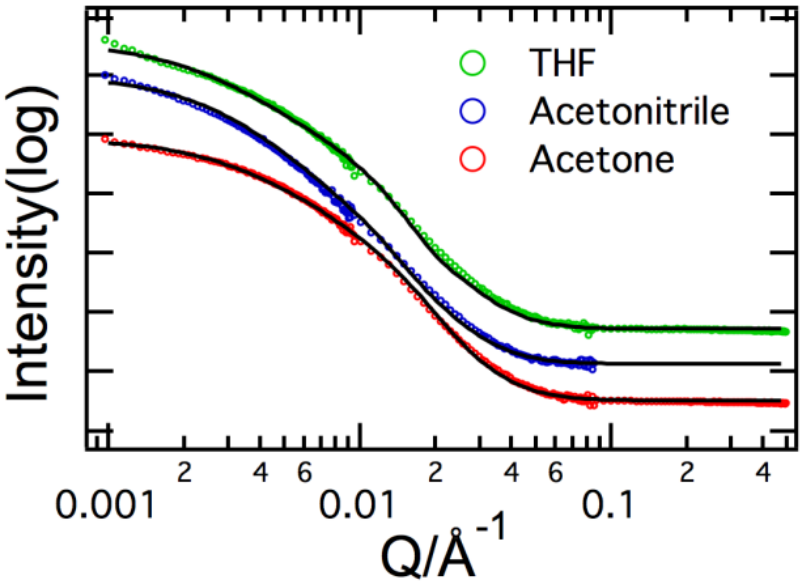


Figure 1. SANS profiles for PLGA-PEG nanoparticles prepared with different solvents via a nanoprecipitation method measured in $D_2O$. Solid lines are the best fit to the data.

| | Solvent used in synthesis | | |
|---|---|---|---|
| | THF | MeCN | Acetone |
| PEG block radius (nm) | 18.5 | 17.5 | 12.5 |
| Polydispersity | 0.29 | 0.38 | 0.39 |
| Aggregation number | 31 | 46 | 32 |
| $R_g$ (nm) | 94.5 | 85.6 | 69.4 |

Table 1 Fitting parameter determined from SANS profiles presented in Figure 1 using a model representing a fractal composed of polydisperse spheres of PEG in a PLGA matrix.

## References

(1) Dinarvand, R.; Sepehri, N.; Manoochehri, S.; Rouhani, H.; Atyabi, F. *Int J Nanomed* **2011**, *6*, 877.

(2) MacKay, J. A.; Deen, D. F.; Szoka, F. C. *Brain Res* **2005**, *1035*, 139.

(3) Cheng, J.; Teply, B. A.; Sherifi, I.; Sung, J.; Luther, G.; Gu, F. X.; Levy-Nissenbaum, E.; Radovic-Moreno, A. F.; Langer, R.; Farokhzad, O. C. *Biomaterials* **2007**, *28*, 869.