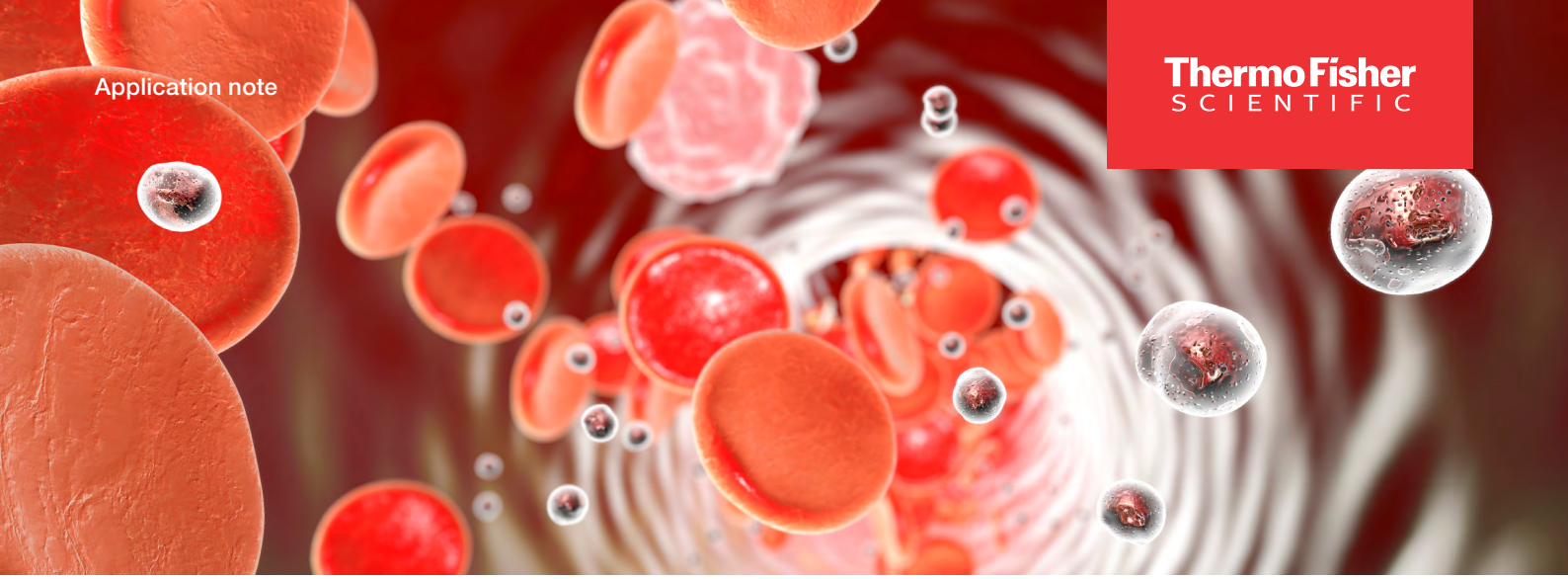

Application note

# Measurement of poly(lactic-co-glycolic acid) nanoparticle concentration with the NanoDrop One Microvolume UV-Vis Spectrophotometer


## Authors

Kejian Li,[1] Elise C. Hoover,[1] and Emily S. Day[1,2,3]

1. University of Delaware, Department of Biomedical Engineering, Newark, DE, 19716, USA
2. University of Delaware, Department of Materials Science and Engineering, Newark, DE 19716, USA
3. Helen F. Graham Cancer Center and Research Institute, Newark, DE, 19713, USA



## Keywords

Nanoparticles, light scattering, NanoDrop, Nanoparticle Tracking Analysis


NanoDrop One Microvolume UV-Vis Spectrophotometer

## Introduction

Poly(lactic-co-glycolic acid) (PLGA) is one of the most popular polymers utilized to formulate polymeric nanoparticles (NPs).[1, 2] It has been approved by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) for human administration, making the potential translation of nanomedicines based on PLGA more feasible. PLGA is also attractive for incorporation into drug delivery systems because it has numerous favorable properties, including biocompatibility, biodegradability, dual polarity, and controlled/sustained release.

To maximize the benefits of PLGA NPs in different biological and medical applications as drug delivery systems, the accurate determination of molar/particle concentration is crucial. The concentrations of PLGA NPs in solution are often estimated using the initial solid content (weight/volume). However, the commonly used emulsification solvent evaporation method for PLGA NP preparation requires several centrifugation steps resulting in consequent loss of NPs. It is hence necessary to develop strategies to accurately measure NP molar/particle concentrations to ensure success in downstream modification, functionalization, and application. Traditional methods to measure polymer NP concentrations, such as electron microscopy, Nanoparticle Tracking Analysis (NTA), and Tunable Resistive Pulse Sensing (TRPS), are labor- and time-consuming and often require stringent sample preparation and dilution.

The Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer has previously been used to accurately measure highly concentrated gold-based nanoparticles via their characteristic surface plasmon resonance (SPR).[3,4] This study investigates the potential of using OD600 (optical light scattering at 600 nm wavelength) measurements via the NanoDrop One instrument for the quantitative analysis of PLGA NPs. NTA was used to measure the concentration of PLGA NPs in solution (number of particles per volume) as a comparison. The results indicate that the NanoDrop One instrument can serve as a simple alternative to traditional methods for measuring PLGA NP concentration via OD600 measurements.

## Experimental procedures

### Nanoparticle synthesis

Poly(lactide-co-glycolide)-Polyethylene glycol-Maleimide (PLGA-PEG-MAL) NPs were synthesized using a single emulsion oil-in-water method. PLGA-PEG-MAL (20 kDa 50:50 PLGA, 5 kDa PEG, Nanosoft Polymers) was dissolved in dichloromethane (DCM) at 5 mg/mL. Then, 500 µL of the PLGA-PEG-MAL/DCM solution was added to a scintillation vial containing 2 mL 0.1% polyvinyl alcohol (PVA) and probe sonicated on ice with a Fisherbrand model 120 Sonic Dismembrator (Fisher Scientific) at 80% amplitude for 90 s (10 s on, 5 s off). The DCM solvent was allowed to evaporate for 3 hours at room temperature under continuous stirring at 800 rpm. Nanoparticles were purified through centrifugation for 15 minutes at 20,000 rcf. The resulting pellet of NPs was washed 3 times with MilliQ water using the same centrifugation settings and then systematically diluted in water to prepare samples ranging from 10 mg/mL to 1 mg/mL (assuming no loss of mass during the centrifugation/purification process).

### Nanoparticle Tracking Analysis (NTA)

PLGA-PEG-MAL NP samples were prepared for NTA measurement by diluting each sample into 1 mL MilliQ water to give a particle count between 20 and 100 particles per frame when introduced to the system. The camera level and focus were adjusted for each sample such that 20% of the visible NPs showed signal saturation and there were few “halos” around the NPs. A syringe pump injected the samples at an infusion rate of 50-60 A.U. Three 30-second videos recorded by the NTA were used to calculate NP counts. The detection threshold was also adjusted so the blue crosshair count was less than five for each frame.

### NanoDrop One Spectrophotometer OD600 measurement

For measurements on the NanoDrop One Spectrophotometer, the optical density at 600 nm (OD600) was recorded 3 times for each sample (prepared at 10 mg/mL to 1 mg/mL as described above) by pipetting 2 µL aliquots directly onto the sample pedestal. Between measurements, the NanoDrop One instrument sample pedestal was cleaned using a lint-free Kimwipe®.

## Results

The synthesized PLGA NPs were diluted in water to prepare samples with concentrations of 1, 2.5, 5, 7.5, and 10 mg/mL (based on the initial solid content). The particle count (**Figure 1A**) and hydrodynamic diameter of the NPs were measured using NTA, which revealed the NPs were 167.5 nm in mode diameter. The corresponding OD600 of the PLGA NPs was measured using the NanoDrop One Spectrophotometer and plotted against the concentration (**Figure 1B**). The plot shows a strong linear relationship over a wide concentration range (1 mg/mL to 10 mg/mL) with an $R^2$ value of 0.9993, which is consistent with the measurements obtained using NTA showing a linear relationship between mg/mL and particle count with an $R^2$ value of 0.9981. The OD600 measurements produced by the NanoDrop One Spectrophotometer were very precise, reproducible, and could be utilized to evaluate the concentration of PLGA NPs. Importantly, no further dilution of samples was required and a very small volume (2 µL) was used for measurements with the NanoDrop instrument.

## Conclusions

OD600 (the optical density measured at a wavelength of 600 nm) is commonly measured to estimate the concentration of bacteria and other microbial cultures to monitor the growth stage of the cultured cells. OD600 is largely based on light scattering rather than light absorption. Light scattering-based approaches have long been used in physicochemical characterization of NPs. The intensity of the scattered light should be proportional to the concentration of NPs in suspension. Since different size NPs will have a different slope, it is recommended researchers looking to use this OD600 method perform a calibration curve to determine the number of NPs per microliter that corresponds to one unit of OD600. The results indicate that OD600 measurements are comparable to NTA measurements and can be used to estimate the NP concentration after the calibration curve is measured.

This study presents an easy and fast method to evaluate the concentration of PLGA NPs via optical light scattering (OD600) using a standard spectrophotometer. The NanoDrop One

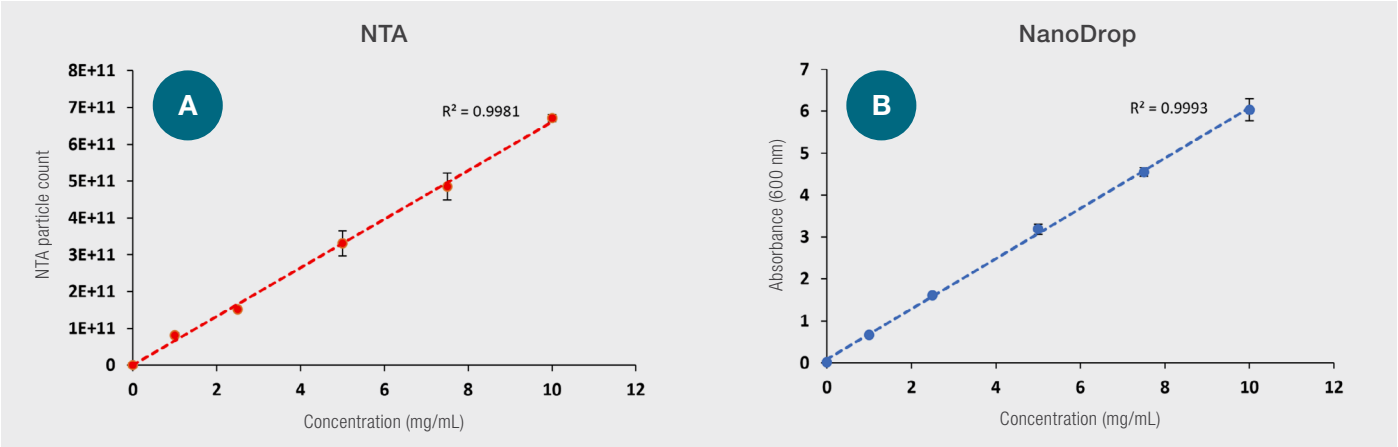


**Figure 1: Nanoparticle concentration (mg/ml) versus (A) particle count as measured by NTA and (B) the OD600 of NPs as measured by NanoDrop One Spectrophotometer. Both measurements show a linear relationship over a large range of 1 mg/mL to 10 mg/mL with an $R^2$ value of 0.9981 (NTA) and 0.9993 (NanoDrop instrument). (n=3 per concentration).**

Spectrophotometer allows users to reliably measure highly concentrated samples in microvolumes (1-2 µL) without dilution, as demonstrated here. These advantages make the NanoDrop One Spectrophotometer an economical instrument for PLGA NP quantification to support quality control, further modification, and other downstream applications.

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