https://doi.org/10.48047/AFJBS.6.14.2024.1822-1832

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Development and evaluation of risperidone-loaded solid lipid nanoparticles

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Volume 6, Issue 14, Aug 2024

Received: 09 June 2024

Accepted: 19 July 2024

Published: 08 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.1822-1832

ABSTRACT:
Solid lipid nanoparticles are a potential way to deliver drugs. They are made up of solid lipids, surfactants, and active drug molecules. The study's goal was to look at the physicochemical features and see how the drug was released. The present study is an attempt to make stable lipid nanoparticles of risperidone using a mix of polymer and lipid components. from the fact that the tests always came up with the same results. The infrared spectra showed that the polymer and risperidone did not interact with each other, which proved that the two molecules are compatible. We made risperidone solid lipid nanoparticles using the solvent injection method, which involved mixing glyceryl monostearate and tween 80 in water to make an emulsion. It was found that increasing the concentration of lipids made the solid lipid nanoparticles better at catching things. Formulation F3 was able to trap 77±0.3% of its targets. The study of the particle sizes of the solid lipid nanoparticles made with risperidone shows that all of the particles in the mixtures were in the nano range. Particles with a size of 278.6 nm were found to be the smallest in tests F3 and F4. A study on drug release that was done in a lab found that adding more lipids also makes the medication break down faster. The results of the dissolving study made it hard to believe that solid lipid nanoparticles could control how quickly the medicine risperidone was released. The current study comes to the conclusion that the dissolving test made it hard for solid lipid nanoparticles to control how fast the drug risperidone was released.

Keywords: Risperidone, solid lipid nanoparticles, drug delivery, solubility.

INTRODUCTION:

Nanotechnology has grown a lot in the past few years. It has become a powerful tool that can be used in many fields, including medicine transport, diagnostics, cosmetics, and non-biological sciences. However, the usual ways of providing therapeutic chemicals, like flavonoids from plants, are limited by problems like not being selective, not working well, and not being bioavailable enough (Yasir, *et al.*, 2017, Yasir and Sara 2013).

SLNs are a way to deliver drugs. They are made up of active drug molecules, solid lipids, surfactants, and/or co-surfactants. They are very good at getting drugs to where they need to go. Some safe ways to give these nanoparticles are by mouth, through a syringe, or on the skin (Behera et al., 2010; Sharma et al., 2016; Keservani et al., 2019; Sharma et al., 2018; Khulbe et al., 2023; Khairnar et al., 2024; Keservani and Gautam, 2022; Keservani and Gautam, 2020; Keservani et al., 2020; Keservani et al., 2010; Keservani & Sharma, 2018; Bharti et al., 2012). The use of SLN applications could improve the treatment of many illnesses by making exact changes to physicochemical properties (Ahire, *et al.*, 2023; Yasir, *et al.*, 2021). Due to their flexibility, they can load both lipophilic and hydrophilic drugs, which improves the drug's properties and increases its length of action and release profiles. As a result, this means that the treatment can be given less often while still being more effective. When it comes to accurately delivering medications, SLNs are much better than traditional delivery methods. This benefit goes beyond their ability to improve drug absorption and release over time (Nataraja et al., 2023; Jaiswal et al., 2023; Komu et al., 2023; Keservani et al., 2024). As an example of how risperidone can be added to nanoparticles, we used simple methods in this work. The study's goal was to look at the physicochemical features and watch how a drug called risperidone is released from solid lipid nanoparticles (HP-SLNs) (Gautam et al., 2015; Khambete et al., 2016).

MATERIAL AND METHODS:

The risperidone was sent by a licensed seller in Mumbai. Polyvinyl chloride and glyceryl monostearate were provided by Loba Chemie. The drugs and reagents that are left are all analytical grade.

Compatibility Study by FTIR:

Infrared spectroscopy was used to check how well the risperidone, which was used to make the nanosuspension, worked with other substances. We used FT-IR spectroscopy to look at how risperidone, polymers, and excipients interacted with each other at the right temperature (Mohammadi, *et al.,* 2021).

Preparation of the HP –SLNs:

The method used to make the HP-SLNs was based on one from Tan et al., with a few small changes. Ultrasonication and high-shear homogenization were used together in a two-step process to make the HP-SLNs. Mixing Tween-80 with 25 milliliters of pure water and then using a water bath to heat it up to 75 degrees Celsius (°C) started the process of making an aqueous phase (Qureshi, *et al.,* 2019).

Characterization of HP –SLNs:

% yield:

To find the percentage yield of solid lipid nanoparticles, the following method was used to compare the mass of the nanoparticles that were made after they were dried (Chalikwar, *et al.,* 2012), gathered, and weighed to the mass of the starting materials:

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}}\ x\ 100$$

Particle Size and Zeta Potential:

The particle size and Zeta Potential readings were found. The Malvern Zetasizer was used to describe the solid lipid nanoparticles. A Zeta sizer (Malvern Zeta sizer) was used to test the Zeta-potential and particle size of solid lipid nanoparticles that had drugs loaded on them. Nanoparticle samples that had been diluted were put into an electrophoretic cell and an electrical field of 15.2 V/cm was applied to measure the zeta potential and particle size (Patel, *et al.,* 2024).

Surface morphology study:

The scanning electron microscope (SEM) method was used to look at the shape of the solid lipid nanoparticle that had the medicine on it. After solid lipid nanoparticles were put on the metal stubs, the sputter coater that was connected to the device was used to cover them with a layer of conductive gold. A Jeol scanning electron microscope was used to take the shots, which were 20kv×25000 in size (Ahirrao, *et al.,* 2022).

Drug content and Entrapment efficiency:

Lipid solids Nanoparticles equal to 100 mg of risperidone were broken up with a glass blender. After the powdered nanoparticles were spread out and dampened with a 7.4 pH Phosphate buffer, the amount of drug they contained was measured. A UV spectrophotometer was used to measure the absorbance at 280 nm, and a 7.4 pH phosphate buffer was used as a blank to find the drug dose (Piazza, *et al.,* 2014).

In-vitro Drug Release study:

Using a dissolving test device, a study was done to look into how solid lipid nanoparticles (equal to 100 mg) that were enclosed in capsules dissolve. The experiment took place in a buffer solution with a pH of 7.4 at 37±0.5°C and 75 rpm to keep the mixture spinning. Over the course of twelve hours, 5 ml samples were taken out at regular one-hour intervals. It was decided how much of the dissolved medium to use to keep the sink state. A UV spectrophotometer was used to measure the amount of drug in the sample at a range of 280 nm. Three separate and independent samples were used for each test, and each sample was used three times (Eisa, *et al.,* 2022; Abd-Elrazek and Elnawawy, 2019; Aher, *et al.,* 2023).

Stability studies:

This study looked at how stable the solid lipid nanoparticles were by putting them in a stability box at 30°C ± 2°C for 90 days using the best formulation. At 0, 1, 2, and 3 months, the samples were checked for drug content and drug release rate (Subedi, *et al.,* 2009).

RESULTS AND DISCUSSION:

Preparation of the HP –SLNs:

To make the lipid phase, HP, soy lecithin, and glyceryl beheaded were mixed in 5 mL of 73 °C ethanol. The lipid-based phase and the water-based phase were carefully mixed together. To make an emulsion, the mixture was homogenized for 10 minutes on a high-speed Ultra Turrax D-500 homogenizer that was set to 7000 turns per minute. The emulsion was put through ultrasonic processing for 15 minutes using an ultrasonic cell breaker to make an HP-SLNs emulsion.

Compatibility study by FT-IR:

The FTIR spectra of the pure risperidone medicine were looked at along with the physical mixes that contained the fillers. The fact that the drug's functional groups show similar peaks across the spectrum shows that there is no contact between the medicine and the polymers. The results from the FTIR spectrum are shown in Figure 1 and Table 1 (Yasir, *et al.,* 2018).

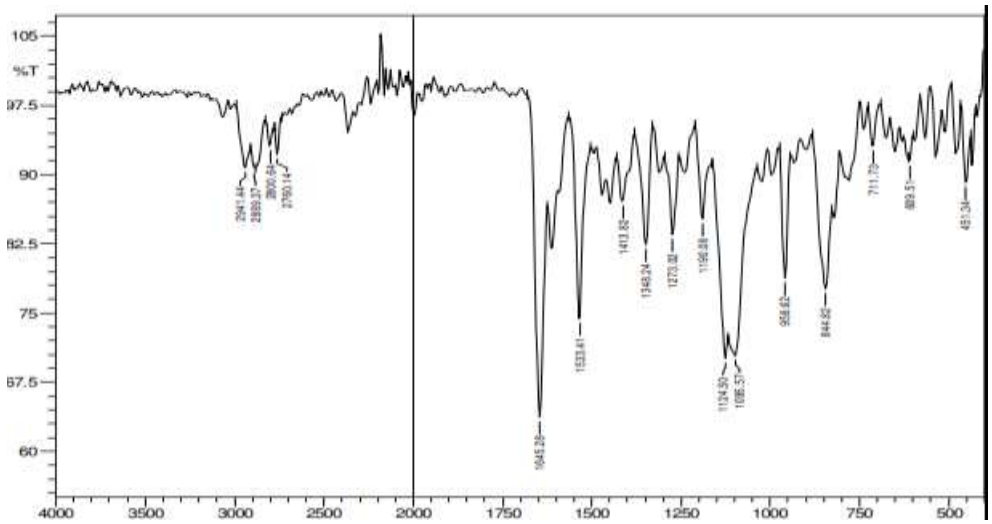

Figure 1: FT-IR Spectra of drug and excipients

Table 1: FT-IR spectra of drug and excipients

Sr. No.	Functional Group	Reported	Observed
1	-OH Stretching	2939	2940
2	-CH Stretching	2804	2801
3	C=C Stretching	1642	1640

4	-CH-O-CH Stretching	1128	1123
5	-CH_2 Scissoring	1531	1532
6	-OH Bending	1349	1345
7	-C=O	1651	1642

Characterization of HP –SLNs:

Percentage yield:

To find the % output, the solid lipid nanoparticles had to be collected, dried out, and evaluated by weight. Table 2 shows the percentage yield that was reached for each batch of formulas that were made (Yasir, *et al.*, 2014).

Table 2: % yield of prepared formulation

Sr. No.	Batches	% Yield (% w/w)
1	F1	83.88
2	F2	85.98
3	F3	87.56
4	F4	73.89
5	F5	72.67
6	F6	77.37

Particle Size and Zeta potential:

A Malvern Zetasizer was used to find out the particle size and polydispersity index of the solid lipid nanoparticles. In Figure 2 and Table 3, you can see the particle size and polydispersity index of the formulas that were made (Filippov, *et al.*, 2021).

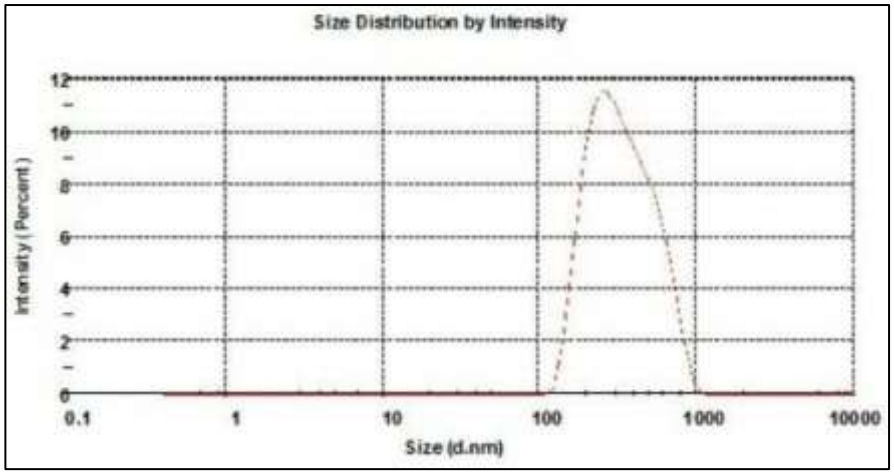

Figure 2: Particle Size of the prepared formulation

Table 3: Particle size and PDI of Prepared formulation

Sr. No.	Batches	Particle size (nm)	PDI
1	F1	310.8	0.455
2	F2	352.9	0.333
3	F3	279.7	0.452
4	F4	316.8	0.466
5	F5	310.6	0.398
6	F6	325.8	0.424

Surface morphology:

The generated nanoparticles' surface look and form were studied with scanning electron microscopy. The shapes and behaviors of the made formulations are shown in Figures 3(a) and (b) (Abou Youssef, *et al.*, 2018).

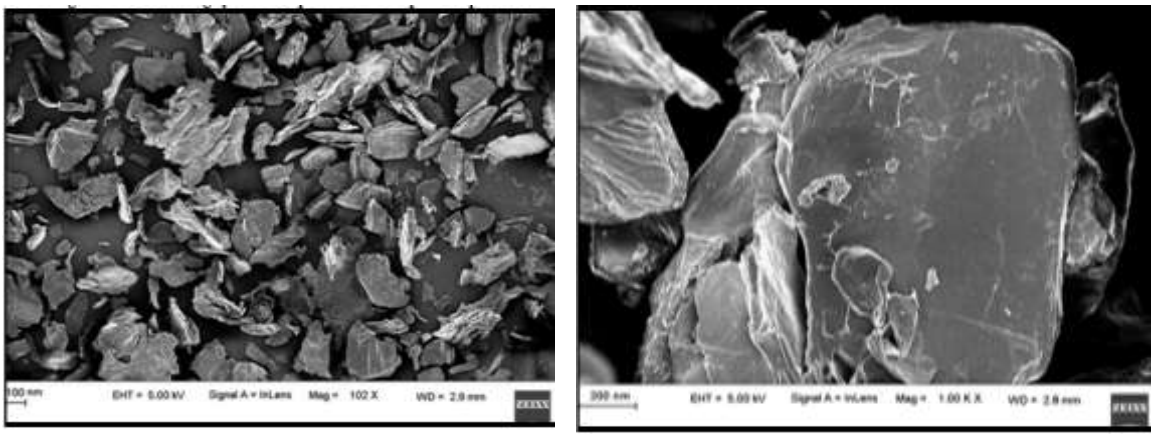

Figure 3 (a), (b): SEM images of the prepared formulations

Drug content and Encapsulation efficiency:

The dose given was between 32.51 mg and 46.36 mg, and the encapsulation rate was between 54.40 mg and 78.69 mg (Table 4). It was seen that as the percentage of polymers went up, so did the entrapment efficiency. This is because the viscosity went up (Mante, *et al.*, 2021).

Table 4: Formulation drug content and encapsulation efficiency

Sr. No.	Batches	Drug content (mg)	Encapsulation Efficiency (%)
1	F1	36.78±0.41	56.01±0.25
2	F2	45.20±0.80	61.55±0.71
3	F3	46.36±0.51	78.69±0.66
4	F4	32.51±0.45	72.31±0.25
5	F5	35.34±0.55	62.55±0.30
6	F6	41.25±0.66	54.40±0.20

In vitro drug release:

After being in a pH 7.4 buffer for nine hours, all of the formulas gave off more than 90% of the medicine. Table 5 and Figure 5(a) and (b) show the results of the in vitro drug release tests on the new preparations. Out of all the formulas (F1 through F6), the F5 one had the best release.

Table 5: *In-vitro* drug release study

Time	F1	F2	F3	F4	F5	F6
1	20.180	19.565	10.120	18.654	20.124	11.324
2	30.900	30.732	20.357	30.879	30.457	22.987
3	41.925	41.947	32.614	40.230	42.369	35.258
4	50.535	52.824	46.578	51.657	56.578	49.021
5	64.168	61.421	56.397	61.378	65.518	60.364
6	75.165	72.942	68.466	72.378	75.825	71.124
7	84.525	83.863	78.814	81.852	85.548	80.367

8	85.186	88.314	86.241	88.897	90.122	88.348
9	89.898	91.523	89.324	92.254	95.356	90.894

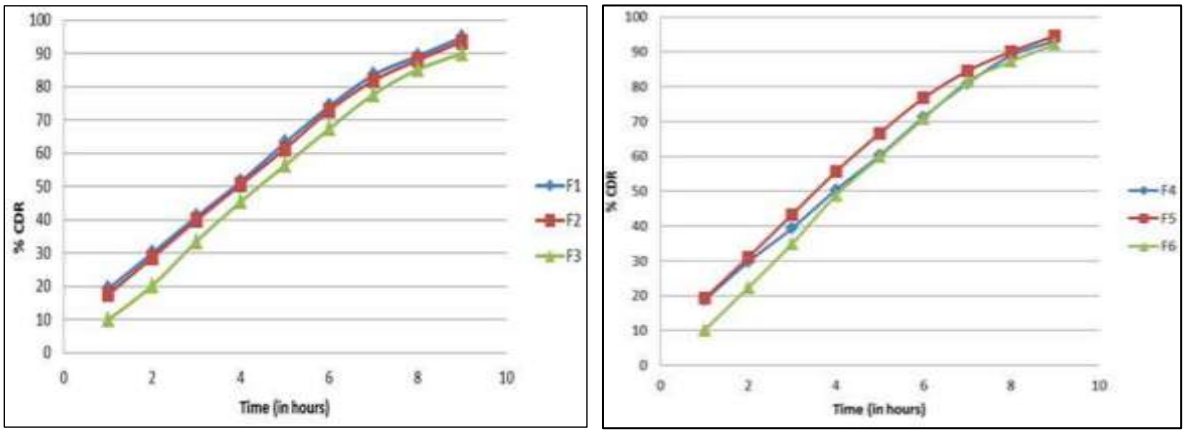

Figure 5(a) and (b): *In-vitro* drug release study

Stability Studies:

The results of the stability study showed that formulation F5's drug content stayed the same during the stability study time. There was no major change in the amount of cumulative medicine release after 60 days of research. Results of the stability study shown in the table number 6.

Table 6: Stability study

Sr. No.	Time (Days)	Drug content (mg)	Drug Release (%)
1	0	46.12	88.12
2	30	45.35	89.35
3	60	45.68	90.89
4	90	45.70	90.94

CONCLUSION:

The present study is an attempt to make stable lipid nanoparticles of risperidone using a mix of polymer and lipid components. The following conclusions can be drawn from the fact that the studies have always led to the same results: The infrared spectra showed that the polymer and risperidone did not interact with each other, which proved that the two molecules are compatible. We made risperidone solid lipid nanoparticles using the solvent injection method, which involved mixing glyceryl monostearate and tween 80 in water to make an emulsion. The solid lipid nanoparticles became better at entrapping things as the quantity of lipids rose. The entrapment rate of Formulation F5 was 78.69±0.66%. A study on drug release that was done in a lab found that adding more lipids also makes the medication break down faster. The results of the dissolving study made it hard to believe that solid lipid nanoparticles could control how quickly the medicine risperidone was released.

Funding:

None

Conflict of Interest:
None

REFERENCES:

Abd-Elrazek, A. and Elnawawy, T., 2019. The Effect of minor doses of olanzapine-solid lipid nanoparticles on an animal model of schizophrenia (neurochemical and behavioral study) and the side effect. *Drug Delivery Letters*, *9*(4), pp.308-320.

Abou Youssef, N.A.H., Kassem, A.A., Farid, R.M., Ismail, F.A., Magda Abd Elsamea, E.M. and Boraie, N.A., 2018. A novel nasal almotriptan loaded solid lipid nanoparticles in mucoadhesive in situ gel formulation for brain targeting: preparation, characterization and in vivo evaluation. *International journal of pharmaceutics*, *548*(1), pp.609-624.

Aher, P., Surana, K., Ahire, E., Patil, D., Sonawane, D. and Mahajan, S., 2023. Development and validation of RP-HPLC method for quantitative determination of 4-amino benzene sulphonamide in sulphonamide hydrochloride. *Trends in Sciences*, *20*(6), pp.5209-5209.

Ahire, E.D., Talele, S.G. and Shah, H.S., 2020. Nanoparticles as a promising technology in microbial pharmaceutics. In *Applied Pharmaceutical Science and Microbiology* (pp. 133-158). Apple Academic Press.

Ahirrao SP, Sonawane MP, Bhambere DS, Udavant PB, Ahire ED, Kanade R, Kuber D. Cocrystal formulation: a novel approach to enhance solubility and dissolution of etodolac. Biosci. Biotechnol. Res. Asia. 2022 Mar 1;19(1):111.

Behera, J., Keservani, R. K., Yadav, A., Tripathi, M., & Chadoker, A., 2010. Methoxsalen loaded chitosan coated microemulsion for effective treatment of psoriasis. International Journal of Drug Delivery, 2(2).

Bharti. AD., Keservani R. K., Sharma. AK., Kesharwani Rajesh, K., & Mohammed GH., 2012, Formulation and in vitro characterization of metoprolol tartrate loaded chitosan microspheres. Ars Pharmaceutica, (53-3), 13-18.

Chalikwar, S.S., Belgamwar, V.S., Talele, V.R., Surana, S.J. and Patil, M.U., 2012. Formulation and evaluation of Nimodipine-loaded solid lipid nanoparticles delivered via lymphatic transport system. *Colloids and Surfaces B: Biointerfaces*, *97*, pp.109-116.

Eisa, A.M., El-Megrab, N.A. and El-Nahas, H.M., 2022. Formulation and evaluation of fast dissolving tablets of haloperidol solid dispersion. *Saudi Pharmaceutical Journal*, *30*(11), pp.1589-1602.

Filippov, S.K., Khusnutdinov, R.R., Inham, W., Liu, C., Nikitin, D.O., Semina, I.I., Garvey, C.J., Nasibullin, S.F., Khutoryanskiy, V.V., Zhang, H. and Moustafine, R.I., 2021. Hybrid nanoparticles for haloperidol encapsulation: Quid est optimum?. *Polymers*, *13*(23), p.4189.

Gautam, S. P., Keservani, R. K., Gautam, T., Gupta, A. K., & Kumar Sharma, A., 2015. An alternative approach for acetylation of amine terminated polyamidoamine (PAMAM) dendrimer. Ars Pharm. 56(3): 155-159.

Jaiswal, M., Gupta, A.A., Singh, AK., Keservani, R.K., Kachave R.N., Amrutkar S.V., 2023. Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide in Bulk Drug and its Tablet Formulation by RP-HPLC, Eur. Chem. Bull., 12 (11), 74 – 86.

Keservani Raj K, Praveena R, Pawar Shubham Vijay, Devi Ritismita, Tiwari Sunita Waila, Patel Virendra Kumar, Guru Prasad V., Uddin Nazim, 2024, Insilico Efficacy of Quercetin As A Therapeutic Potential For Colorectal Cancer, Afr.J.Bio.Sc. 6(Si2), 3322-3329.

Keservani, R. K., & Sharma, A. K., 2018. Nanoemulsions: Formulation Insights, Applications, and Recent Advances. Nanodispersions for Drug Delivery, 71-96.

Keservani, R. K., Bandopadhyay, S., Bandyopadhyay, N., & Sharma, A. K., 2020. Design and fabrication of transdermal/skin drug-delivery system. In *Drug Delivery Systems* (pp. 131-178). Academic Press.

Keservani, R. K., Sharma, A. K., & Ramteke, S., 2010. Novel vesicular approach for topical delivery of baclofen via niosomes. Lat Am J Pharm, 29, 1364-1370.

Keservani, R. K., Sharma, A. K., &Kesharwani, R. K. (Eds.). 2019. Nanocarriers for brain targeting: principles and applications. CRC Press.

Keservani, Raj K. and Gautam, Surya Prakash., 2020. Formulation and evaluation of baclofen liposome vesicles using lecithin, ARS Pharmaceutica, 61 (3), 175-180.

Keservani, Raj K. and Gautam, Surya Prakash., 2022. Skeletal muscle relaxant activity of different formulation of span 60 niosome, ARS Pharmaceutica, 63 91, 32-44.

Khairnar, S. J., Ahire, E. D., Jagtap, M. R., Surana, K. R., Kshirsagar, S. J., & Keservani, R. K., 2024. Management and Prevention of Diseases by Flavonoids. In Advances in Flavonoids for Human Health and Prevention of Diseases (pp. 47-71). Apple Academic Press.

Khambete, H., Keservani, R. K., Kesharwani, R. K., Jain, N. P., & Jain, C. P., 2016. Emerging trends of nanobiomaterials in hard tissue engineering. Nanobiomaterials in Hard Tissue Engineering, 63-101.

Khulbe, P., Singh, D. M., Aman, A., Ahire, E. D., & Keservani, R. K., 2023. The emergence of nanocarriers in the management of diseases and disorders. Community Acquired Infection, 10.

Komu, L.T., Dharmalingam, S., Kumar, S., Keservani, R. K., Joshi, N., Rajesh, K.S., Sakarkar, S.N., Vippamakula, S., Mehta, F.F., 2023. Formulation Development and Evaluation of Thermo-Responsive BIOGELS: Nose to Brain Approach, JCHR, 13(3), 839-844.

Mante, P.K., Adomako, N.O., Antwi, P., Kusi-Boadum, N.K. and Osafo, N., 2021. Solid-lipid nanoparticle formulation improves antiseizure action of cryptolepine. *Biomedicine & Pharmacotherapy*, *137*, p.111354.

Mohammadi, P., Mahjub, R., Mohammadi, M., Derakhshandeh, K., Ghaleiha, A. and Mahboobian, M.M., 2021. Pharmacokinetics and brain distribution studies of perphenazine-loaded solid lipid nanoparticles. *Drug Development and Industrial Pharmacy*, *47*(1), pp.146-152.

Nataraja, B. T., Dharmalingam, S., Keservani, R. K, Gupta, A. K, Pulipati, S, Bhor R. J., Singh, A.K., Singh, S.K., Boddeda, B., 2023. Silver Nanoparticle Formulation Development and Evaluation: In-Vitro Anti-Microbial Evidences, JCHR, 13(3), 1389-1396.

Patel, A.D., Modi, C.D., Thakkar, V.T., Rana, H.B. and Chavda, D.D., 2024. Development and Characterization of Lipid Nanoparticles Loaded with Antipsychotic Drugs using Central Composite Design. *Current Nanomedicine (Formerly: Recent Patents on Nanomedicine)*, *14*(2), pp.155-168.

Piazza, J., Hoare, T., Molinaro, L., Terpstra, K., Bhandari, J., Selvaganapathy, P.R., Gupta, B. and Mishra, R.K., 2014. Haloperidol-loaded intranasally administered lectin functionalized poly (ethylene glycol)–block-poly (d, l)-lactic-co-glycolic acid (PEG–PLGA)

nanoparticles for the treatment of schizophrenia. *European Journal of Pharmaceutics and Biopharmaceutics*, *87*(1), pp.30-39.

Qureshi, M., Aqil, M., Imam, S.S., Ahad, A. and Sultana, Y., 2019. Formulation and evaluation of neuroactive drug loaded chitosan nanoparticle for nose to brain delivery: in-vitro characterization and in-vivo behavior study. *Current Drug Delivery*, *16*(2), pp.123-135.

Sharma, A. K., Keservani, R. K., & Kesharwani, R. K. (Eds.). (2018). Nanobiomaterials: applications in drug delivery. CRC Press.

Sharma, V. K., Koka, A., Yadav, J., Sharma, A. K., & Keservani, R. K., 2016. Self-micro emulsifying drug delivery systems: A strategy to improve oral bioavailability.

Subedi, R.K., Kang, K.W. and Choi, H.K., 2009. Preparation and characterization of solid lipid nanoparticles loaded with doxorubicin. *European journal of pharmaceutical sciences*, *37*(3-4), pp.508-513.

Yasir, M. and Sara, U.V.S., 2013. Preparation and optimization of risperidone loaded solid lipid nanoparticles by Box–Behnken design. *Journal of pharmacy research*, *7*(6), pp.551-558.

Yasir, M. and Sara, U.V.S., 2014. Solid lipid nanoparticles for nose to brain delivery of haloperidol: in vitro drug release and pharmacokinetics evaluation. *Acta Pharmaceutica Sinica B*, *4*(6), pp.454-463.

Yasir, M., Chauhan, I., Zafar, A., Verma, M., Noorulla, K.M., Tura, A.J., Alruwaili, N.K., Haji, M.J., Puri, D., Gobena, W.G. and Dalecha, D.D., 2021. Buspirone loaded solid lipid nanoparticles for amplification of nose to brain efficacy: Formulation development, optimization by Box-Behnken design, in-vitro characterization and in-vivo biological evaluation. *Journal of Drug Delivery Science and Technology*, *61*, p.102164.

Yasir, M., Sara, U.V.S. and Som, I., 2017. Development of a new HPLC method for in vitro and in vivo studies of risperidone in solid lipid nanoparticles. *Brazilian Journal of Pharmaceutical Sciences*, *53*, p.e16047.

Yasir, M., Sara, U.V.S., Chauhan, I., Gaur, P.K., Singh, A.P., Puri, D. and Ameeduzzafar, 2018. Solid lipid nanoparticles for nose to brain delivery of donepezil: Formulation, optimization by Box–Behnken design, in vitro and in vivo evaluation. *Artificial cells, nanomedicine, and biotechnology*, *46*(8), pp.1838-1851.