

Correlation Analysis of Refractive Index (dn/dc) for PLGAs with Different Ratios of Lactide to Glycolide

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Introduction

A gel permeation chromatography triple detection (GPC-TD) system, consisting of a static light scattering, viscometer, and refractive index (RI) detector in tandem, is a powerful tool for both qualitative and quantitative analysis of polymer properties. Static light scattering provides a means for accurate molecular weight determination without the need for calibration using standard reference materials. It is also critical in obtaining other useful information including the degree of branching for hyper branched polymers, e.g., star-shaped poly(lactide-co-glycolide) (PLGA). To obtain accurate measurements, however, an accurate dn/dc (incremental refractive index) value for each PLGA is needed. The literature value for dn/dc of PLGA in tetrahydrofuran (THF) varies around 0.05 mL/g, but with no specific information on the PLGA used. We examined dn/dc values of various PLGAs having different ratios of lactide to glycolide (L:G ratios). To increase the dn/dc for PLGA, we also tested acetone (ACE) and acetonitrile (ACN) as alternatives to THF.

Methods

Solutions of PLGAs with various molecular weights and L:G ratios were prepared in THF, ACE, and ACN at concentrations of 1, 2, 3, 4, and 5 mg/mL. Solvents were first filtered using 0.2 μ m-filters, and then left stirring overnight with a loose cap to allow dissolved oxygen and atmospheric water to reach equilibration.

Solutions were directly perfused into Wyatt T-rEX RI detector using a syringe pump. RI was measured at 658.0 nm. After a baseline response of each solvent (THF, ACE, and ACN) was established, PLGA solutions were measured as the concentration was changed from low to high. This was followed by blank THF/ACE/ACN to reestablish the baseline. Wyatt Astra software was used to calculate dn/dc for each set of PLGA solutions. Triplicate preparations of solutions were used to measure dn/dc and average of triplicate analysis is reported except where specified. GPC-TD system consisted of Agilent 1260 Infinity II HPLC connected to Dawn Heleos II (MALLS) coupled to Dynapro Nanostar DLS via optical cable, Optilab T-rEX (RI detector) and Viscostar III viscometer operated by Astra 7 software. GPC analysis performed by injecting 50 ml of 2.5 mg/ml polymer solution. Separation performed with a linear gradient column (Tosoh Bioscience LLC) at 0.6 ml/min flow of indicated solvent.

Results

Figure 1 shows linear increase in differential RI as a function of the PLGA concentration. An important trend noted in Table 1 is that the dn/dc value of PLGAs is linearly dependent on the L:G ratio. As the L:G ratio increases, the dn/dc value decreases. The linear relationship makes it possible for using accurate dn/dc values as long as the L:G ratio is known.

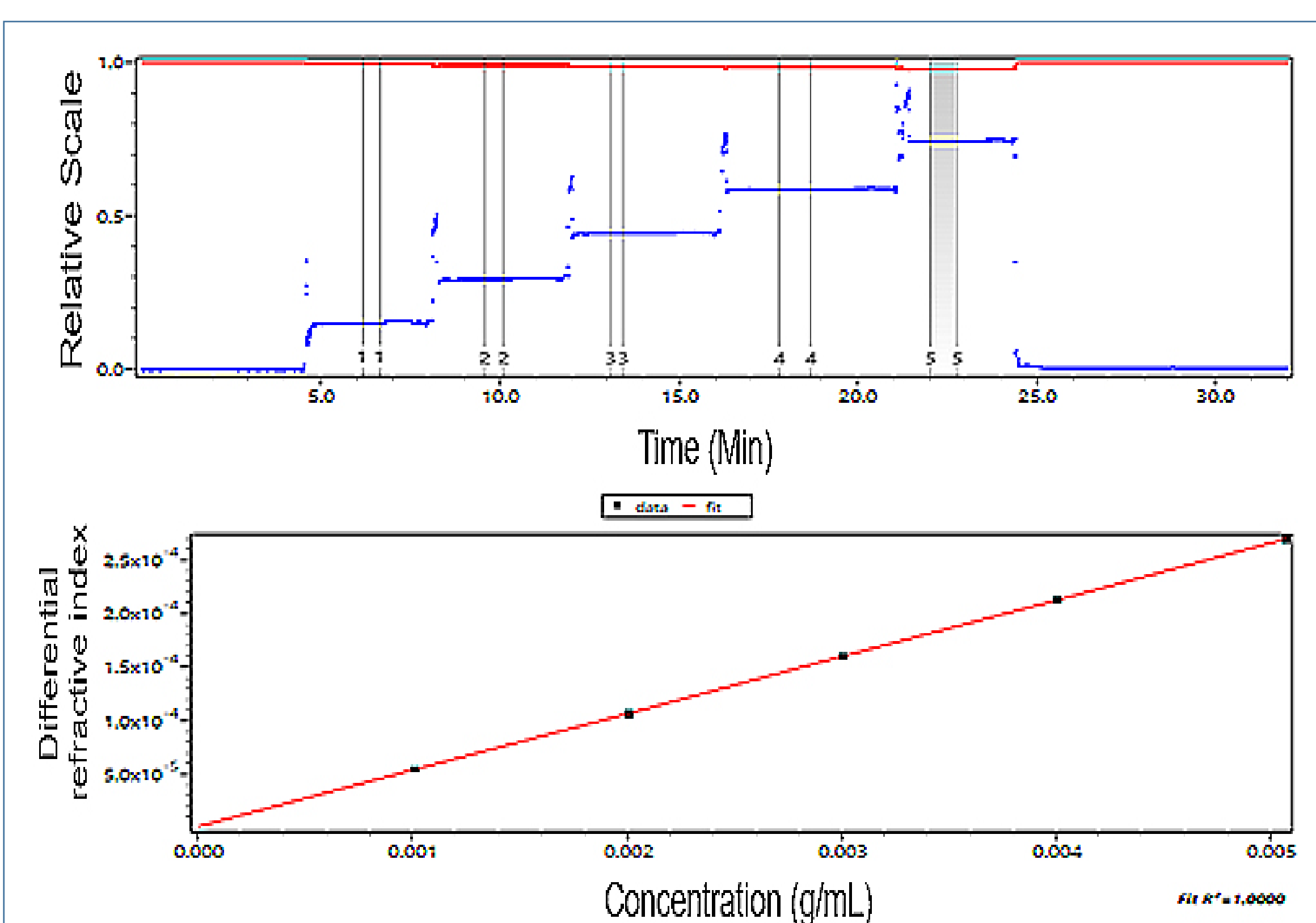


Fig. 1: Results from batch dn/dc run using Wyatt Astra software.

Figure 2 displays this linear relationship between L:G ratio and dn/dc . Two commonly used PLGAs, e.g., 50:50 and 75:25, show a dn/dc difference of 10%, which is significant enough to affect the quantitative data of measured PLGA properties. All three solvents (ACE, THF, and ACN) display a similar linear trend for dn/dc for PLGAs. Compared to THF, ACE provides nearly a two-fold increase in dn/dc . Accurate dn/dc values are critical for a full characterization of PLGA having different L:G ratios. Using accurate dn/dc values allows correct interpretation of GPC-TD data regarding the PLGA structures.

Work with ACE and ACN were pursued due to their lower absolute refractive index. This allows for greater dn/dc response while maintaining adequate chromatographic separation. ACE was chosen over ACN due to solvent profile being available in the Astra software. The solvent profile in the Astra software contains values such as thermal expansion, viscosity, and Rayleigh ratio which compensate for fluctuations in GPC system conditions. This permitted the on-line measurement of hydrodynamic radius (R_h) through differential light scattering (DLS). This is performed by the Astra software as Quasi-Elastic Light Scattering (QELS) by connecting a DLS module via fiberoptic cable through one of the static light scattering detectors.

Initial online DLS experiments did not provide meaningful correlation data for accurate measurement of R_h , as seen in Fig. 3. This is in part due to the lower dn/dc of PLGA in THF as compared to ACE or ACN. Using ACE to generate stronger DLS correlation values, displayed in Fig. 4 resulted in improved R_h data.

Conclusion

The published dn/dc values for PLGA are not accurate enough to be used in the analysis of PLGA using GPC-TD, as the type of PLGA used is not usually known.

The linear correlation between the accurate dn/dc values and the L:G ratio is useful for the study of PLGA properties. This information is especially important in the analysis of not only the PLGA quantity from RI values but also the branching index of star shaped-PLGAs, such as glucose-PLGA.

Acknowledgements

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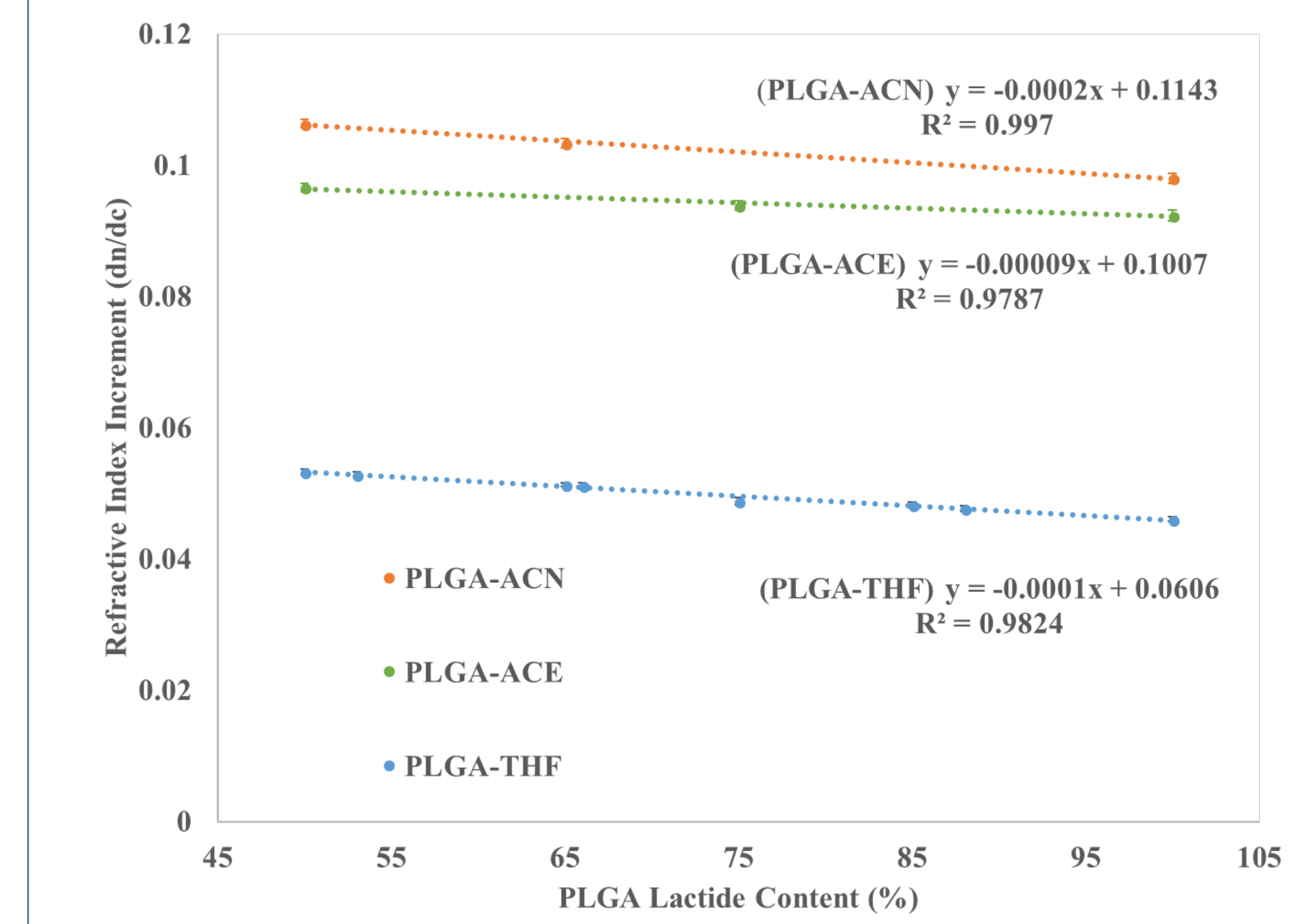


Fig. 2. Correlation of dn/dc with the lactide content.

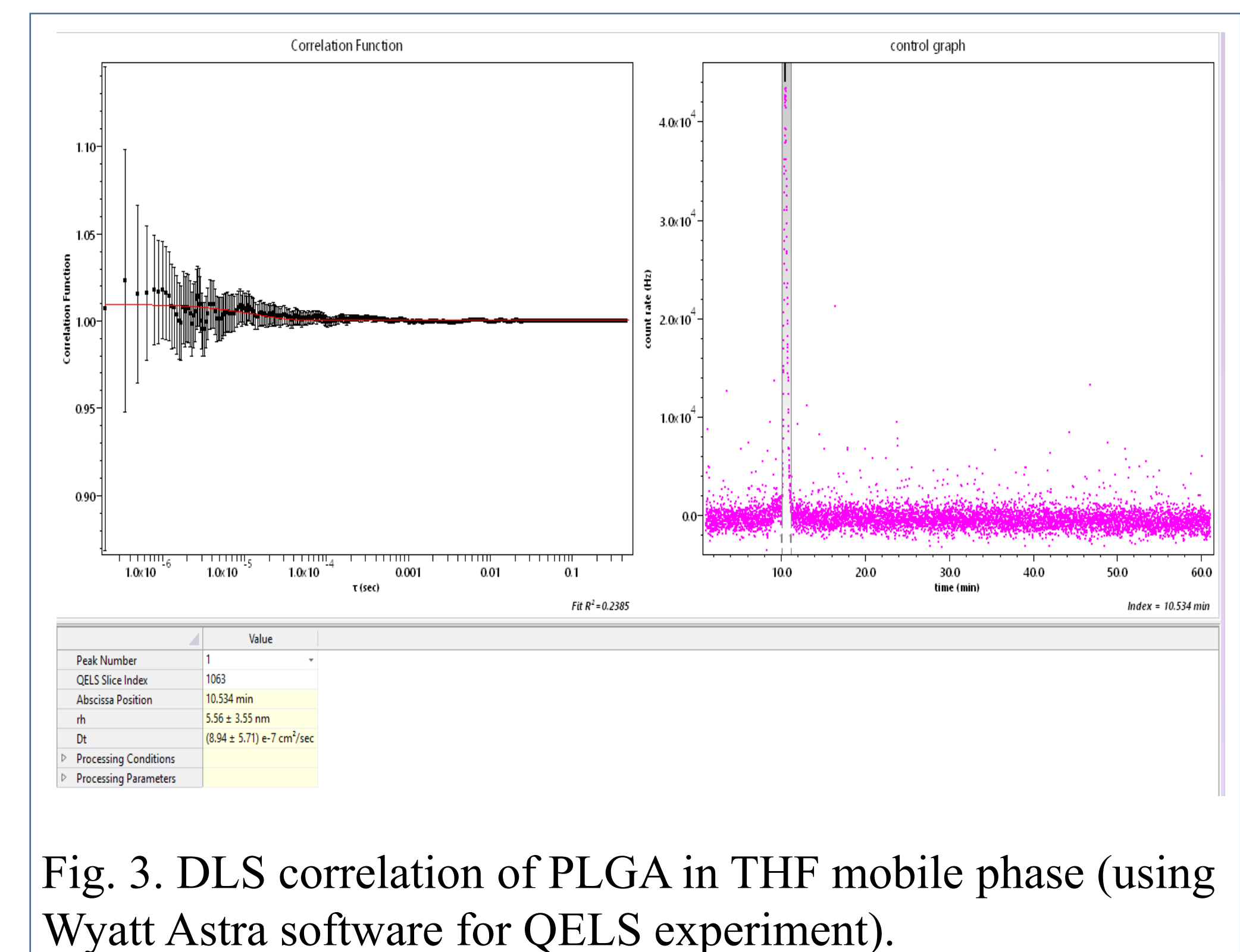


Fig. 3. DLS correlation of PLGA in THF mobile phase (using Wyatt Astra software for QELS experiment).

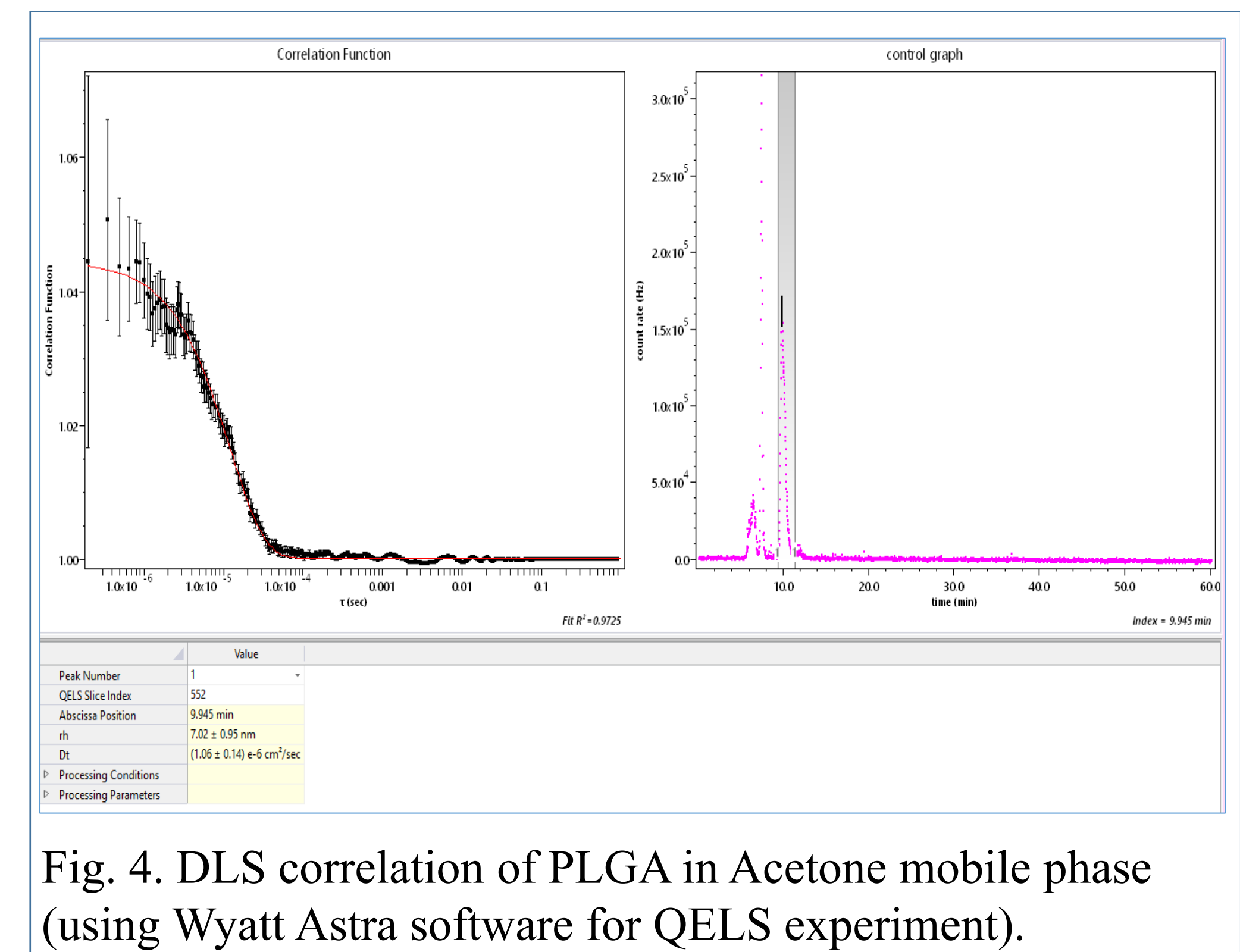


Fig. 4. DLS correlation of PLGA in Acetone mobile phase (using Wyatt Astra software for QELS experiment).

Table 1. Experimentally determined dn/dc values for various PLGAs. (Value is average \pm STDEV with n=3 unless otherwise specified. *n=1)

Polymer	L:G Ratio (NMR, molar)	M.W. (GPC determined)	dn/dc (mL/g) THF	dn/dc (mL/g) Acetone	dn/dc (mL/g) Acetonitrile
Linear Polymers					
Evonik RG504H	50:50	60,889	0.0531 \pm 0.0006	0.0977 \pm 0.0014	0.1063*
Evonik RG503H	50:50	33,983	0.0535 \pm 0.0003	NA	NA
Evonik RG502H	50:50	12,237	0.0532 \pm 0.0003	NA	NA
Lactel 50:50 PLGA-E	53:47	69,629	0.0528 \pm 0.0004	NA	NA
Lactel 65:35 PLGA-E	65:35	49,850	0.0511 \pm 0.0005	NA	NA
Evonik RG653H	65:35	34,944	0.0513 \pm 0.0003	0.0968 \pm 0.0008	0.1034*
Evonik R756S	75:25	107,234	0.0487*	NA	NA
Evonik R755S	75:25	72,176	0.0480*	0.0950 \pm 0.0010	NA
Lactel 75:25 PLGA-E	76:24	83,973	0.0490 \pm 0.0005	NA	NA
RG858S	85:15	179,454	NA	0.0930 \pm 0.0014	NA
Lactel 85:15 PLGA-E	86:14	75,554	0.0486 \pm 0.0004	NA	NA
AP071 170706AMS-B	100:0	57,374	0.0457*	0.0911 \pm 0.0015	0.0980*
Evonik R205S	100:0	76,949	NA	0.0917 \pm 0.0004	NA
Branched Polymers					
Purasorb PDLG5505G	55:45	69,351	0.0535 \pm 0.0004	0.0996 \pm 0.0026	NA
Evonik 5545 DLG 5Glu	55:45	62,762	NA	0.0987 \pm 0.0012	0.1069 \pm 0.0008
AP227	55:45	59,686	0.0540*	0.0980 \pm 0.0007	NA
AP228	55:45	59,275	NA	0.0980 \pm 0.0012	NA
AP229	55:45	57,854	0.0533*	0.0973 \pm 0.0007	NA