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Improvement of Rebamipide Solubility via optimized Microsponge formulation

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Abstract
Rebamipide (REB) is a novel gastroprotective anti-ulcer drug; is used for mucosal protection and treatment of gastritis. It works by enhancing mucosal defense works by enhancing endogenous prostaglandin synthesis. REB possesses low aqueous solubility and poor oral absorption; listed class IV in biopharmaceutics classification system. The aim of this study is to improve pharmaceutical properties of REB including solubility by preparation of microsponge. Microsponges are tiny sponge-like spherical polymeric delivery systems composed of porous microspheres with a large surface area. Twelve formulas of REB microsponges were prepared by the quasi-emulsion solvent diffusion method using Eudragit L100 as main polymer and glycerol as plasticizer dissolved in ethanol while polyvinyl alcohol as a stabilizer in the external phase. The prepared formulas were utilized to optimize preparation variable include; a different drug to polymer ratio, stirring rate, stirring time, and internal phase volume. The products were subjected to evaluation regarding saturated solubility, FT-IR, DSC, and SEM. The results show that the best drug: polymer ratio was 10:1, and the best stirring rate was 500rpm for 1h regarding the optimum properties of microsponge and solubility enhancement. Also, the best volume of internal phase was 5mL. The selected formula prepared (F7) shows 4.62 folds enhancement of solubility compared to pure REB. It can be concluded that the optimized formula of REB microsponge is promising drug delivery with improved pharmaceutical properties.

INTRODUCTION

Microsponges are at the forefront of the rapidly developing field of a unique novel drug delivery technology [1]. In the beginning, it is mainly used for topical administration but now a day as per recent information according to many researchers to applying microsponge in oral administration and biopharmaceutical drug delivery [2].

Microsponges are polymeric delivery systems composed of porous microspheres [3]. They are tiny sponge-like spherical particles with a large porous surface [4]. This technology has many favorable characteristics which make it a versatile drug delivery vehicle [5].

Now a day solubility of the drug is the critical factor that controls its formulation development, as well as its therapeutic efficacy [6]. Microsponge delivery system enhances the rate of solubilization of poorly water-soluble drugs by entrapping such drugs in microsponge pores [7]. Dissolution rates of the drugs are related to the shape as well as the particle size. Therefore, decrease in particle size by micronization of such drugs result in an increase in dissolution rate [8, 9].

Rebamipide [2-[(4-chlorobenzoyl)amino]-3-(2-oxo-1H-quinolin-4-yl) propanoic acid] [10] (REB) is a novel anti-ulcer drug; works by enhancing endogenous prostaglandin synthesis, scavenging oxygen-derived free radicals, and decreasing neutrophil activity and inflammation process [11]. REB possesses low aqueous solubility and poor oral absorption; listed class IV in biopharmaceutics classification system [12].

The aim of the present research was to design microsponges as an innovative carrier for a practical water insoluble drug (REB) in order to enhance the solubility and the dissolution of REB. This study consisted of preparation, optimization, and evaluation of REB microsponges.

MATERIALS

Rebamipide powder was purchased from Shijiazhuang aopharm medical technology Co. Ltd. China, Eudragit L100 powder was obtained from Barlocher-GMBH-Germany and polyvinyl alcohol (PVA) from Barcelona Espana. All other materials used in this study were of analytical grade.

METHODS

Preparation of Rebamipide microsponges

The microsponges enclosing REB were fabricated by quasi-emulsion solvent diffusion method, using an organic internal phase comprising of Eudragit L100 and glycerol dissolved in ethanol. Glycerol was added to improve the plasticity of the polymer. Further REB was added to the solution and dissolved through ultrasonication at 35 °C for 15 minutes. Followed by dropwise addition of this mixture into an aqueous solution of PVA (external phase) with stirring for 0.5, 1, or 2 h at room temperature. Subsequently, microsponges were formed due to ethanol removal from the system by evaporation. Prepared microsponges then filtered, washed with distilled water and subjected to drying at 40 °C for 12 hs and stored for further investigation [13, 14, 15]. Various microsponge formulations were prepared as per Table (1).

Evaluation of REB Microsponges

Saturation Solubility Studies

The saturation solubility studies were carried out for both unprocessed pure drug and different formulas of microsponge. Excess amounts of REB or microsponge formulas were dispersed in 5 ml of HCl buffer (pH 1.2). The suspensions were sonicated for 15 min and shaken at constant temperature (37 ± 0.5 °C) in a water bath. After 72 hs; a time which was sufficient to attain equilibrium, the suspensions were filtered through a 0.45µm cellulose membrane filter and appropriately diluted with HCl buffer (pH 1.2) [16]. The amount of dissolved REB was quantified by UV spectroscopy at 227nm [17].

Fourier Transform Infrared (FTIR) analysis

FTIR spectra of the pure REB, Eudragit L100, the physical mixture of REB: Eudragit L100 at 1:1 ratio and selected microsponge formula were obtained to determine the compatibility between the REB and excipients. All samples were grounded and mixed thoroughly with potassium bromide and, the analysis was done using FTIR – 8300 Shimadzu, Japan. The spectrum obtained was in between the wave number of 4000-400 cm^{-1} [18].

Differential scanning calorimetric (DSC) analysis

DSC can be used to determine the compatibility between REB and excipients, and can also use to evaluate the crystalline state of RIB especially in the prepared formula. Thermal analysis using DSC were carried out on the same samples used in FTIR by using DSC-60 plus Shimadzu, Japan. Accurately weighed samples (5mg) were loaded in non-hermetically aluminum pans and heated to the rate of 10 °C/min against an empty aluminum pan as a reference covering a temperature range of 40-350 °C in an atmosphere of nitrogen [19].

Table (1): The composition of Rebamipide microsponge formulas

Formula No.	Internal phase					External phase		Stirring rate (rpm)	Stirring time (h)
	Drug: Polymer ratio	Drug (gm)	Eudragit L100 (gm)	Ethanol (ml)	Glycerol (ml)	Water (ml)	PVA (gm)		
F1	1:1	0.1	0.1	5	1	100	0.1	750	1
F2	2:1	0.2	0.1	5	1	100	0.1	750	1
F3	3:1	0.3	0.1	5	1	100	0.1	750	1
F4	5:1	0.5	0.1	5	1	100	0.1	750	1
F5	10:1	1	0.1	5	1	100	0.1	750	1
F6	15:1	1.5	0.1	5	1	100	0.1	750	1
F7	**10:1**	**1**	**0.1**	**5**	**1**	**100**	**0.1**	**500**	**1**
F8	10:1	1	0.1	5	1	100	0.1	1000	1
F9	10:1	1	0.1	5	1	100	0.1	500	0.5
F10	10:1	1	0.1	5	1	100	0.1	500	2
F11	10:1	1	0.1	10	1	100	0.1	500	1
F12	10:1	1	0.1	15	1	100	0.1	500	1

Scanning electron microscope (SEM) study

SEM was used to study the morphology and surface topography of the selected microsponge formula. SEM was confirmed by coated the sample with gold-palladium under an argon atmosphere at room temperature. The samples were visualized using a scanning electron microscope (VEGA3 Tescan Czech republic, UK) [20].

RESULT AND DISCUSSION

Quasi-emulsion solvent diffusion method was used because of its feasibility, ease of preparation and reproducibility. Microsponges with desired morphology were obtained with this method [21].

The formation of porous surface results due to the rabid diffusion of ethanol into the aqueous medium might reduce the solubility of the polymer in the droplets. The instant mixing of ethanol and water at the interface of the droplets induced precipitation of the polymer, thus encapsulating shell has formed as the polymer is insoluble in water. The finely dispersed droplets of the drug-polymer solution were solidified in the aqueous phase via diffusion of the solvent [22]. The saturation solubility of the pure REB in HCl buffer (pH 1.2) was 0.327µg/ml.

Effect of the drug to polymer ratio on the saturated solubility of REB microsponges formulations

The drug: polymer ratio has a considerable effect on the saturated solubility of microsponges as shown in the table (2) and figure (1). It was observed that increasing the drug to polymer ratio increased the saturation solubility of REB in HCl (pH 1.2). It was observed that as the drug to polymer ratio was increased, the particle size decreased. At higher drug to polymer ratio (formula F6), the amount of polymer available per microsponge was comparatively lower [23, 24]. Therefore, the most suitable formula F5 was picked out as the selected formula for further studies.

Table (2): Effect of the drug to polymer ratio on the saturated solubility of REB microsponge formulations

Formula No.	Solubility (µg/ml)	Folds of solubility enhancement
F1 (1:1)	0.238	0.73
F2 (2:1)	0.301	0.92
F3 (3:1)	0.382	1.17
F4 (5:1)	0.585	1.79
F5 (10:1)	**1.281**	**3.92**
F6 (15:1)	1.202	3.67

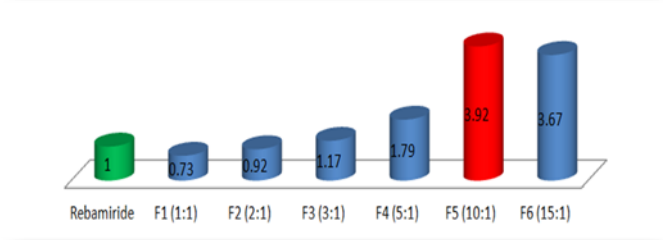

Figure (1): Effect of the Drug to Polymer ratio on the Saturated Solubility of REB Microsponge Formulations

Effect of stirring rate on the saturated solubility of REB microsponge formulations

The dispersion of the drug and polymer into aqueous phase has found to be dependent on the agitation speed. Therefore, the stirring rate had a significant effect on the saturated solubility of microsponges as shown in the table (3) and figure (2).

It was indicated that at the higher stirring rate, the saturation solubility of REB in HCl buffer (pH 1.2) was decreased. This could probably be due to the fact that the higher the rate of stirring the higher the early solvent drug for diffusion and accordingly the agglomeration of microsponge. It was hypothesized that with the increase in speed, the precipitation of polymer solution droplets gradually became slower, allowing more time for agglomeration [14, 25]. Therefore, the most suitable formula F7 was picked out as the selected formula for further studies.

Table (3): Effect of the stirring rate on the saturated solubility of REB microsponge formulations

Formula No.	Solubility (µg/ml)	Folds of solubility enhancement
F5 (750rpm)	1.281	3.92
F7 (500rpm)	**1.513**	**4.62**
F8 (1000rpm)	1.131	3.46

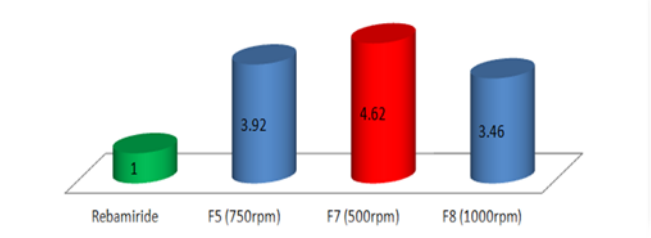

Figure (2): Effect of the Stirring Rate on the Saturated Solubility of REB Microsponge Formulations

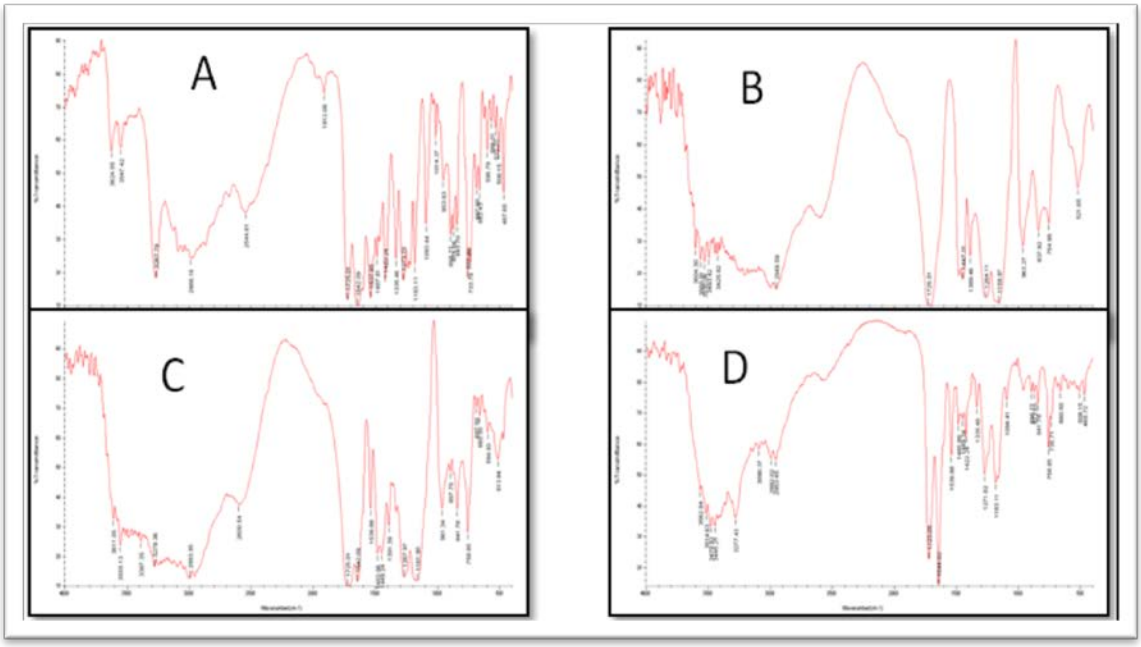

Figure (5): FTIR Spectrum of A) Pure REB, B) Eudragit L100, C) Physical Mixture D) Selected REB Microsponges Formula F7

Effect of stirring time on the saturated solubility of REB microsponge formulations

The stirring time has a considerable effect on the saturated solubility of microsponges as shown in the table (4) and figure (3). One hour stirring was appropriate for the preparation and additional stirring time has no significant effect on the saturated solubility of microsponges. In this respect, the optimum stirring time was selected as one hour [26]. Therefore, the most suitable formula F7 was picked out as the selected formula for further studies.

Table (4): Effect of the stirring time on the saturated solubility of REB microsponge formulations

Formula No.	Solubility (µg/ml)	Folds of solubility enhancement
F7 (1hr)	**1.513**	**4.62**
F9 (0.5hr)	1.357	4.14
F10 (2hr)	1.502	4.59

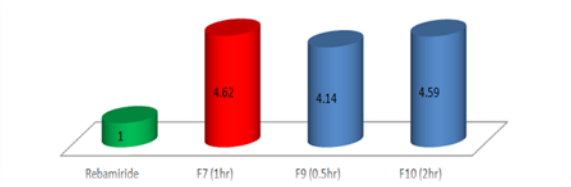

Figure (3): Effect of the Stirring Time on the Saturated Solubility of REB Microsponge Formulations

Effect of internal phase volume on the saturated solubility of REB microsponge formulations

An increment in solvent volume (ethanol) was lead to decrease the saturation solubility of REB in HCl (pH 1.2) as shown in the table (5) and figure (4).

Further enhancement in the volume of the inner phase as in (F9 and F10), failure to form microsponges was observed, this may be related to the deficient elimination of the inner phase solvent with the production globules that could not consolidate as most of the inner phase stay in it. So the inner phase must be used in its optimum volume (5 ml) to ensure the production of quasi-emulsion globules, and consolidation of the remedy and polymer thereafter [27, 28]. From all these studies, F7 considered as the optimized REB microsponges formula.

Table (5): Effect of the internal phase volume on the saturated solubility of REB microsponge formulations

Formula No.	Solubility (µg/ml)	Folds of solubility enhancement
F7 (5ml)	**1.513**	**4.62**
F9 (10ml)	1.379	4.22
F10 (15ml)	1.298	3.97

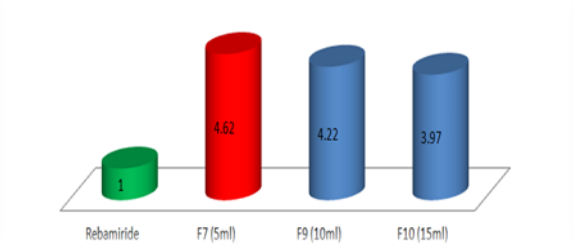

Figure (4): Effect of the Internal Phase Volume on the Saturated Solubility of REB Microsponge Formulations

Fourier Transform Infrared (FTIR) analysis

FTIR spectroscopic study revealed that there was no appearance of any new peak or disappearance of characteristic peaks of the drug, which indicated that there was no chemical interaction between the drug and polymer used [29]. The FTIR spectrum of pure REB, Eudragit L100, physical mixtures, and the selected microsponge formula F7 are given in figure (5). FTIR spectroscopic study revealed that their FTIR spectrum of the pure drug showed characteristic sharp peaks of amines stretching (N–H and CH_2) vibration at 3420.32 –3379.48 cm^{-1} and alkane stretching (–CH_3,–CH_2 and –CH) vibration at 2938.73 cm^{-1}. Also exhibited C = O stretch at 1740.2 cm^{-1} due to aldehydes and C=O – NH stretching at 1650.90 cm^{-1}. A selective stretching vibration at 1580.57 cm^{-1} and 1525.80 cm^{-1} for primary and secondary amine were observed also [30].

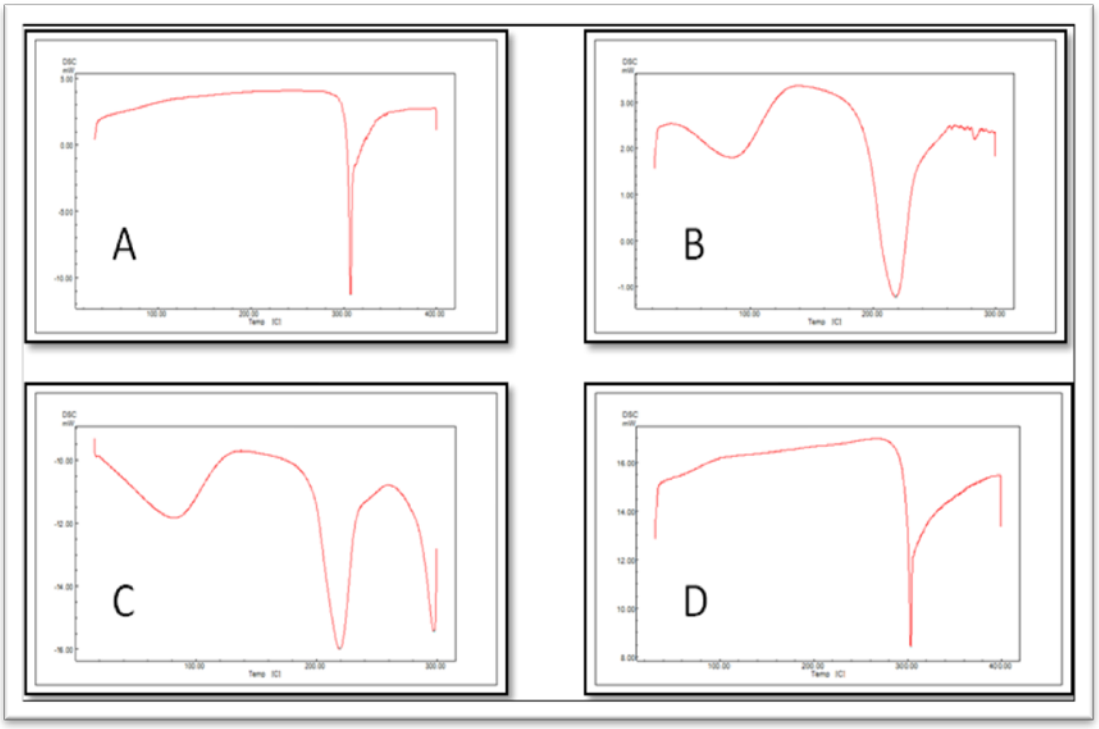

Figure (6): DSC of A) Pure REB, B) Eudragit L100, C) Physical Mixture, D) Selected REB Microsponges Formula F7

Hence IR spectroscopy results showed that the drug was compatible with selected polymer and excipients. This suggested that REB was compatible with selected polymers and it was apparently stable in the microsponges.

Differential scanning calorimetric (DSC) analysis

The DSC curve figure (6) of REB presented a sharp characteristic endothermic melting peak at 305 °C, in accordance with the literature. The thermogram of the physical mixture showed that the drug was in its crystalline form, and there was no interaction between them. The DSC curve of formula F7 showed only typical signal for the drug crystals. The disappearance of polymer peaks mainly due to the lower amount of polymer used in the preparation of F7 in comparison to the amount of REB, in addition to the amorphous nature of the polymer. Such results showed no interaction between REB and polymers, indicating that microsponge production process used for the preparation of REB microsponges did not change the nature of the drug in microsponges [30, 31].

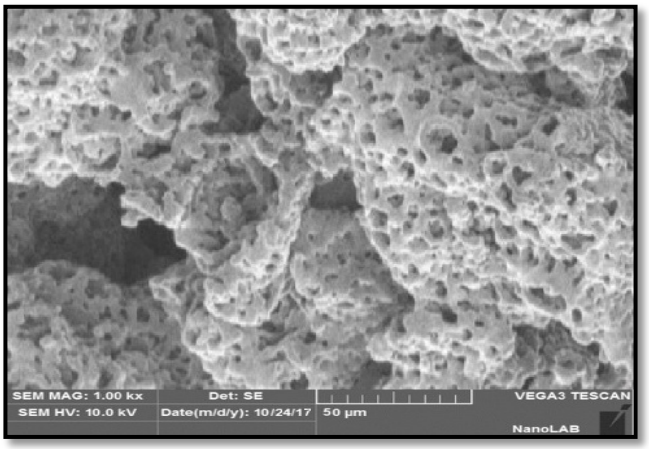

Figure (7): SEM for Selected REB Microsponges Formula F7

Scanning electron microscope (SEM) study

SEM picture of the selected formula F7 is presented in figure (6) at 1000X magnification. It was observed that the microsponges were fine, smooth, porous structure. The surface topography reveals that REB microsponges contained tiny pores. The pores were induced by diffusion of the volatile solvent (ethanol) from the surface of the microparticles [26, 32].

CONCLUSIONS

From the results of this study; it can be concluded that the microsponges formulation have enhanced the saturated solubility of Rebamipide in HCL significantly and the optimal drug to polymer ratio that can give higher increment in solubility among the used ratios was 10:1, the stirring rate 500 rpm, the stirring time by one hour and the internal phase volume of 5 ml. In addition, the FTIR and DSC of optimized formula (F7) show stability of drug during formulation, which gives consideration in that the microsponge could be a promising carrier in enhancement of solubility of insoluble drugs.

REFERENCES

1. Sandhyarani R. Sagavkar, Dr. Shrinivas K Mohite. INNOVATIVE AND NOVEL STRATEGY: MICROSPONGES DRUG DELIVERY SYSTEM. International Journal of Universal Pharmacy and Bio Sciences ISSN: 2319-8141 3(4):2014.
2. Pavankumar Ade, Pravin Bhalerao, Arshiya Khan, S.R Shahi, Imran Tadwee. Microsponge Drug Delivery System: A Review. IJPDerma ISSN: 2456-9070 Volume 1, Issue 1, 35-44, 2017.
3. Dharmendra Solanki, Sandeep Patidar, Nikesh Kag, Mohit Motiwale, Lalit Kushwah and Akhilesh Mewade. An Overview of Microsponge as a Novel Tool in Drug Delivery. ISSN: 2349-7203 Volume 9, Issue 2, 2017.
4. Desai, Siddhi; Talele, Ajay N. A REVIEW ON MICROSPONGE DRUG DELIVERY SYSTEM: AN INNOVATIVE APPROACH. Pharma Science Monitor. Volume 9 Issue 1, p598-609, 2018.
5. Saroj kumar Pradhan. Microsponges as the versatile tool for drug delivery system. International Journal of Research in Pharmacy and Chemistry 1(2):243-258, 2011.
6. Ashwini Pravin Gowardhane, Nilesh Vishnu Kadam and Saptrishi Dutta. Review on Enhancement of Solubilization Process. American Journal of Drug Discovery and Development, Volume 4 (2): 134-152, 2014.

7. Rahul Shivaji Patil, Vishnu Uddhav Kemkar, S. S. Patil. Microsponge Drug Delivery System: A Novel Dosage Form. American J. PharmTech Res. ISSN: 2249-3387; 2(4), 2012.
8. Mitra Mosharraf and Christer Nyström. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. International Journal of Pharmaceutics 122(s 1–2):35–47, 1995.
9. PrakashKhadka, JieunRo, HyeongminKim, IksooKim, Jeong TaeKim, HyunilKim, Jae MinCho, GyiaeYun, JaehwiLee. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian Journal of Pharmaceutical Sciences, Volume 9, Issue 6, Pages 304-316, 2014.
10. Mohan H Kudur and Manjunath Hulmani. Rebamipide: A Novel Agent in the Treatment of Recurrent Aphthous Ulcer and Behcet's Syndrome. Indian J Dermatol. 58(5): 352–354, 2013.
11. Kazushi Sakurai, Hiroyuki Sasabe, Toshihisa Koga and Hiroyuki Sasabe. Mechanism of Hydroxyl Radical Scavenging by Rebamipide: Identification of Mono-hydroxylated Rebamipide as a Major Reaction Product. Free Radical Research 38(5):487-94, 2004.
12. Nguyen-Thach Tung, Chun-Woong Park, Tack-oon Oh, Ju-Young Kim, Jung-Myung Ha, Yun-Seok Rhee, Eun-Seok Park. Formulation of solid dispersion of rebamipide evaluated in a rat model for improved bioavailability and efficacy. JPP 2042-7185, 2011.
13. Parikh B.N., Gothi G.D., Patel T.D., Chavda H.V., and Patel C.N., Microsponge as Novel Topical Drug Delivery System. Journal of Global Pharma Technology 2(1):17-29, 2010.
14. Madhuri Desavathu, Raghuveer Pathuri, Mounika Chunduru. Design, Development and Characterization of Valsartan Microsponges by Quasi Emulsion Technique and the Impact of Stirring Rate on Microsponge Formation. Journal of Applied Pharmaceutical Science, ISSN: 2231-3354, Volume 7 (01), pp. 193-198, 2017.
15. Saboji, J. K., Manvi, F. V., Gadad, A. P., Patel, B. D. Formulation and evaluation of ketoconazole microsponge gel by quassi emulsion solvent diffusion. Journal of cell and tissue research, 11(1): 2691-2696, 2011.
16. Edit Baka. Development and examination of solubility measurement methods for drug solubility determination. Thesis of PhD, Semmelweis University, Doctoral School of Pharmaceutical and Pharmacological Sciences, 2010.
17. Praveen K. Srivastava, Archana Roy. Development and Validation of a New Uv Method for the Analysis of Rebamipide. International Journal of PharmTech Research, ISSN: 0974-4304, Vol.3, No.3, pp1270-1274, 2011.
18. Vikas Jain, Ranjit Singh. Development and characterization of Eudragit Rs 100 loaded microsponges and its colonic delivery using natural polysaccharides. Acta poloniae pharmaceutica-drug research. 67(4): 407-415, 2010.
19. Bodmeier R, Chen H. Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen, and ketoprofen. J Control Release, 10:167-175, 1989.
20. Mohan kumar V, Veena N M, Manjula B P. Formulation and evaluation of microsponges for topical drug delivery of mupirocin. International Journal of Pharm Tech Research. 5(3): 1434-1440, 2013.
21. V. V. Pande, N. A. Kadnor, Rupali N. Kadam, and S. A. Upadhye. Fabrication and Characterization of Sertaconazole Nitrate Microsponge as a Topical Drug Delivery System. Indian J Pharm Sci. 77(6): 675–680, 2015.
22. R´e, M.I., Biscans, B. Preparation of microspheres of ketoprofen with acrylic polymers by a quasi-emulsion solvent diffusion method. Powder Technol. 101: 120–133, 1999.
23. Nokhodchi A, Jelvehgari M, Siahi MR, Mozafari MR. Factors affecting the morphology of benzoyl peroxide microsponges. Micron. 38(8): 834-840, 2007.
24. Priyanka S. Pagar, A. D. Savkare. FORMULATION AND EVALUATION OF OMEPRAZOLE MICROSPHERES BY DIFFERENT TECHNIQUES. Indo American Journal of Pharmaceutical Research, ISSN: 2231-6876, 2017.
25. Nivedita M., Formulation and Evaluation of Diacere in Loaded Microsponges in Capsule. Master's thesis, College of Pharmacy Madras Medical College, Chennai. 2016.
26. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. Int J Pharm. 318: 103–117, 2007.
27. Sareen R, Nath K, Jain N, Dhar KL, Curcumin loaded microsponges for colon targeting in inflammatory bowel disease: Fabrication, optimization, and in vitro and pharmacodynamic evaluation, BioMed research international, 2014.
28. Yang MS, You BG, Fan YL, Wang L, Yue P, Yang H, Preparation of sustained-release nitrendipine microspheres with Eudragit RS and Aerosil using quasiemulsion solvent diffusion method, International journal of pharmaceutics, 259, 103-113, 2003.
29. Riyaz Ali M.Osmani Nagesh H.Aloorkar Dipti J. Ingale Parthasarathi K. Kulkarni UmmeHani Rohit R. Bhosale DandasiJayachandra Dev. Microsponges based novel drug delivery system for augmented arthritis therapy. Saudi Pharmaceutical Journal, Volume 23, Issue 5, Pages 562-572, 2015.
30. P. Kanteepan and D.V.R.N. Bhikshapathi. DESIGN AND EVALUATION OF MICROSPHERES LOADED WITH REBAMIPIDE. IAJPS 05 (02), 952-961, ISSN: 2349-7750, 2018.
31. Sonali S. Bharate, Sandip B. Bharate and Amrita N. Bajaj. Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review. J. Excipients and Food Chem. 1 (3):1-26, 2010.
32. Crotts G, Park TG, Preparation of porous and nonporous biodegradable polymeric hollow microspheres; Journal of Controlled Release, 35, 91-105, 1995.