International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 5, 2023

Short Communication

TARGETING ANGIOGENESIS WITH FLUPHENAZINE-ZINC OXIDE NANOCONJUGATES: A POTENTIAL MECHANISM FOR IMPROVING ANTIPSYCHOTIC EFFICACY

NAGASEN DASARI[a,b], SUJIYA BALLA[a,b], PYDIRAJU KONDRAPU[a,b], RAMAKRISHNA GUMMADI[a,b], NOOKARAJU SURADA[a,b*], UMA MAHESWARI KONDRU[a,b], SAI KIRAN S. S. PINDIPROLU[a,b*]

[a]Aditya Pharmacy College, Surampalem-533437, Andhra Pradesh, India. [b]Jawaharlal Nehru Technological University Kakinada, Kakinada, Andhra Pradesh, India

[*]Corresponding author: Sai Kiran S. S. Pindiprolu; [*]Email: pindiprolusskiran@gmail.com

Received: 28 May 2023, Revised and Accepted: 03 Jul 2023

ABSTRACT

Objective: This study aimed to develop a more effective formulation of Fluphenazine (FLP) for the management of psychosis. Antipsychotics are widely used for the treatment of severe mental disorders such as schizophrenia and bipolar disorder. However, their clinical use is limited due to various side effects and low efficacy in a large number of patients. Nanoparticle-based drug delivery systems have shown great potential in improving the pharmacokinetics and pharmacodynamics of various drugs, including antipsychotics. Zinc oxide nanoparticles (ZnO NPs) have emerged as a promising carrier for drug delivery due to their unique physicochemical properties, biocompatibility, and low toxicity.

Methods: In this study, we reported the preparation and characterization of FLU-encapsulated ZnO NPs (FLU-ZnO-NPs) for the management of psychosis. The synthesized FLU-ZnO-NPs were characterized using various techniques, such as X-Ray Diffractometer, Energy Dispersive X-Ray analysis, Transmission Electron Microscopy, and Zetasizer (Malvern).

Results: The characterization results showed that the synthesized FLU-ZnO-NPs had improved solubility, enhanced bioavailability, targeted delivery, and reduced toxicity.

Conclusion: The development of FLU-ZnO-NPs could provide a more effective and safe treatment option for patients with mental disorders.

Keywords: Antipsychotics, Fluphenazine, Zinc oxide nanoparticles, Psychosis, Drug delivery systems

DOI: https://dx.doi.org/10.22159/ijap.2023v15i5.48317. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

Antipsychotics are widely used for the treatment of severe mental disorders such as schizophrenia and bipolar disorder. These disorders significantly impact the world population and have significant socioeconomic consequences, morbidity, and mortality. Antipsychotics can be classified into three categories based on their underlying mechanism of action: typical antipsychotics, atypical antipsychotics, and dopamine partial agonists [1]. While these medications have been effective in managing symptoms, they are associated with numerous undesirable adverse effects, such as extrapyramidal symptoms (EPS) and lack of efficacy in a large number of patients. Furthermore, non-adherence to antipsychotic medication is common due to potential adverse effects and forgetfulness [2].

Fluphenazine (FLP) is a medication that belongs to the class of typical antipsychotic drugs. It is primarily used for the treatment of schizophrenia and other psychotic disorders [3]. Fluphenazine works by blocking certain neurotransmitters in the brain, which helps to reduce the symptoms of psychosis such as delusions and hallucinations. However, the clinical use of FLP is limited due to its poor solubility, low bioavailability, and side effects. Therefore, there is a need to develop a more effective formulation of FLP that can improve its therapeutic efficacy and minimize its side effects [4]. In recent years, researchers, therefore, focused on development of antipsychotic drug formulations fewer or negligible side effects, and which can increase patient compliance [5].

Nanoparticle-based drug delivery systems have shown great potential in improving the pharmacokinetics and pharmacodynamics of various drugs, including antipsychotics [6]. The development of fluphenazine nanoparticles can address the limitations of conventional formulations and provide several benefits, such as improved solubility, enhanced bioavailability, targeted delivery, and reduced toxicity [7].

Among various nanoparticle formulations, zinc oxide nanoparticles (ZnO NPs) have emerged as a promising carrier for drug delivery due to their unique physicochemical properties, biocompatibility, and low toxicity [8]. ZnO NPs have a large surface area-to-volume ratio, which allows for high drug loading capacity and controlled release of drugs. Additionally, ZnO NPs have been shown to enhance the cellular uptake and transport of drugs across the blood-brain barrier, which is crucial for the treatment of central nervous system disorders such as psychosis [9, 10].

We hypothesize, the development of FLU-encapsulated ZnO NPs (FLU-ZnO-NPs) can provide a more effective and safe treatment option for patients with mental disorders. In the present study we reported the preparation and characterization of FLU-ZnO-NPs for management of psychosis [11, 12].

The synthesis of ZnO nanoparticles was carried out using a sol-gel method. In a clean beaker, an aqueous solution of ZnCl2 (0.2M ZnCl2) was prepared, followed by the addition of 2 ml of glacial acetic acid. The mixture was stirred at 400 RPM at room temperature. Drop-wise addition of 8M NaOH was carried out until the pH of the solution reached 7, resulting in a gradual color change from transparent to a full white milk color. The gel obtained was stirred for 1 hour, filtered, and washed with distilled water 4-5 times. The above procedure was repeated for every molar %of x, and the obtained precipitate was air-dried for one day. The dried powder was calcinated at 500 °C for the observation of crystallinity. The resulting powder was used for the subsequent characterization of the synthesized material [13-15].

From the TEM it is clear that the shape of FLU-ZnO-NPs were spherical (fig. 1).

Particle size plays key role in solubility, dissolution rate and bioavailability of the drug. Smaller the particle size greater the dissolution rate. The optimized ZnO nanoformulation had a mean (z-average) particle size of 97.0 nm and poly dispersity index (PDI) was found to be 1.3±0.06, which indicates the particles are in uniform distribution. An increase or decrease in the particle size of the drug in a formulation can affect its *in vitro* release and subsequently its bioavailability.

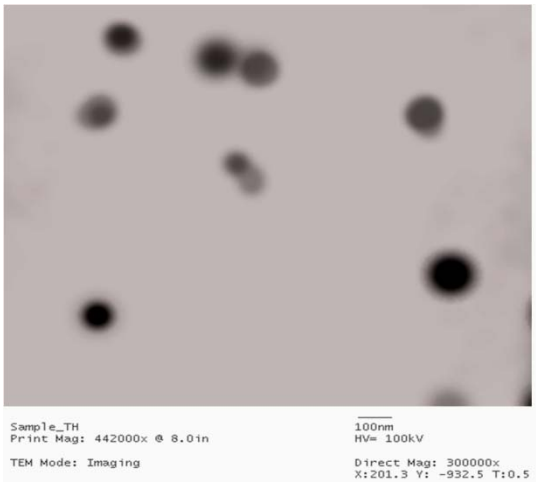

Fig. 1: TEM

Zeta Potential was determined by using Microtrac Zetatrac analyser. The zeta potential is potential at the hydrodynamic shear plane and can be determined from particle mobility and under electric field. The mobility will depend on surface charge and electrolyte concentration. Drug particles dispersed within a liquid continuous medium are stabilized by steric and electrostatic mechanisms, or by a combination of both (i.e., electrostatic mechanism) via carbohydrate. Zeta potential greater than+30mV and smaller than-30mV is normally considered stable. Zeta potential of the nanoformulation was found to be+31.8 mV. Greater the zeta potential value indicates greater the stability of the nanoparticles. The UV spectra of ZnO-FLU-NPs were given in table 1 and fig. 2,3 and 4.

Drug entrapment efficiency and % drug loading of different ZnO nanoformulations were found to be 92.13±0.06 to 93.04±0.56 and 4.54±0.01 to 4.59±0.07 respectively.

A comparative *in vitro* drug release study was performed in pH 6.8 phosphate buffer for pure drug and nanoformulations was performed. The dissolution experiments were conducted in triplicate. ZnO nano formulation exhibited higher rates of dissolution than pure drug may be due to reduction of particle size and greater surface area. Therefore, it may be concluded that optimized nano formulation not only has superior dissolution profile than pure drug and but also has much better release profile when compared to pure drug (PD) (fig. 5).

Table 1: UV spectra for fluphenazine, Zno and formulation (ZnO-FLU-NPs)

Concentration (µg)	Absorbance		
	Fluphenazine	Zno	Formulation (ZnO-FLU-NPs)
0.5	0.011	0.001	0.002
1	0.014	0.002	0.003
1.5	0.015	0.003	0.005
2	0.018	0.003	0.007
2.5	0.036	0.005	0.009

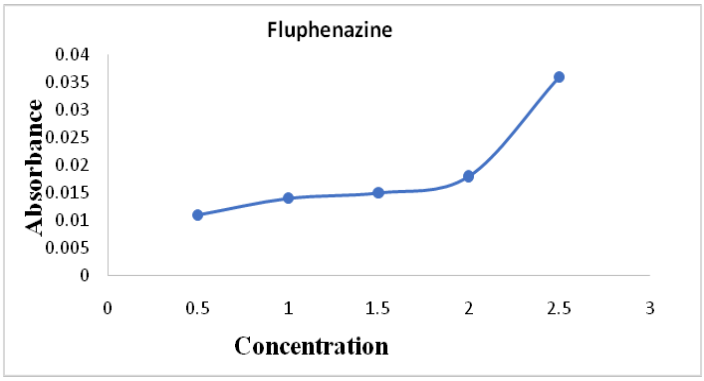

Fig. 2: UV spectra for fluphenazine

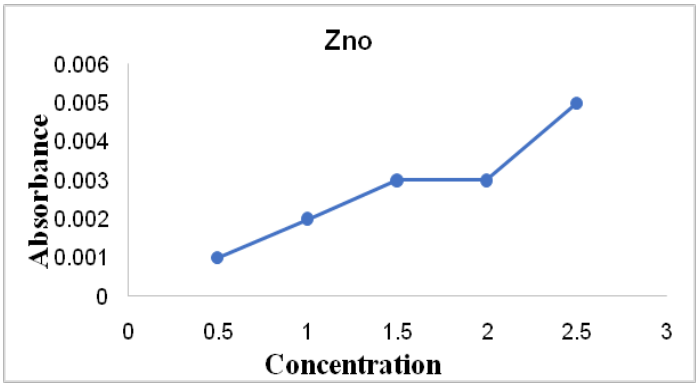

Fig. 3: UV spectra for Zno

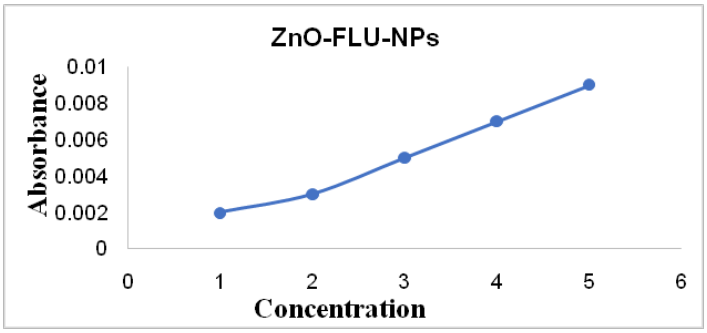

Fig. 4: UV spectra for formulation (ZnO-FLU-NPs)

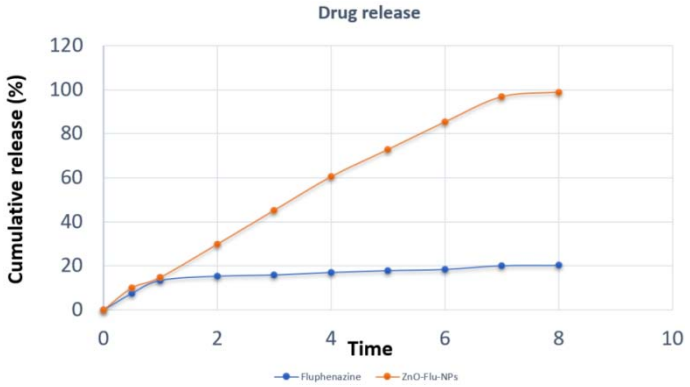

Fig. 5: % Drug release of fluphenazine and FLU-ZnO-NPs

The crystalline phase of the ZnO nanoparticles was analyzed using an X-Ray Diffractometer with CuKα radiation source. Composition analysis was performed using Energy Dispersive X-Ray analysis (EDAX) data. Particle size was estimated through Transmission Electron Microscopy. Morphology of the particles was examined using Zetasizer (Malvern) [16-18].

Analysis was performed at an accelerating voltage of 200kV. The sample was placed onto carbon-coated copper grid (200 mesh) to create thin film. Then samples were negatively stained with 2% w/v phosphotungstic acid solution. Excess droplets were removed. The grid was allowed to dry. Soft imaging viewer software was used to capture the image of samples [19].

TLC is a chromatographic technique used to separate organic compounds and assess their purity. The technique is based on principles of adsorption and/or partition chromatography, which depend on the nature of the adsorbent, its treatment, and the solvent used. In this study, we utilized silica gel-coated TLC plates to separate fluphenazine and a formulated drug [20].

The procedure began by preparing the TLC plates and allowing them to dry. A mobile phase was then prepared by mixing Ethyl Acetate, methanol, and water in the ratio of 8.1:1.0:0.81 ml per 10 ml, respectively. The drugs were dissolved in water, and a capillary tube was used to spot them onto the TLC plate. The plate was then placed in the mobile phase and allowed to run up to 3/4th of the plate. The separated compounds were then detected using UV and iodine chambers, and the Rf value for each compound was calculated using the formula:

$$\text{Rf} = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$$ [21].

Entrapment Efficiency and Drug Loading: Entrapment efficiency is the percentage of actual amount of drug entrapped in the carrier relative to the initial amount of loaded drug. The % entrapment efficiency is calculated by:

$$\text{Entrapment efficiency} = \frac{(W1 - W2)}{W1} \times 100$$

W1= total amount of the drug used in preparation; W2 = amount of the drug

For theoretical drug loading it was assumed that entire drug gets entrapped in ZnO core. For practical drug loading, an accurately weighed 10 mg of nanoparticles were dissolved in 10 ml of pH 6.8 phosphate buffer. Then the solution was transferred to 100 ml of 0.05 N NaOH solution and sonicated for 20 min. Then, the solution was measured the absorbance at 259.8 nm by UV-Visible spectrophotometer.

$$\%\ \text{Drug loading} = \frac{[\text{Weight of the loaded drug} - \text{Weight of unentrapped drug}]}{\text{Total weight of aquasomes}} \times 100$$

$$\text{Entrapment efficiency} = \frac{(W1 - W2)}{W1} \times 100$$

W1= total amount of the drug used in preparation; W2 = amount of the drug

In vitro dissolution studies of the pure drug and ZnO Nano formulations were carried out using USP-Type II dissolution apparatus. 900 ml of pH 6.8 phosphate buffer was used as dissolution media and temperature was maintained at 37 °C±0.5 °C with paddle rotation speed at 50 rpm. Aliquots of 5 ml were withdrawn at various intervals and were replaced with same quantity of fresh dissolution medium to maintain the sink condition. Samples were filtered through wattman filter paper and analysed UV–Vis spectrophotometrically. The dissolution experiments were conducted in triplicate and the cumulative percentage of drug release was calculated [22].

The fertilized eggs were kept in a humidified incubator at 37 °C for 48 h. They were then placed in a horizontal position and rotated several times. The eggs were divided into groups based on the test sample and sprayed with 70% ethanol and air-dried to minimize contamination from the egg surface. On day 7-9, a sterile blade was used to create a window in the egg shell of chick embryos, and the test substances were applied to the extra-embryonic membrane through this window. The shell was then removed with sterile forceps under Laminar airflow. After capturing photographs of the

embryo, the window was closed using cellophane tape. The embryos were allowed to incubate for another 48 h, after which photographs of the CAM were taken to analyze the effects of the various test samples [23-25].

CAM assay was performed to evaluate the angiogenic activity of ZnO. CAM assay proved that ZnO enhanced the formation of new blood vessel formation that shows the pro-angiogenic effect in comparision with that of control in fig. 6.

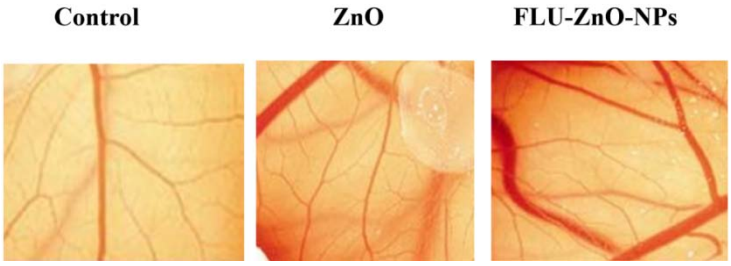

Fig. 6: Evaluation of anti-angiogenic activity in chick CAM model

Our study suggests that the developed FLU-ZnO-NPs can overcome the limitations of conventional formulations and provide several benefits, such as improved solubility, enhanced bioavailability, targeted delivery, and reduced toxicity. Additionally, ZnO NPs have been shown to enhance the cellular uptake and transport of drugs across the blood-brain barrier, which is crucial for the treatment of central nervous system disorders such as psychosis.

In conclusion, the present study successfully developed and characterized FLU-ZnO-NPs for the management of psychosis. The results demonstrate that the developed formulation has the potential to provide a more effective and safe treatment option for patients with mental disorders. Future studies are needed to evaluate the *in vivo* efficacy and safety of the developed FLU-ZnO-NPs.

ACKNOWLEDGEMENT

The authors would like to thank Aditya Pharmacy College for their kind support during the investigation.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Nagasen Dasari-Conceptualization, Methodology, Investigation, Writing-Original Draft, Visualization, Sujiya Balla-Methodology, Investigation, Data Curation, Writing-Review and Editing, Pydiraju Kondrapu-Resources, Formal Analysis, Writing-Review and Editing, Supervision, Ramakrishna Gummadi-Validation, Writing-Review and Editing, Project Administration, Nookaraju Surada-Formal Analysis, Data Curation, Visualization, Writing-Original Draft, Uma Maheswari Kondru-Resources, Writing-Review and Editing, Supervision, Sai Kiran S. S. Pindiprolu-Conceptualization, Methodology, Validation, Writing-Original Draft.

CONFLICT OF INTERESTS

The authors have no conflict of interest regarding this investigation.

REFERENCES

1. Zuchowska A, Kasprzak A, Dabrowski B, Kaminska K, Poplawska M, Brzozka Z. Nanoconjugates of graphene oxide derivatives and meso-tetraphenyl porphyrin: a new avenue for anticancer photodynamic therapies–cell-on-a-chip analysis. New J Chem. 2020;44(43):18770-9. doi: 10.1039/D0NJ04189K.
2. Zhao H, Wang D, Gao C, Liu H, Han L, Yin Y. Ultrafine platinum/iron oxide nanoconjugates confined in silica nanoshells for highly durable catalytic oxidation. J Mater Chem A. 2016;4(4):1366-72. doi: 10.1039/C5TA09215A.
3. Yousuf S, Arjmand F, Siddique HR, Ali MS, Al-Lohedan HA, Tabassum S. Biophysical binding profile with ct-DNA and cytotoxic studies of a modulated nanoconjugate of umbelliferone cobalt oxide loaded on graphene oxide (GO) as drug carrier. J Biomol Struct Dyn. 2022;40(10):4558-69. doi: 10.1080/07391102.2020.1860821, PMID 33331234.
4. Yang C. Bactericidal functionalization of wrinkle-free fabrics via covalently bonding TiO 2@Ag nanoconjugates. Journal of Materials Science 2009;44:1894-901.
5. Yadav N, Kumar N, Prasad P, Shirbhate S, Sehrawat S, Lochab B. Stable dispersions of covalently tethered polymer improved graphene oxide nanoconjugates as an effective vector for siRNA delivery. ACS Appl Mater Interfaces. 2018;10(17):14577-93. doi: 10.1021/acsami.8b03477, PMID 29634909.
6. Wang N, Yu X, Zhang K, Mirkin CA, Li J. Upconversion nanoprobes for the ratiometric luminescent sensing of nitric oxide. J Am Chem Soc. 2017;139(36):12354-7. doi: 10.1021/jacs.7b06059, PMID 28844137.
7. Turin Moleavin IA, Fifere A, Lungoci AL, Rosca I, Coroaba A, Peptanariu D. *In vitro* and *in vivo* antioxidant activity of the new magnetic-cerium oxide nanoconjugates. Nanomaterials (Basel). 2019;9(11):1565. doi: 10.3390/nano9111565, PMID 31690040.
8. Turiel Fernandez D. Ultrasmall iron oxide nanoparticles cisplatin (IV) prodrug nanoconjugate: ICP-MS based strategies to evaluate the formation and drug delivery capabilities in single cells. Anal Chim Acta. 2021;1159:338356. doi: 10.1016/j.aca.2021.338356.
9. Pindiprolu SKSS, Avasarala H, Dinakaran SK. Phenylboronic acid modified lipid nanocarriers mediated co-delivery of immunocytokine TRAIL and gamma-secretase inhibitor to triple-negative breast cancer cells and cancer stem cells. Med Hypotheses. 2021;157:110716. doi: 10.1016/j.mehy.2021.110716. PMID 34731681.
10. Anshika S, Rajora A, Mazumder R, Padhi S. Effect of ZEIN on ciprofloxacin floating tablets. Int J Appl Pharm. 2022;14(6):137-47.
11. Pindiprolu SKSS, Madhan J, Srinivasarao DA, Dasari N, Phani Kumar CS, Katta C. Therapeutic targeting of aberrant sialylation for prevention of chemoresistance and metastasis in triple negative breast cancer. J Drug Deliv Sci Technol. 2023 Jun 14;86:104617. doi: 10.1016/j.jddst.2023.104617.
12. Moya C, Escudero R, Malaspina DC, de la Mata M, Hernandez Saz J, Faraudo J. Insights into preformed human serum albumin corona on iron oxide nanoparticles: structure, effect of particle size, impact on mri efficiency, and metabolization. ACS Appl Bio Mater. 2019;2(7):3084-94. doi: 10.1021/acsabm.9b00386, PMID 35030800.
13. Sarkar P, Das S, Majee SB. Solid dispersion tablets in improving oral bioavailability of poorly soluble drugs. Int J Curr Pharm Sci. 2022;14(2, Mar):15-20. doi: 10.22159/ijcpr.2022v14i2.1961.
14. Chintamaneni PK, Nagasen D, Babu KC, Mourya A, Madan J, Srinivasarao DA. Engineered upconversion nanocarriers for synergistic breast cancer imaging and therapy: current state of the art. J Control Release. 2022 Dec;352:652-72. doi: 10.1016/j.jconrel.2022.10.056, PMID 36328078.

15. Maru AD, Lahoti SR. Formulation and evaluation of ointment containing sunflower wax. Asian J Pharm Clin Res. 2019;12(8 Aug):115-20. doi: 10.22159/ajpcr.2019.v12i18.33199.
16. Chintamaneni PK, Nagasen D, Babu KC, Mourya A, Madan J, Srinivasarao DA. Engineered upconversion nanocarriers for synergistic breast cancer imaging and therapy: current state of the art. J Control Release. 2022;352:652-72. doi: 10.1016/j.jconrel.2022.10.056. PMID 36328078.
17. Chen F, Chen D, Deng T, Li J. Combination of alkaline phosphatase/graphene oxide nanoconjugates and D-glucose-6-phosphate–functionalized gold nanoparticles for the rapid colorimetric assay of pathogenic bacteria. Biosens Bioelectron. 2022;216:114611. doi: 10.1016/j.bios.2022.114611. PMID 35985060.
18. Chauhan RP, Mathur R, Singh G, Kaul A, Bag N, Singh S. Evaluation of folate conjugated superparamagnetic iron oxide nanoparticles for scintigraphic/magnetic resonance imaging. J Biomed Nanotechnol. 2013;9(3):323-34. doi: 10.1166/jbn.2013.1548, PMID 23620987.
19. Chakraborty SP, Mahapatra SK, Sahu SK, Chattopadhyay S, Pramanik P, Roy S. Nitric oxide-mediated staphylococcus aureus pathogenesis and protective role of nano conjugated vancomycin. Asian Pac J Trop Biomed. 2011;1(2):102-9. doi: 10.1016/S2221-1691(11)60005-1, PMID 23569737.
20. Veronica EF, Dwiastuti R. Formulation and evaluation of wound healing gel of white lead tree (Leucaena leucocephala (lam.) DE wit.) leaves extract. Int J App Pharm. 2022;14:275-80. doi: 10.22159/ijap.2022v14i1.42126.
21. Ali MS, Tabassum S, Al-Lohedan HA, Farah MA, Al-Anazi KM, Usman M. Fluorescent delivery vehicle containing cobalt oxide–umbelliferone nanoconjugate: DNA/protein interaction studies and anticancer activity on MF7 cancer cell line. RSC Adv. 2019;9(45):26503-18. doi: 10.1039/c9ra02412c, PMID 35531044.
22. Joshi S, Siddiqui R, Sharma P, Kumar R, Verma G, Saini A. Green synthesis of peptide-functionalized reduced graphene oxide (rGO) nano bioconjugate with enhanced antibacterial activity. Sci Rep. 2020;10(1):9441. doi: 10.1038/s41598-020-66230-3, PMID 32523022.
23. Gonzalez Rodriguez R, Sizemore E, Naumov AV. Graphene oxide-iron oxide nanoconjugates for drug transport, biosensing and bimodal fluorescence/magnetic resonance imaging. Electrochemical Society Meeting Abstracts. 2018;01(6):669. doi: 10.1149/MA2018-01/6/669.
24. Armenia I, Marcone GL, Berini F, Orlandi VT, Pirrone C, Martegani E. Magnetic nano conjugated teicoplanin: a novel tool for bacterial infection site targeting. Front Microbiol. 2018;9:2270. doi: 10.3389/fmicb.2018.02270, PMID 30386305.
25. Achadu OJ, Britton J, Nyokong T. Graphene quantum dots functionalized with 4-Amino-2, 2, 6, 6-tetramethylpiperidine-n-oxide as fluorescence "Turn-ON" Nanosensors. J Fluoresc. 2016;26(6):2199-212. doi: 10.1007/s10895-016-1916-y. PMID 27624186.