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FORMULATION OF ZINGIBERENE LOADED MICROPARTICLES FOR EXTENDEDRELEASE

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ABSTRACT

The objective of the present research work was to encapsulate zingiberene in polymeric microparticles in order to improve the half life of zingiberene. Zingiberene was isolated from ginger oil using column chromatographic technique using hexane-diethyl ether (97:3,v/v) as the eluting solvent. The absorption maximum of zingiberene in UV spectroscopy was found to be 232 nm It was found that zingiberene was miscible with organic solvents whereas immiscible with water. Stirring time, stirring speed and the concentration of surfactant were pivotal for obtaining the particles of optimum particle size and spherical shape and the polymer concentration of 10 %w/v was able to produce denser particles. The yield of the zingiberene loaded dried microspheres was found to be 74% and the average particle size of the drug loaded microparticles was found to be 37 ± 1.8 µm. The percentage of drug loading in the microparticles was found to be 6% with entrapment efficiency of 66%. The *in vitro* drug release suggested a sustained release of zingiberene from the microparticles with around 63 % drug released in the medium over a period of 10h.

KEYWORDS: Zingiberene, microspheres, release, column chromatography, sodium dodecyl sulfate.

INTRODUCTION

Zingiberene is a monocyclic sesquiterpene that is the predominant constituent of the oil of ginger (*Zingiber officinale*).[1] It is a natural antioxidant isolated for *Zingiber officinale* and is known to possess a large number of pharmacological actions. The antioxidant potential of the molecule is mainly responsible for almost all of its effect on the human body. The half life of the molecule is though low (3.5h) which limits the use of the molecule in therapy. It is very evident from the literature that the encapsulation of drug in microspheres,

microsponges or microparticles causes an increase in the release kinetics of most of the drugs.[2-8] Hence it is often considered to be a very good approach for extended release of drug molecules especially those with a shorter half life. An increase in the release duration increases the inherent efficacy of the drug molecule and also reduces the overall cost of the regimen due to a decreased dosing frequency. It was therefore envisioned that encapsulating zingiberene in microparticles may be able to increase the half life of the molecule thereby reducing its dose and therefore enhancing its antioxidant potential.

MATERIAL AND METHODS

Ginger oil was procured from Meraki essentials, New Delhi and Zingiberene was isolated from ginger oil in laboratory. Eudragit RL 100 was obtained from Yarrow Pharma, Mumbai; sodium dodecyl sulfate, dichlormethane, methanol, ethanol, acetone, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, hexane and diethyl ether were procured from Oxford Fine Chemicals LLP, Mumbai. Any other chemicals used in the study were of laboratory grade and used as received without any purification. Distilled water was freshly prepared in laboratory using glass distillation unit.

Isolation of Zingiberene from ginger oil[9]

In order to prepare the oil-sample for fractionation, ginger oil was dissolved in ethanol at a ratio of 2:1(v/w) of ethanol-to-ginger oil, and then folded in a capful of silica gel for mixed. This mixture was allowed to cool in refrigerator and kept in a desiccator until required. The columnfor chromatography was packed by pouring the activated silica gel into the glass column (60×2.0cm i.d.). The prepared sample was applied to the stationary phase. The contents of the column were eluted by employing hexane-diethyl ether (97:3,v/v) as the eluting solvent. The eluate was collected in fraction size of 4 mL, subjected to TLC identification. The fractions that exhibited same Rf value were pooled together, analyzed by UV-visible spectroscopy for absorption at 232 nm (λ_{max} for zingiberene) and dried by rotary evaporation respectively. The second step fractionation process was performed as the above process taking the pooled samples with absorption at 232 nm.

Solubility Profile

A qualitative analysis of the miscibility of the sample of zingiberene was performed by adding a small volume of the sample to various solvents in the test tubes. The formation of separate layers indicated immiscibility (insolubility) of the sample.

Calibration curve of zingiberene by HPLC[10]

Exactly measured volume of zingiberene was dissolved in methanol to obtain a stock solution of 1 mg/mL. The stock solution was appropriately diluted with methanol to obtain working standard solutions of concentration 1-10 µg/mL. A hypersil ODS C18 column was used at column temperature of 25℃ and the injection volume was 10 µL. A solvent mixture of methanol-water (7:3) was used as mobile phase and the flow rate of 1.0 mL/min was used for elution and the detection wavelength was set to 230 nm.

Optimization of microparticles[11]

The required quantity of Eudragit RL 100 was weighed into a glass bottle and dissolved in 20 mlof dichloromethane. The surfactant, SDS, was dissolved in 50 mL of distilled water in a 200 mL beaker. The beaker containing surfactant solution was partly immersed into ice to minimize solvent evaporation during emulsification. An overhead paddle stirrer was lowered into the polymer solution and the surfactant solution was poured in and mixed with high speed stirring. After mixing at the required speed for the required period of time, the beaker was transferred to a water bath (50°C) and stirring continued at 200 rpm until the solvent was completely evaporated (about 60 min). All the microparticle samples were kept in solution for further characterization.

Effect of polymer concentration on particle size

A solution containing 2.5, 5, 7.5 or 10% w/v Eudragit RL 100 was emulsified into 50 mL of 1% w/v SDS solution for a period of 10 minutes at 350 rpm, followed by solvent evaporation at 200 rpm.

Effects of mixing speed on particle size

Two concentrations of polymer solution were used, 2.5% and 10% w/v Eudragit RL 100. Solutions were emulsified into 50 mL of 1% w/v SDS for a period of 10 minutes at 350, 500 and 700 rpm, followed by solvent evaporation. Two batches of microspheres were produced for each polymer concentration/speed combination.

Effects of stirring time on particle size

2.5 & 10% w/v Eudragit RL 100 solutions were used for study. The initial emulsification stage was carried out for 5, 10, 20, 30 and 40 minutes into 50 mL of 1% w/v SDS solution, at a mixing speed of 350 rpm. Two batches of microparticles were produced for each combination of polymer and surfactant solution.

Effects of SDS concentration on particle size

20 ml of 5% w/v Eudragit RL 100 solution was emulsified into 50 ml of distilled water containing 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 % w/v SDS, at 350 rpm. Two batches of microparticles were produced for each concentration of surfactant.

Formulation of zingiberene loaded microspheres

The optimized parameters were used for formulating the zingiberene loaded microspheres. Zingiberene (1 mL) was dissolved in 20 mL of a 10% w/v solution of Eudragit RL 100 in dichloromethane. The drug: polymer mixture was then dispersed into 100 ml of 1% w/v aqueous SDS solution at 500 rpm for 5 minutes using an overhead stirrer. The dichloromethane was evaporated at by stirring at 350 rpm at room temperature.

Characterization of microparticles[12]

Determination of Yield

The dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formulae given below:

$$\% \text{ yield} = \frac{\text{Mass of the dried microspheres obtained} * 100}{\text{Total Weight of drug and polymer}}$$

Determination of particle size of microspheres

The particle size of the microspheres was determined by using an Olympus microscope, employing the calibrated eye piece and stage micrometer method.

Encapsulation Efficiency and drug loading

Drug containing microspheres were vortexed for 15 minutes. After agitation, the suspension was centrifuged at 10,000 rpm and the drug content in the microparticles was determined by using the HPLC method as previously described. Briefly, 1 mg of accurately weighed microparticles were dissolved in 1 mL of ethyl m using a bench top centrifuge (Remi, Mumbai) for 15 min. The supernatant was collected and subjected for HPLC analysis for determining the zingiberene content as described earlier. Zingiberene loading and encapsulation efficiency were determined by the following equations respectively.

$$\% \, Drug \; loading = \frac{Amount \; of \; drug \; in \; microparticles}{Weight \; of \; microparticles} \; X \; 100$$

$$\% \, EE = \frac{Actual \; drug \; loading \; in \; microparticles}{Theoretical \; drug \; loading} \; X \; 100$$

In vitro release of zingiberene

In vitro zingiberene release from the microparticles was determined by weighing lyophilized the microparticles (10 mg) and dispersing them in 10 mL of phosphate buffer (100 mM, pH 7.4) (PBS) and incubated at 37°C under gentle shaking at 100 rpm. At appropriate intervals, 1 mL of samples was centrifuged at 10,000 rpm for 15 min. The supernatant was withdrawn and assayed for zingiberene using HPLC method described in the previous section. The medium was replenished with 1 mL of fresh PBS solution after each sample withdrawal.

RESULTS AND DISCUSSION

The zingiberene isolated from the ginger oil was obtained as clear oil and its identity was confirmed by determining the absorption maximum wavelength by UV spectroscopy and laterwas subjected to mass spectroscopic analysis. The absorption maximum of 232 nm was obtained which was found in tandem to previously reported value for zingiberene. The organoleptic properties were examined and the isolated zingiberene was found to be clear, colorless oil with a characteristic strong spicy odor. The literature also reports similar organoletpic profile for zingiberene.[13] The isolated zingiberene was found that zingiberene was miscible with organic solvents whereas immiscible with water. The calibration curve of zingiberene was constructed by analyzing the peak area obtained in the HPLC chromatogram of several dilutions of known concentration and plotting the concentration against the peak area value. The retention time of zingiberene using the selected eluting solvent system was found to be 7.4 min.

Effect of polymer concentration on particle size

The effect of changing the polymer concentration on the size of microspheres is presented in Figure 1. It was observed that the particle size increased proportionally with the polymer concentration. Particles of around 25 µm were obtained using the procedure that was followedfor formulation.

Effect of stirring speed on particle size

The influence of mixing speed on the size of particles that are obtained is shown in Figure 2. It was observed that there was a large difference in size between the two polymer concentrations when stirring was done at 350 rpm whereas the difference in particle size at the higher mixing speeds was small. The small size difference between the samples stirred at 500 and 700 rpm, suggested that the 10% w/v polymer solution produced denser microspheres compared to the 2.5% w/v solution at higher mixing speed.

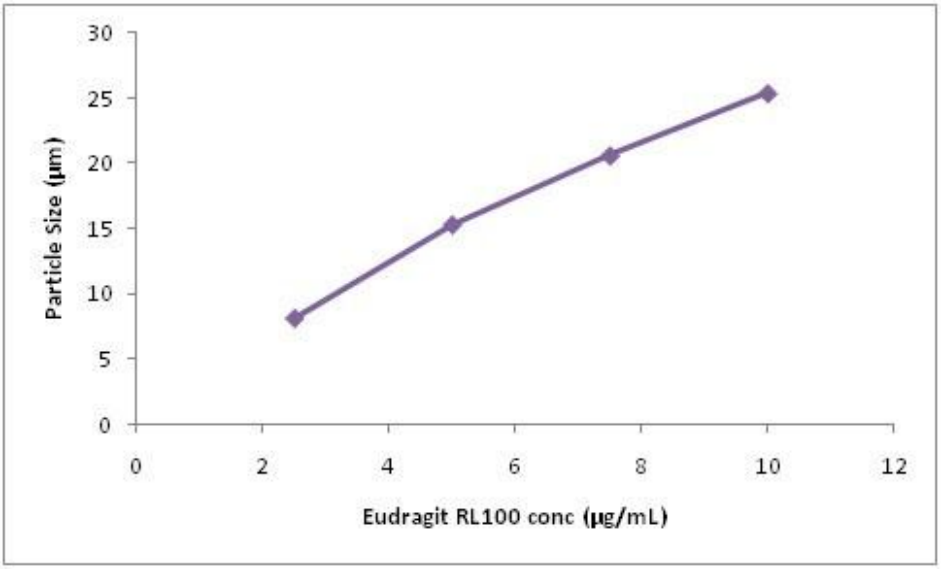

Figure 1: Effect of polymer concentration on particle size.

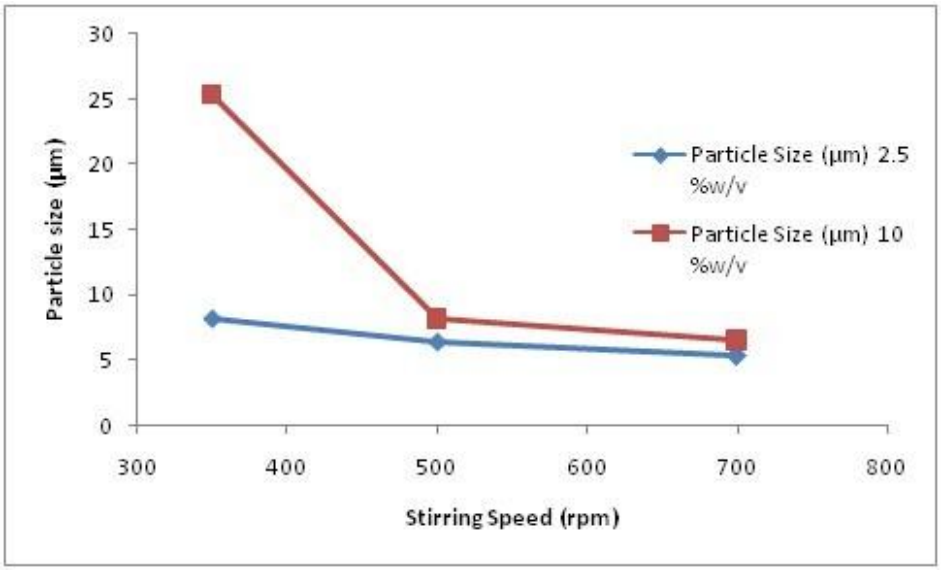

Figure 2: Effect of stirring speed on particle size.

Effect of stirring time on particle size

It was witnessed that increasing the mixing time reduced the particle size rapidly as it was thecase with stirring speed. The results show that mixing time of 5 min produced particles morethan 50 and 100 µm in size using 2.5 and 10 %w/v polymeric solution respectively. Increasing the mixing time caused particles of almost equal size n both the concentration of polymers. The result is presented in Figure 3.

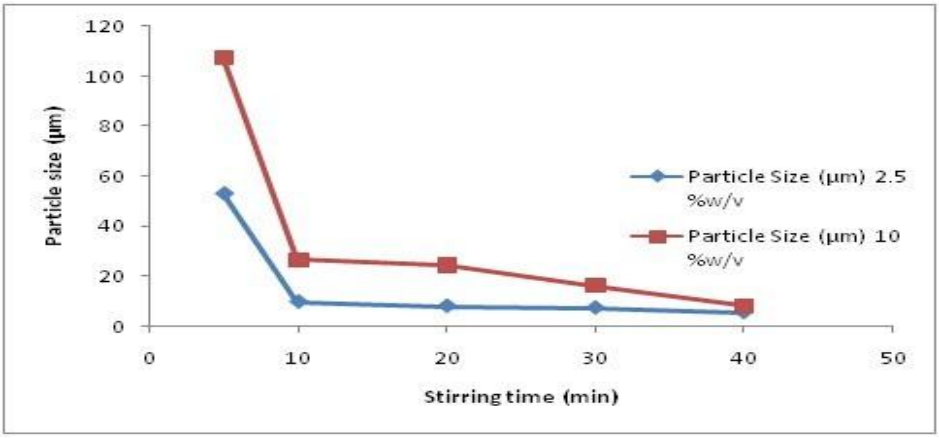

Figure 3: Effect of stirring time on particle size.

Effect of SDS concentration on particle size

The effect of surfactant concentration on the particle size is shown in Figure 4. It was seen that an initial rapid decrease in size with increasing surfactant concentration occurred which was followed by a sudden, marked increase in size at 4 and 5% w/v SDS. The increase in size at higher SDS concentration suggests the formation of agglomerates at higher surfactant concentration.

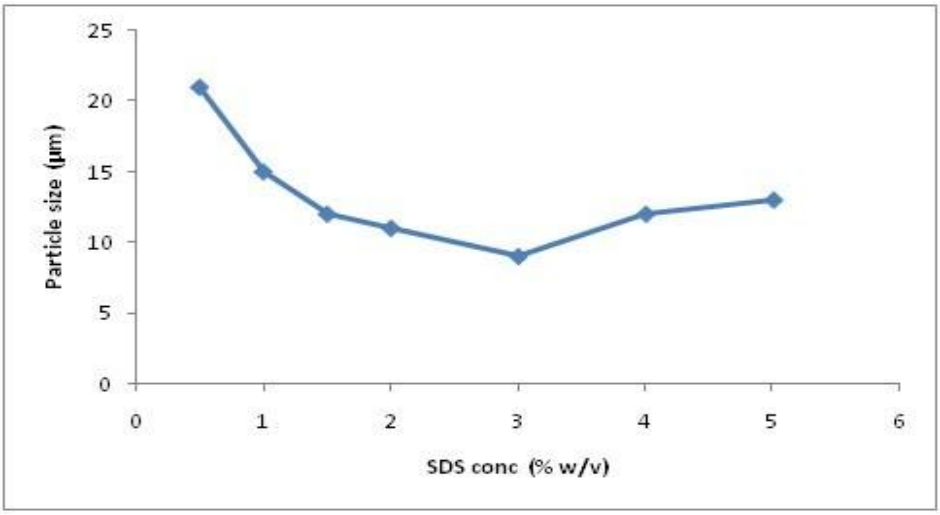

Figure 4: Effect of SDS concentration on particle size.

Formulation of zingiberene loaded microspheres

From the optimization experiments it was evident that the stirring time, stirring speed and the concentration of surfactant were pivotal for obtaining the particles of optimum particle size and spherical shape. Hence stirring time of 5 min at 500 rpm was selected for formulating the drug loaded microspheres. The polymer concentration of 10 %w/v was able to produce denser particles and therefore it was selected the production of final batch of microspheres. The microspheres obtained using the optimized variables were subjected to characterization for yield, encapsulation efficiency, particle size determination and in vitro release of zingiberene.

The microspheres were dried by lyophyllization before using for characterization. The yield ofthe dried microspheres was found to be 74% as calculated from the weight of dry microspheres to that of the drug and polymer used. The average particle size of the drug loaded microparticles was found to be 37 ± 1.8 µm and the theoretical loading was calculated to be 9%.

The percentage of drug loading in the microparticles was calculated by determining the amount of zingiberene in 1 mg of dried microspheres and it was found to be 6%. The entrapmentefficiency of zingiberene in the microspheres was found to be 66%.

The *in vitro* release study was carried out in PBS pH 7.4 solution for a period of 10 h and the cumulative release of zingiberene from the microspheres was calculated. The release data was mathematically treated using zero order, Higuchi and Korsemeyer Peppas models and release pattern is depicted in Figure 5-7.

The *in vitro* drug release suggested a sustained release of zingiberene from the microparticleswith around 63 % drug released in the medium over a period of 10h.

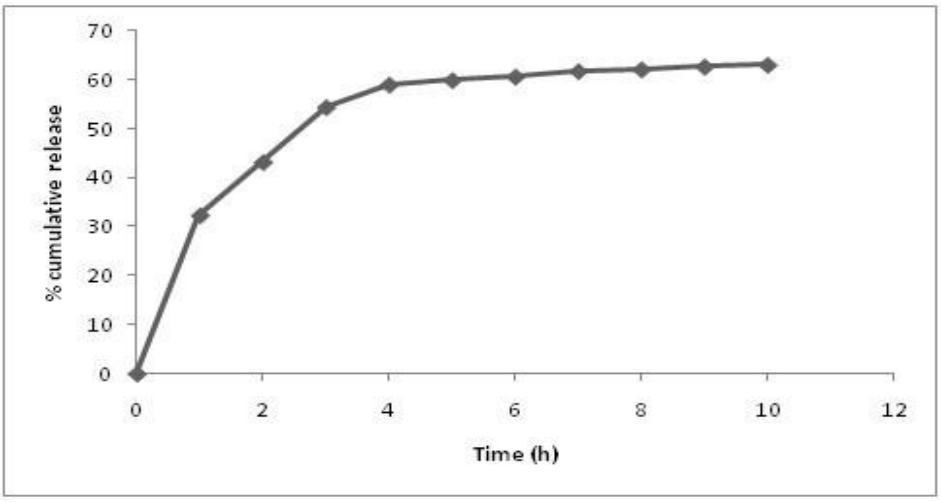

Figure 5: Zero order release profile of zingiberene from microspheres.

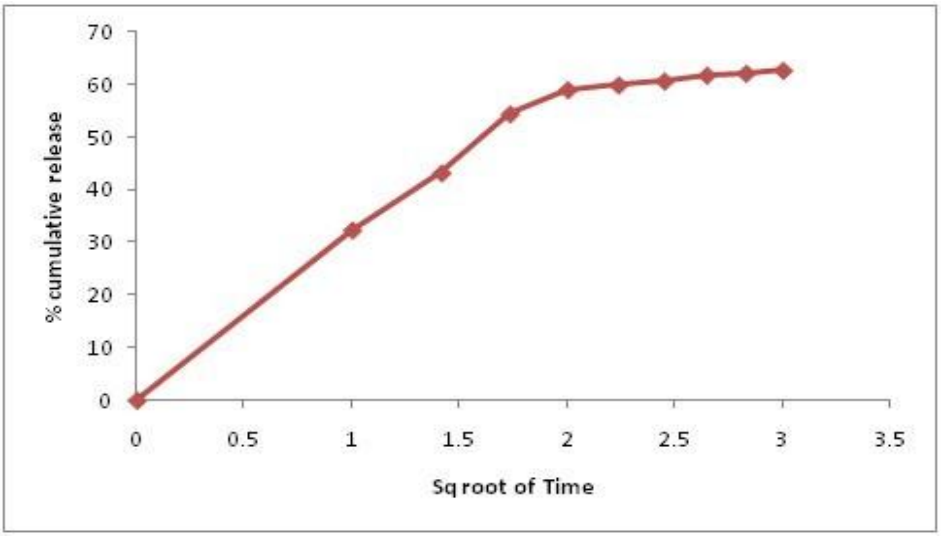

Figure 6: Higuchi release model of zingiberene from microspheres.

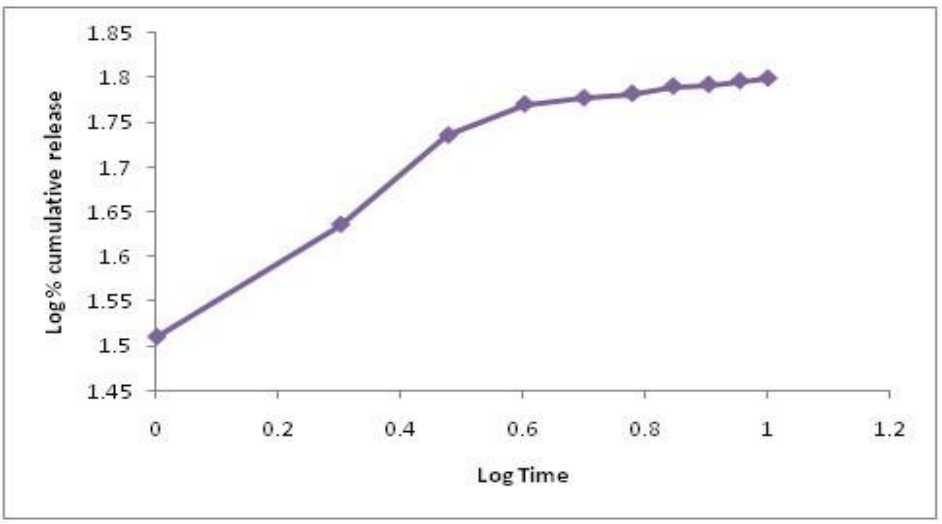

Figure 7: Korsemeyer-Peppas release model of zingiberene from microspheres.

The linear regression constant for zero order, Higuchi and Korsemeyer-Peppas model suggest that release of zingiberene from the microparticles followed Higuchi's release kineticssuggesting that the release is controlled by diffusion and followed Fick's law.

CONCLUSION

The present investigation was undertaken with an objective to improve the half-life of zingiberene by formulating it as microspheres. The improved half life was envisioned to improvise the bioavailability of the drug thereby enhancing its antioxidant potential. It was concluded from the study that the half life of zingiberene indeed increased by formulating as micro sized particles which will definitely improve the antioxidant potential of zingiberene making it more useful therapeutically.

REFERENCES

1. Mahboubi M. Zingiber officinale Rosc. essential oil review, a review on its composition and bioactivity. Clinical phytoscience, 2019; 5. https://doi.org/10.1186/s40816-018-0097- 4.
2. Dang T, Cui Y, Chen Y, Meng X, Tang B and Wu J. Preparation and Characterization of Colon-Specific Microspheres of Diclofenac for Colorectal Cancer. Tropical J. Pharma. Res., 2015; 14: 1541-1547.
3. Zhang W-Q, Chen L-B, Zhe A, Liu G-H, Zhang L, Wang Y-H, Shao Y-K and Liu YY. Gastroretentive Floating Microspheres of Carvedilol: Preparation, In Vitro and In Vivo Characterization. Journal of Biomaterials and Tissue Engineering, 2016; 6: 74- 78.
4. Situ W, Li X, Liu J and Chen L. Preparation and characterization of glycoproteinresistant starch complex as a coating material for oral bioadhesive microparticles for colon-targeted polypeptide delivery. J. Agric. Food Chem, 2015; 63: 4138-4147.
5. Kutmalge M, Mp R, Jadhav A, Wattamwar M, Bangar J and Chaudhari S. Formulation and in-vitro evaluation of lornoxicam floating microsphere. Der Pharm Lett, 2014; 6: 169- 83.
6. Kakar S and Singh R. Preparation of Magnetic microspheres of mesalamine by Phase separation emulsion polymerisation technique. African journal of pharmacy and pharmacology, 2014; 8: 246-258.
7. Diaf K, El Bahri Z, Chafi N, Belarbi L and Mesli A. Ethylcellulose, polycaprolactone, and eudragit matrices for controlled release of piroxicam from tablets and microspheres. Chem. Pap, 2012; 66: 779-786.

8. Dipankar O, Dubey BK, Gupta M. Formulation and evaluation of extended release microspheres loaded with labetalol. J Pharmacol Biomed, 2021; 5(2): 295-303.
9. Wang Y, Du A, Du A. Isolation of Zingiberene from Ginger Essential Oil by two-step intermittent Silica Gel Column Chromatography. Adv Mater Res., 2012; 550-553: 1666- 1670.
10. Watts, Peter James Microspheres for drug-delivery to the colon. PhD thesis, University of Nottingham. http://eprints.nottingham.ac.uk/13455/1/334792.pdf, 1992.
11. Srivastava AK, Ridhurkar DN, Wadhwa S. Floating microspheres of cimetidine: Formulation, Characterization and *In Vitro* Evaluation, Acta Pharm, 2005; 55: 277-285.
12. Pandey GK, Pandey D, Jain D, Joshi A, Dubey BK. Design and optimization of Lamivudine floating microspheres. J Pharmacol Biomed, 2017; 1(1): 15-29.
13. http://www.thegoodscentscompany.com/data/rw1037961.html.