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MOLECULAR WEIGHT MEASUREMENT OF AMPHIPHILIC BLOCK COPOLYMERS BY ^{1}H - NMR

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Introduction

In the therapy of many diseases, great benefit can be earned from targeting of the drug to specific sites. Thus, for improving the site-specific delivery of drugs it is possible to entrap them in particulate drug carriers such as polymeric nanospheres. Among this nanospheres, amphiphilic blocks copolymers have been investigated because show good structural stability, dissociate slowly to free polymeric chains, enable an easy control of particle size and allow solubilization of hydrophobic drugs.
To evaluate their potential as a drug carrier it is very important to measure the molecular weight, characteristic that influence the time release of the drugs, i.e., lower the molecular weight more amorphous the structure and faster the control release of the drug. The present work focuses on the measurement of molecular weight of amphiphilic block copolymeric based on pluronics, poly-(ethylene glycol) and poly-(ε-caprolactone), using ^{1}H – NMR spectroscopy.

Materials and Methods

The studied copolymers were synthesized aiming a reduction of the molecular weight. It were carried out different reactions for obtaining the desire copolymers based on Pluronic F127, poly-(ethylene glycol) (PEG) and poly-(ε-caprolactone)(PCL).
The determination of molecular weight of copolymers was carried out using Nuclear Magnetic Resonance (^{1}H NMR) data. Some data were compared to data obtained by Gel Permeation Chromatography (GPC).
^{1}H NMR spectra were acquired in a VARIAN NMR spectrometer, model 400 MR, operating at 400 MHz frequency for hydrogen. In 5 mm NMR tubes, were prepared solutions containing ~8 mg of the different copolymers and 600 µL of deuterated chloroform with tetramethylsilane (TMS) as reference standard for chemical shift. The following parameters were used for acquisition: temperature 25°C, spectral width 6410 Hz, acquisition time 2.55 s, relaxation delay 2 s.

Results and Discussions

The measurement of molecular weights was carried out as indicated: 1- were obtained qualitative ^{1}H NMR spectra. Figures 1 and 3 show the spectra of synthesized copolymers using Pluronic F127 / PCL and PEG/ PCL, respectively. The identification of signals can also be observed, 2- proceeded with integration (Figures 2 and 4) of spectra and finally, 3) applied equation 1 for calculation.

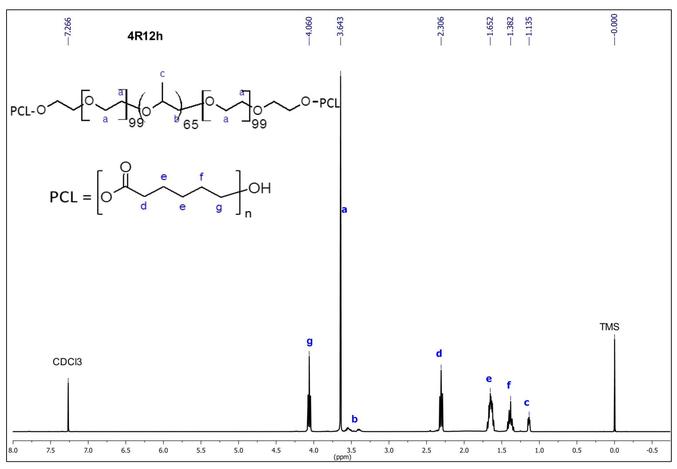

Figure 1 - ^{1}H NMR spectrum of copolymer Pluronic F127 / PCL (Reaction 4R12h).

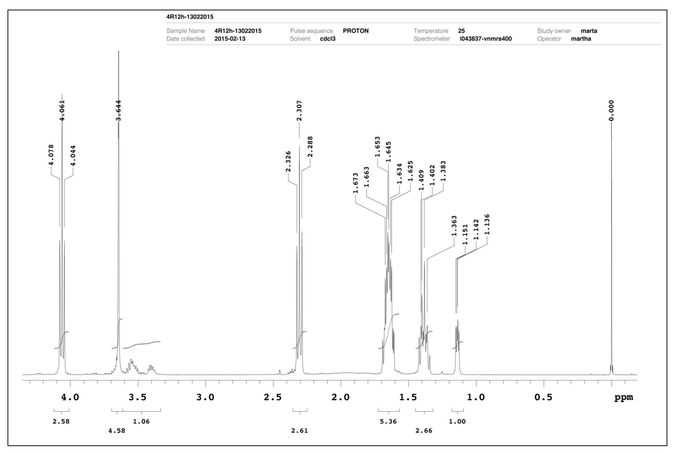

Figure 2 - Integrated ^{1}H NMR spectrum of copolymer Pluronic F127 / PCL (Reaction 4R12h).

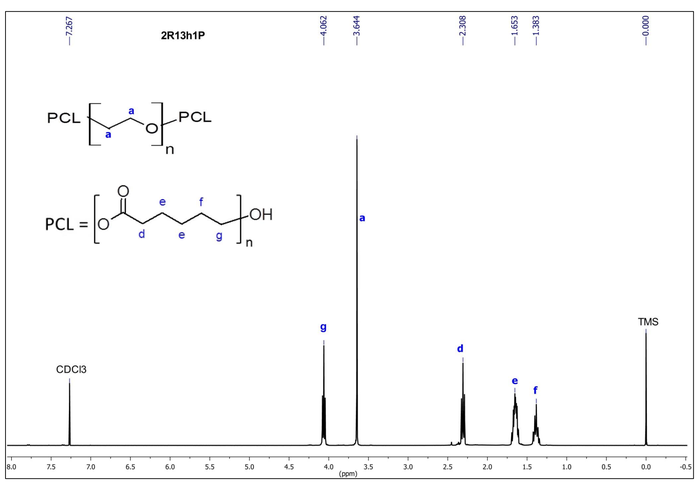

Figure 3 - ^{1}H NMR spectrum of copolymer PEG/ PCL (Reaction 2R13h1P).

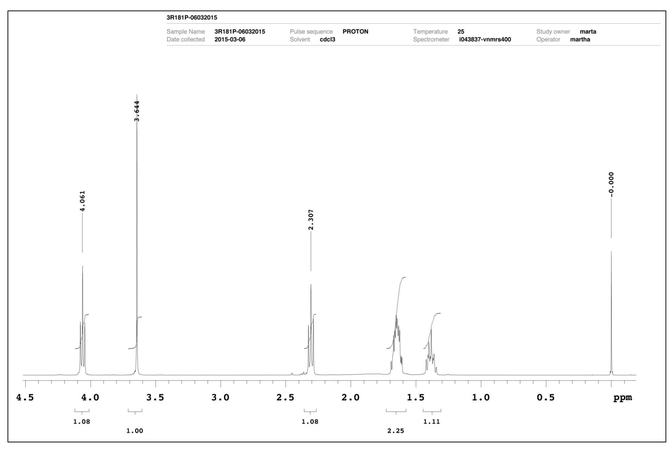

Figure 4 - Integrated ^{1}H NMR spectrum of copolymer PEG / PCL (Reaction 3R18h1P).

Table 1. Comparison of molecular weights calculated by NMR and GPC.

Reaction	Material	M.M. (g.mol^{-1}) by NMR	M.M. (g.mol^{-1}) by GPC	M.M. Theoretical Prediction
2R12H	PEG	37 548	-	25 976
2R18H	PEG	36 923	-	25 976
3R18H	PEG	39 734	-	25 885
4R12H	F127	41 610	-	32 555
4R18H	F127	30 717	17 800	32 555
5R4H	F127	20 491	-	18 804
6R4H	PEG	8 498	-	8 893
7R4H	PEG	7 874	-	8 822
8R4H	F127	16 156	16 800	1 680
9R3#	F127	17 268	-	18 854
9R5#	F127	17 046	16 900	18 854
10R3#	PEG	8 186	-	8 978
10R5#	PEG	8 186	8 000	8 978

Molecular weight measurement of Pluronic F127/PCL

The number-average molecular weight of copolymer ($M_{n\ of\ copolymer}$) is:

$$M_{n\ of\ copolymer} = M_{n\ of\ Pluronic} + 2L_{PCL} * 114 \qquad (1)$$

Where:
- $M_{n\ of\ Pluronic}$ = 12 600 g.mol^{-1} of Pluronic F127
- L_{PCL} = number of monomers of caprolactone
- Molecular weight of caprolactone monomer: 114
- $L_{pluronic}$ = number of monomers of pluronic given by supplier (65)

Thus, for sample 4R12h the $M_{n\ of\ copolymer}$ was calculated considering that:
- ✓ The intensity of signal of CH_3 group in PPO of Pluronic at 1.13 ppm was 1
- ✓ The number of protons of this signal is 195 (i.e., 3* $L_{Pluronic}$)
- ✓ $2L_{PCL}$= (195 * intensity of CH_2 at 2.30 ppm (α to the carbonyl))/2
- ✓ $2L_{PCL}$ = (195*2.61)/2 = 254.47
- ✓ $2L_{PCL}$ * 114 = 29 010 g.mol^{-1}
- ✓ $M_{n\ of\ copolymer}$= 12 600 g.mol^{-1} + 29 010 g.mol^{-1}
- ✓ $M_{n\ of\ copolymer}$= 41 610 g.mol^{-1}

Molecular weight measurement of PEG/PCL

The number-average molecular weight of copolymer ($M_{n\ of\ copolymer}$) is:

$$M_{n\ of\ copolymer} = M_{n\ of\ PEG} + 2L_{PCL} * 114 \qquad (1)$$

Where:
- $M_{n\ of\ PEG}$ = 6 000 g.mol^{-1} of PEG
- L_{PCL} = number of monomers of caprolactone
- Molecular weight of caprolactone monomer: 114
- L_{PEG} = number of monomers of PEG given by supplier (137)

Thus, for sample 3R18h the $M_{n\ of\ copolymer}$ was calculated considering that:
- ✓ The intensity of signal of CH_2CH_2O group in PEG at 3.60 ppm was 1
- ✓ The number of protons of this signal is 548 (i.e., 4* L_{PEG})
- ✓ $2L_{PCL}$= (548 * intensity of CH_2 at 2.30 ppm (α to the carbonyl))/2
- ✓ $2L_{PCL}$= (548*1.08)/2 = 592 protons/2= 296
- ✓ $2L_{PCL}$* 114 = 33 744 g.mol^{-1}
- ✓ $M_{n\ of\ copolymer}$= 6 000 g.mol^{-1} + 33 744 g.mol^{-1}
- ✓ $M_{n\ of\ copolymer}$= 39 744 g.mol^{-1}

Conclusions

•The results show a good agreement among the theoretical predictions, the molecular weights calculated by GPC and the molecular weights calculated by ^{1}H NMR.

•Simplicity, short analysis time, no solvent use and no residues generation are some of advantages of the NMR method over the other most commonly used, GPC.

•NMR method is fairly accurate because allows direct determination considering that the areas under the resonance peaks in the spectra are proportional to the molar concentration of the species in the sample, whereas GPC principle is based on hydrodynamic volume that is a not molecular weight measurement.

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