International Journal of Research in Pharmaceutical Sciences

Studying the effect of variables on baclofen floating microsponge

Faten Q. Ibraheem[*], Anmar A. Abdelrazzaq

Department of Pharmaceutics, College of Pharmacy, University of Israa, Baghdad, Iraq

Abstract

The aim of the present work was to develop a microsponge delivery system of baclofen to control its release and thereby reducing dosing frequency and enhancing patient compliance. The microsponge was produced by oil in oil emulsion solvent diffusion method. The effects of drug/polymer ratio, stirring time and type of Eudragit polymer on the physical characteristics of microsponges were investigated. The prepared microsponges was characterized for production yield (PY), loading efficiency (LD), particle size, surface morphology, and *in vitro* drug release. The results showed that the microsponge formula with Eudragit RS100 had optimum physical properties with PY % equal to 97 %, and LD % equal to 81% and controlled drug release (75% of drug release in 8 hours) when compared with other formulas and pure BFN. Therefore, the non-aqueous emulsion solvent diffusion method is a promising method to produce baclofen microsponges.

*Corresponding Author

Name: Faten Q. Ibraheem
Phone:
Email: fatenalatraqchi@gmail.com

INTRODUCTION

Conventional tablet dosage form is administered several times a day, to avoid unnecessary repetitive management, a higher treatment cost and other undesirable characteristics of the conventional dosage forms, controlled release systems were designed, as they require less frequent drug intake, more therapeutic effects and less side effects. These dosage forms are designed to release medication continuously over a long period of time (Ridhima *et al.*, 2012). Gastro-retentive delivery systems are designed to be kept in the stomach for a long time and release their active ingredients thus enabling continuous and long-lasting input of the drug to the top of the GIT (Gharti *et al.*, 2012). There are two types of designing a floating-dose model. These are single-unit systems and multiple-unit systems (Gangadharappa *et al.*, 2011; Ahmed *et al.*, 2018). One of the new ways of the gastro-retentive dosage form is the floating microsponge. It significantly increases the residence time of medication in the stomach, improves bioavailability, improves patient compliance by reducing frequency doses, reduces drug waste (Patel *et al.*, 2016). The microsponges are small spherical particles consisting of innumerable interconnected spaces under a non-folding structure with a large porous surface through which active components are released in a controlled manner. Microsponge could encapsulate a wide range of hydrophilic and hydrophobic drugs (Kaity *et al.*, 2010). The solubility of BFN decreases with increasing pH, having maximum solubility at pH 1.2, which equal to 26 mg/ml. It has short plasma half-life, which is about 2–4 hr. (Abdelkader *et al.*, 2007). Baclofen has a narrow absorption window in the small intestine because, on arrival to the colon, the absorption becomes low or nonexistent (Jivani *et al.*, 2010). The primary objective of the present investigation was to develop and optimize the BFN floating microsponge formula

to control the release rate of the drug and subsequent evaluation of different variables affecting it.

MATERIALS AND METHODS

Baclofen (Hyperchem company, China), Eudragit polymers RS100 and RL100 (Rhom pharma, Germany), liquid paraffin (Solvochem company, United Kingdom), n-hexane (Chemlab, Belgium).

Preparation of BFN microsponges

The BFN microsponge formulas were produced by oil in oil emulsion solvent diffusion technique. The internal phase consisted of polymer in the organic solvent. Then the BFN was added gradually to the internal phase with the addition of magnesium stearate, and the mixture stayed in the ultrasonic bath for 5 minutes to obtain a homogenous dispersion. Magnesium stearate was added as a stabilizer for reduction of particle aggregation. Then, the mixture was poured gradually into liquid paraffin and stirred by using a magnetic stirrer. The oil in oil emulsion formed was stirred for a different duration at different stirring speed. During the stirring period, the solvent diffused into liquid paraffin will be evaporated, leaving spherical porous particles. The solidified microsponges were filtered by using Whatman filter paper and washed five times with 60 ml of n-hexane, dried at room temperature for 12 h and stored in a desiccator for further investigations (Othman *et al.*, 2017).

Characterization and evaluation of BFN microsponge

Determination of the percent production yield (PY)

The percent production yield of the prepared BFN microsponge formula was determined by dividing the final weight of microsponge formula on the initial weight of the raw material multiplied by 100 (Dineshmohan and Gupta, 2015).

$$PY\% = \frac{Practical\ weight\ of\ microsponge}{Theoretical\ weight\ (polymer\ +\ drug)} \\ x\ 100$$

Determination of percent loading efficiency (LD)

The drug content in all prepared microsponge formulas was determined spectrophotometrically, in which 10 mg of the prepared microsponge formula was dissolved in 100 ml of 0.1N HCl (pH 1.2) and kept for 12 hr. The solution was diluted suitably with 0.1N HCl and analyzed spectrophotometrically at λ max of BFN. The drug content was calculated from the calibration curve equation and expressed as the loading efficiency (%) (Osmani *et al.*, 2015).

$$LD\% = \frac{Actual\ weight\ of\ Baclofen\ in\ microsponge}{Theoretical\ weight\ of\ Baclofen} \\ *100$$

Particle size measurement

The particle size of microsponge was determined using an optical microscope. Calibration of the eyepiece micrometer with a stage micrometer was done. A minute quantity of microsponges was spread on a clean glass slide, the particle diameters of around 100 microsponges were measured randomly, and the average particle size of BFN microsponge was determined (Dhakar *et al.*, 2010).

$$D\ (average)\frac{\sum nd}{\sum n}$$

Where n = number of microsponge observed, d = middle value (μm), D is the average diameter of particles (μm).

Buoyancy study

Buoyancy study is done by placing the microsponge formula in 100 ml beaker of 0.1N HCl and remained for 12 hr. the floating and sinking particles filtered and left to dry overnight, the % buoyancy calculated by dividing the weight ratio of the floating particles to the sum of floating and sinking particles.

Scanning electron microscope (SEM) study

The surfaces morphology of the microsponge formula was analyzed by SEM. Sprinkling the microsponge on adhesive tape stuck to aluminum stub and by using a gold sputter module for coating this stub with gold, and then this coated sample was scanned, and photomicrographs were taken by SEM (Sanjivani *et al.*, 2011).

In-vitro drug release studies of microsponge formulations

Baclofen microsponge formulas were subjected to an in-vitro drug releasee study by using dissolution testing apparatus type ⯑⯑ (paddle). The dissolution test was carried out, utilizing 900 ml of 0.1N HCl (pH 1.2). A specified weight of microsponge corresponding to 15 mg of BFN was taken and by using semipermeable membrane rotated at 50 rpm at 37 $\pm$ 0.5 0C. A sample of 5 ml was collected every hour for 10 hrs and immediately was displaced with 5 ml of fresh dissolution medium (0.1N HCl) for preserving sink conditions, after that the sample was filtrated through 0.45 μm filter syringe. The absorbance of the filtrate was measured by UV spectrophotometer at the corresponding λ_{max} of BFN (Charagonda *et al.*, 2016; Trivedi *et al.*, 2017). This procedure was done in triplicate for each formula to take the mean value.

Fourier transforms infrared (FTIR) analysis

Infrared spectroscopy was conducted using FTIR spectrophotometer (Biotech, England) and the spectrum was recorded in the wavelength region of (4000 - 400) cm^{-1}. The procedure consisted of dispersing the sample (drug alone, selected polymer alone, physical mixture of drug with polymer and the optimized formula) in the KBr disc to certify compatibility (Abdellatif *et al.*, 2018).

Differential scanning calorimetric (DSC) analysis

DSC analysis of pure drug, selected polymer, physical mixture of drug and selected polymer and the optimized microsponge formula were done to indicate thermal compatibility between drug and polymer during the formulation of microsponges and to assess the crystalline state of the drug (Amrutiya *et al.*, 2009).

Statistical analysis

The results of the experiments were given as a mean of three samples $\pm$ standard deviation. Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1) using ANOVA test to assess significant differences among means, $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of the drug: Polymer ratio on the microsponges

The drug-polymer ratio had a considerable effect on the nature of microsponges, as shown in Table 1. It was indicated that increasing the drug: polymer ratio to certain limit increased the PY and LD. Since the available polymer was sufficient to encapsulate more amount of drug resulted in high LD.

Further increase in drug to polymer ratio have reverse effect on both the PY and LD as shown in BF7, the reason for decrease in both the PY and LD with increasing drug to polymer ratio is due to the amount of polymer was not sufficient to encapsulate all amount of drug (Nawal and Mohammad, 2016).

It was observed that as the ratio of drug to polymer was increased. The particle size increased due to increase the viscosity of the internal phase and therefore, will be hardly broken into small droplets (Resmi *et al.*, 2018). It was detected that the formulation of microsponge using Eudragit RS 100 showed higher PY and LD. This may be due to the differences between these polymers, Eudragit RS100 contains 5% quaternary ammonium group which is less than that contained in Eudragit RL100 (10%), and therefore these polymers differ in their permeability. Moreover, Eudragit RS100 preferable over Eudragit RL100 in microsponges preparation using oil in oil emulsion solvent diffusion method (Rizkalla *et al.*, 2011). Statistically, this increment in the PY, LD, and mean particle size with the increasing drug: polymer ratio was significant ($P < 0.05$) when using a one-way ANOVA test.

Effect of internal phase volume on the BFN microsponges

The amount of solvent volume needs to be controlled within an appropriate range during microsponge preparation due to its effect not only on the formation of emulsion droplets at the initial stage but also on the solidification of drug and polymer in the droplets.

BF15 and BF17 fabricated with 10ml acetone, resulted in finely dispersed spherical emulsion droplets during agitation, but as the stirring was discontinued emulsion droplets adhered together and coalesce. Consequently, no microsponges could be formed with an increasing volume of acetone to 10 ml (Jain and Singh, 2010).

The role of the solvent was acting as porogen (pore-forming agent) since the evaporation of solvent lead to formation of pores into which the drug is loaded, and this was the reason for increasing the LD (Maheshwari *et al.*, 2017) associated with increasing the volume of internal phase solvent from 5 ml to 7.5 ml. Larger particle size was associated with lower internal phase volume (5 ml) which may be to high viscous phase would be difficult to split the droplets to smaller ones when poured into the external paraffin phase. The effect of acetone volume on the particle size was shown in Figure 1. The increase solvent volume from 5 ml to 7.5 ml showed a significant effect on LD and mean particle size as p-value <0.05 when using the one-way ANOVA.
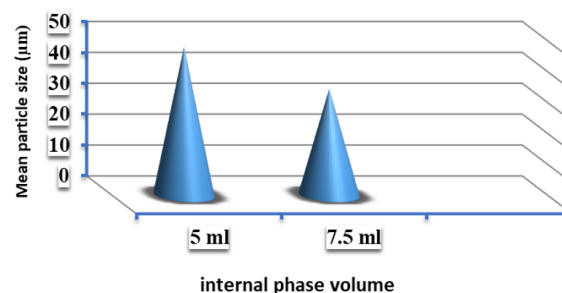
Figure 1: Histogram showing effect of internal phase volume on mean particle size. Effect of stirring speed on BFN *microsponges*

The dispersion of the internal phase of drug and polymer into the droplets in the external phase depended on the agitation speed of the systems. As

Table 1: Effect of Drug Polymer Ratio on Physical Properties of the Microsponge

Formulas	Drug: polymer ratio	Polymer type	PY % ± SD	LD % ± SD	Mean particle size (μm) ± SD
BF1	1:1	Eudragit RS100	89.66±0.577	66.831±0.359	47.7±1.7
BF2	2:1	Eudragit RS100	90.834±0.213	70.812±1.35	60.3±0.8
BF3	3:1	Eudragit RS100	93.83±0.288	75.728±1.09	68.5±0.8
BF4	1:1	Eudragit RL100	87.43±1.15	66.57±0.14	50±0.17
BF5	2:1	Eudragit RL100	89.665±0.47	71.35±0.07	57.2±1.7
BF6	3:1	Eudragit RL100	95.31±1.02	76.03±1.15	70.4±0.8
BF7	4:1	Eudragit RS100	83.73±0.23	62.452±0.594	72.53±1

agitation speed increased, the size of microparticles was reduced due to rapid division of the formed droplets at high stirring speed, which may have less chance of coalescing into bigger droplets with production of more uniform and spherical particles while at lower stirring speed particles suffered from coalescence and aggregation (Desavathu *et al.*, 2017). The microsponges formulated with 1500 rpm had higher LD %. So, it was selected as the optimum stirring speed. Statistically, the effect of stirring speed on the PY % was non- significant but produces a significant effect (P-value < 0.05) on both the LD% and mean particle size.

**Effect of stirring duration on the BFN microsponges**

To find the most appropriate stirring time for fabrication of BFN microsponges, different stirring duration was used 0.5 hr., 1 hr. And 2hr. in BF12, BF1 and BF13 respectively, the results are listed in Table 2 . Stirring duration of 2h in BF13 resulted in low PY% and LD % due to adherence of polymer to beaker during fabrication of microsponges, in addition, a longer time of stirring there was more chance for the drug to be leached. Accordingly, it was adopted that the optimum stirring time is 1 hr. Since 0.5 hr. The stirring duration was associated with lower PY% and LD%. This finding was similar to previously reported by (Nief and Hussein, 2014).

Increase stirring duration from 0.5 hr. to 1 hr. Produced a significant effect on PY% and LD% as P value < 0.05 when using the one-way ANOVA test.

Effect of solvent type on BFN microsponges

Acetone was the preferable solvent for the oil in oil emulsion solvent diffusion method due to its dielectric constant (20.7), so it was poorly miscible with paraffin, that would lead to the slow diffusion of the solvent out of the emulsion droplets to the exter-

nal paraffin medium, resulted in slow precipitation of polymer matrix, and subsequent separation of a microsphere with a spongy structure (Rizkalla *et al.*, 2011). Microsponge preparation by using acetone gave higher PY%, LD% than that of ethanol, as illustrated in Figure 2.
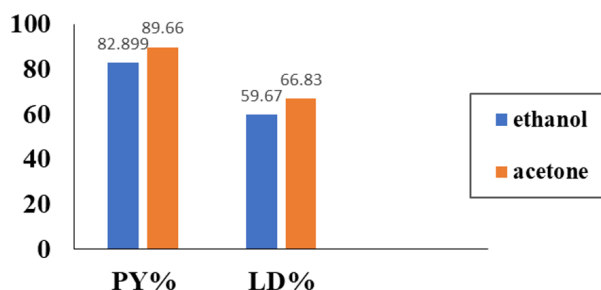
**Figure 2: Effect of solvent type on physical properties of micro-sponges**
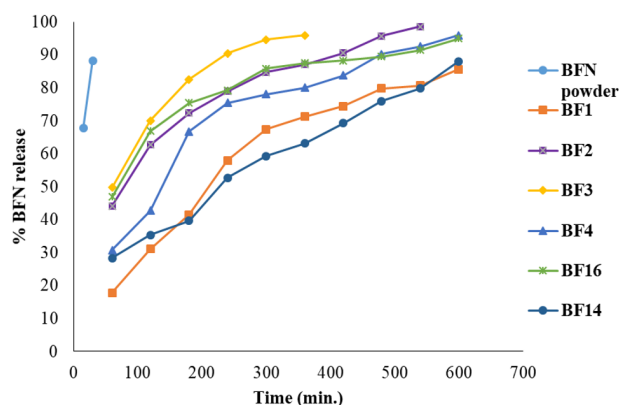
Figure 3: Dissolution profile of BFN from different microsponge formula in 0.1N HCl (pH1.2) at 37ºC

In- vitro drug release study of microsponges

Dissolution was done for different microsponge formulas and pure BFN, as illustrated in Figure 3.

Table 2: Effect of Stirring Duration on Physical Prosperities of Microsponges

Formula	drug: poly-mer ratio	type of poly-mer	acetone (ml)	paraffin (ml)	stirring speed(rpm)	stirring dura-tion(hr.)	PY% ±SD	LD% ±SD	Mean particle size±SD (μm)
BF1	1:1	Eudragit RS100	5	100	1500	0.5	77.6± 0.51	60.83± 3.05	42.83± 0.61
BF12	1:1	Eudragit RS100	5	100	1500	1	89.66± 0.577	66.831± 0.35	47.7± 1.7
BF13	1:1	Eudragit RS100	5	100	1500	2	80.9± 0.17	63.686± 1.8	47.36± 1.7
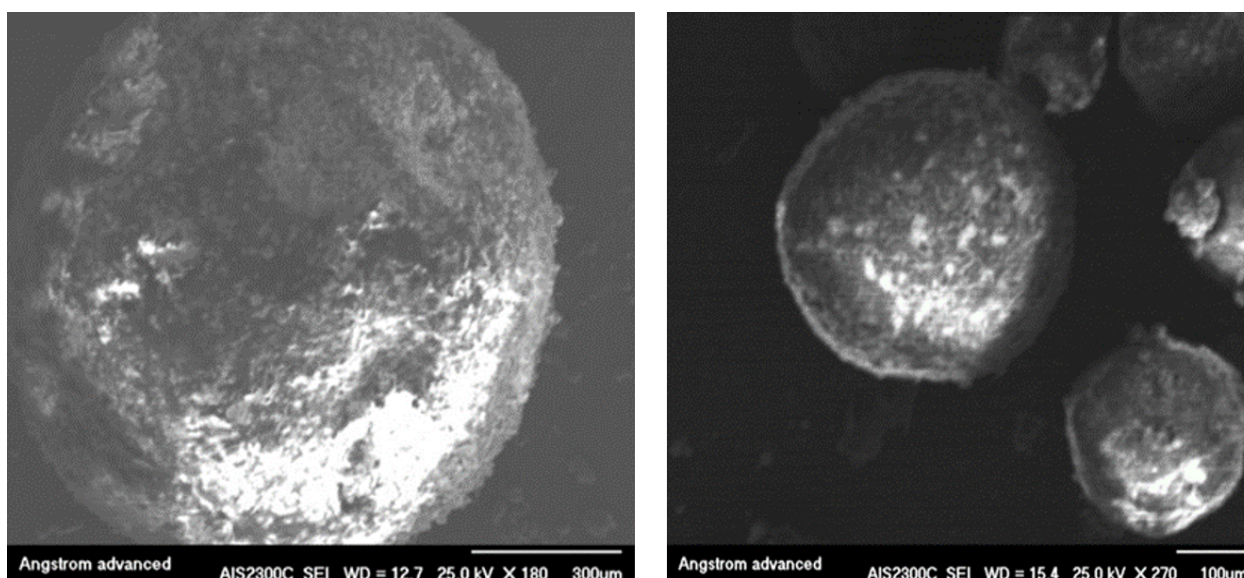
Figure 4: SEM of the selected microsponge formula BF14 at (Left) 310 X magnification and (right) 270 X magnification

Faster and greater drug release was noticed from BF16 than that of BF14 which may be related to higher drug amount compared to the amount of polymer which resulted in more porosity and consequently, more drug release was obtained.

The amount of polymer available per microsponge showed a realistic effect on drug release. So, as the amount of polymer became equal to the amount of drug, increase in the thickness of the polymer matrix was obtained that led to longer diffusion path and ultimately to decreased drug release.

BF14 was determined as the optimum formula because it showed control drug release (75% of drug release in 8 hr.), acceptable PY and LD, so it was subjected to further investigation.

Buoyancy studies

The In-vitro buoyancy test was carried out to investigate the buoyancy of prepared floating microsponges. The BF14 showed the good floating ability of 88.11%.

Evaluation of the shape and surface morphology by scanning electron microscope (SEM)

The SEM result of the selected microsponge formula showed (Figure 4) a spherical nature of the microsponge, uniform size with sufficient pores that loaded with the drug.

Fourier transforms infrared spectroscopy

The spectrum of pure BFN showed characteristic peaks at 1398 cm^{-1} (O-H bending), 1244cm^{-1}(C-O stretching), 831cm^{-1} (C-Cl stretching), which considered as fingerprint of BFN, the FTIR of BFN also showed broad peak at 2590 and extended up to 3100cm^{-1}(O-H of alcohol and carboxylic acid stretching). The spectrum of the physical mixture was as that of the drug, indicating no chemical interaction or complexation. The spectrum of the

selected formula BF14 exhibited similar peaks, no appearance or disappearance of peaks and/or shift of their positions and therefore BFN was apparently stable in the microsponges.

Differential scanning electron microscopy (DSC)

The thermal behavior of pure BFN showed a sharp endothermic peak at 213.24°C corresponding to BFN melting temperature with the onset of the peak at 208.74°C and end set at 217.35°C. This indicates that the drug was in the pure crystalline state.

The thermogram of the physical mixture of BFN and Eudragit RS100 at equal ratio (1:1), and the selected BFN microsponge formula (BF14) exhibited endothermic peaks at 211.84 and 192.94 °C respectively.

CONCLUSION

Microsponges of BFN were successfully formed by the non-aqueous emulsion solvent diffusion method, and the microsponge formulas exhibited excellent floating ability that remained buoyant and extended drug release successfully.

REFERENCES

Abdelkader, H., Abdalla, O. Y., Salem, H. 2007. Formulation of controlled-release baclofen matrix tablets: influence of some hydrophilic polymers on the release rate and in vitro evaluation. *AAPS PharmSciTech*, 8.

Abdellatif, A. A. H., Zayed, G. M., Kamel, H. H., Mohamed, A. G., Arafa, W. M., Khatib, A. M., Sayed, O. M. 2018. A novel controlled release microsponges containing Albendazole against Haemonchus contortus in experimentally infected goats. *Journal of Drug Delivery Science and Technology*, 43:469–476.

Ahmed, A. M., Jwaid, A. H., Younus, M. M., Fawzi, H. A. 2018. Pharmacovigilance study of the Penicillin's adverse drug reactions and their seriousness in the Iraqi hospitals. *International Journal of Research in Pharmaceutical Sciences*, 9:1051–1055.

Amrutiya, N., Bajaj, A., Madan, M. 2009. Development of microsponges for topical delivery of mupirocin. *AAPS PharmSciTech*, 10:402–411.

Charagonda, S., Puligilla, R., Ananthula, M., Bakshi, V. 2016. Formulation and evaluation of famotidine floating microsponges. *Int Res J Pharm*, 7(4):62–69.

Desavathu, M., Pathuri, R., Chunduru, M. 2017. Design, Development and Characterization of Valsartan Microsponges by Quasi Emulsion Technique and the Impact of Stirring Rate on Microsponge Formation. *J Appl Pharm Sci*, 23:62–74.

Dhakar, R. C., Maurya, S. D., Sagar, B. P., Bhagat, S., Prajapati, S. K., Jain, C. P. 2010. Variables Influencing the Drug Entrapment Efficiency of Microspheres: A Pharmaceutical Review . *Der Pharmacia Lettre*, 2:102–116.

Dineshmohan, S., Gupta, V. R. M. 2015. Formulation and In-Vitro Evaluation of Fluconazole Loaded Microsponge Gel For Topical Sustained Delivery. *IOSR Journal of Pharmacy and Biological Sciences*, 10:15–20.

Gangadharappa, H., Biswas, S., Getyala, A., Gupta, V., Kumar, P. 2011. Development, In vitro and In vivo Evaluation of Novel Floating Hollow Microspheres of Rosiglitazone Maleate. *Der Pharmacia Lettre*, 3:299–316.

Gharti, K. P., Budhathoki, U., Thapa, P., Bhargava, A. 2012. Formulation in vitro evaluation of floating tablets of hydroxypropyl methylcellulose and polyethylene oxide using ranitidine hydrochloride as a model drug. *Journal of Young Pharmacists*, 4:201–208.

Jain, V., Singh, R. 2010. Development and characterization of eudragit RS 100 loaded microsponges and its colonic delivery using natural polysaccharides. *Acta Pol Pharm*, 67:407–422.

Jivani, R., Patel, C., Patel, D., Jivani, N. 2010. Development of a Novel Floating In-situ Gelling System for Stomach Specific Drug Delivery of the Narrow Absorption Window Drug Baclofen. *Iranian journal of pharmaceutical research*, 9(4):359–368.

Kaity, S., Maiti, S., Ghosh, A. K., Pal, D., Ghosh, A., Banerjee, S. 2010. Microsponges: A novel strategy for drug delivery system. *J Adv Pharm Technol Res*, 1:283–290.

Maheshwari, R., Sharma, P., Tekade, M., Atneriya, U., Dua, K., Hansbro, P. M., Tekade, R. K. 2017. Microsponge Embedded Tablets for Sustained Delivery of Nifedipine. *Pharm Nanotechnol*, 5:192–202.

Nawal, A. R., Mohammad, S. J. 2016. Formulation and in vitro evaluation of piroxicam microsponge as a tablet. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8:104–118.

Nief, R., Hussein, A. 2014. Preparation and evaluation of meloxicam microsponges as transdermal delivery system. *Iraqi J Pharm Sci*, 23:62–74.

Osmani, R. A. M., Aloorkar, N. H., Ingale, D. J., Kulkarni, P. K., Hani, U., Bhosale, R. R., Dev, D. J. 2015. Microsponges based novel drug delivery system for augmented arthritis therapy. *Saudi Pharma-*

ceutical Journal, 23:562–572.

Othman, M. H., Zayed, G. M., El-Sokkary, G. H., Ali, U. F., Abdellatif, A. A. 2017. Preparation and Evaluation of 5-Fluorouracil Loaded Microsponges for Treatment of Colon Cancer. *Journal of Cancer Science & Therapy*, 9:307–320.

Patel, S., Aundhia, C., Seth, A., Shah, N., Pandya, K., Patel, D., Sheth, H. 2016. Microsponge: a novel approach in gastro-retention drug delivery system (GRDDS). *Indo American Journal of Pharmaceutical Research*, 6:6430–6436.

Resmi, D., Mathew, P., Dev, A. P., Abraham, E. 2018. Formulation and Evaluation of Topical Econazole Nitrate Microsponge Loaded Hydrogel. *International Journal of Pharmacognosy and Phytochemical Research*, 12:28–64.

Ridhima, D., Shweta, P., Upendra, K. 2012. Formulation and evaluation of floating microspheres of Curcumin. *Int J Pharm Pharm Sci*, 4:334–340.

Rizkalla, C. M. Z., Aziz, R. L., Soliman, I. 2011. In vitro and in vivo evaluation of hydroxyzine hydrochloride microsponges for topical delivery. *AAPS PharmSciTech*, 12(989):989–1001.

Sanjivani, A., Swapnila, S., Shalaka, D., Uddhav, B., Jagdish, S. 2011. Development and evaluation of hollow microspheres of clarithromycin as a gastroretentive drug delivery system using eudragit polymers. *Int J Pharma Bio Sci*, 2:344–58.

Trivedi, R., Borkar, J., Taksande, J., Awandekar, N., Wadher, K., Umekar, M. 2017. Development and characterization of stomach specific mucoadhesive drug delivery system of baclofen. *Int J Res Pharm Sci*, 8:608–623.