'Nanobots' for Drug Delivery
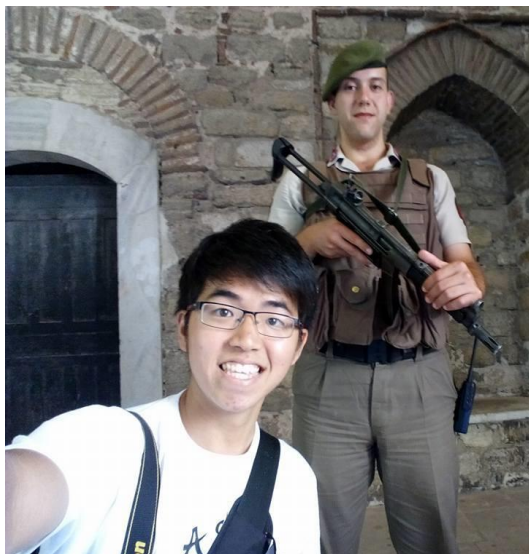
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The science of pharmaceutics is what makes drugs into medicines. However, this is not always an easy task. Take cancer treatment for an example. Systemic chemotherapy remains the mainstay treatment for various types of cancer. Due to poor targeting, systemic chemotherapy has debilitating side effects as it affects all rapidly dividing cells, regardless of it being healthy or malignant. Hair follicles are affected which resulted in hair loss and would impact upon the patient psychologically; myelosuppression would cause the patient to be anaemic and a suppressed immune system would eventually expose the patient to life-threatening neutropenic sepsis. In 2010, there were 716 neutropenic sepsis related deaths in England and Wales, giving us an idea of its severity.[1] Chemotherapy related nausea and vomiting was also reported to significantly interfere patient's quality of life and daily functioning.[2,3]

Given the various problems associated with off-target effects of drugs such as chemotherapy, highly target-specific drug delivery is inarguably the holy grail of pharmaceutical sciences. Abundant efforts are ongoing at the moment to develop better and more efficient ways of delivering therapeutic compounds to the site of interest in the human body. This in turn would give maximum treatment effectiveness and reduce off-site related risk to the patient. These efforts include the development of nanoparticles or microparticles for drug delivery with examples of successes such as liposomal doxorubicin (Doxil), the heat-triggered release liposomal doxorubicin (Thermodox), polymer-protein conjugate (Oncaspar) and polymer-drug conjugate (Opaxio).[4,5] These new approaches to drug delivery have the potential to revolutionise 21st century drug delivery through ground-breaking research.

The aim of my project is to produce nano-sized polymer delivery systems using tri-block co-polymers of low polydispersity index (PDI) consisting of hydrophilic and hydrophobic polymer blocks. Polymers with low PDI (values of 1~1.2) are very useful for therapeutic purposes such as delivering drugs. A chain of hydrophilic polymer is first synthesised and blocks of hydrophobic polymers are grown at each end of the hydrophilic polymer to give a linear polymer with two hydrophobic ends. Under suitable conditions, several of these chains will assemble to give a flower-shaped micelle system. Drugs and targeting ligands could then be attached to these nano/microparticles. Targeting ligands such as folic acid and varying combinations of glycomaterials would allow delivery of drugs to cancer cells in which folic acid receptors are highly expressed and specific cells that exhibit certain lectins on its surface respectively.
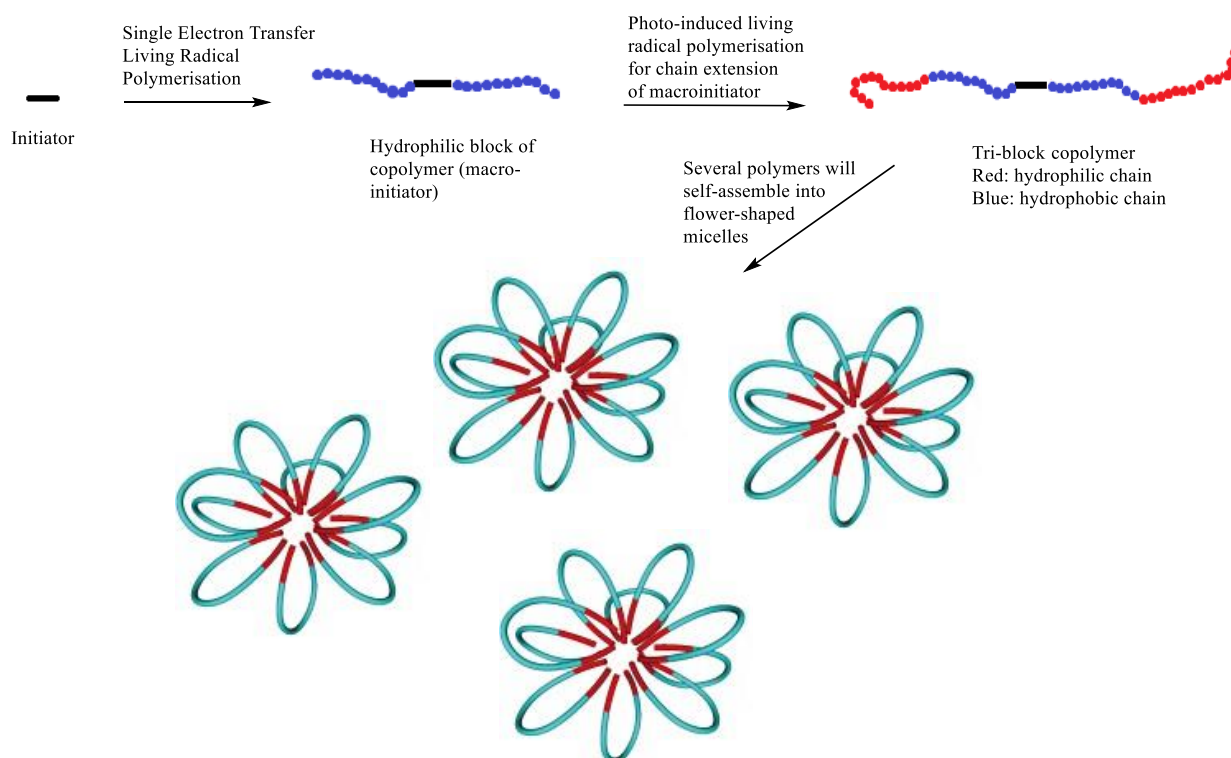
Fig. 1: A brief outline of the project, which aimed to produce flower-shaped nanoparticles for development into drug delivering nanoparticles.[6]

Synthesis of Hydrophilic Block of Co-polymer a.k.a. macroinitiator

The approach to hydrophilic polymer synthesis was Single Electron Transfer Living Radical Polymerisation (SET LRP) under zero oxygen condition. Synthesis protocol was adapted from Q. Zhang (2013), using diethylene bromoisobutyrate as the initiator and N-hydroxyethylacrylamide as the monomer at a ratio of 1:40.[7] The reaction involved disproportionation reaction of copper(I) bromide to give copper metal and copper(II) ions which in complex with tris 2-(dimethylamino)ethylamine (Me$_6$TREN) ligand, acted as the catalyst for this reaction. As this was a free radical polymerisation reaction, degassing procedure involving argon and nitrogen using a Schlenk line was carried out to rid of oxygen in the system.

1H NMR analysis was used to study polymer conversion at various time intervals. At full conversion, the polymer product was purified via dialysis method and lyophilisation which was then characterised using GPC with aqueous cationic solution as eluent. The protocol was repeated for a second time until a sufficient amount of the macroinitiator was obtained. The first polymer product was coded SA026 whereas second was coded SA026(1).
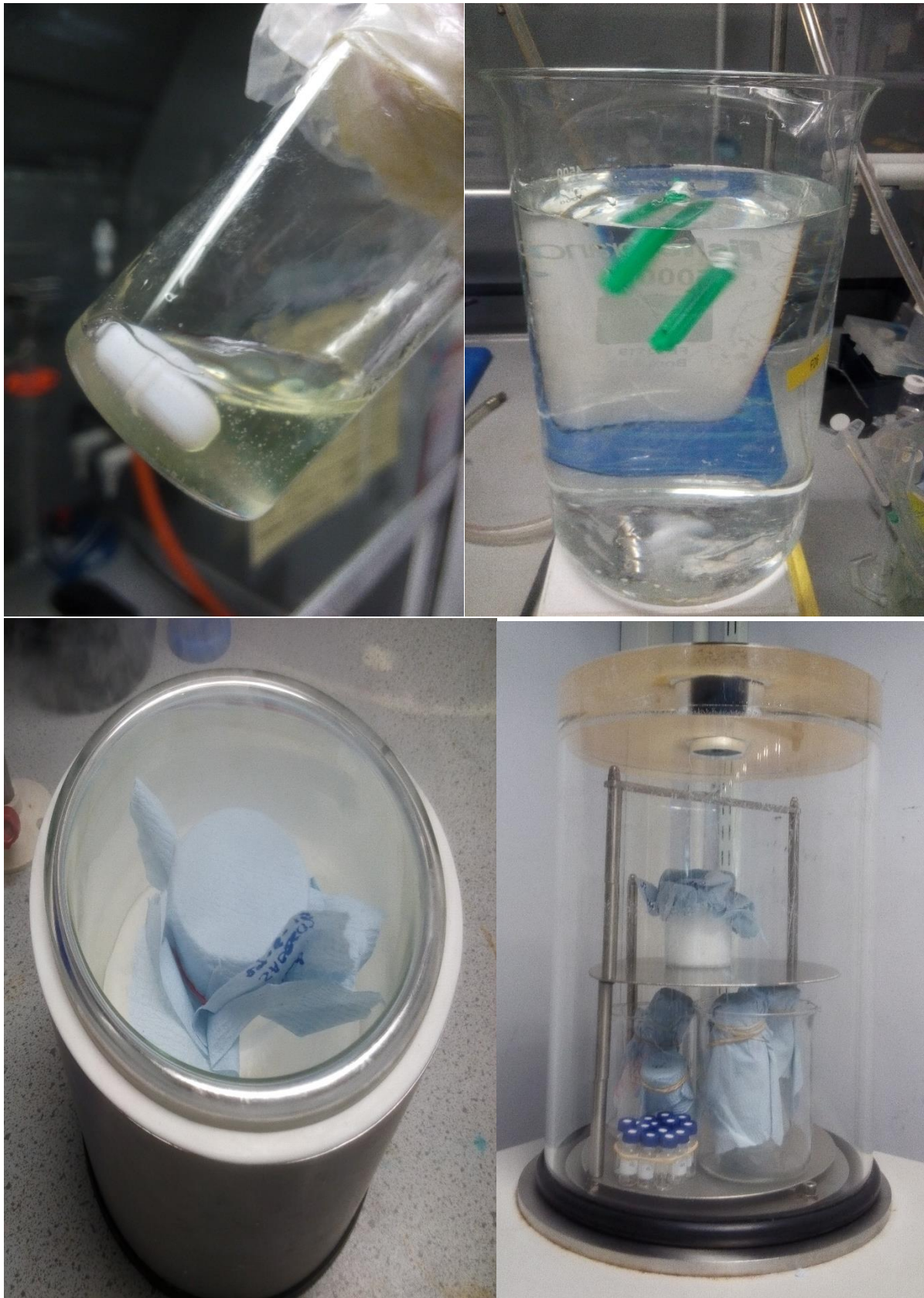
Fig. 2: The purification process. Top left: Polymer product after termination of the reaction. Top right: dialysis process. The polymer was retained in the cellulose membrane. Bottom left: freezing of polymer product (dissolved in deionised water) using liquid nitrogen. Bottom right: lyophilisation machine.
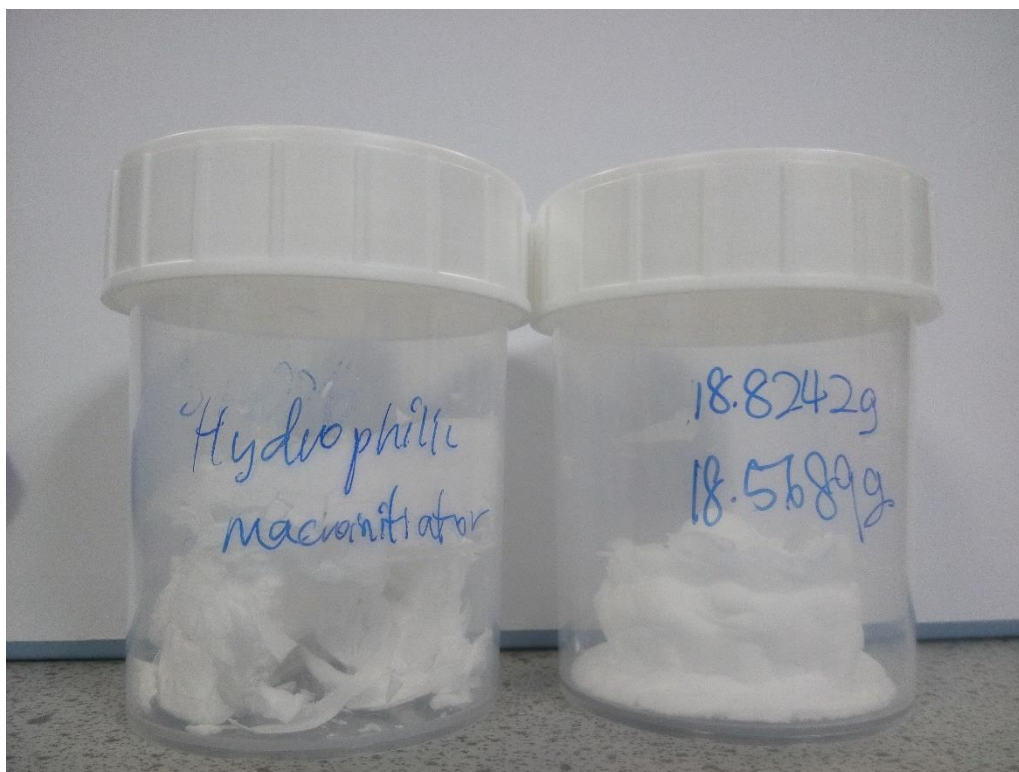
Fig. 3: Macroinitiator product obtained post-purification.

Outcome:

Polymer Code	Duration (h)	Conversion	PDI
SA026	1.5	99%	1.185
SA026(1)	1.5	99%	1.183

Table 1: Conversion rate at t=0.5 hours and PDI of hydrophilic polymer SA026 and SA026(1).

Near 100% conversion rate was achieved for both SA026 and SA026(1) at t=0.5 hours as seen from the complete disappearance of the acrylate signal (Fig. 4). PDI for both SA026 and SA026(1) were considered to be narrow (<1.2). The next step of the synthesis would be to chain extend these hydrophilic polymers with hydrophobic monomers using photo-induced LRP. Prior to that, optimisation of the reaction (duration, catalyst concentration) was required.
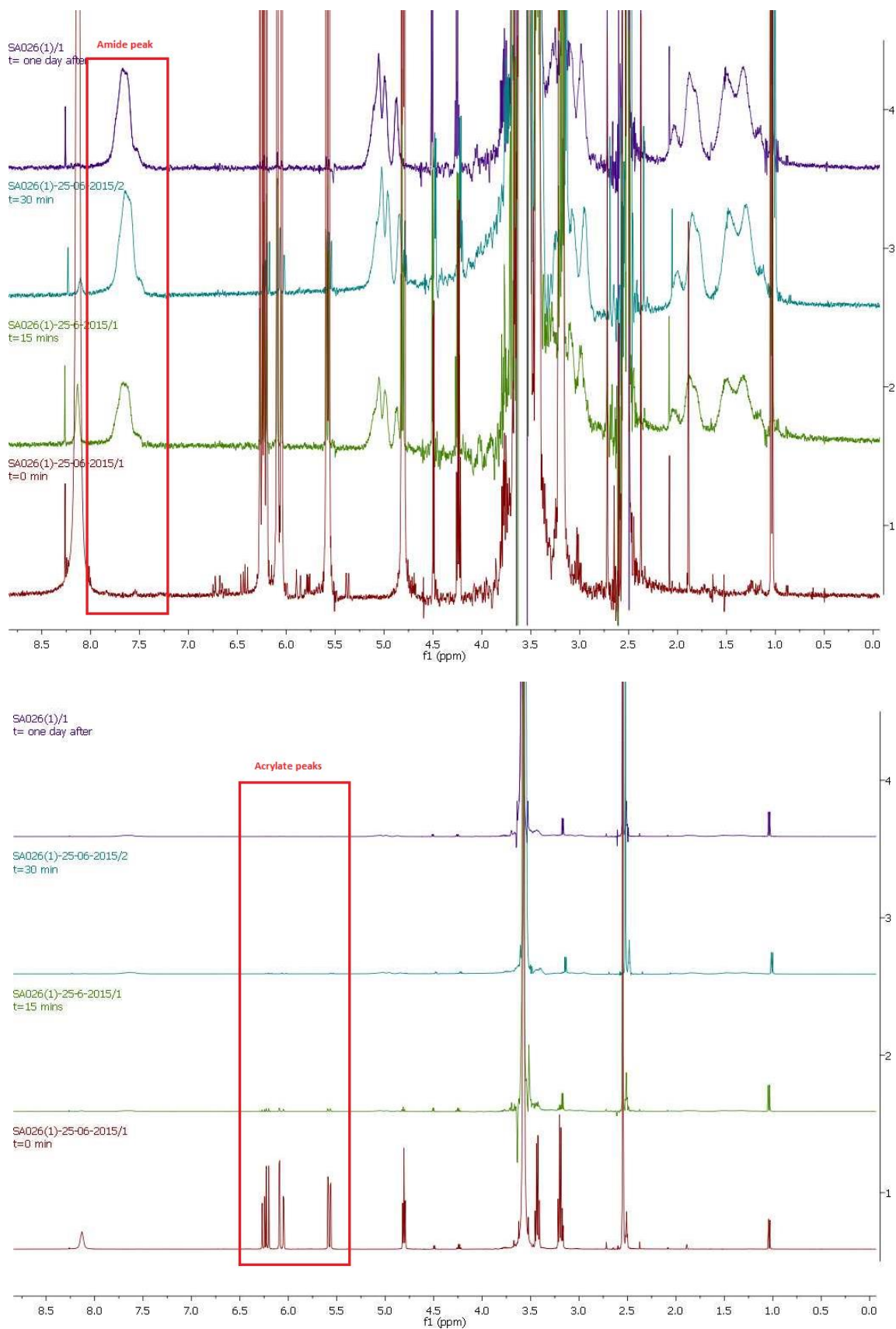
Fig. 4: NMR signals obtained at various time of reaction. Strength of amide peak at 7.7ppm increased gradually (top) whereas acrylate peaks at 6~6.5ppm diminished slowly (below) as the reaction progressed. At t=0.5 hours, acrylate signal disappeared completely.

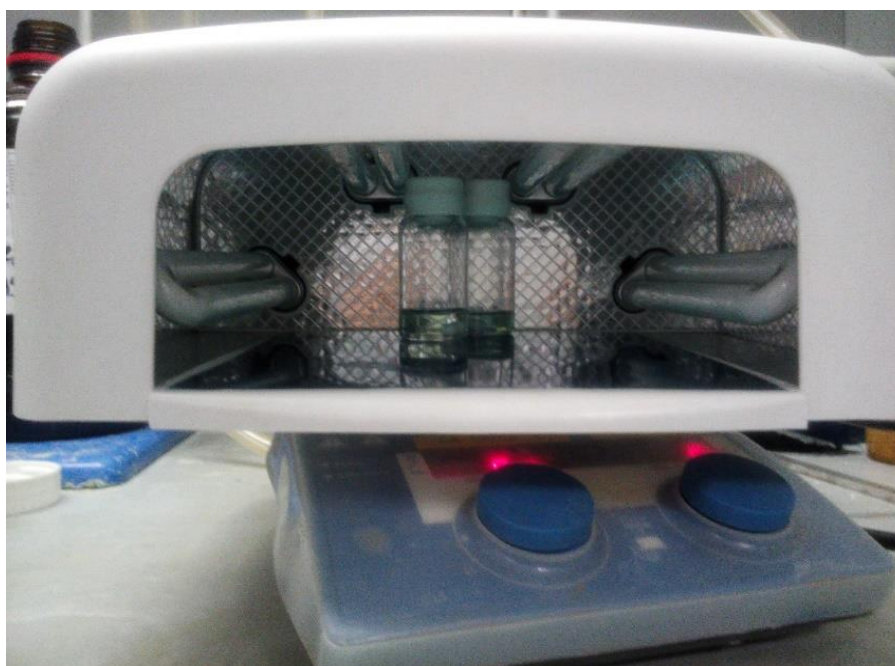**Optimisation of Photo-Induced Living Radical Polymerisation**

Fig. 5: Vials inside a UV lamp for photo-induced LRP.

This preliminary reaction was conducted to determine the optimum initiator:monomer:copper(II) catalyst:ligand ratio and reaction duration of the macroinitiator chain extension reaction. Photo-induced LRP of two different initiator:monomer:copper(II) catalyst:ligand ratio of 1:50:0.01:0.06 (ZY01) and 1:50:0.02:0.12 (ZY02) were conducted in an ultraviolet lamp using diethylene bromoisobutyrate (BIB) as the initiator, n-butyl acrylate (n-BA) as the monomer, Copper(II) bromide as the catalyst and Me_6TREN as the ligand after degassing the system. DMSO was the solvent for the reaction at a DMSO:n-butyl acrylate ratio of 50:50. The protocol of synthesis was adapted from A.Anastasaki (2013) in which high conversion rate of monomer to polymer was reported.[8] Duration of reaction was also made a variable. Samples were dissolved in tetrahydrofuran (THF) post-reaction and 1H NMR analysis in d-chloroform was conducted. As the reaction progressed, the ester peak (~4ppm) which corresponded to the monomer ester functional group diminished gradually. Meanwhile, a new ester peak (~3.9ppm) which corresponded to the polymer ester functional group emerged and the signal strength increased over time. This shift was due to the change in the proton environment as a result of polymerisation. The shift allowed the conversion rate of monomer to polymer to be determined via integration of the ester peak in the NMR spectra. A kinetic plot was then made to study the conversion rate over time.

Polymer products were then purified by running them through an aluminium oxide column to remove the copper catalyst, rota-vaporisation to remove THF and lastly decanting off DMSO. Samples were prepared for polymer characterisation using GPC with chloroform as eluent to determine PDI.

Fig. 6: Vial contents of photo-induced LRP under varying reaction durations (from left: 1.5 hours, 2 hours and 3 hours) dissolved in THF in order to obtain a homogenous sample for NMR.
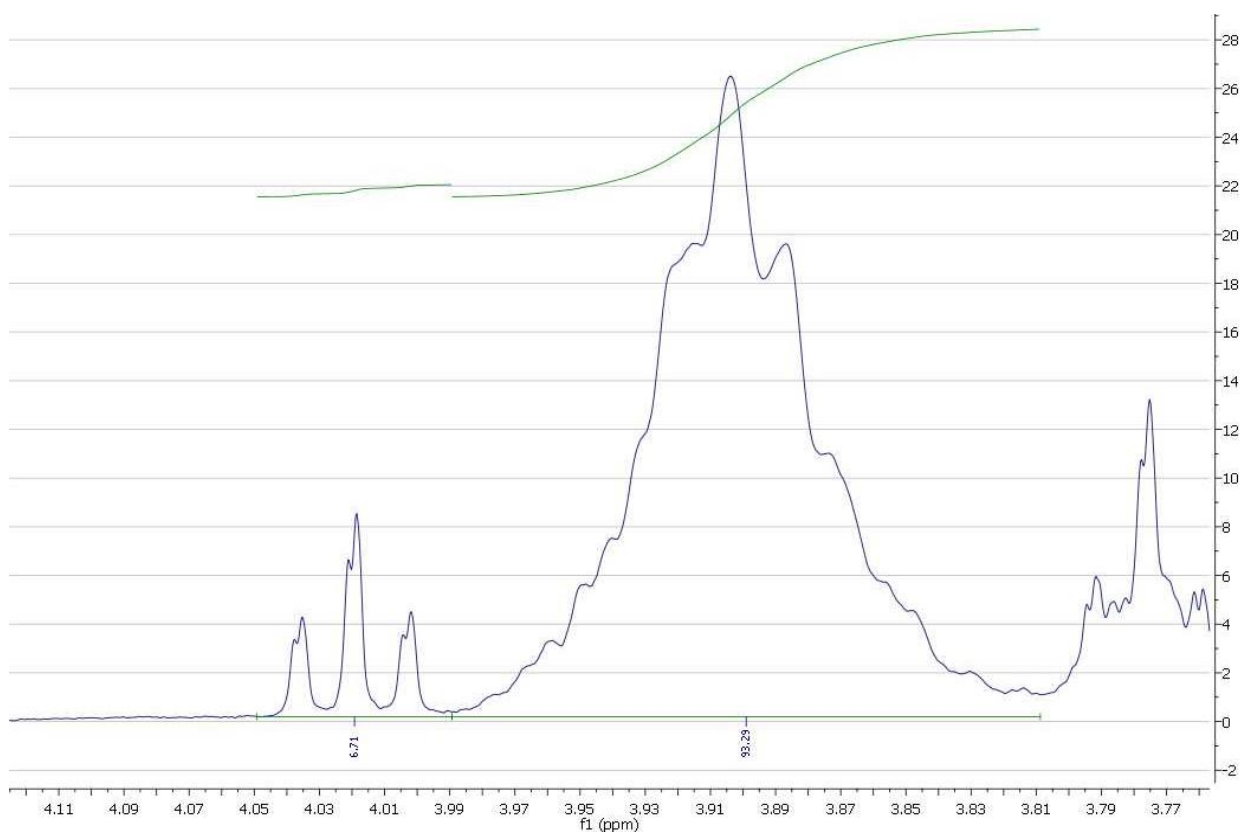
Outcome:

Fig. 7: NMR spectra for ZY01(3). The small peaks at 4.05~3.99ppm corresponded to the monomer ester functional group whereas the broad peak at 3.99~3.81ppm corresponded to the polymer ester functional group. Using integral method, the combined Area Under Curve (AUC) of the peaks was set at 100(%). AUC for each peak (monomer and polymer) was then determined. The AUC of polymer peak was the conversion rate. The same method was used to work out the conversion rate for all similar reactions.
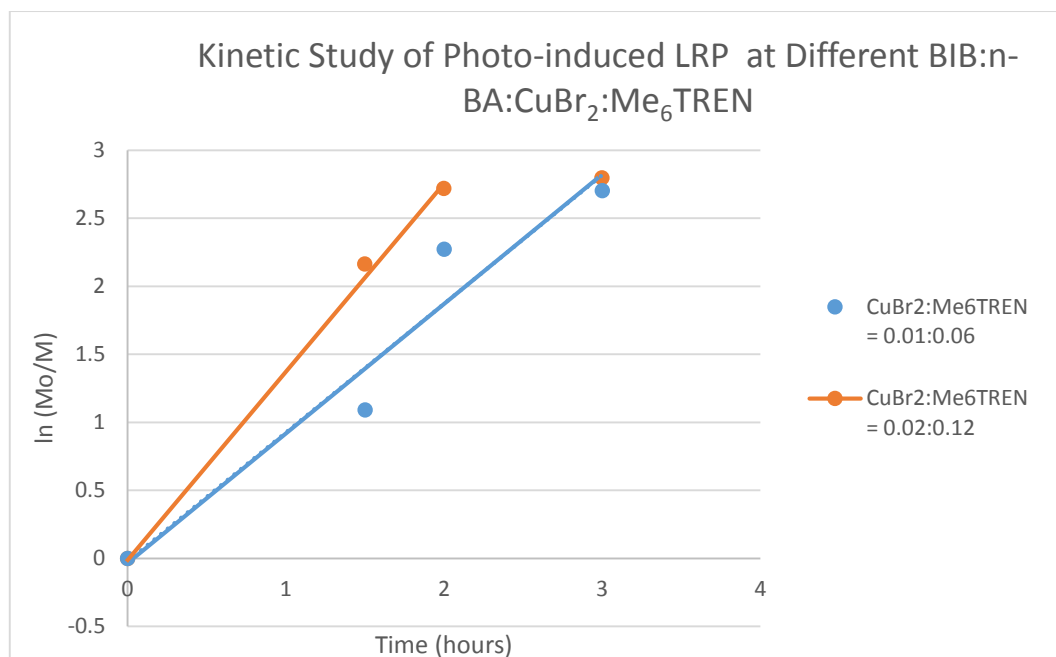
Fig. 8: Polymerisation showed first order kinetics for the two different BIB:n-BA:CuBr$_2$:Me$_6$TREN. For ZY02, the graph reached a plateau from t=2 hours to t=3 hours.

Polymer Code	CuBr$_2$	Me$_6$TREN	Duration (h)	Conversion	PDI
ZY01 (1)	0.01	0.06	1.5	66.4%	1.16
ZY01(2)	0.01	0.06	2	89.7%	1.11
ZY01 (3)	0.01	0.06	3	93.3%	1.12
ZY02(1)	0.02	0.12	1.5	88.5%	1.10
ZY02(2)	0.02	0.12	2	93.4%	1.15
ZY02(3)	0.02	0.12	3	93.9%	1.12

Table 2: Conversion rate and PDI of hydrophobic polymers synthesised using different amount of CuBr$_2$ and Me$_6$TREN for various durations (t=1.5 hours, t=2 hours and t=3 hours).

At t=1.5 hours, conversion rate for ZY02 was significantly higher (88.5%) as compared to ZY01 (66.4%). However, as the reaction approached 100% conversion rate, there seemed to be a saturation point for the reaction at approximately 94% conversion rate as seen from the lack of reaction progress in ZY02 after t=2 hours. The saturation point could possibly be due to very low concentration of monomers which failed to achieve sustainable collisions with the polymer macrostructure for the reaction to progress. Polymer-monomer collisions might be made worse by the entanglement of polymers, resulting in the inaccessibility of the polymer end chain.

At t=3 hours, conversion rates of the two different CuBr$_2$:Me$_6$TREN reactions were approximately the same. There was no evidence suggesting that doubling the amount of CuBr$_2$ and Me$_6$TREN would significantly increase the polymer conversion rate to achieve 100%. Doubling the amount of catalyst and ligand would only save 1 hour time and possibly result in a less controlled polymerisation reaction due to higher rate of reaction. PDI was low (<1.2) for both [CuBr$_2$]:[Me$_6$TREN] at all reaction times. BIB:n-BA:CuBr$_2$:Me$_6$TREN ratio of 1:50:0.01:0.06 was sufficient to achieve >90% conversion rate at t=3 hours, polymerisation proceeded at a controlled rate (first-order kinetics) and polymers of low PDI were synthesised. Therefore, the chain extension reaction was to be conducted at a BIB:n-BA:CuBr$_2$:Me$_6$TREN ratio of 1:50:0.01:0.06 for a duration of 3 hours.

Chain Extension of Macroinitiator via Photo-induced Living Radical Polymerisation

Photo-induced living radical polymerisation was conducted with Macroinitiator:n-BA:CuBr$_2$:Me$_6$TREN ratio of 1:50:0.01:0.06 for 3 hours. During the first reaction attempt (ZY03), separation occurred due to significantly large differences in lipophilicity of the macroinitiator (hydrophilic) and n-butylacrylate (hydrophobic). DMSO:n-butyl acrylate ratio of 50:50 was not sufficient to solubilise n-butylacrylate and the ratio was increased to 75:25. After 3 hours, the reaction was terminated and the sample was dissolved in THF and observations were made.

NMR sampling was conducted in d-DMSO to confirm presence of macroinitiator chain extension. Unfortunately, for the trial experiment (ZY03), NMR analysis could not be done as the sample was too diluted with non-deuterated-DMSO. The conversion rate for ZY03 was not able to be determined. The issue was discussed and the experiment was repeated (ZY04) using d-DMSO instead of DMSO as the solvent system. NMR analysis was conducted and AUC was determined using integration method whereby amide peak at 7.6ppm was set as reference at an integral value of 40. This is because on average, each hydrophilic polymer chain should have 40 monomers (initiator:monomer = 1:40). Conversion rate was estimated by comparison of AUC of acrylate peak at 5.8~6.3ppm before and after reaction. After 3 hours, the polymer product was purified via dialysis method and lyophilisation as described in Fig. 2. The pure polymer product was then characterised using GPC with dimethylformaldehyde as eluent.
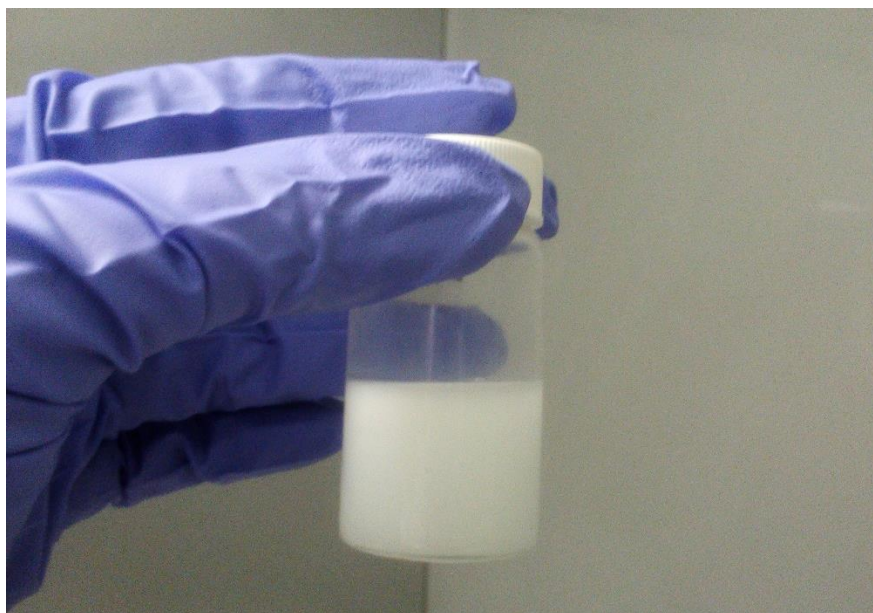
Outcome:

 Fig. 9: Formation of cloudy solution in ZY03 upon addition of THF which did not separate over time.

Upon addition of THF, cloudy solution was formed, indicating presence of co-polymer. This claim was supported by A. Stimula (2015) as the team also reported the formation of cloudy solution and presence of micelles was confirmed using Transmission Electron Microscopy (TEM).[9]
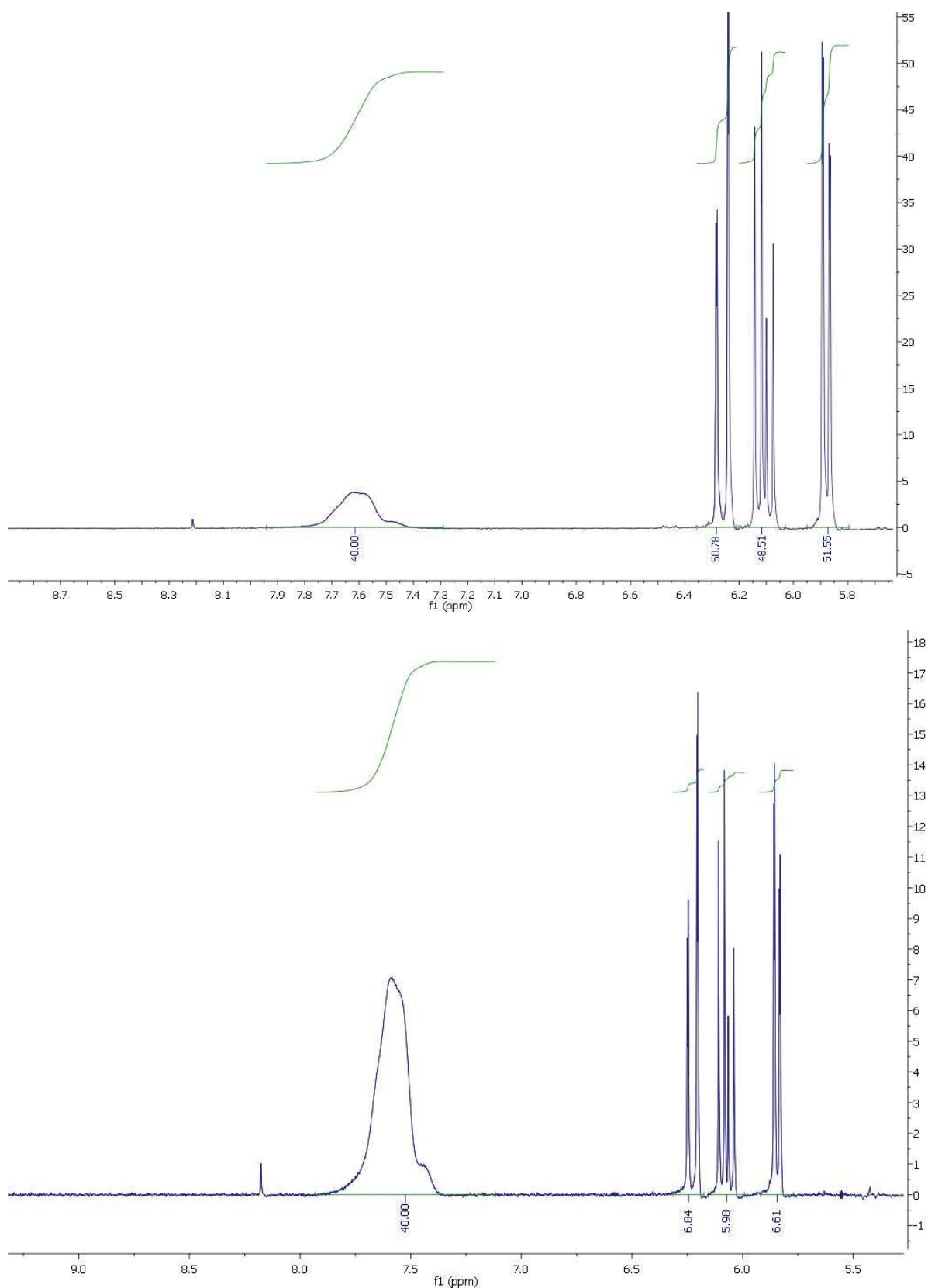
Fig. 10: Peak at 7.6ppm corresponded to the amide functional group in the macroinitiator whereas the 3 peaks at 5.8~6.3ppm corresponded to the acrylate functional group of n-butylacrylate. At t=0 hour (above), ratio of macroinitiator:monomer was approximately 40:50. At t=3 hours (below), ratio of macroinitiator:monomer was 40:6.48 (average AUC value for all three peaks). AUC for the acrylate peaks at 5.8~6.3ppm decreased by 43.52, implying that conversion rate was 87%.

Polymer Code	CuBr$_2$:Me$_6$TREN	Duration (h)	Conversion	Polydispersity Index
ZY04	0.01:0.06	3	87.0%	n.d.

Table 3: Conversion rate at t=3 hours and PDI of ZY04.

Unfortunately, Dimethylformaldehyde eluent GPC was not operational due to a technical breakdown and hence PDI for ZY04 was not able to be determined. That said, however, a tri-block co-polymer ZY04 was successfully synthesised at a considerably high conversion rate of 87%. After leaving the vial for 3 days, NMR analysis was conducted. It was found that conversion rate remained at 87% after 3 days post-reaction. There seemed to be a saturation point in the chain extension reaction as well, but lower (87%). In addition to the possible explanations as described previously, chain-extended macroinitiator might have self-assembled, causing the hydrophobic polymer end chain to be burrowed deep within the core of the micelle structure thus preventing reaction with monomers.

Conclusion

The project was successful in synthesising the tri-block co-polymer but its characterisation was not achieved due to technical difficulties with the GPC and time constraint. Future work should be focused on further optimisation of the protocol or exploring new ways of synthesis in order to achieve near 100% conversion rate, desired polymer molecular weight and low PDI.

Acknowledgement

References

1. Simmons T. Neutropenic sepsis: prevention and management of neutropenic sepsis in cancer patients [Internet]. 1st ed. National Institute for Health Clinical Excellence (NICE); 2012 [cited 9 October 2015]. Available from: https://www.nice.org.uk/guidance/cg151/resources/cg151-neutropenic-sepsis-needs-assessment2

2. Cohen L, de Moor C, Eisenberg P, Ming E, Hu H. Chemotherapy-induced nausea and vomiting—incidence and impact on patient quality of life at community oncology settings. Support Care Cancer. 2006;15(5):497-503.

3. Ballatori E, Roila F, Ruggeri B, Betti M, Sarti S, Soru G et al. The impact of chemotherapy-induced nausea and vomiting on health-related quality of life. Support Care Cancer. 2006;15(2):179-185.

4. Celsion.com. ThermoDox® — Celsion [Internet]. 2015 [cited 10 October 2015]. Available from: http://celsion.com/docs/technology_thermodox

5. Duro-Castano A, Conejos-Sánchez I, Vicent M. Peptide-Based Polymer Therapeutics. Polymers. 2014;6(2):515-551.

6. Nature.com. Self-assembly: Flower Power [Internet]. 2010 [cited 10 October 2015]. Available from: http://www.nature.com/am/journal/2010/201010/full/am2010166a.html

7. Zhang Q, Wilson P, Li Z, McHale R, Godfrey J, Anastasaki A et al. Aqueous Copper-Mediated Living Polymerization: Exploiting Rapid Disproportionation of CuBr with Me 6 TREN. J Am Chem Soc. 2013;135(19):7355-7363.

8. Anastasaki A, Nikolaou V, Zhang Q, Burns J, Samanta S, Waldron C et al. Copper(II)/Tertiary Amine Synergy in Photoinduced Living Radical Polymerization: Accelerated Synthesis of ω-Functional and α,ω-Heterofunctional Poly(acrylates). J Am Chem Soc. 2014;136(3):1141-1149.

9. Simula A, Nurumbetov G, Anastasaki A, Wilson P, Haddleton D. Synthesis and reactivity of α,ω-homotelechelic polymers by Cu(0)-mediated living radical polymerization. European Polymer Journal. 2015;62:294-303.