

Synthesis of 4-cyanopentanoic acid dithiobenzoate (CPADB).

4-cyanopentanoic acid dithiobenzoate (CPADB) was synthesized as reported by Y. Mitsukami et al (Mitsukami, Donovan et al. 2001). Briefly, the dithiobenzoic acid (DTBA) was firstly prepared using sodium methoxide (30 % solution in methanol, 45 g, 250 mmol), elemental sulphur (8.0 g, 250 mmol), anhydrous methanol (62.5 g) and benzyl chloride (15.75 g, 125 mmol). The reaction mixture was heated in an oil bath at 67 °C for 10 h. After this time, the reaction mixture was cooled to 7 °C using an ice bath. The precipitated salt was removed by filtration and the solvent removed in vacuum. The residue was dissolved in deionized water (125 mL). The crude sodium dithiobenzoate solution was acidified with 1.0 N HCl (125 mL) and extracted with diethyl ether (50 mL). Deionized water (75 mL) and 1.0 N NaOH (150 mL) were added, and sodium dithiobenzoate was transferred to the aqueous phase.

The sodium dithiobenzoate solution (87.5 mL) and potassium ferricyanide (III) (8.23 g, 25 mmol) were mixed in deionized water (150 mL) under vigorous stirring. The red precipitate was filtered and washed with deionized water until the washings became colourless. The solid was filtered and dried in vacuum at room temperature overnight. The product, di(thiobenzoyl) disulphide, was recrystallized from ethanol.

Finally, the synthesis of 4-cyanopentanoic acid dithiobenzoate (CPADB) was carried out by dissolving 4,4'-azobis(4-cyanopentanoic acid) (2.92 g, 11.5 mmol) and di(thiobenzoyl)disulfide (2.13 g, 7 mmol) in 40 mL of distilled ethyl acetate. The reaction solution was heated at reflux for 18h and ethyl acetate was removed in vacuum. The crude product was isolated by column chromatography (silicagel 60 Å, 70-230 mesh) using ethyl acetate hexane (2:3) as eluent. Fractions that were red in colour were combined and dried over anhydrous sodium sulphate overnight. The solvent mixture was removed in vacuum, and the red oily residue placed in a freezer at -20 °C,

whereupon it crystallized. The target compound was recrystallized with an acetate:hexane (2:3) mixture (melting point 98 °C, measured in a Perkin Elmer DSC7 apparatus from 20 to 180 °C at a constant rate of 10 °C/min. Yield was 78%). Melting point obtained was in a good concordance with the value described in bibliography (Thang, Chong et al. 1999).

The $^1\text{H-NMR}$ spectra of this reagent is shown in figure 1, where the resonance signals at 7.4 – 8.0 ppm corresponds to the aromatic protons designed as a, b, c, d and e in the chemical structure, the signals at 2.5 – 3.0 ppm represent the methylene protons (g,h) from the cyanopentanoic acid fragment and the signals at 2 ppm correspond to the protons from the methyl group in the same fragment (f).

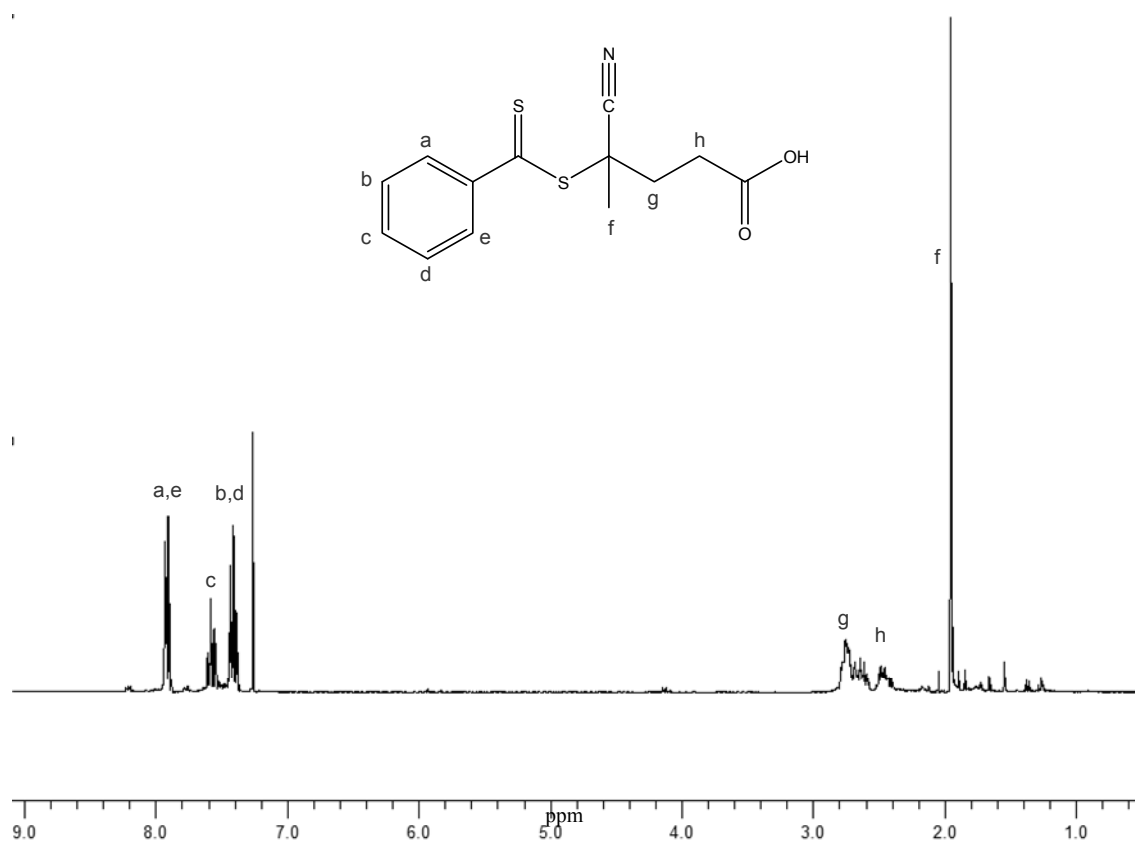


Figure 1. CPADB RAFT agent $^1\text{H-NMR}$ spectra characterisation.

Synthesis of PMMA macroRAFT agent.

The synthesis of PMMA macroRAFT agent (P1) was carried out using CPADB as RAFT agent (Fig. 2). The theoretical molecular weight can then be calculated using $M_n = [M]/[RAFT] * M_{\text{Monomer}} * c + M_{\text{Raft}}$ where $[M]$ and $[RAFT]$ are the initial concentrations of the monomer and the RAFT agent, respectively, and c is the conversion.

The polymerization of a second monomer, [2-(methacryloyloxy)ethyl] trimethylammonium, in the presence of poly(methylmethacrylate) macroRAFT agent leads to chain extension and therefore to the formation of amphiphilic block copolymers PMMA-*b*-PMAETMA (Fig. 3).

CPADB did not introduce any decreasing of the polymerization rate of MMA. Conversion of MMA was calculated gravimetrically and corroborated by $^1\text{H-NMR}$, obtaining the same value with both techniques. It was observed a linear increase of conversion with time, showing a good control of the polymerization. After 24h, 97% conversion of PMMA was obtained with a $\overline{M}_n = 55000$, PDI = 1.24 (figure 4).

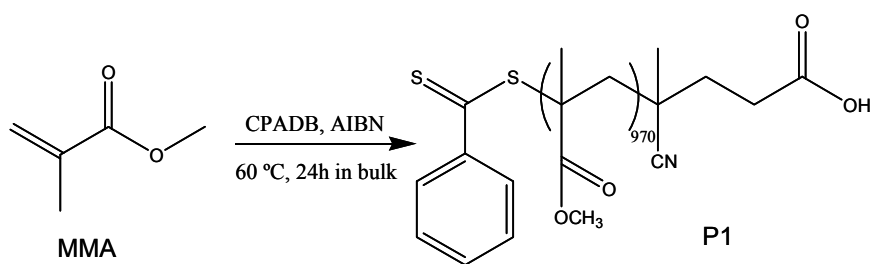


Figure 2. MMA polymerization scheme using CPADB RAFT agent to obtain the P1.

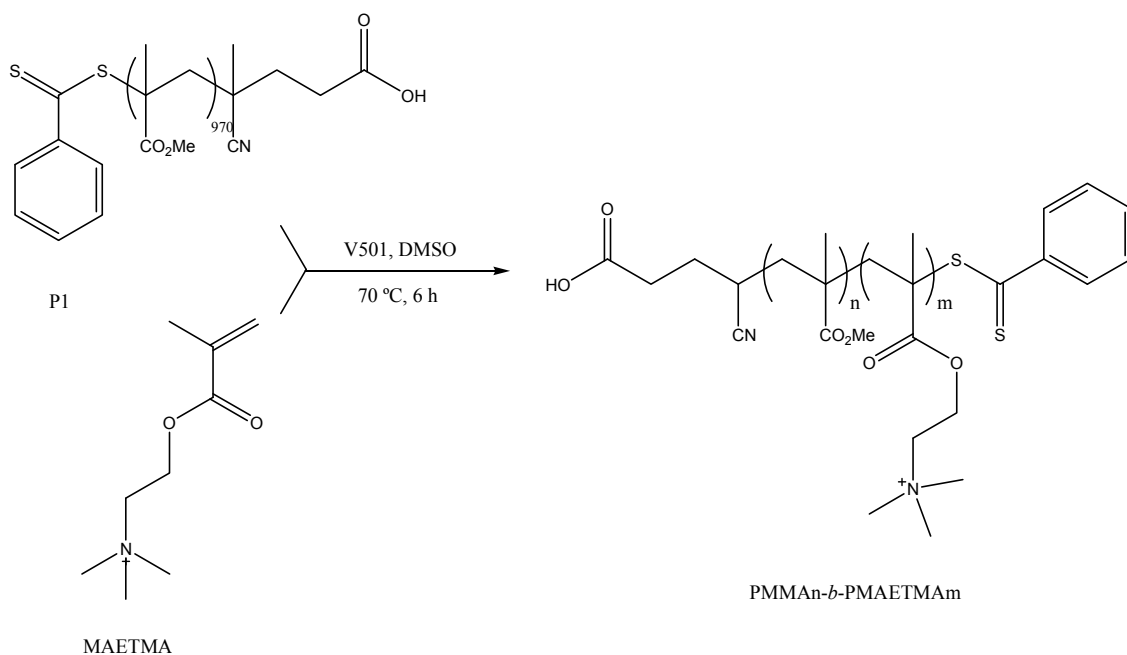


Figure 3. MAETMA polymerization scheme using P1.

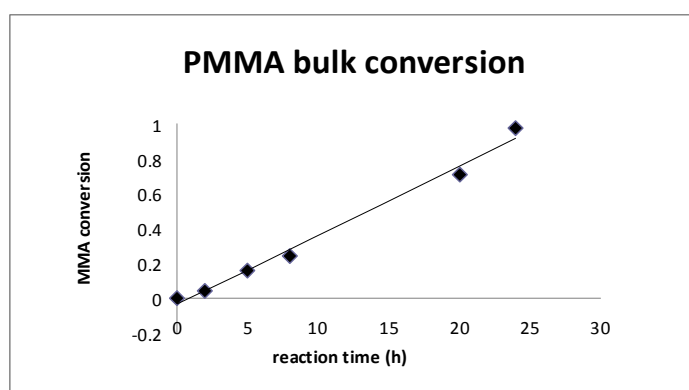


Figure 4. Kinetic plot of MMA macro-RAFT agent polymerization.

GPC elution curves (Fig. 5a) clearly showed a peak shift to higher molecular weights with increasing polymerization time. The curves were all unimodal with no sign of coexisting low and high molecular weight species that may be yielded from uncontrolled polymerization.

The increase in M_n with conversion (Fig. 5b) was linear and the resulting polydispersity was narrow ($M_w/M_n = 1.24$).

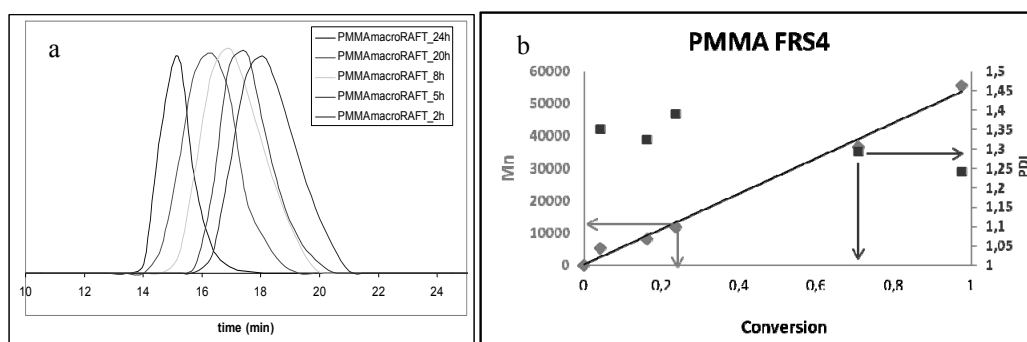


Figure 5. Molecular weight evolution of P1 agent with time and conversion.

$^1\text{H-NMR}$ spectrum of P1 without previous purification is shown in figure 6 with the characteristic assignement of signals. Resonance signals that correspond to the protons from MMA residual monomer: $\delta \sim 5.6 - 6.1$ ppm (CH_2) and $\delta \sim 3.75$ ppm (OCH_3) are also indicated.

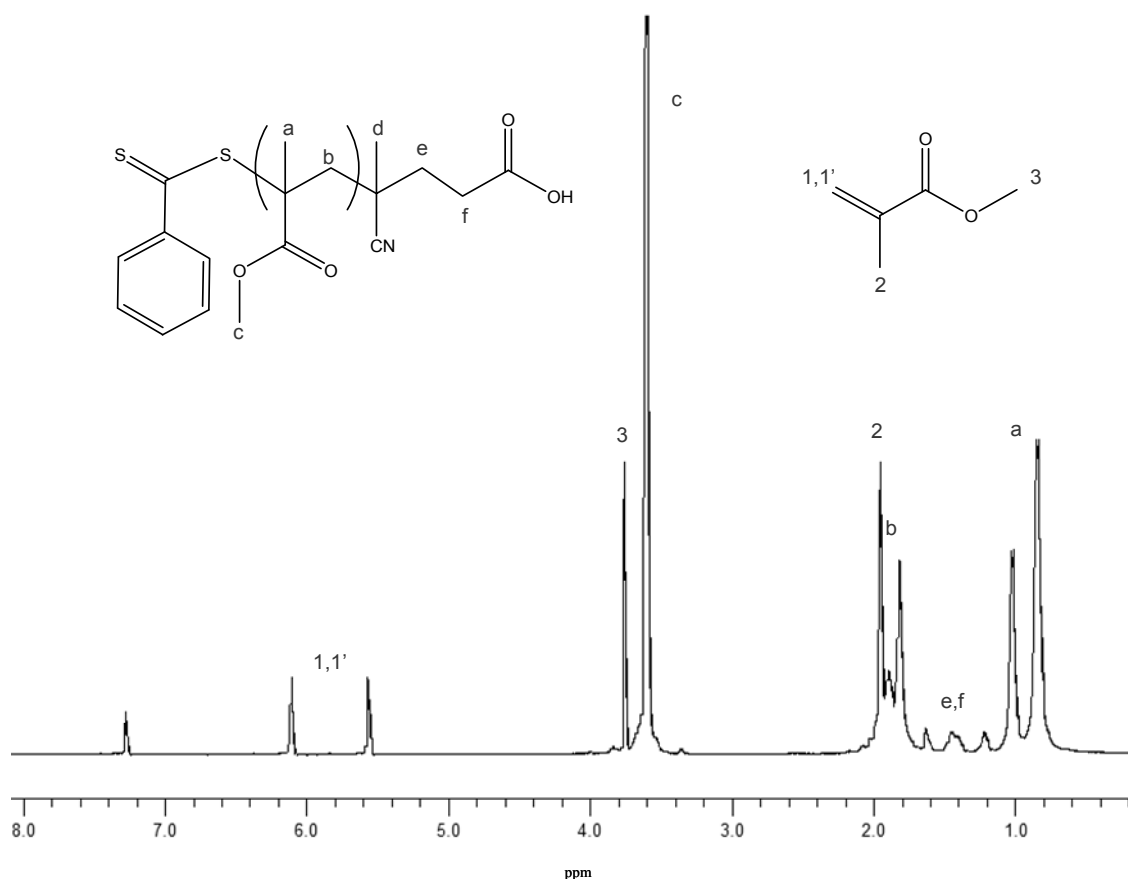


Figure 6. PMMA macroRAFT agent $^1\text{H-NMR}$ spectrum after 24h of bulk polymerisation.

Fourier transform infrared spectroscopy fitted with attenuated total reflectance (ATR-FTIR)

ATR-FTIR spectra were recorded on a Perkin-Elmer-Spectrum One spectrophotometer. Infrared (IR) spectra were obtained between 4000 and 400 cm^{-1} by 32 scans and with a scanning resolution of 4 cm^{-1} .

FTIR characterization of NP systems.

The FTIR spectra (Fig. 7) of nanoparticle systems charged with bempiparin showed two different carbonyl group signs, the first one at 1650 cm^{-1} belongs to bempiparin and the second one at 1750 cm^{-1} or 1720 cm^{-1} belong to PLGA or the ionic polymers respectively. Besides, at 3500 cm^{-1} the nanoparticle systems showed a wide band similar to bempiparin spectra, that belongs to the OH groups of bempiparin.

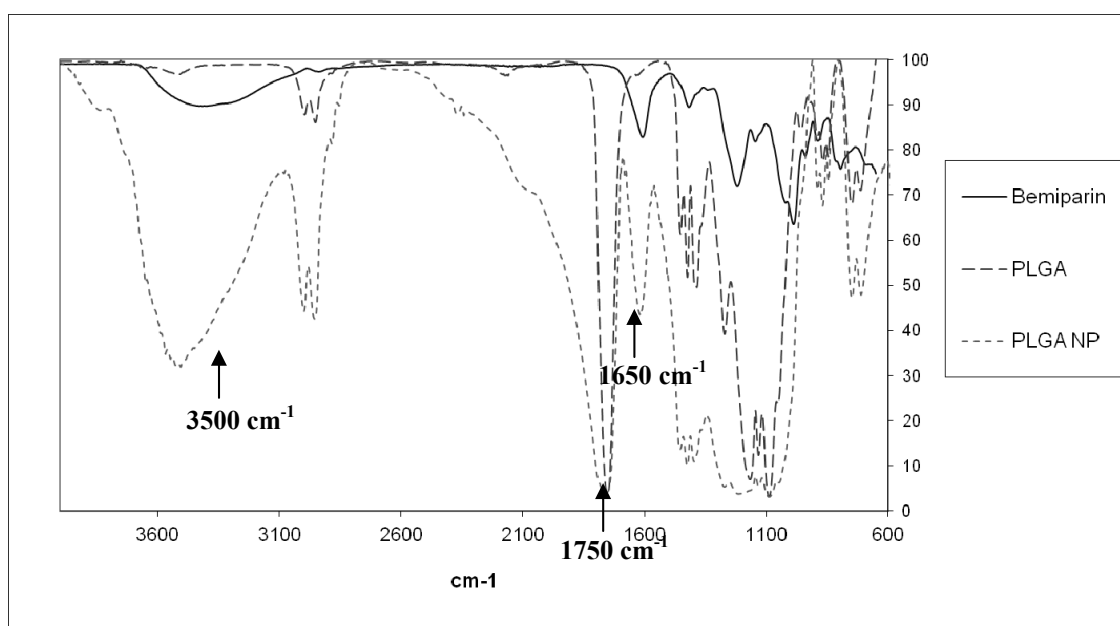


Figure 7. FTIR spectra of bempiparin, PLGA and nanoparticle system of PLGA charged with bempiparin.

References

- Mitsukami, Y., M. S. Donovan, et al. (2001). "Water-Soluble Polymers. 81. Direct Synthesis of Hydrophilic Styrenic-Based Homopolymers and Block Copolymers in Aqueous Solution via RAFT." Macromolecules **34**(7): 2248-2256.
- Thang, S. H., Y. K. Chong, et al. (1999). "A novel synthesis of functional dithioesters, dithiocarbamates, xanthates and trithiocarbonates." Tetrahedron Letters **40**(12): 2435-2438.