RESEARCH PAPER

Formulation and Evaluation of Imatinib Nanosuspension

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Abstract

Imatinib is an oral drug used to treat acute lymphoblastic leukaemia and chronic myeloid leukaemia. It falls under the kinase inhibitor category. By lowering the activity of proteins that regulate cell division, growth, and survival, they stop tumours from spreading. Imatinib will be prepared as a nanoformulation as part of the trial to improve its solubility. This drug's nanosuspension formulation was created utilising the nanoprecipitation process. SEM analysis and zeta potential analysis were used to characterise the imatinib nanoformulation. Studies on the stability of nanoformulations have been done. The nanosuspension was used to investigate in vitro anticancer property with the appropriate cell lines.

Keywords: Nanosuspension, Imatinib, Anticancer

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Introduction

In all branches of science, engineering, and technology, nanotechnology is a growing field. The results of research and development on nanotechnology have an impact on bio-products, tools, gadgets, and materials. With nanotechnology, better medical treatments, more suitable building materials, and more helpful products will be created. Other life sciences and medicine will be impacted by nanotechnology. Research studies on drugs modified by nanotechnology for the treatment of cancer are growing daily. Better cancer detection, more effective drug delivery to tumour cells, and molecularly tailored cancer therapy are all possible because the advancement in nanotechnology. [1]

A formulation or device that facilitates the entrance of a therapeutic material into the body and enhances its efficacy and safety by managing the rate, time, and location of drug release in the body is referred to as a drug delivery system. The primary benefit of nanometric particles is their enhanced physical and chemical characteristics. The major parameters in drug delivery includes particle size, surface area, hydrophobicity, surface charge and crystallinity.

Drug particles are colloidally dispersed at submicron sizes in nanosuspension. Pharmaceutical nanosuspension is a term used to describe extremely finely colloid, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1m stabilised by surfactants and polymers generated by acceptable procedures for drug delivery applications. The problem associated with the administration of medications that are poorly water soluble and poorly water and lipid soluble has been demonstrated to be amenable to solution by nanosuspension. It increases bioavailability and absorption while assisting in lowering the dosage of standard oral dosing forms. The Noyes Whitney equation states that decreasing drug particle size increases surface area, which in turn increases the rate of dissolution. The particle size reduction caused by a rise in saturation solubility. Nanosuspension not only solve the problem of poor solubility and poor bioavailability but also alter the pharmacokinetics of the drug and improve the drug safety and efficacy.

Advantages:[2]

A. Most economical.
b. Beneficial for medicines with limited solubility.
c. Liposomes are physically more unstable.
d. Make manufacturing simple and allow for large-scale production and rapid tissue targeting and disintegration.
f. Lessening of tissue irritancy and
Higher bioavailability for medication administration via inhalation and the eyes.

Formulation techniques[3]

Techniques for the formulation of nanosuspension can be classified into two.
Top-down process
Bottom-up process

Top-down processes are mechanical and involve homogenization and grinding. When using a bottom-up approach, molecules are dissolved in a solvent and precipitated using a variety of techniques, including the addition of a solvent, spray freezing, evaporative precipitation, and liquid solvent switching. Most processes are top-down. The disadvantages of mechanical processes include their time and energy requirements, potential for contaminants, and insufficient control over particle size

Media milling[4]

Using pearl mills or high-shear media mills, nanosuspensions are created. The milling chamber,

recirculation chamber, and milling shaft make up this component. Balls or pearls formed of ceramic sintered zirconium oxide or aluminium oxide are used as milling media. Milling medium, water, medication, and stabiliser are added to the milling chamber. The sample is affected by balls rotating at a high shear rate while being kept at a constant temperature. Particle size is reduced as a result of both frictional and impact forces, and nanosized particles are produced.

Homogenisation in non-aqueous media

In water-free media or a water mixture, it is homogenised. The temperature will be zero degrees Celsius or even below. Thus, it is often referred to as deep freeze homogenization. For thermolabile compounds, it is the most effective technique.

Nanoedge

This technique will be similar to homogenization method or precipitation method. It is considered as the combination of this two methods which leads to the better stability and bioavailability. The suspension obtained by this method will be again homogenised to reduce the particle size and prevent crystal growth. In precipitation method there will be chances of crystal growth and long term stability problems. Nanotechnology will solve such problems. An evaporation technique is also included in the nano edge technology for the better production of nanosuspension which will result in the solvent free modified starting material.

Precipitation technique

Precipitation method has been used for long years for the formulation of submicron particles. It is mainly used for the poorly soluble drugs. First drug is dissolved in a suitable solvent. This solution is then mixed with a miscible antisolvent system in the presence of surfactants. Rapid addition of drug solution in to the antisolvent leads to the sudden supersaturation of drug in the mixed solution forms ultrafine drug solids. Precipitation method involves two phases – nuclei formation & crystal growth. When preparing a stable suspension with the minimum particle size, a high nucleation rate and but low growth rate is necessary. Both rate are depend on temperature. In this technique the drug needs to be soluble in at least one solvent which is miscible with non solvent.

EXPERIMENTAL METHODS

Pre formulation study[5]

Prior to the development of dosage forms with a new drug candidate, it is essential that certain fundamental physical and chemical properties of drug powder are determined. This information will dictate many of subsequent events and possible approaches in formulation development.

Organoleptic properties

The colour, nature and odour of the drug were characterised and recorded using descriptive terminology.

Solubility studies[6]

The solubility studies was used to identify the suitable solvent that possess good solubilizing capacity of imatinib mesylate. Solubility of Imatinib mesylate in various solvent like DMSO,propylene glycol, water,n-octanol, acetonitrile and acetone were determined by adding excess of imatinib mesylate in each selected solvents in each conical flask containing 20 ml and is shaken for 24 hours using rotary shaking apparatus. Then the solubility of drug in each solvent is observed visually and by UV spectrophotometrically at maximum wavelength 281 nm after relevant dilutions.

Preparation of standard stock solution of Imatinib

Accurately weighed 100 mg of Imatinib mesylate transferred to 100 ml volumetric flask. It was dissolved in distilled water and was shaken manually for 10 minutes. The volume was made up to the mark with same solvent to obtain final strength of 100 mcg /ml.

Study of linearity

Appropriate known volumes of aliquots from standard stock Imatinib mesylate solution were transferred to separate 10 ml volumetric flask. The volume was adjusted to the mark with distilled water to a series of concentration in the range of2-28 mcg/ml. Absorbance of these solutions were recorded at 281 nm and caliberation curve was plotted absorbance vs concentration.

Preparation of Imatinib nanosuspension[7]

100 mg of drug dissolved in 10 ml of propylene glycol. In another beaker, take 10 ml water and 1g tween 20 and maintain at 850 c in water bath. Cool it for 10 minutes. Subject it to magnetic stirring for 2 hours. Add the drug solution drop by drop to above solution to form nanosuspension. Subject it to homogenisation for 1 hour and sonication for 15 minutes.[7]

Evaluation of imatinib nanosuspension

Zetapotential analysis [8]

The zeta potential of nanoparticle were analyzed by photon correlation spectroscopy. Samples were placed into disposable plastic cuvette.The elecctrophoretic mobility measurements were made using the Malvern Zetasizer (Nano ZS90, Malvern Instruments) at 25ºC.

Scanning electron microscope (SEM) analysis[9]

The surface morphology of the prepared Imatinib NS was determined for by using scanning electron microscopy (JEOL model JSM-6390LV). A drop of Imatinib NS was placed on a carbon film coated copper grid for SEM. Studies were performed at 80kv using JEOL model JSM-6390LV, Japan equipped with electron diffraction pattern (SAED). The copper grid was fixed in to sample holder and placed in a vacuum chamber of the scanning electron microscope and observed under low vacuum and SEM images were recorded.[8]

Solubility studies[10]

Saturation solubility is defined as the maximum quantity of a compound (solute) that can be dissolved in a certain quantity of a specific solvent at a specified temperature. However it is well known that the saturation solubility also depends upon the modification of polymorphic compounds and the particle size if the particles is less than 1µm are present. Solubility studies were performed using

a shaker (orbital shaker incubator,). Excess of pure quercetin was added in 20 ml DI water and stored at 37 ± 0.5 0C. After 24 hours of shaking, suspensions were filtered and analyzed using UV spectrophotometer at 370 nm (UV- 1700Pharmaspec, Shimadzu). Experiments were carried out in triplicate, and solubility data were found

In vitro antioxidant study [11]

Antioxidant activity can be evaluated by scavenging of the stable DPPH radical. The model is extensively used as it is less time consuming than the other methods. DPPH can accept an electron and hydrogen radical and can be converted in to a stable diamagnetic molecule. DPPH contain an odd electron, and so it has a strong absorption at 517nm.when this electron becomes paired off, the absorption decreases with respect to the electro take up. Such change in the absorbance produced in this reaction has been widely applied to assess the capacity of numerous molecules to act as free radical scavengers. Free radical scavenging activities of all the prepared Imatinib nanosuspension formulation were determined by DPPH assay method.

Preparation of solutions[12,13]

Preparation of 1000µg/ml stock solution of Imatinib Nanosuspension

10mg of Nanosuspension was taken and dissolved in PBS. The volume was made up to 10mL with PBS.

Preparation of 0.1%v/v, 1%v/v and 10%v/v formulated nanosuspension

From the above solution 1.0mL aliquot was transferred to a 10mL standard flask and the volume was made up to 10mL with PBS for 10%v/v solution. From the stock solution 1.0mL and 0.1mL aliquots were transferred to a 100mL standard flask and the volume was made up to 100mL with PBS for 1%v/v and 0.1%v/v solution.

Preparation of 0.2mM DPPH solution

0.00789g of DPPH was taken in a 100mL standard flask and dissolved in 100mL of methanol. The final volume was made up to 100ml with methanol.

Preparation of 0.1%v/v, 1%v/v and 10%v/v standard solution:

10mg of ascorbic acid was taken in a 10mL standard flask and dissolved in water. The volume was made up to 10mL with water. From this solution 1.0 ml aliquot was transferred to a 10mL standard flask and the volume was made up to 10 ml with methanol for 10%v/v solution. From the stock solution 1.0mL and 0.1mL aliquots were transferred to a 100 ml standard flask and the volume was made up to 100 ml with water for 1%v/v and 0.1%v/v solution.

Procedure for the evaluation of antioxidant activity:[14,15]

1.5ml of 2.0mM of DPPH solution was added to 1.5mL of different concentration of the formulated nanosuspension. Another series of solution were prepared by taking 1.5mL of PBS and 1.5ml of different concentrations of formulated nanosuspension. The above solutions were allowed to react at room temperature for 30min. after 30 min the absorbance values were measured at 517nm. Imatinib was used as the reference standard. Percentage of scavenging activity was calculated by using the following formula.

% scavenging = (Ab +As)-Am/Ab×100

Ab = absorbance of 1.5mL of DPPH +1.5mL of methanol at 517nm.

Am= absorbance of 1.5mL of DPPH +1.5mL of Nanosuspension at 517nm.

As= absorbance of 1.5mL of PBS +1.5mL of Nanosuspension at 517nm

Stability studies of Imatinib NS [16,17],

Stability is an important factor in case of nanosuspension. In case of suspensions the possibility of sedimentation and cake formations are more. The particles will settle down and it would not redisperse uniformly after shaking. So the dose will be changed, bioavailability will be less which leads to the decreased pharmacological output. When we are formulating the drug in to nanometer range the particle size will be less than 1µm, so it would not sediment rapidly it will suspend in the solvent more time and redispersion will be fast. The stability was measured at different temperature and find out at which temperature it would be more stable. The prepared formulation was kept at room temperature and 40c for six months at specific time period (0,3,6 months). A portion of the sample were taken and subjected to particle size analysis for the determination of stability.

IN VITRO anticancer study[18]

MTT Assay

The 3-(4, 5 – dimethylthiazol-2, 5-diphenyltetrazolium bromide) dye reduction assay was conducted to diagnose the cytotoxic activity of the formulated nanosuspension. HCT-116 cells were plated onto 48 wells plates, 18 hours before the commencement of the test. Growth medium used was DMEM with 10% Foetal Bovine Serum. The plates were incubated in an animal cell culture incubator, maintained at 37ºC with 5% carbon dioxide. The wells achieved 70% confluency at the time of testing. The original growth medium in the 48 well plate was removed and the samples prepared (Imatinib nanosuspension, in the concentration of 0.1%, 1% & 10%) were added to into the appropriate wells. The plates were returned to the incubator for 96 hours. At the end of 96 hours, the media in the wells were carefully removed and fresh complete growth media was added. To each well MTT solution (5 mg/ml of MTT dissolved in PBS) was added and replaced in the incubator for 3 hours. After 3 hours, the medium was carefully removed from the wells, and DMSO was added to each well and kept on a rocking platform for efficient mixing and extraction of formazan dye from the cells by DMSO. After 30 minutes, the absorbance of the DMSO was measured at 570 nm, in a multi-well spectrophotometer. The average absorbance of the "control" wells was taken as 100% and all other absorbance values were calculated.

Results and discussions

Pre formulation studies

Organoleptic properties

Colour : white to off white
Nature : crystalline powder
Odour : none

Imatinib Mesylate is very soluble in DMSO, propylene glycol and it is also soluble in water due to its salt form. So, we select propylene glycol as solvent for further studies as its drug solubility(fig 1)

Solubility studies

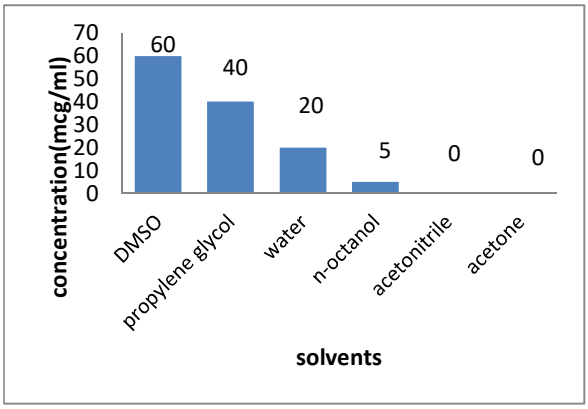

Fig 1: Solubility study

Preparation of Standard Curve of Imatinib

The calibration curve was obtained for a series of concentration in the range of 2-28µg/ml. It was found to be linear and hence suitable for the estimation of the drug. (fig 2)

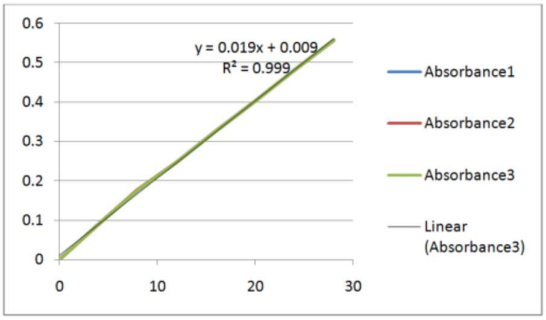

Fig 2: Caliberation curve of Imatinib mesylate

Zeta potential analysis

The zeta potential values of formulated Imatinib nanosuspension were obtained as (2.78mV). The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. In order to obtain a nanosuspension exhibiting good stability, for a nanosuspension a minimum zeta potential of ±30mV is required.(fig 3)

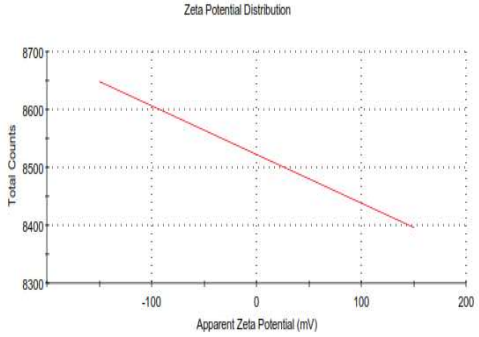

Fig 3: Zeta potential analysis

SEM analysis

Particle size of the Imatinib NS was found to be within the range of 140-180 nm. Therefore the formulation is in nanoscale (fig 4)

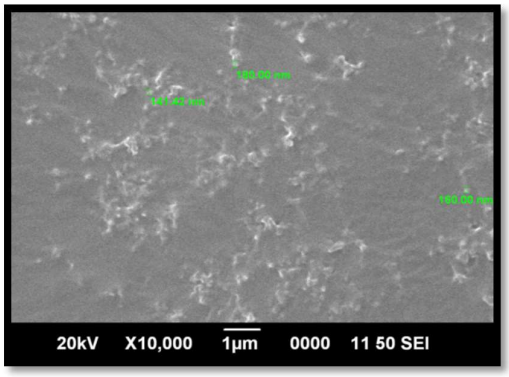

Fig 4: SEM analysis

Solubility studies

Solubility of Imatinib after 24 hr shaking was found to be 4.5±0.2081 µg/ml. Solubility of Imatinib nanosuspension after 24 hr shaking was found to be 10.51 ±0.6683mcg/ml. It was observed that the solubility of prepared nanosuspension has been increased, due to the formation of stabilized nanoparticles. The study indicated that nanosuspension increase the saturation solubility as well as dissolution velocity. From the solubility of Imatinib was extremely low being only 4.5± 0.2081, there was the significant enhancement in the solubility of the nanosuspension produced by homogenization method. (fig 5)

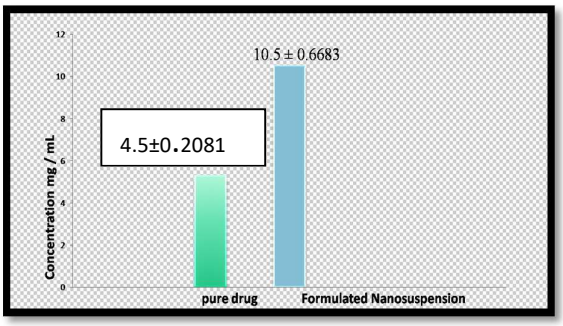

Fig 5: Solubility study of Imatinib nanosuspension

Stability study

Nanosuspensions were stored at 4 0c and at room temperature respectively. The size of the particle were measured over six months. At lower temperature and at room temperature there were no significant changes in the particle size. It indicates that the formulated nanosuspension was stable in cold condition and also in room temperature.

In vitro antioxidant study

Vitamin c has potent antioxidant activity. The in vitro study of formulated nanosuspension shows greater percentage of scavenging activity compared to vitamin c. Hence the formulated nanosuspension has antioxidant activity.(fig 6)

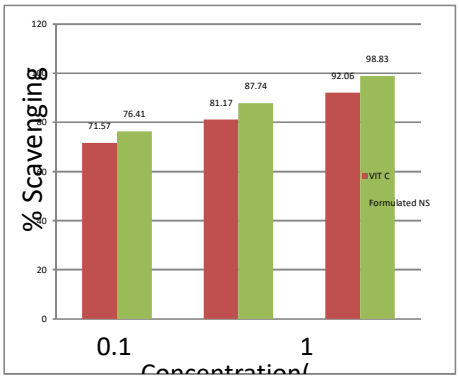

Fig 6: In vitro antioxidant study

Invitro anticancer study

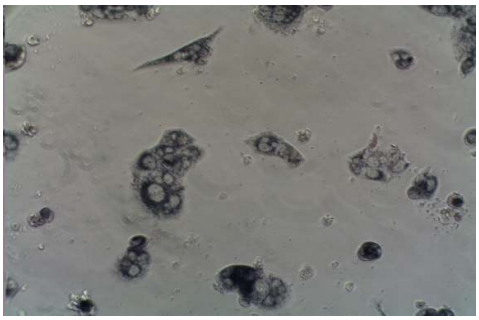

Figure 7 control (Live cells of HCT-116)

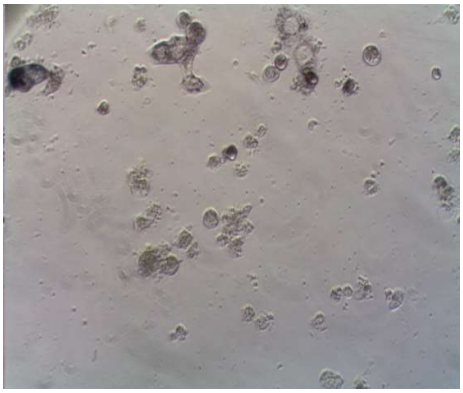

Figure 8 Cells treated with Imatinib nanosuspension(Drug treated cells (noncancer cells)

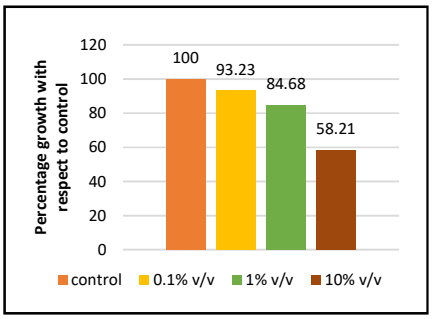

Figure 9 : Percentage cytotoxicity of imatinib nanosuspension

The percentage cytotoxicity of different concentration of formulated nanosuspension were plotted on the graph. From this the Formulated nanosuspension at 10% v/v showed significant cytotoxicity activity. Nanosuspension of imatinib exhibited significant anticancer activity against HCT- 116 cell line at dose dependent manner. Anticancer study reveals that the formulated nanosuspension has got the ability to destroy the cancer cells (fig 7,8,9)

Conclusion

Nanosuspensions are mainly seen as vehicles for administering poorly watersoluble drugs. Nanosuspension formulation have been largely solved the solubility as well as dissolution problems to improve drug absorption. It has therapeutic advantages, such as simple method of preparation, less requirement of excipients, increased saturation solubility and dissolution velocity of drug. Imatinib Nanosuspension was prepared by using homogenization method. The particle size distribution of formulated QNS showed range from ~ 140 to 180 (d.nm) . The zeta potential values of formulated Imatinib nanosuspension were obtained as (2.78mV) using homogenization method. Solubility study indicated that Imatinib NS enhances the solubility of the imatinib produced by the homogenization method. Stability study showed that at low temperature and at room temperature the stability of nanosuspension can be maintained for six months. In addition, Imatinib NS exhibited significant antioxidant activity using DPPH method and anti-tumour activity.

References

1. Yih TC, Al-Fandi M. Engineered nanoparticles as precise drug delivery systems. Journal of

Cellular Biochemistry. 2006;97(6):1184-1190. DOI: 10.1002/jcb.20796; PMID: 16421834.
2. Arias JL, Ruiz MA, López-Viota M, Delgado AV. Poly(alkylcyanoacrylate) colloidal particles as vehicles for antitumour drug delivery: a comparative study. Colloids and Surfaces B: Biointerfaces. 2008;62(1):64-70. DOI: 10.1016/j.colsurfb.2007.09.014; PMID: 17954015.
3. Alvarez-Román R, Naik A, Kalia YN, Guy RH, Fessi H. Skin penetration and distribution of polymeric nanoparticles. Journal of Controlled Release. 2004;99(1):53-62. DOI: 10.1016/j.jconrel.2004.06.015; PMID: 15342180.
4. Dai J, Nagai T, Wang X, Zhang T, Meng M. pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A. International Journal of Pharmaceutics. 2004;280(1-2):229-240. DOI: 10.1016/j.ijpharm.2004.05.019; PMID: 15265563.
5. Moussaoui N, Cansell M, Denizot A. Marinosomes, marine lipid-based liposomes: physical characterization and potential application in cosmetics. International Journal of Pharmaceutics. 2002;242(1-2):361-365. DOI: 10.1016/s0378-5173(02)00216-5; PMID: 12176274.
6. Svenson S, Tomalia DA. Dendrimers in biomedical applications—reflections on the field. Advanced Drug Delivery Reviews. 2005;57(15):2106-2129. DOI: 10.1016/j.addr.2005.09.009; PMID: 16305813.
7. Gilles ER, Fréchet JMJ. Dendrimers and dendritic polymers in drug delivery. Drug Discovery Today. 2005;10(1):35-43. DOI: 10.1016/S1359-6446(04)03243-5; PMID: 15639634.
8. Jones MC, Leroux JC. Polymeric micelles - a new generation of colloidal drug carriers. European Journal of Pharmaceutics and Biopharmaceutics. 1999;48(2):101-111. DOI:
16. Pharmaceutics. 1995;125(1):91-97. DOI: 10.1016/0378-5173(95)00122-X.
17. Peters K, Leitzke S, Diederichs JE, Borner K, Hahn H, Müller RH, Ehlers S. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. Journal of Antimicrobial Chemotherapy. 2000;45(1):77-83. DOI: 10.1093/jac/45.1.77; PMID: 10629031.
18. Senthil Kumar C, Vedha Hari BN, Sharavanan SP, Subramanian N, Punitha S, Senthil Kumar V. Novel metronidazole nanosuspension as a controlled drug delivery system for anthelmintic activity. Journal of Pharmacy Research. 2010;3(10):2422-2425.
19. Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in drug therapy: Rationale for 10.1016/s0939-6411(99)00031-1; PMID: 10452949.
9. Boudier A, Aubert A, Gérardin C, Devoisselle JM, Begu S. pH-sensitive double-hydrophilic block copolymer micelles for biological applications. International Journal of Pharmaceutics. 2009;379(2):212-217. DOI: 10.1016/j.ijpharm.2009.05.061; PMID: 19524021.
10. Müller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. European Journal of Pharmaceutics and Biopharmaceutics. 2000;50(1):161-177. DOI: 10.1016/s0939-6411(00)00087-4; PMID: 10840199.
11. Kumar MNV. Nano and microparticles as controlled drug delivery devices. Journal of Pharmacy & Pharmaceutical Sciences. 2000;3(2):234-258. PMID: 10996417.
12. Lenhardt T, Vergnault G, Grenier P, Scherer D, Langguth P. Evaluation of nanosuspensions for absorption enhancement of poorly soluble drugs: in vitro transport studies across intestinal epithelial monolayers. The AAPS Journal. 2008;10(3):435-442. DOI: 10.1208/s12248-008-9049-y; PMID: 18696222.
13. Arunkumar N, Deecaraman M, Rani C. Nanosuspension technology and its applications in drug delivery. Asian Journal of Pharmaceutics. 2009;3(3):168-173. DOI: 10.4103/0973-8398.56193.
14. Pu X, Sun J, Li M, He Z. Formulation of nanosuspensions as a new approach for the delivery of poorly soluble drugs. Current Nanoscience. 2009;5(4):417-427. DOI: 10.2174/157341309789375422.
15. Liversidge GG, Cundy KC. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: absolute oral bioavailability of nanocrystalline danazol in beagle dogs. International Journal of

development and what we can expect for the future. Advanced Drug Delivery Reviews. 2001;47(1):3-19. DOI: 10.1016/s0169-409x(00)00118-6; PMID: 11251241.