SYNTHESIS OF METHACRYLATE- ACRYLAMIDE BASED COPOLYMER VIA REVERSE ADDITION-FRAGMENTATION CHAIN TRANSFER POLYMERIZATION WITH THIOL- CONJUGATION AND THERMORESPONSIVE STUDIES

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DEDICATION

Specially dedicated to my amazing parents
Encik Sailan Bin Samingin and Puan Rukimah Binti Rosdan
My husband, Rozaidi Bin Mazlam
All my family members and friends.

"Thank you for the endless love, support and everything "

I love you, Lillahi Taala

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ABSTRACT

The potential of poly(2-hydroxyethyl methacrylate)-*random*-poly(2-chloroethyl methacrylate)-*block*-poly(*N*-isopropylacrylamide) (PHEMA-*r*-PCEMA-*b*-PNIPAM) for biological purpose was investigated. In this study, the synthetic strategies to prepare this copolymer were developed. Initially, PHEMA and PNIPAM were synthesized via reversible addition-fragmentation chain transfer (RAFT) radical polymerization to obtain a controlled molecular weight of the polymer. 4-Cyanopentanoic acid dithiobenzoate (CPADB) and 4,4'-azobis(4-cyanopentanoic acid) (ACPA) were chosen as the RAFT agent and initiator due to its compatibility with both monomers. In order to replace hydroxyl group (-OH) in PHEMA macroRAFT with chlorine (-Cl), thionyl chloride ($SOCl_2$) was used as chlorinating agent and copolymer of PHEMA-*r*-PCEMA was successfully synthesized. The targeted copolymer, PHEMA-*r*-PCEMA-*b*-PNIPAM, was successfully synthesized by polymerizing PHEMA-*r*-PCEMA with *N*-isopropylacrylamide (NIPAM) monomer. The change of lower critical solution temperature (LCST) points for PNIPAM macroRAFT, PHEMA-*b*-PNIPAM and PHEMA-*r*-PCEMA-*b*-PNIPAM were observed. A decreasing trend of temperature was spotted as the copolymer hydrophobicity in aqueous solution was increased. Both PHEMA-*r*-PCEMA and PHEMA-*r*-PCEMA-*b*-PNIPAM were reacted with thiolglycerol to form a thioester *via* thiol-halogen S_N2 nucleophilic substitution. PHEMA-*r*-PCEMA-*b*-PNIPAM potential as protein-polymer modification material was investigated by reacting PHEMA-*r*-PCEMA-*b*-PNIPAM with cysteine. Based on the ^1H NMR result, chlorine (-Cl) was successfully substituted with cysteine. Thus, PHEMA-*r*-PCEMA-*b*-PNIPAM can potentially be used for biological applications.

ABSTRAK

Keupayaan poli(2-hidroksietil metakrilat)-*rawak*-poli(2-kloroetil metakrilat)-*blok*-poli(*N*-isopropilakrilamida) (PHEMA-*r*-PCEMA-*b*-PNIPAM) untuk tujuan biologi telah dikaji. Dalam kajian ini, strategi sintesis untuk menyediakan kopolimer ini telah dibangunkan. Pada mulanya, PHEMA dan PNIPAM telah disintesis melalui pempolimeran radikal perpindahan rantai penambahan-penyerpihan berbalik (RAFT) dengan tujuan untuk mendapatkan polimer dengan berat molekul yang terkawal. Asid 4-sianopentanoik ditiobenzoat (CPADB) dan 4,4'-azobis(4-asid sianopentanoik) (ACPA) telah dipilih sebagai agen RAFT dan pemula kerana keserasiannya dengan kedua-dua monomer. Untuk menggantikan kumpulan hidroksil (-OH) dalam makroRAFT PHEMA dengan kumpulan klorin (-Cl), tionil klorida ($SOCl_2$) digunakan sebagai ejen pengklorinan dan PHEMA-*r*-PCEMA kopolimer telah berjaya dihasilkan. Kopolimer yang disasarkan, PHEMA-*r*-PCEMA-*b*-PNIPAM, telah berjaya disintesis dengan pempolimeran PHEMA-r-PCEMA dengan monomer *N*-isopropilakrilamida (NIPAM). Perubahan titik suhu larutan genting lebih rendah (LCST) makroRAFT PNIPAM, PHEMA-*b*-PNIPAM dan PHEMA-*r*-PCEMA-*b*-PNIPAM telah diperhatikan. Penurunan suhu telah dikesan apabila tahap hidrofobik kopolimer di dalam berair ditingkatkan. Kedua-dua PHEMA-*r*-PCEMA dan PHEMA-*r*-PCEMA-*b*-PNIPAM telah ditindakbalaskan dengan tiolgliserol untuk membentuk tioester melalui penggantian S_N2 nukleofilik thiol-halogen. Keupayaan PHEMA-*r*-PCEMA-*b*-PNIPAM sebagai bahan pengubahsuaian protein-polimer telah dikaji dengan melakukan tindak balas PHEMA-*r*-PCEMA-*b*-PNIPAM dengan sisteina. Berdasarkan keputusan ^{1}H NMR, klorin (-Cl) telah berjaya digantikan dengan sisteina. Dengan itu, PHEMA-*r*-PCEMA-*b*-PNIPAM berpotensi digunakan untuk aplikasi biologi.

TABLE OF CONTENT

LIST OF TABLE

PNIPAM-*b*-PHEMA MacroRAFT **(10)** and PHEMA-*r*-PCEMA-*b*-PNIPAM MacroRAFT **(11)**

LIST OF FIGURE

PHEMA macroRAFT in DMSO: H_2O (1:4) at ~9°C.

LIST OF SCHEME

PNIPAM macroRAFT **(11)** with cysteine **(15)**

LIST OF ABBREVIATIONS

^{1}H NMR	-	Nuclear Magnetic Resonance
ACPA	-	4,4'-Azobis(4-cyanopentanoic acid)
ATR-FTIR	-	Attenuated total reflectance fourier transform infrared
ATRP	-	Atom transfer radical polymerization
$CDCl_3$	-	Deuterated Chloroform
CEMA	-	2-Chloroethyl methacrylate
CPADB	-	4-cyanopentanoic acid dithiobenzoate
CRP	-	Controlled radical polymerization
d-DMSO	-	Deuterated dimethyl sulfoxide
DMF	-	Dimethylformamide
DMSO	-	Dimethyl sulfoxide
FRP	-	Free radical polymerization
HEMA	-	2-Hydroxyethyl methacrylate
LCST	-	Lower critical solution temperature
NIPAM	-	*N*-isopropylacrylamide
NMP	-	Nitroxide mediated polymerization
PCEMA	-	Poly(2-chloroethyl methacrylate)
PHEMA	-	Poly(2-hydroxyethyl methacrylate)
PHEMA-*b*-PNIPAM		Poly(2-hydroxyethyl methacrylate)-*block*-poly(N-isopropylacrylamide
PHEMA-*r*-PCEMA	-	Poly(2-hydroxyethyl methacrylate)-*random*-poly(2-chloroethyl methacrylate)
PHEMA-*r*-PCEMA-*b*-PNIPAM		Poly(2-hydroxyethyl methacrylate)-*random*-poly(2-chloroethyl methacrylate)-*block*-poly(N-isopropyl-acrylamide)

PNIPAM	-	Poly(*N*-isopropylacrylamide)
RAFT	-	Reversible addition–fragmentation chain transfer
$SOCl_2$	-	Thionyl chloride

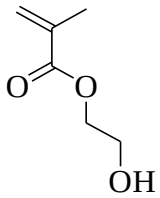
CHAPTER 1

INTRODUCTION

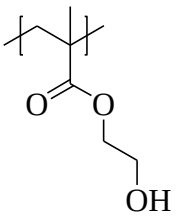
1.1 Background of Study

The evolution of polymer's application, especially biocompatible polymer in biomedical field attracted researcher's attention to work on it. Since there was no valid method to evaluate the material's biocompatibility, the acceptance of body towards a material was the key to evaluate its compatibility to living organisms [1].

From 2-hydroxyethyl methacrylate (HEMA) **(1)**, poly(2-hydroxyethyl methacrylate) (PHEMA) **(2)** had been synthesized through various polymerisation method. PHEMA **(2)** was known to be a non-toxic polymer which was favourably compatible with human's tissue (biocompatible) and had high content of water. Through chemical modification, its physical properties could be easily manipulated [2, 3]. PHEMA **(2)** and its hydrogels had been reported to be the first synthetic hydrogel used in the pharmaceutical and medical fields [4].



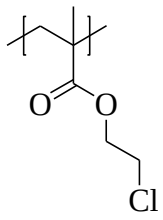
(1)



(2)

Since 1960, PHEMA **(2)** had been widely used in a wide numbers of application including the soft contact lenses production, drug delivery, artificial corneas and degradable scaffolds for tissue engineering [5, 6]. Although PHEMA **(2)** was known as a hydrophilic type of polymer and had a high degree of hydration, it was insoluble in water [7]. PHEMA **(2)** swelled considerably in water due to the water affinity towards the hydrophilic hydroxyl sites [6].

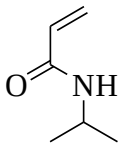
One of the products from the chemical modification of PHEMA **(2)** is poly(2-chloroethyl methacrylate) (PCEMA) **(3)**. By using chlorination agent, such as thionyl chloride ($SOCl_2$) [8], hydroxyl group of PHEMA **(2)** was substituted by chlorine to obtain PCEMA **(3)**. As the result, the physical properties of PHEMA **(2)** tuned from hydrophilic to hydrophobic of poly(2-chloroethyl methacrylate) (PCEMA) **(3)**.



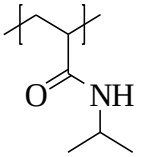
(3)

Many researchers paid overwhelming attention to build up new copolymer based on the thermoresponsive polymer over many decades ago. This modification of copolymers based on thermoresponsive polymer had been reported to increase the biocompatible and tuneable response of the properties of the copolymers [9].

From its monomer, *N*-isopropylacrylamide (NIPAM) **(4)**, a novel thermoresponsive and biocompatible polymer, poly-(*N*-isopropylacrylamide) (PNIPAM) **(5)** could be synthesized via various methods of polymerization. Interestingly, PNIPAM **(5)** has novel characteristic which is insoluble in aqueous as the temperature of the solution increase more than 32°C. This characteristic was different from other polymers which have their solubility increase as the temperature increases. Due to its LCST was approximately around human body temperature which is 37°C, the application of PNIPAM (**5**) had been widely explored including in tissue engineering, drug and gene delivery [10].



(4)



(5)

Controlled radical polymerization (CRP) had been paid off with great interest recently in polymer synthesis due to the ability to form well-defined structure of polymers with narrow polydispersities [11]. Reverse addition-fragmentation chain transfer (RAFT) polymerization, one of the CRPs methods, had been widely known for its application in polymerisation process. Due to its tolerance to huge range of monomer's functional group, it was attractive for its simplicity but can afford to synthesize complex architecture of polymers [12] such as stars polymers, hyperbranched polymers, block copolymers and higher order supramolecular structure.

Most proteins were known to be chemically and physically unstable, which became a barrier on its application in pharmaceutical interest [13]. Due to this shortcoming, polymer modification on protein had been done and successfully overcame the shortcomings in pharmaceutical concern [14]. Thus, huge potential of polymer- protein modified material in biomedical interest could be discovered further.

1.2 Problems Statement

Many researchers had put much interest in biocompatible and biodegradable polymer and their application in medical field. The biocompatibility of the polymer gave a lot of benefit especially in modern medical field.

Although a lot of polymers were biocompatible and biodegradable, most of them had been found to be lacked in terms of degradation and also compatibility to human body. Thus, most of them need to be modified so that its properties meet the desired need. Poly(2-hydroxyethyl methacrylate) (PHEMA) **(2)** and poly(*N*-isopropylacrylamide) (PNIPAM) **(5)** has been widely known as biocompatible polymer. By incooperating PHEMA **(2)** with another polymer will result increase in mechanical strength and improved their biocompatibility. Copolymer consist of PNIPAM **(5)** had turned to have tuneable response. The response can be altered based on the different types and properties of copolymers attached on PNIPAM **(5)**. The copolymer consists of PCEMA **(3)** and PNIPAM **(5)** will exhibit different properties from another thermoresponsive polymer. The hydrophobicity of PCEMA **(3)** may alter the hydrophilic properties of PNIPAM **(5)** to form a hydrophobic copolymer.

Due to limitations of conventional polymerization method, it is difficult to synthesize polymer to meet specific application. It was because, in conventional polymerization method, they have weak control over the polymerization process. Because of that, controlled

radical polymerization (CRP) was chosen to overcome the limitations. Herewith, reverse addition fragmentation chain transfer (RAFT) polymerization method is chosen as the main polymerization method. It has good control in synthesized polymer with small range molecular weight.

Since most of the polymers were not easily being modified, the applications of the polymers were fixed to a number of applications. By added a good leaving functional group such as chlorine (-Cl), further modification can be introduced that lead to the increasing of polymer application.

1.3 Objectives

The objective for this experiment is as follow;

1. To synthesis and characterise homopolymer of PHEMA macroRAFT **(2)** and PNIPAM macroRAFT **(5)**.
2. To synthesis block copolymer PHEMA-*r*-PCEMA **(9)**, PHEMA-*b*-PNIPAM **(10)** and PHEMA-*r*-PCEMA-*b*-PNIPAM **(11)** by using reverse addition fragmentation chain transfer (RAFT) polymerisation.
3. To compare the change of lower critical solution temperature (LCST) of PNIPAM macroRAFT **(5)** and its block copolymer, PHEMA-*b*-PNIPAM **(10)** and PHEMA-*r*-PCEMA-*b*-PNIPAM **(11)** in aqueous solution.

4. To investigate the reaction of PHEMA-*r*-PCEMA **(9)** and PHEMA-*r*-PCEMA-*b*-PNIPAM **(11)** with thiol and cysteine **(15)** via thiol-halogen S_N2 nucleophilic substitute for modification of polymer as precursor for further functionalization.

1.4 Scope of Research

In this study, we focused on the synthesis of homopolymer of PHEMA macroRAFT **(2)** and PNIPAM macroRAFT **(5)** along with block copolymer of PHEMA-*r*-PCEMA **(9)**, PHEMA-*b*-PNIPAM **(10)** and PHEMA-*r*-PCEMA-*b*-PNIPAM **(11)** by using reverse addition fragmentation chain transfer (RAFT) polymerisation.

All the homopolymers were characterised by using ^{1}H NMR and attenuated total reflectance fourier transform infrared (ATR-FTIR). While the block copolymers were characterized by using ^{1}H NMR and HMQC 2D NMR. The response of block copolymers, PHEMA-*b*-PNIPAM **(10)** and PHEMA-*r*-PCEMA-*b*-PNIPAM **(11)** compared to PNIPAM **(5)** in term of their LCST point were observed to discover any changes of PNIPAM after being modified.

Then, the presence of chlorine atom in PHEMA-*r*-PCEMA **(9)** and (PHEMA-*r*-PCEMA-*b*-PNIPAM **(11)** as good leaving group were tested on polymer modification by using thiol-halogen S_N2 nucleophilic substitution approached. PHEMA-*r*-PCEMA-*b*-

PNIPAM) **(11)** ability to react with amino acid containing thiol (-SH), cysteine, was discovered.

1.5 Significant of Research

Since, natural polymer made up from natural resource such as carbohydrate and protein have limitation which easily being degraded enzymatically and lose their bioactivity, polymer- protein modified material was favourable to be used in medical application such as in tissue engineering and drug delivery. This was because, polymer- protein modified material had longer circulating lifetime, reduced immunogenicity and easier to handle. Thus, synthetic polymers made up from HEMA **(1)** and NIPAM **(4)** had been chosen to be used in this polymer- protein modified material because it had widely been known to be biocompatible and widely used in medical applications.

Besides that, as the formulation and molecular weight of the synthetic polymers can be controlled by the polymerization synthesis, such as reverse addition fragmentation chain transfer (RAFT) polymerization, therefore, the properties of the polymers could met their specific applications. RAFT polymerization has been chosen as the polymerization synthesis method due to its ability to control the molecular weight of the polymers although in complex building of polymer.

The presence of chloride (-Cl) which was a good leaving group, will let the final product easily being modified to its desired need. As we targeted the final product to be introduced in medical field application as polymer- protein modified material, amino acid and protein containing sulphur such as cysteine and its residue were used due to it is possible for chlorine (-Cl) to be substituted by sulphur (-SH) to form a thioester. Thus the application of the polymer could be optimized. By having good leaving group on polymer- protein modified material, the properties of the material can be altered to its specific need by introducing specific polymer.

REFERENCE

1. Chen, H., Yuan, L., Song, W., Wu, Z., and Li, D. (2008), Biocompatible polymer materials: Role of protein–surface interactions. *Progress in Polymer Science*, **33**(11): 1059-1087.

2. Saini, R., Bajpai, J., and Bajpai, A. (2012), Synthesis of Poly (2-Hydroxyethyl Methacrylate) (PHEMA) Based Nanoparticles for Biomedical and Pharmaceutical Applications. *In M. Soloviev (Ed.), Nanoparticles in Biology and Medicine*, **906**: 321-328.

3. Wang, X.-H., Cao, L.-Q., Zhang, L.-J., and Wang, J.-D. (2012), Precipitation polymerization of 2-hydroxyethyl methacrylate in supercritical carbon dioxide. *Polymers for Advanced Technologies*, **23**(3): 529-533.

4. Giglio, E. D., Cafagna, D., Giangregorio, M. M., Domingos, M., Mattioli-Belmonte, M., and Cometa, S. (2011), PHEMA-based thin hydrogel films for biomedical applications. *Journal of Bioactive and Compatible Polymers*.

5. Paterson, S. M., Brown, D. H., Chirila, T. V., Keen, I., Whittaker, A. K., and Baker, M. V. (2010), The synthesis of water-soluble PHEMA via ARGET ATRP in protic media.

Journal of Polymer Science Part A: Polymer Chemistry, **48**(18): 4084-4092.

6. Plummer, R., Hill, D. J. T., and Whittaker, A. K. (2006), DOSY NMR Studies of Chemical Exchange Behavior of Poly(2-hydroxyethyl methacrylate). *Macromolecules*, **39**(11): 3878-3889.

7. Robinson, K. L., Khan, M. A., Paz Báñez, M. V., Wang, X. S. and Armes, S. P. (2001) *Controlled Polymerization of 2-Hydroxyethyl Methacrylate by ATRP at Ambient Temperature*. Macromolecules, **34**: 3155-3158.

8. Holata, J., Ševčík, S., and Štamberg, J. (1974), Chemical transformations of polymers. XVI. Preparation and splitting of poly (methacryloyloxyethyltrimethyl-ammonium hydroxide). *Journal of Polymer Science: Polymer Symposia*, **47**(1): 335-343.

9. Roy, D., Brooks, W. L. A., and Sumerlin, B. S. (2013), New directions in thermoresponsive polymers. *Chemical Society Reviews*, **42**(17): 7214-7243.

10. Park, Y. I., Zhang, B., Kuo, C.-Y., Martinez, J. S., Park, J., Mallapragada, S., and Wang, H.-L. (2013), Stimuli-Responsive Poly-N-isopropylacrylamide: Phenylene

Vinylene Oligomer Conjugate. *The Journal of Physical Chemistry C*, **117**(15): 7757-7763.

11. Islam, M. R., Bach, L. G., Park, J. M., Hong, S.-S., and Lim, K. T. (2013), Synthesis and characterization of poly(HEMA-*co*-MMA)-*g*-POSS nanocomposites by combination of reversible addition fragmentation chain transfer polymerization and click chemistry. *Journal of Applied Polymer Science*, **127**(3): 1569-1577.

12. Chiefari, J., Chong, Y. K., Ercole, F., Krstina, J., Jeffery, J., Le, T. P. T., Mayadunne, R. T. A., Meijs, G. F., Moad, C. L., Moad, G., Rizzardo, E. and Thang, S. H. (1998) Living Free-Radical Polymerization by Reversible Addition-Fragmentation Chain Transfer: The RAFT Process. *Macromolecules*, **31**: 5559-5562.

13. Maulik, V. T., Jennifer, S. L., and Teruna, J. S. (2009), The Role of Thiols and Disulfides on Protein Stability. *Current Protein & Peptide Science*, **10**(6): 614-625.

14. Kochendoerfer, G., (2003), Chemical and biological properties of polymer-modified proteins. *Expert Opinion on Biological Therapy*, **3**(8): 1253-1261.

15. Michael M. Coleman, P. C. P. (1997), *Fundamentals of Polymer Science: An Introductory Text.* Lancaster, PA: Technomic Pub.

16. Wade, L. G. (2010), *Organic Chemistry* (7th ed.). United States, America: Pearson Prentice Hall.

17. Harris, F. W. (1981), Introduction to Polymer Chemistry. *Journal of Chemical Education*, **58**(11): 837-843.

18. Matyjaszewski, K., and Spanswick, J. (2005), Controlled/living radical polymerization. *Materials Today*, **8**(3): 26-33.

19. Willcock, H., and O'Reilly, R. K. (2010), End group removal and modification of RAFT polymers. *Polymer Chemistry*, **1**(2): 149-157.

20. Moad, G., Rizzardo, E., and Thang, S. H. (2006), Living Radical Polymerization by the RAFT Process—A First Update. *Australian Journal of Chemistry*, **59**(10): 669-692.

21. Moad, G., Chong, Y. K., Postma, A., Rizzardo, E., and Thang, S. H. (2005), Advances in RAFT polymerization: the synthesis of polymers with defined end-groups. *Polymer*, **46**(19): 8458-8468.

22. Liu, L., Wu, C., Zhang, J., Zhang, M., Liu, Y., Wang, X., and Fu, G. (2008), Controlled polymerization of 2-(diethylamino)ethyl methacrylate and its block copolymer with N-isopropylacrylamide by RAFT polymerization. *Journal of Polymer Science Part A: Polymer Chemistry*, **46**(10): 3294-3305.

23. Chou, K. F., Lee, S. and Han, C. C. (2000), Water transport in crosslinked 2-hydroxyethyl methacrylate. *Polymer Engineering & Science*, **40**(4): 1004-1014.

24. Zeng, R. C., Cheng, J., Xu, S. P., Liu, Q., Wen, X. F., and Pi, P. H. (2014), *Preparation of antimicrobial PHEMA-g-PCBMAE hydrogels with improved mechanical properties*. *Applied Mechanics and Materials*, **576**: 49-53.

25. Babu, M., Yadav, H. K. S., Moin, A., and Shivakumar, H. G. (2011), In vitro–in vivo evaluation of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel implants containing cisplatin. *Acta Pharmaceutica Sinica B*, **1**(4): 261-267.

26. Samsonova, O., Pfeiffer, C., Hellmund, M., Merkel, O. M., and Kissel, T. (2011), Low Molecular Weight pDMAEMA-block-pHEMA Block-Copolymers Synthesized via RAFT-Polymerization: Potential Non-Viral Gene Delivery Agents, *Polymers*, **3**(2): 693.

27. Dobić, S. N., Filipović, J. M., and Tomić, S. L. (2012), Synthesis and characterization of poly(2-hydroxyethyl methacrylate/itaconic acid/poly(ethylene glycol) dimethacrylate) hydrogels. *Chemical Engineering Journal*, **179**: 372-380.

28. Deng, N., Ni, X., and Shen, Z. (2015), Syntheses and properties of amphiphilic poly (diethyl vinylphosphonate-*co*-2-chloroethyl methacrylate) copolymers. *Designed Monomers and Polymers*, **18**(5): 470-478.

29. Salsamendi, M., Cormack, P. A. G., and Graham, D. (2013), Template-directed synthesis of uniformly-sized silver nanoparticles with high colloidal stability. *New Journal of Chemistry*, **37**(11): 3591-3594.

30. Ono, Y., and Shikata, T. (2007), Contrary Hydration Behavior of N-Isopropylacrylamide to its Polymer, P(NIPAm), with a Lower Critical Solution Temperature. *The Journal of Physical Chemistry B*, **111**(7): 1511-1513.

31. Zhao, H., Chen, H., Li, Z., Su, W., and Zhang, Q. (2006), The synthesis of temperature-sensitive PMMA-coating PNIPAM particles via a rapid microwave-assisted polymerization. *European Polymer Journal*, **42**(9): 2192-2198.

32. Schild, H. G. (1992), Poly(N-isopropylacrylamide): experiment, theory and application. *Progress in Polymer Science*, **17**(2): 163-249.

33. Sierra-Martin, B., Retama, J. R., Laurenti, M., Barbero, A. F., and Cabarcos, E. L. (2014), Structure and polymer dynamics within PNIPAM-based microgel particles. *Advances in Colloid and Interface Science,* **205**: 113-123.

34. Yu, T. L., Lu, W. C., Liu, W. H., Lin, H. L., and Chiu, C. H. (2004), Solvents effect on the physical properties of semi-dilute poly(N-isopropyl acryl amide) solutions. *Polymer*, **45**(16): 5579-5589.

35. Convertine, A. J., Ayres, N., Scales, C. W., Lowe, A. B., and McCormick, C. L. (2004), Facile, Controlled, Room-Temperature RAFT Polymerization of N-Isopropylacrylamide. *Biomacromolecules,* **5**(4): 1177-1180.

36. Tang, T., Castelletto, V., Parras, P., Hamley, I. W., King, S. M., Roy, D., Perrier, S., Hoogenboom, R. and Schubert, U. S. (2006) Thermo-responsive Poly(methyl methacrylate)-*block*-poly(*N*-isopropylacrylamide) Block Copolymers Synthesized by RAFT Polymerization: Micellization and Gelation. *Macromolecular Chemistry and Physics*, **207**(19): 1718-1726.

37. Cooperstein, M. A., and Canavan, H. E. (2013), Assessment of cytotoxicity of (*N*-isopropyl acrylamide) and Poly(*N*-isopropyl acrylamide)- coated surfaces. *Cooperstein and Canavan Biointerphases*, **8**(1): 19.

38. Pelton, R. (2010), Poly(*N*-isopropylacrylamide) (PNIPAM) is never hydrophobic. *Journal of Colloid and Interface Science*, **348**(2): 673-674.

39. Ohya, S., Sonoda, H., Nakayama, Y., and Matsuda, T. (2005), The potential of poly(N-isopropylacrylamide) (PNIPAM)-grafted hyaluronan and PNIPAM-grafted gelatin in the control of post-surgical tissue adhesions. *Biomaterials*, **26**(6): 655-659.

40. Boutureira, O., and Bernardes, G. J. L. (2015), Advances in Chemical Protein Modification. *Chemical Reviews*, **115**(5): 2174-2195.

41. Krishna, O. D., and Kiick, K. L. (2014), Protein- and Peptide-Modified Synthetic Polymeric Biomaterials. *Biopolymers*, **94**(1): 32-48.

42. Monji, N., and Hoffman, A. S. (1987), A novel immunoassay system and bioseparation process based on thermal phase separating polymers. *Applied Biochemistry and Biotechnology*, **14**(2): 107-120.

43. Desponds, A., and Freitag, R. (2005), Light-responsive bioconjugates as novel tools for specific capture of biologicals by photoaffinity precipitation. *Biotechnology and Bioengineering*, **91**(5): 583-591.

44. Chilkoti, A., Chen, G., Stayton, P. S., and Hoffman, A. S. (1994), Site-Specific Conjugation of a Temperature-Sensitive Polymer to a Genetically Engineered Protein. *Bioconjugate Chemistry*, **5**(6): 504-507.

45. Pennadam, S. S., Lavigne, M. D., Dutta, C. F., Firman, K., Mernagh, D., Górecki, D. C., and Alexander, C. (2004), Control of A Multisubunit DNA Motor by a Thermoresponsive Polymer Switch. *Journal of the American Chemical Society*, **126**(41): 13208-13209.

46. Li, M., De, P., Li, H., and Sumerlin, B. S. (2010), Conjugation of RAFT-generated polymers to proteins by two consecutive thiol–ene reactions. *Polymer Chemistry*, **1**(6): 854-859.

47. Karim, K. J. A. (2013), *Polymeric Platinum Conjugated Drug for Cancer Treatment*. The University of New South Wales (UNSW) Australia, Australia.

48. Vega-Rios, A. and Licea-Claveríe, A. (2011), Controlled Synthesis of Block Copolymers containing N-

isopropylacrylamide by Reversible Addition-Fragmentation Chain-Transfer (RAFT) Polymerization. *J. Mex. Chem. Soc.*, **55**(1): 21-32.

49. Matsugi, M. and Kita, Y. (2007). 4,4′-Azobis(4-cyanopentanoic acid), *Encyclopedia of Reagents for Organic Synthesis*. John Wiley & Sons, Ltd.

50. Yan, Q., Yuan, J., Kang, Y., Cai, Z., Zhou, L., and Yin, Y. (2010), Dual-sensing porphyrin-containing copolymer nanosensor as full-spectrum colorimeter and ultra-sensitive thermometer. *Chemical Communications,* **46**(16): 2781-2783.

51. Pavia, D. L., Lampman, G. M., & Kriz, G. S. (2010). *Spectroscopy*: Brooks/Cole, Cengage Learning.

52. Berg, J.M., T. J. and Stryer L. (2002). *Biochemistry* (5th ed.). New York: W. H. Freeman.

53. Boyer, C., Huang, X., Whittaker, M., Bulmus, V., and Davis, T. (2011). *An overview of protein–polymer particles* (Vol. 7)