Educational Administration: Theory and Practice
2024, 30(5), 14309 - 14316
ISSN: 2148-2403
https://kuey.net/

Research Article

Novel Tyrosine And Pectin-Based Nanoparticles For Anticancer Application

Yogesh Kumar Sood[1], Maninder Kaur[2*], , Rohini Dharela[3]

[1]sood05yogesh@Gmail.Com, Department Of Chemistry, School Of Sciences, A P Goyal Shimla University, Shimla, Himachal Pradesh, India-171013
[2]kaurmaninder.87@Gmail.Com, Department Of Chemistry, School Of Sciences, A P Goyal Shimla University, Shimla, Himachal Pradesh, India-171013
[3]Rohinidharela@Gmail.Com, Department Of Chemistry, School Of Sciences, A P Goyal Shimla University, Shimla, Himachal Pradesh, India-171013

Citation: Maninder Kaur et al. (2024), Novel Tyrosine And Pectin-Based Nanoparticles For Anticancer Application, Educational Administration: Theory And Practice, 30(5), 14309 - 14316
Doi: 10.53555/kuey.v30i5.6563

ARTICLE INFO

ABSTRACT

Nanoparticles with particle size capable of penetration into deep-seated body tissue are excellent candidates for site-specific therapeutic applications. The present work focuses on the synthesis, characterisation and in vitro MTT assay of novel tyrosine-pectin-based nanoparticles against HeLa Cells. The nanoparticles were synthesized under ultrasonication at 75°C employing citric acid as crosslinker and acrylamide as co-monomer. The synthesized tyrosine-pectin-cl-citric acid-poly(acryl amide) nanoparticle [(Ty-Pc-cl-CA-poly(AAm)] were characterised using techniques such as FT-IR, particle size analysis and FESEM to get evidence of successful nanoparticle synthesis. The characterization results indicated that the nanoparticles of size below 100nm were successfully synthesised. These nanoparticles were employed for anticancer study against HeLa cells and proved to be better cytotoxic agents than the control. They exhibited the IC_{50} value (µg/ml) of 359.8 ± 0.14.

Keywords: Anti-cancer, Citric acid, Pectin, Nanoparticle, Tyrosine

1.Introduction

A novel class of anticancer therapies may be developed with nanotechnology. These compounds medicinal qualities are improved and their toxicity is decreased using nanotechnology. A nanoparticle is a tiny particle that can be produced artificially or organically, and depending on its intended application, it can have many different forms. These tiny particles have several benefits and can be utilized to deliver drugs. [1] Nanoparticles act as excellent particulate dispersions or solid particles sized between 10-1000 nm.[2] Drug dispersion, entrapment, encapsulation, or attachment to a nanoparticle matrix can all be accomplished with particulate dispersion or solid particles with sizes between 10 and 1000 nm.[3]Nanoparticles are used in a wide range of fields, including medicine and industry. These tiny carriers have special qualities as drug delivery systems, such as increased reactive area and the capacity to pass through bodily barriers. [4,5]With the emphasis now being shifted towards the use of biodegradable and biocompatible materials for varied applications the use of biobased materials has increased many folds. Of late these materials have been explored for the preparation of matrices for anticancer applications hoping they have better penetration, biocompatibility, lower toxicity and lower side effects than the traditional matrices.

Pectin is one such biopolymer being explored as the material for the preparation of biocompatible nanoparticles or nano-composites for drug delivery and other applications. Pectin nanoparticles are being synthesized following varied methods. For use as glucose biosensors, pectin-coated polyaniline nanoparticles were created. These nanoparticles functioned as stable matrices that promoted improved enzyme functionalization and increased biosensing stability.[6].Pectin-based nanomaterials can be used for drug delivery owing to their innate stability, biodegradability and biocompatibility. These supports could act as devices that deliver hydrophilic and hydrophobic drugs to various organs.[7]The average size of pectin-based nanoparticles generated with iron oxide ions was approximately 14–17 nm for 10 weight percent of pectin, suggesting that these entities may find use in the future in site-specific medication delivery, even to the most remote areas of the human body.[8]Drug delivery using natural pectin-based nanoparticles containing galactose residues demonstrated active targeting of hepatocellular cancer.[9]The water solubility, stability,

and bioactivities of curcumin were also improved by pectin-stabilized selenium nanoparticles used for curcumin encapsulation.[10]. Liu et al.[11] reported on folic acid-modified pectin-based nanoparticles for dual-targeted control in combination therapy for liver cancer. Self-assembled pectin-tannic acid nanocomplexes for drug delivery in pancreatic cancer with for efficient drug delivery have also been reported.[12]Nanoparticles based on gellan gum/pectin blends for colon-targeted delivery were prepared by Fabiola et. al.[13]For anti-cancer applications, negatively charged pectin nanoparticles containing paclitaxel demonstrated extended plasma retention and significant accumulation in the liver.[14]New hybrid nanoparticles that self-assembled using pectin-doxorubicin conjugates showed lessened doxorubicin adverse effects when used to deliver drugs in vivo.[15] Pectin is increasingly used as a vehicle for therapeutic delivery of nanoparticles that showed potential for various administration routes.Another important agent that has been explored for antimicrobial application and shows excellent promise as anticancer agent is tyrosine.Tyrosine had been essentially employed for skin pigmentogenesis and with time came to be studied for antimicrobial applications. Tyrosine-containing medications have shown promise as SARS-CoV-2 inhibitors, and more research is advised before using them as antibacterial agents.[16]According to reports, tyrosine has been added to these silver nanoparticles to improve their antibacterial capabilities.[17]Polyacrylates based on tyrosine have strong antibacterial properties against a range of diseases. Natural antimicrobial peptide mimics that work well against MRSA, S. flexneri, and other microorganisms.[18] Tyrosine synthesized nanoparticles exhibited antibacterial activity against gram-positive bacteria and showed toxicity towards bacterial cell walls.[19]Tyrosine-based prodrugs have been used for antiviral, not antimicrobial, purposes.[20]Tyrosine derivatives have applications in preventing bacterial infections.[21]

Keeping in view the wide spectrum of applications of pectin and tyrosine, tyrosine and pectin based nanoparticles were synthesized following a noval protocol and studied for action against HeLa cells. In order to aid in cancer research, the HeLa cell line was developed. Even when contrasted with other cancer cells, these cells multiply excessively quickly. During cell division, HeLa cells, like many other cancer cells, have an active form of telomerase that repeatedly duplicates telomeres. Hence these cell lines were selected for the anti-cancer assay.

2.Experimental

2.1.Materials

Tyrosine [HIMEDIA],Ammonium Persulphate (APS) [Loba Chemie], Sodium Dodecyl Sulphate (SDS Needle shape extra pure 91%, MW 288.38) [Loba Chemie], Citric acid(CA) [HIMEDIA], Pectin [HIMEDIA],Acrylamide(AAm) [Fisher Scientific India Pvt. Ltd.], Acetone (99% MW 58.08) [Qualikems Fine Chem Pvt. Ltd.], Ethyl alcohol[Qualikems Fine Chem Pvt. Ltd.] and an Ultrasonicator (Digital Ultrasonic Cleaner, 80W) were used as procured.

2.2.Method

30 ml of distilled water was used to solubilize 1 gm of pectin, followed by the addition of 1 gm of acrylamide (AAm) dissolved in a minimal amount of distilled water to the same. To create a homogeneous solution, 0.500g of tyrosine, 0.05g of citric acid (a crosslinking agent), 0.02g of initiator APS, and 0.100g of SDS were added to the above mixture while it was continuously stirred on a magnetic stirrer for approximately 5–6 minutes. Using an ultrasonicator, the homogeneous solution was ultrasonically heated at 75°C for four hours. The synthesized nanoparticles thus obtained were designated as tyrosine incorporated-pectin-cl-CA-poly(AAm) nanoparticles [(Ty-Pc-cl-CA-poly(AAm)](**Scheme 1)** and were extracted through acetone and ethyl alcohol used in the ratio of 60:40.The extracted nanoparticles were dried in a hot air oven at 40°C for 36h without disturbing. **Figure 1.** depicts the image of the synthesized Ty-Pc-cl-CA-poly(AAm) nanoparticle.

3. Characterization

The synthesized Ty-Pc-cl-CA-poly(AAm) nanoparticles were characterised using techniques such as Fourier transform infrared spectroscopy(FT-IR) and Field Emission Scanning Electron Microscopy (FESEM) to get evidence of successful nanoparticle synthesis. The Fourier Transform Infra-Red (FTIR) Spectra were reported in a wavenumber range of 400-4000 cm^{-1} as a KBr pellet by a FTIR spectrometer (Nicole 6700, Thermo-Scientific, USA). The Field Emission Scanning Electron Microscope (FESEM) images were recorded by ZEISS. The particle size diameter was recorded by Dynamic Light Scattering (DLS) in a Nano Zeta sizer instrument with a (BI-SVK92) and scattering angle of 173° at 25°C**.**

4. Results and Discussion

The nanoparticles were characterized by (FTIR) Fourier Transform InfraRed Spectroscopy (**Figure 2**), Particle size analysis (**Figure 3**)and FESEM **(Figure 4)** which gave evidence of successful synthesis.

The FTIR data showed characteristic stretching frequencies that pointed towards the successful incorporation of tyrosine into the nanoparticle matrix(**Figure 2**). The stretching peaks at 3400cm^{-1} and 3000cm^{-1} for –O-H, 2930cm^{-1} for –C-Hand 16050 cm^{-1} or –N-H are observed that all correspond to tyrosine. The broad band centred at 3400cm^{-1} arises due to the presence of $-NH_2$ and –OH groups in the amino acid. The peaks observed at 1750, 1617, and 1440cm^{-1} correspond to the stretching modes of the –C=O, $-NH_2$, and –COO– groups, respectively. The particle size analysis indicated that the nanoparticles synthesized are of size below 100nm (**Figure 3**)also supported by the FESEM **(Figure 4)** images. In the FESEM images, it is observed that the agglomerated nanoparticles are spherical and almost of the same size with average nanoparticle size being between 10-50nm. The results indicated that size and shape-controlled synthesis was achieved following this noval approach.

5. Application of Synthesized Nanoparticle for Anticancer Study

5.1.Materials

Synthesized nanoparticles namely Ty-Pc-cl-CA-poly(AAm). HeLa (Immortal human cells that were taken from a cervical cancer, procured from NCCS Pune), Dimethyl sulphoxide (DMSO) Dulbecco's Modified Eagle Medium-AT149-1L-(DMEM/HIMEDIA), Fetal Bovine Serum (FBS) (HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781).

5.2.MTT Assay

Cytotoxicity of the synthesised nanoparticles namely Ty-Pc-cl-CA-poly(AAm) on HeLa (Immortal human cells that were taken from a cervical cancer, procured from NCCS Pune) cell line was determined by MTT Assay[22-24]. Employing DMEM (Dulbecco's Modified Eagle Medium-AT149-1L-HIMEDIA) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781), the cells (10000 cells/well) were grown in 96-well plates for 24 hours at 37°C with 5% CO_2. Next day cells were treated from different concentrations of the nanoparticle Ty-Pc-cl-CA-poly(AAm) (1,10,50,100,250,500 and 1000 µl) (**Figure 4)**. Dimethyl sulfoxide (DMSO) was used to generate a stock solution of the nanoparticles, which was then further diluted to get various quantities in incomplete cell culture medium (without FBS). The cell culture was cultured for a full day before adding MTT Solution (5 mg/ml) and continuing for an additional two hours. Cells without MTT were referred to as Blank, and cells receiving no treatment were referred to as Control. After the experiment was over, the cell layer matrix was dissolved in 100 µl of DMSO, the culture supernatant was withdrawn, and the plate reader (Elisa, Biorad, USA) was used to read the results at 540 and 660 nm. IC_{50} was calculated by using software Graph Pad Prism -6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS) (**Figure 4)**. 50% inhibitory concentration (IC_{50}) was presented as Mean ± SEM (Standard Error of Mean). The following formula was used to carry out the calculations:

% Viable cells = (A_{test} / $A_{Control}$) *100

(A_{test} = Absorbance of test sample)
($A_{Control}$ = Absorbance of Control)

5.3.Inference

The in vitro MTT assay for the synthesized Ty-Pc-cl-CA-poly(AAm) nanoparticles against the HeLa cell line when the same was exposed to different concentrations of the nanoparticles indicated that the nanoparticles exhibited cytotoxic activity against the cell line. The study was carried out to estimate for Ty-Pc-cl-CA-poly(AAm) nanoparticles the 50% inhibitory concentration.

An effective drug's half-maximum inhibitory concentration (IC_{50}) is the most commonly used and informative metric. It provides a measure of an antagonist medication's potency in pharmacological research by indicating the amount of drug required to block a biological process by half. Ty-Pc-cl-CA-poly(AAm) was found to be cytotoxic at IC_{50} value (µg/ml) of 359.8 ± 0.14. That means IC_{50} is the concentration of Ty-Pc-cl-CA-poly(AAm) nanoparticle formulation at which the viable cells concentration is reduced by half (**Figure 4**).

6. Conclusion

The successful synthesis of the noval Ty-Pc-cl-CA-poly(AAm) nanoparticles was carried out following an innovative approach. The synthesized nanoparticles on characterization revealed the particle size lying between 50-100 nm with individual particles being of spherical geometry. These nonentities were successfully employed for cytotoxic in vitro studies against HeLa cells. They proved to exhibit excellent cytotoxicity against the cell lines with 50% inhibitory concentration of 359.8 ± 0.14. These nanoparticles show potential for application in anti-cancer studies against other carcinogenic cell lines.

AUTHOR CONTRIBUTIONS

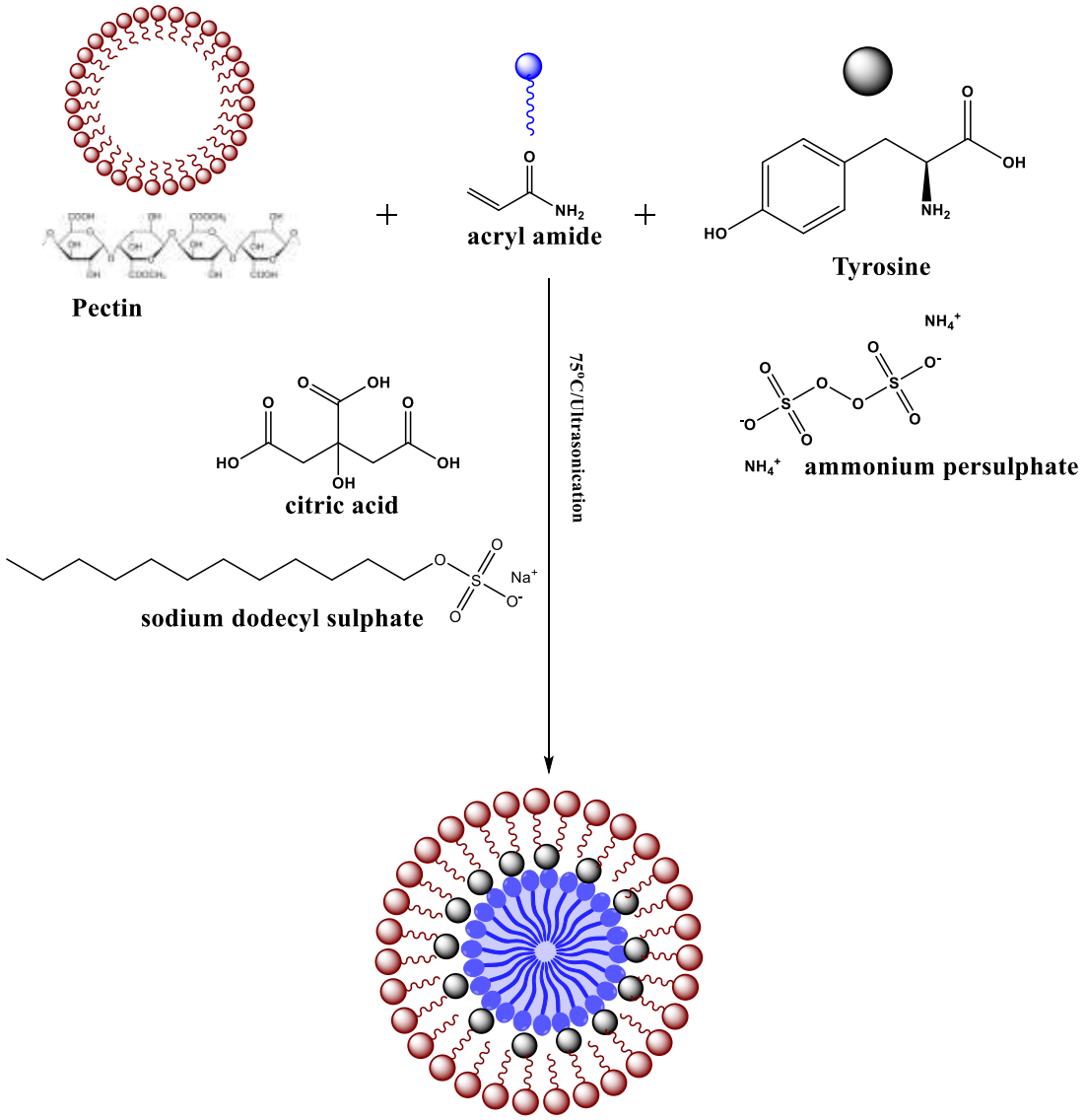
All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Maninder Kaur http://orchid.org/0000-0002-2375-9335
Yogesh Kumar Sood http://orchid.org/0009-0486-3563
Rohini Dharela http://orchid.org/0000-0002-8526-7585

7. References

1. Kadian, R. (2018). Nanoparticles: A promising drug delivery approach. Asian Journal of Pharmaceutical and Clinical Research, 11(1), 30–35. https://doi.org/10.22159/AJPCR.2018.V11I1.22035
2. Suganeswari, M., Shering. M. A., Bharathi, P. & JayaSutha J.(2011). Nano particles: A novel system in current century. International Journal of Pharmaceutical &Biological Archive, 2(3),847-854.
3. Khan, I., Saeed, K. & Khan, I.(2019).Nanoparticles: Properties, applications and toxicities, Arabian Journal of Chemistry, 12(7), 908-931. https://doi.org/10.1016/j.arabjc.2017.05.011.
4. Khandbahale, S. V. & Saudagar, R. B. (2017). Nanoparticle- A review. Asian Journal of Research in Pharmaceutical Science, 7(3), 162–172. https://doi.org/10.5958/2231-5659.2017.00026.1
5. Kumbani, J., Tank, C., Upadhyay, J., Darshit, R., Nirali, T., & Savaliya, H. (2016). Nanoparticle: A Promising carrier for Novel Drug Delivery. Hetal. International Journal of Pharma Research and Review, 5(2),27-40.
6. Thakur, B., Amarnath, C. A., & Sawant, S. N.(2014). Pectin coated polyaniline nanoparticles for an amperometric glucose biosensor. RSC Advances, 4, 40917-40923. https://doi.org/10.1039/C4RA05264A
7. Kedir, W. M., Deresa, E. M. & Diriba, T. F.(2022). Pharmaceutical and drug delivery applications of pectin and its modified nanocomposites.Heliyon,8(9):e10654. https://doi.org/10.1016/j.heliyon.2022.e10654
8. Zakharova, N., Kydralieva, K., Khudaibergenova, E., Gorbunova, N., Pomogailo, S. I., Dzhardimalieva, G. I., Pomogailo, A. & Jorobekova, S.(2012). Synthesis and Characterization of Nanosized Pectin-Based Formulations.Macromolecular Symposia, 317(1),175-179. https://doi.org/10.1002/masy.201100126
9. Yu, C.-Y., Wang, Y. -M., Li, N.-M., Liu, G.-S., Yang, S., Tang, G.-T., He, D.-X., Tan, X.-W. & Hua, W. (2014). In Vitro and in Vivo Evaluation of Pectin-Based Nanoparticles for Hepatocellular Carcinoma Drug Chemotherapy. Molecular Pharmaceutics, 11(2),638-644. https://doi.org/10.1021/mp400412c
10. Wu, Y., Hong, L., Zhihua, Li., Huang, D., Nong, L., Ning, Z., Hu, Z., Xu, C., & Yan, J.-K. (2019). Pectin-decorated selenium nanoparticles as a nanocarrier of curcumin to achieve enhanced physicochemical and biological properties. IET Nanobiotechnology, 13(8),880-886. https://doi.org/10.1049/iet-nbt.2019.0144
11. Liu, Yanxue., Liu, Yanxue., Zong, Yuheng., Yang, Zixuan., Luo, Min., Guiliang, Li., Wang, Yingsa., Cao, Yongli., Xiao, Meng., Kong, Tianjiao., Jing, He., Xingyong, Liu., Jiandu, Lei. & Jiandu, Lei. (2019). Dual-Targeted Controlled Delivery Based on Folic Acid Modified Pectin-Based Nanoparticles for Combination Therapy of Liver Cancer. ACS Sustainable Chemistry & Engineering, 7(3),3614-3623. https://doi.org/10.1021/acssuschemeng.8b06586
12. Chauhan, S. S., Shetty, A. B., Hatami, E., Chowdhury, P. & Yallapu, M. M.(2020). Pectin-Tannic Acid Nano-Complexes Promote the Delivery and Bioactivity of Drugs in Pancreatic Cancer Cells. Pharmaceutics, 12(3),285. https://doi.org/10.3390%2Fpharmaceutics12030285
13. Prezotti., F., Boni, F. I., Ferreira, N. N., Silva, D. S., Almeida, A., Vasconcelos, T., Sarmento, B., Gremião, M. P. D., & B. S. F. Cury. (2020). Oral nanoparticles based on gellan gum/pectin for colon-targeted delivery of resveratrol. Drug Development and Industrial Pharmacy, 46(2), 236-245. http://dx.doi.org/10.1080/03639045.2020.1716374
14. Verma, A. K. & Kumar, A.(2013). Pharmacokinetics and biodistribution of negatively charged pectin nanoparticles encapsulating paclitaxel. Cancer Nanotechnology, 4(4),99-102. https://doi.org/10.1007/s12645-013-0041-8
15. Ye, P.-J., Huang, C., Yang, S., Gao, P., Li, Z.-P., Tang, S.-Y., Xiang, Y., Liu Y.-F., Chen, Y.-P., He, D.-X. & Yu, C.-Y. (2018). Facile fabrication of a novel hybrid nanoparticles by self-assembling based on pectin-doxorubicin conjugates for hepatocellular carcinoma therapy.Artificial Cells Nanomedicine and Biotechnology, 46. https://doi.org/10.1080/21691401.2018.1505745
16. Abdelbaky, M., Abdelghany., A.M., Oraby., A.H., Abdelrazek. E. M. & Rashad M. M. (2023). Degradation of Crystal Violet Using Gadolinium Zirconium Oxide ($Gd_2Zr_2O_7$) Nanoparticles. Journal of Inorganic and Organometallic Polymers and Materials,33,3304-3314. https://doi.org/10.1007/s10904-023-02770-1
17. Chakraborty, S., Sagarika, P., Rai, S., Sahi, C. & Mukherjee, S. (2021). Tyrosine-Templated Dual-Component Silver Nanomaterials Exhibit Photoluminescence and Versatile Antimicrobial Properties through ROS Generation. ACS Applied Materials & Interfaces, 13(31),36938-36947. https://doi.org/10.1021/acsami.1c10520

18. Datta, L.P., Dutta, D., Chakraborty, A. & Das., T. K. (2019). Tyrosine based cationic acrylates as potent antimicrobial agents against shigellosis. Biomaterials Science, 7(6),2611-2622. https://doi.org/10.1039/C8BM01588K
19. Daima, H. K., Selvakannan, PR., Homan, Z., Bhargava, S. K., & Bansal, V. (2011). Tyrosine Mediated Gold, Silver and Their Alloy Nanoparticles Synthesis: Antibacterial Activity Toward Gram Positive and Gram Negative Bacterial Strains. 1-6. https://doi.org/10.1109/NSTSI.2011.6111779
20. Charles, E., McKenna., Boris, A., Kashemirov., Ivan, S., Krylov., Michaela, Serpi., Valeria, M., Zakharova., Larryn, W., Peterson., John, & M., Hilfinger. (2011). Tyrosine-based prodrugs of antiviral agents.[Patent]
21. Kuban-Jankowska, A., Kostrzewa, T. & Gorska-Ponikowska, M. (2022). Bacterial Protein Tyrosine Phosphatases as Possible Targets for Antimicrobial Therapies in Response to Antibiotic Resistance. Antioxidants (Basel). 11(12),2397. https://doi.org/10.3390%2Fantiox11122397
22. Morgan, D. M. (1998). Tetrazolium (MTT) assay for cellular viability and activity. Methods in Molecular Biology, 79,179-183. https://doi.org/10.1385/0-89603-448-8:179
23. Meerloo, J. v., Kaspers G. J. L., & Cloos, J.(2011). Cell sensitivity assays: The MTT assay. In: Cree, I. (eds) Cancer Cell Culture. Methods in Molecular Biology, vol 731. Humana Press. . https://doi.org/10.1007/978-1-61779-080-5_20
24. Fotakis, G. & Timbrell, J. A.(2006). In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicology Letters, 160(2), 171-177. https://doi.org/10.1016/j.toxlet.2005.07.001



Tyrosine incorporated pectin and poly acryamide based citric acid crosslinked nanoparticle

Scheme 1: Graphical representation of the synthesis of Ty-Pc-cl-CA-poly(AAm) nanoparticles

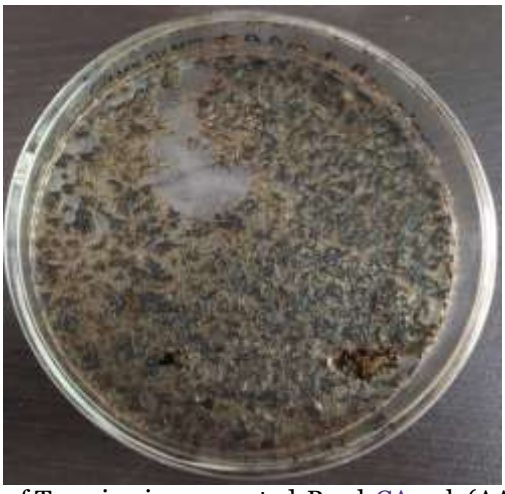


Figure 1: Image of Tyrosine incorporated-Pc-cl-CA poly(AAm) Nanoparticle

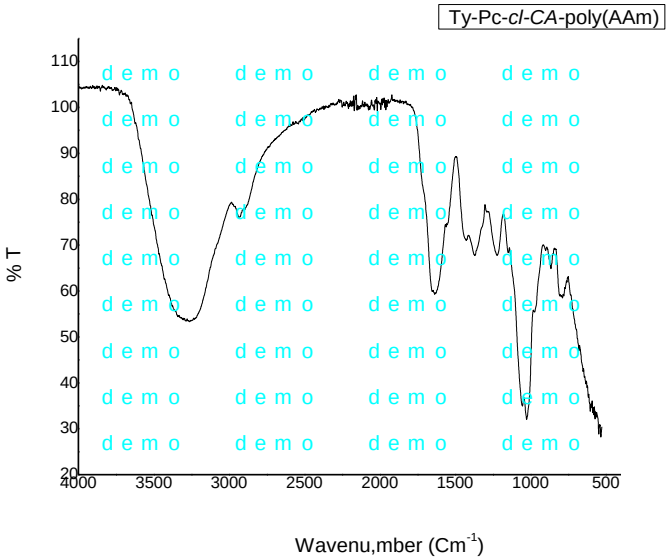


Figure 2: FTIR of Tyrosine incorporated-Pc-cl-CA poly(AAm) Nanoparticle

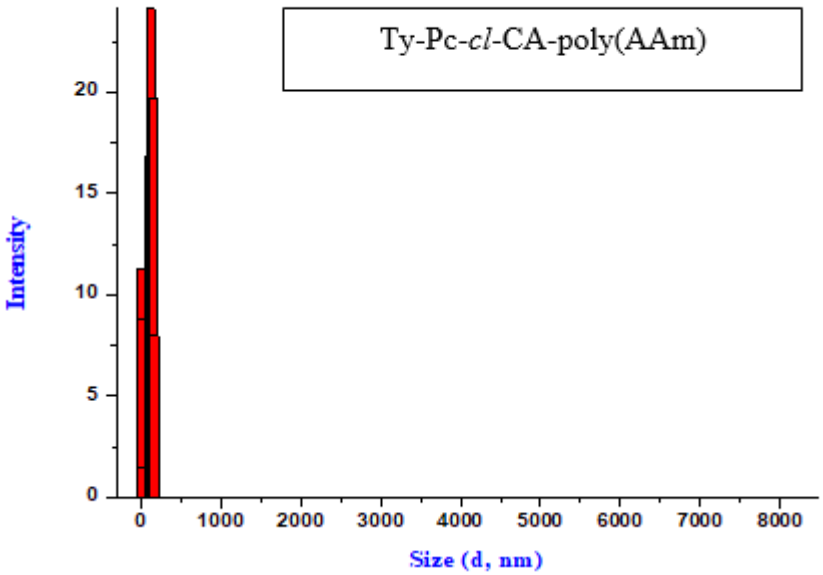


Figure 3: Particle size analysis of Tyrosine incorporated-Pc-cl-CA poly(AAm) Nanoparticle

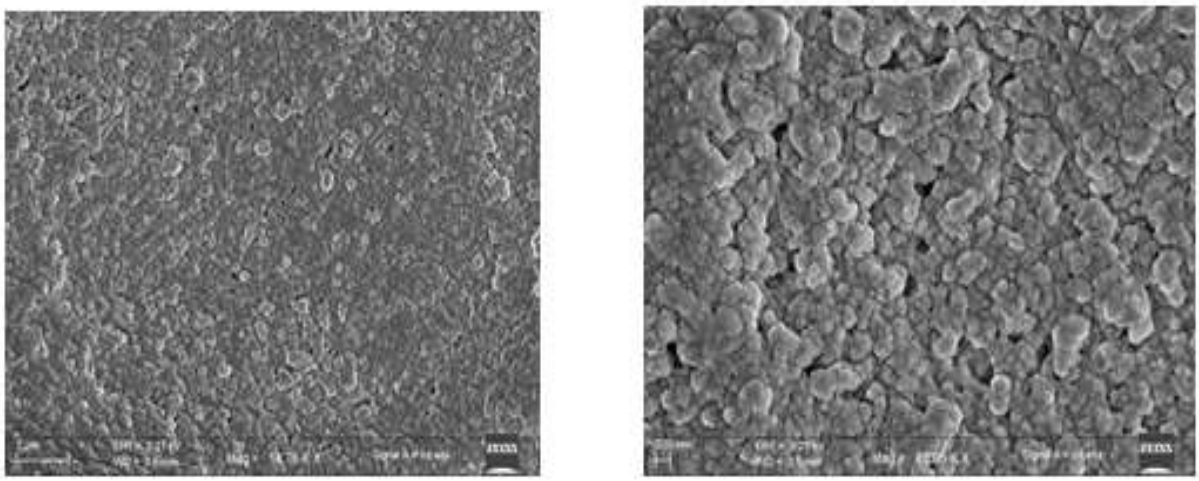
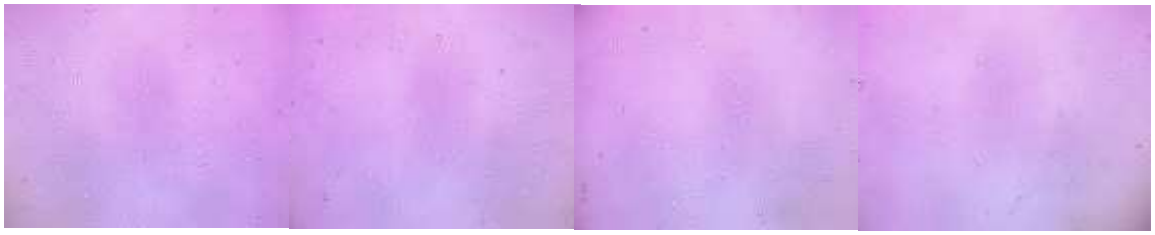


Figure 4: FESEM of Tyrosine incorporated-Pc-cl-CA poly(AAm) Nanoparticle

Images of the HeLa Cells during MTT assay at various dose concentration of Ty-Pc-cl-CA-poly(AAm) nanoparticle			
% viable cells w.r.t. control	100	75.11	71.56
Concentration of Ty-Pc-cl-CA-poly(AAm) nanoparticle used in µg/ml	0	10	50

Images of the HeLa Cells during MTT assay at various dose concentration of Ty-Pc-cl-CA-poly(AAm) nanoparticle				
% viable cells w.r.t. control	66.67	60	47.11	40.89
Concentration of Ty-Pc-cl-CA-poly(AAm) nanoparticle used in µg/ml	100	250	500	1000

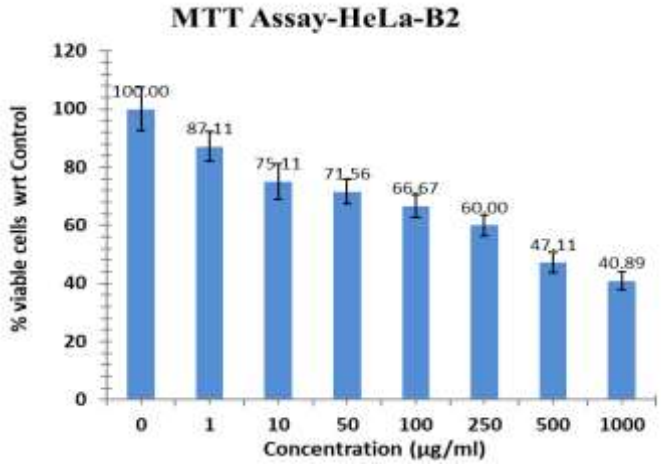


Figure 4:MTT assay of the nanoparticle Ty-Pc-cl-CA-poly(AAm) against HeLa Cells.