Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (1) January-March, pp.116-119/Aslam et al.
Research Article

SYNTHESIS OF POLYMERIC NANOPARTICLE "PNIPAM" POLY (N-ISOPROPYLACRYLAMIDE) AND THEIR TOXICITY ASSAY ON SWISS ALBINO MICE *Mus musculus*

Imtiyaz Aslam*, Rohit Kumar Verma and S. P. Roy
**P.G. Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur-812007*
**Author for Correspondence*

ABSTRACT
Through the application of Nanotechnology for development of functional nanoparticles with nanometric dimensions has attracted growing interests in fundamental as well as applied sciences. The nanoparticles are highly potent and are widely used in Medical Sciences as drug carriers. In the present study the polymeric nanoparticle viz- PNIPAM, a thermo-sensitive polymer has been synthesized. The characterization result of prepared nanoparticle reveals that the nanoparticle is about 150 nm in diameter. The toxicity assay of the nanoparticle has been carried out in Swiss albino mice *Mus musculus* for finding the possible applications of these nanoparticles as targeted drug delivery system. The nanoparticles have large surface area per unit volume and this characteristic is explored for the application of targeted drug delivery to the various organs. The ligands are adsorbed on the nanoparticle for the targeted delivery. The tolerance of the amount by the organisms should be determined before using the nanoparticle for the drug delivery vehicle and the permissible level should be administered to achieve the desired effectiveness of the drug.

Key Words: *Nanotechnology, Nanoparticle, PNIPAM, Mus musculus, Ligands*

INTRODUCTION
The PNIPAM i.e. Poly (N-isopropylacrylamide) is a thermoresponsive polymer. It forms a three-dimensional hydrogel when crosslinked with *N,N'*-methylene-bis-acrylamide (MBAm).It is also known as Smart Polymers. The unique feature of this polymer in respond to small changes in the environmental conditions has made this class of materials very promising for several applications in the field of Nanoscience, and Nanomedicine. PNIPAM responds according to the change in temperature. It has Lower Critical Solution Temperature (LCST) at 32^{o} C. By using this unique property of this polymeric nanoparticle researches are going on to use PNIPAM as carrier of drug delivery. The PNIPAM has hydrophobic core and hydrophilic outer shell and can be used for delivery of any hydrophobic molecule. Using nanoparticle as a carrier for drug delivery is helpful in the enhancement of the therapeutic effects and reduction in the side effects. In past years many drug delivery carriers have been employed by using Nanotechnology which is Liposome, Dendrimers etc. The size of the particle in nanometer, their surface charges etc. are the beneficial parameters which directs their pharmokinetics. The application of the smart polymers for drug delivery shows great promise due to modulated or pulsatile drug release pattern to mimic biological demand. Stimuli occurring externally or internally include temperature, pH, and metabolic chemicals.

The property of swelling or shrinking of smart hydrogel beads in response to pH or temperature can be used successfully to control drug release. While integration of Smart polymers into microcapsule wall or a liposomal lipid bilayer, the conformational transition of the polymer affects the integrity of the microcapsule or liposome and allows the regulated release of the drug loaded into the microcapsule or liposome. In a temperature sensitive polymer, a dilute solution (1-3%) of the polymer is watery liquid, while on warming to body temperature the solution gels, becoming viscous and clinging to surface in a 'bioadhesive' form. The hydrogel therefore provides an effective way to administer drugs. By the application of such drug formulations incorporated into hydrogels pharmaceutical companies will be able

to increase the efficiency, cost-effectiveness and range of applications for existing therapeutics. The application of smart polymers for development of glucose-sensitive insulin-releasing system for diabetes therapy has become a popular model.

MATERIALS AND METHOD

PNIPAM Synthesis

Materials used in PNIPAM synthesis

NIPAAM [N-isopropyl acrylamide as Monomer] MW 113.16 ,99% pure, N-VINYL PYRROLIDONE (monomer)99% pure, MW 111.14, MBA (N,N′-methylene-bis-acrylamide) MW 154.17 and TEMED, MW 116.21.

Method of Synthesis

In a round bottom flask10 ml double distilled water was taken and 90 mg NIPAAM (monomer) were added .Then 10 (µL) and 14 (µL) of N-vinyl pyrrolidone and MBA- N, N′-methylene-bis-acrylamide (49 mg/ml in distilled water) for 0.6% cross linking were added respectively. After this the mouth of the flask was covered with Para film and nitrogen gas (N_2) was passed through the solution at a steady speed to attain an inert environment. The assembly was then transferred to a water bath maintained at 35^0C and the round bottom flask was immersed in it. After 10 minutes, 20 µL of TEMED (N, N, N`, N`-Tetra methyl ethylene diamine) was added to polymeric solution and Nitrogen flush (4 bubbles/s) was continued for 10 minutes. Finally, 20 µL of 20% Ammonium per sulfate was added. The whole set up was then kept for 24 hours with constant N_2 flow (4 bubbles/s [100sccm]). After 24 hours the polymeric nanoparticle was taken out from the flask and transferred to an airtight container. The percentage yield was found to be around 40%.

Model Animal

Twenty four female Swiss albino mice *Mus musculus* strain balb C with average body weight ranging from 25.0g to 30.0g were obtained from Animal house of Department of Zoology, T.M. Bhagalpur University, Bhagalpur, India. Food and water to mice were provided *ad libitum* (prepared mixed formulated feed by the laboratory itself). Animals were housed in colony rooms with 12 hrs light/dark cycle at 30 ± 2^oC. Approval of Institutional Ethical Committee was sought prior to the commencement of experiment.

Treatment Protocol

The animals were grouped into five test groups along with one Control was treated with five different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) for five test groups and with one control. Each test group consists of 4 mice. The tests were conducted for 30 days. The water solution of the PNIPAM is given orally at once a day to each test group. The number of deaths in each test group was estimated on day basis.

Table 1: Different Treated Test groups for LD_{50}

	Conc. (mg/ml)	**No. of Deaths**	**Day**
control	0.0	0.0	30
Test 1	0.2	0.0	30
Test 2	0.4	1.0	28
Test 3	0.6	1.0	25
Test 4	0.8	1.0	20
Test 5	1.0	2.0	18

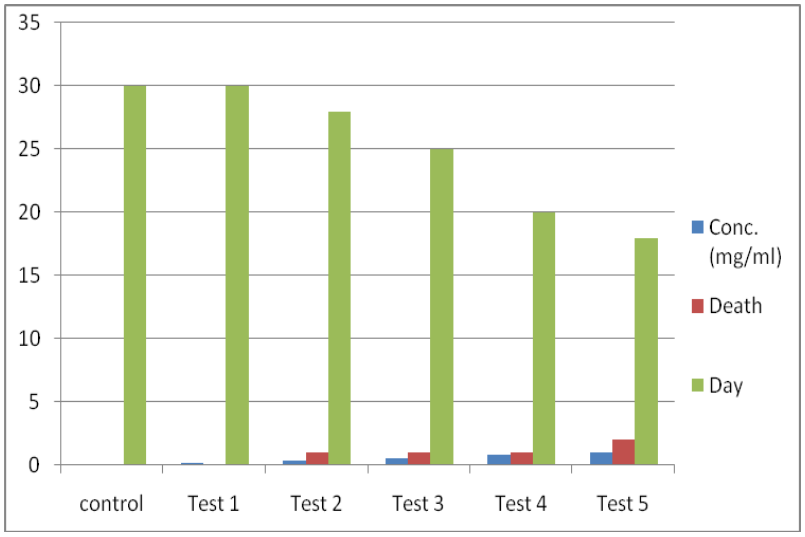

Figure 1: Graph showing LD50 for different Test groups

RESULTS

The nanoparticle synthesized is somewhat larger than the normal range but has reduced diameter in comparison to the normal compound. The characterization of the synthesized particle shows globular nature with a diameter of 150nm.The particle has hydrophilic exterior and hydrophobic interior surface which makes it soluble into polar and slightly polar solvents. The test groups showed abnormal behavior which corresponds to the amount of compound fed to them. The death of the test groups shows that PNIPAM is tolerable at low concentration /kg body weight. The Test group 1 shows no significant effect in comparison to other Test groups. The estimation of LD_{50} suggests that a concentration of 1.0 mg/ml kg b.w. is lethal for half of the population at treatment for 18 days.

DISCUSSION

The PNIPAM is tolerable at the lower concentration but the higher concentration has lethal effect in quick time. The death of the test groups may be due to accumulation of the PNIPAM in the vital organs such as Liver, Spleen and Kidney etc. The histopathological and quantitative estimation of the PNIPAM in these organs may give better detailed conclusion and may further be explored.

ACKNOWLEDGEMENT

The Authors are thankful to University Grants Commission, New Delhi for providing the financial assistance and P.G. Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur for providing the laboratory facility.

REFERENCES

Brazel CS and Peppas NA (1996). Pulsatile local delivery of thrombolytic and antithrombotic agents using poly (N isopropylacrylamide- comethacrylic acid) hydrogels. *Journal of Controlled Release* **39** 57-64.

Chung JE, Yokoyama M and Okano T (2000). Inner core segment design for drug delivery control of thermo-responsive polymeric micelles. *Journal of Controlled Release* **65** 93-103.

Hay DNT, Rickert PG, Seifert S and Firestone MA (2004). Thermoresponsive nanostructures by self-assembly of a poly (N-isopropylacrylamide)– lipid conjugate. *Journal of the American Chemical Society* **126** 2290-2291.

Jeong B and Gutowska A (2002). Lessons from nature: Stimuli-responsive polymers and their biomedical applications. *Trends in Biotechnology* **20** 305-311.

Kujawa P, Aseyev V and Tenhu H (2006). Temperature-Sensitive Properties of Poly(N-isopropylacrylamide) Mesoglobules Formed in Dilute Aqueous Solutions Heated above Their Demixing Point. *Macromolecules* **39** 7686-7693.

Ramanan RMK, Chellamuthu P and Tang L (2006). Development of a Temperature-Sensitive Composite Hydrogel for Drug DeliveryApplications. *Biotechnology Progress* **22** 118-125.

Topp MDC, Dijkstra PJ, Talsma H and Feijen J (1997). Thermosensitive micelle-forming block copolymers of poly(ethylene glycol) and poly(N-isopropylacrylamide). *Macromolecules* **30** 8518-8520.

Weil CS (1952). Tables for convenient calculation of medium effective dose (LD_{50} or ED_{50}) and instruction in their use. *Biometrics* **8** 263-294.

Yoshida R, Uchida K and Kaneko Y (1995). Combtype grafted hydrogels with rapid de-swelling response to temperature-changes. *Nature* **374** 240-242.

Zhang XZ and Zhuo RX (2001). Dynamic properties of temperature-sensitive poly(N-isopropylacrylamide) gel cross-linked through siloxane linkage. *Langmuir* **17** 12-16.