FABAD J. Pharm. Sci., 36, 93-100, 2011

RESEARCH ARTICLE

Investigation of the Parameters Influencing the Drug Release from Lipid Microspheres Prepared by Congealable Hydrophobic Disperse Phase Encapsulation Method

Eda GÖKBULUT*, Nurten ÖZDEMIR*°

Investigation of the Parameters Influencing the Drug Release from Lipid Microspheres Prepared by Congealable Hydrophobic Disperse Phase Encapsulation Method

Summary

The aim of this study was to prepare ibuprofen microspheres by congealable hydrophobic disperse phase encapsulation method using hydrogenated cotton seed oil (HCSO) as the lipid material. With in vitro dissolution studies, the drug release influenced by the amount of lipid material, the amount and types of dispersing agents, water and oil soluble auxiliary substances were investigated. Static dissolution method was applied by using pH 7.2 phosphate buffer as medium. Different dissolution models were applied to drug release data in order to evaluate release mechanisms and kinetics. According to in vitro dissolution studies, using different molecular weights of polyvinyl alcohol (PVA) as dispersing agent had no significant effect on the drug release, besides, using polyethylene glycoles (PEGs) as water soluble, glyceryl monostearate (GMS), stearyl alcohol (SAL) and stearic acid (SAS) as oil soluble auxiliary substances increased drug release. In order to enhance the solubility and drug release, solid dispersions of β-cyclodextrin (1:1) were prepared but contrarily the drug release decreased.

Key Words: *Lipid microsphere, Ibuprofen, Controlled release, Congealable hydrophobic disperse phase encapsulation*

Received:19.11.2012
Revised:11.12.2012
Accepted:13.12.2012

Dondurulabilir Hidrofobik Dispers Faz Enkapsülasyonu Yöntemi ile Hazırlanan Lipid Mikrokürelerden Etkin Madde Çıkışını Etkileyen Parametrelerin İncelenmesi

Özet

Bu çalışmada lipid materyal olarak hidrojene pamuk tohumu yağı (HPTY) kullanılarak dondurulabilir hidrofobik dispers faz enkapsülasyonu yöntemi ile ibuprofen mikrokürelerin hazırlanması amaçlanmıştır. Formüllere ilave edilen lipid materyal miktarının, farklı tip ve miktarlarda kullanılan dispersiyon ajanlarının, suda ve yağda çözünen yardımcı madde tip ve miktarlarının etkin madde açığa çıkışı üzerine etkileri, çözünme hızı tayinleri yapılarak incelenmiştir. Bu amaçla, çözünme ortamı olarak pH 7,2 fosfat tamponu seçilerek statik çözünme hızı yöntemi kullanılmıştır. Elde edilen veriler değişik kinetiklere uyumları yönünden incelenmiştir.

In vitro çözünme hızı çalışmalarına göre, dispersiyon ajanı olarak kullanılan polivinil alkolün (PVA) farklı molekül ağırlıklarının kullanımının çıkışı değiştirmediği, suda çözünen yardımcı ajanlardan polietilen glikollerin (PEG), yağda çözünen yardımcı maddelerden gliseril mono stearat (GMS), stearil alkol (SAL) ve stearik asitin (SAS) ilavesi ile etkin madde çıkışının arttığı bulunmuştur. Etkin madde çözünürlüğünü ve formüllerden açığa çıkışını artırmak amacıyla etkin maddenin β-siklodekstrin ile 1:1 molar oranda katı dispersiyonları hazırlanmış ancak beklenenin aksine çıkışın azaldığı saptanmıştır.

Anahtar Kelimeler: *Lipid mikroküre, İbuprofen, Kontrollü salım, Dondurulabilir hidrofobik dispers faz enkapsülasyonu*

* Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Turkey
° Corresponding Author E-mail: nozdemir@pharmacy.ankara.edu.tr

INTRODUCTION

One of the most significant advantages of dosage forms which allow for controlled release is that they have the optimum therapeutic impact while minimizing the side effects. In particular, it is pointed out that it is possible to eliminate the gastrointestinal (GI) side effects observed in long-term oral use of anti-inflammatory drugs by preparing multiparticular dosage forms providing controlled release (1,2). Ibuprofen, is an anti-inflammatory agent with analgesic-antipyretic effect, the major side effect of which is GI irritation (3,4). In the present study, the aim was to prepare the multiparticular dosage form of this substance which has longer effect in lower doses and which has minimized GI side effects by preparing its controlled release microspheres. It is possible to use various methods and polymers to prepare microspheres (5-8). In this study, hydrogenated cotton seed oil, which has a lipid structure, was used in the formulations, and they were prepared by congealable hydrophobic disperse phase encapsulation technique, for it does not require the use of organic phase. A major advantage of this technique is that the drug melted with the lipid material undergoes homogeneous dispersion in the matrix structure without showing recrystallization behavior (9,10).

MATERIAL AND METHODS

Materials

Hydrogenated cotton seed oil (HCSO) was obtained from Henkel, glyceryl monostearate (GMS) from BDH Chemicals, stearic acid (SAS), stearyl alcohol (SAL), polyethylene glycols (PEG) from Merck, polyvinyl alcohols (PVA) and β-cyclodextrine (β-CD) from Sigma, polyvinyl pyrrolidone K30 (PVP K30) from ICN Biomedicals, and ibuprofen (IB) from Atabay Inc.

Preparation of Microspheres

The formulations were prepared by congealable hydrophobic disperse phase encapsulation method. To this end, HCSO was melted in 73-75 ºC, and the drug was dispersed in this oil phase. The aqueous solution of dispersing agent was prepared and heated up to approximately 5 ºC above the melting point of the oil phase. The oil phase heated up to approximately 10 ºC above the melting point was added to the water phase stirred at 1100 rpm (Stir-pak, model 50002-35, Cole Parmer Instruments Co., USA) to obtain o/w emulsion. The emulsion was stirred for an additional 5 minutes, and in the meantime, distilled water at +4 ºC was added by the emulsion volume so that the inner phase of the emulsion hardened and microsphere development was achieved. The microsphere suspension was poured onto +4 ºC distilled water of twice the total volume which was stirred at 500 rpm and stirred for another 10 minutes. The microspheres obtained were filtered, washed with cold water for three times, and dried in a 37 ºC incubator over night.

To investigate the alteration of the drug release from the lipid microspheres, oil and water soluble auxiliary substances were used. Oil soluble auxiliary substances (GMS, SAL and SAS) were added directly to the oil phase, while the water soluble ones (PEGs) were first melted and mixed with drug prior to addition to oil phase. In addition, solid dispersions were prepared by means of 1:1 drug-β-CD molar ratio with kneading technique. For this purpose, drug-β-CD mixture was dissolved in 50% ethanol and dried at 37 ºC for 24 h. A series of formulations were prepared by also using these solid dispersions with the method indicated above.

Determination of Drug Substance Content of Microspheres

Theoretically, microsphere containing 30 mg of drug was weighed, and dissolved by adding ethanol and applying heat. The solution was filtered, and a spectrophotometric quantification was carried out at 263.5 nm.

In vitro release studies

The microspheres prepared were separated into 250-500 µm particular weight fractions to be used in dissolution rate studies. Data concerning dissolution rate was obtained by using static method (11,12). 100 mL phosphate buffer (pH 7.2), the lowest volume meeting the sink condition, was used as the dissolution medium. Microspheres placed in cellophane membrane were exposed to dissolution rate tests at 37 ± 0.5 ºC for 3 days. The data on

dissolution rate was analyzed in terms of zero-order, first-order and Q→t kinetics. All studies were performed in triplicate.

RESULTS AND DISCUSSION

Preformulation studies

First, preformulation studies were conducted to determine the type and amount of the dispersing agent, drug-lipid ratio, and stirring rate to be used in the study. During the formation of initial emulsion in lipid microsphere formulations, inner phase droplets were quite viscose and liable to flocculation. This could be prevented by adding dispersing agent suitable for the emulsion medium and applying the ideal stirring rate (13,14). For this purpose, PVP K30 with 2.5%, 5%, 7.5% and 10% w/v ratios was used as the dispersing agent to prepare the formulations. An investigation of the prepared formulations in terms of microsphere formations, particle sizes and drug loading ratios revealed that 7.5% w/v PVP K30 was the optimum ratio. When formulations were prepared for the same purpose by using PVA's of varying molecular weights (mol. Wt. 30000-700000, 70000-100000) and 7.5% w/v ratio (FPVA1, FPVA2), no significant difference between the drug releases was identified (Figure 1). 7.5% w/v PVP K30 was used as the dispersing agent, and hollow microspheres were prepared by using such different stirring rates 800 rpm, 1100 rpm, 1500 rpm and 1800 rpm. The results demonstrated that particle sizes of microspheres prepared at 1100, 1500 and 1800 rpm did not differ significantly (64 µm), whereas the particle size of those prepared at 800 rpm increased (120 µm). Therefore, the consequent formulations were prepared using the stirring rate, 1100 rpm. Optical microscopic analysis of the formulations with drug-lipid ratios of 1:1, 1:2, 1:3, 1:4, 1:5 revealed that microsphere formation did not occur in 1:1, 1:2 drug-lipid ratios. On the other hand, it was observed that using polymer at such high ratios as 1:4, 1:5 results in particle expansion (81.3 µm). Since the 1:3 (64 µm) ratio was identified as the ratio that yields smaller particle sizes, it was also identified as the lowest ratio by which microsphere was obtained. For this reason, and also for the high efficacy achieved (99%), the drug-lipid ratio to be used in the following studies was decided to be 1:3 (FEM 1:3). Table 1 shows the formulations prepared in the study.

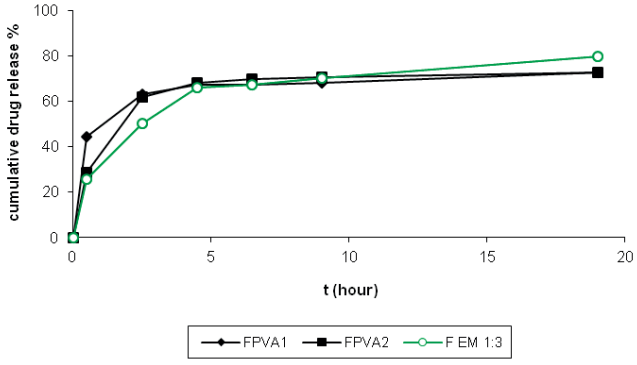

Figure 1. Effect of dispersing agents with varying molecular weights on drug release

Drug release studies

Effect of water soluble auxiliary substances on drug release

Analyzing the kinetics of drug release from FEM 1:3, FEM 1:4, FEM 1:5 formulations containing only drug and polymer, zero-order kinetics were expected because of the low water solubility of the drug. By contrast, higher tendency to first-order kinetics was observed which indicates that drug release is a diffusion controlled release (Table 2). Based on this data, PEGs, which have the channel forming function, can increase diffusion and act as water soluble auxiliary agents, were added to the formulations (15). To this end, PEGs with different molecular weights (4000, 6000, 10000 and 20000) and different ratios (10%, 20%, 30%) were used. Drug release from formulations containing PEG demonstrated significant increase only when compared with the formulations containing polymer (FEM 1:3) (Figure 2-5). It was observed that the higher the PEG percentages in the formulations, the higher the drug releases were. PEG ratios by percentages in PEG 4000-containing formulations were summarized graphically together with dissolution rate constants of Q→t kinetics, which show the greatest kinetic (k) consistence, and a linear relationship was observed (Figure 6). However, it was found out that the increase in drug release correlates negatively with the increase in PEG molecular weights in formulations. As can be seen in Figure 7, as the molecular weights

Table 1. Composition of microsphere formulations.

Formulations	IB (mg)	HCSO (mg)	PEG 4000 (mg)	PEG 6000 (mg)	PEG 10000 (mg)	PEG 20000 (mg)	βCD (mg)	GMS (mg)	SAL (mg)	SAS (mg)	PVP*	PVA**
FEM 1:3	1250	3750									15	
FEM 1:4	1000	4000									15	
FEM 1:5	833	4167									15	
FPVA1	1250	3750										15
FPVA2	1250	3750										15
FPEG41	1250	3350	400								15	
FPEG42	1250	2950	800								15	
FPEG43	1250	2550	1200								15	
FPEG61	1250	3350		400							15	
FPEG62	1250	2950		800							15	
FPEG63	1250	2550		1200							15	
FPEG11	1250	3350			400						15	
FPEG12	1250	2950			800						15	
FPEG13	1250	2550			1200						15	
FPEG21	1250	3350				400					15	
FPEG22	1250	2950				800					15	
FPEG23	1250	2550				1200					15	
FEM/CD1:3	1250	3750					230				15	
FEM/CD1:4	1250	5000					230				15	
FEM/CD1:5	1250	6250					230				15	
FGMS40	1250	3350						400			15	
FGMS80	1250	2950						800			15	
FGMS120	1250	2550						1200			15	
FSAL40	1250	3350							400		15	
FSAL80	1250	2950							800		15	
FSAL120	1250	2550							1200		15	
FSAS40	1250	3350								400	15	
FSAS80	1250	2950								800	15	
FSAS120	1250	2550								1200	15	

* : The amount of PVP K30 in 200 mL distilled water (g)
**: The amount of PVA in 200 mL distilled water (g)

Table 2. Data on kinetics relating to formulations

Formulations	0 Order		1. Order		Q→t	
	k_0 (mg/sa)	r^2	k_r (sa^{-1})	r^2	k (mg/sa)	r^2
FEM 1:3	1.40	0.783	0.073	0.964	11.0	0.904
FEM 1:4	2.33	0.807	0.151	0.984	11.6	0.853
FEM 1:5	2.10	0.739	0.162	0.989	10.6	0.910
FPVA1	0.823	0.532	0.023	0.648	5.62	0.717
FPVA2	0.934	0.400	0.022	0.521	7.10	0.579
FPEG41	7.64	0.933	0.262	0.988	23.4	0.850
FPEG42	12.9	0.934	1.04	0.867	38.1	0.982
FPEG43	27.7	0.994	1.01	0.959	58.4	0.988
FPEG61	6.10	0.914	0.140	0.970	20.8	0.975
FPEG62	9.30	0.945	0.246	0.987	31.5	0.986
FPEG63	10.8	0.965	0.285	0.992	36.3	0.991
FPEG11	4.72	0.939	0.112	0.969	15.9	0.980
FPEG12	10.3	0.967	0.251	0.990	32.2	0.990
FPEG13	12.5	0.979	0.573	0.879	39.0	0.996
FPEG21	4.42	0.931	0.071	0.957	15.0	0.981
FPEG22	5.70	0.912	0.162	0.962	19.5	0.973
FPEG23	4.42	0.986	0.102	0.985	14.6	0.986
FEM/CD1:3	1.53	0.825	0.053	0.933	11.5	0.938
FEM/CD1:4	1.02	0.694	0.054	0.908	9.48	0.856
FEM/CD1:5	1.50	0.836	0.101	0.988	12.6	0.720
FGMS40	1.57	0.749	0.062	0.928	11.6	0.892
FGMS80	2.45	0.556	0.095	0.808	15.3	0.776
FGMS120	9.85	0.833	0.763	0.916	34.0	0.934
FSAL40	1.14	0.751	0.073	0.861	9.51	0.892
FSAL80	1.60	0.483	0.082	0.761	10.1	0.689
FSAL120	5.64	0.573	0.574	0.907	24.1	0.746
FSAS40	1.71	0.485	0.069	0.639	10.9	0.712
FSAS80	1.90	0.358	0.079	0.535	9.98	0.635
FSAS120	4.03	0.579	0.504	0.935	17.0	0.750

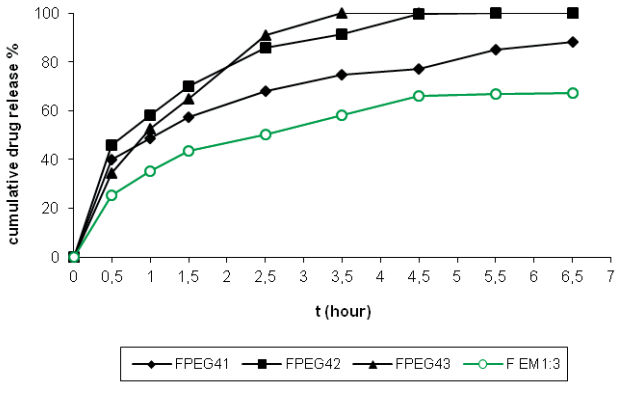

Figure 2. Drug release from formulations prepared by 10, 20 and 30% PEG 4000 additions

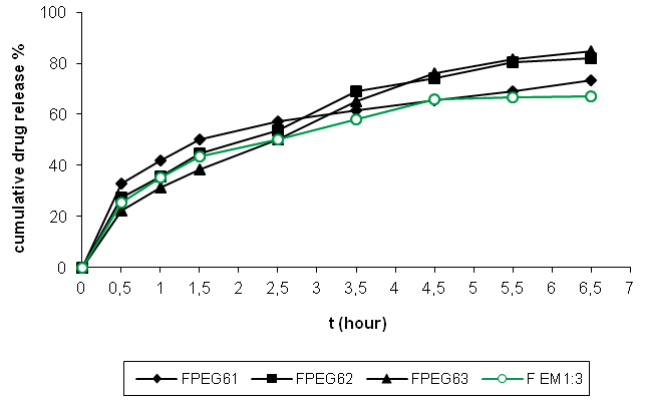

Figure 3. Drug release from formulations prepared by 10, 20 and 30% PEG 6000 additions

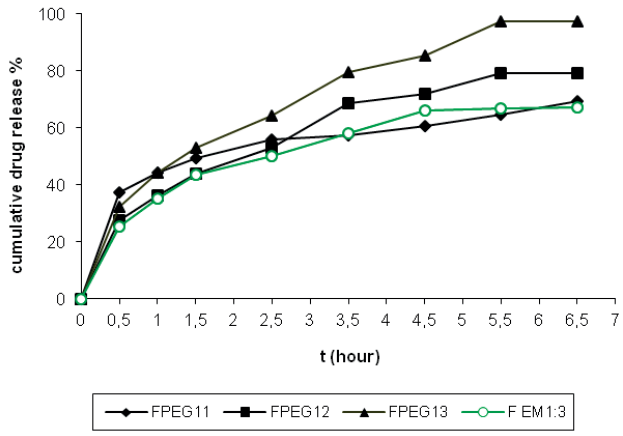

Figure 4. Drug release from formulations prepared by 10, 20 and 30% PEG 10000 additions

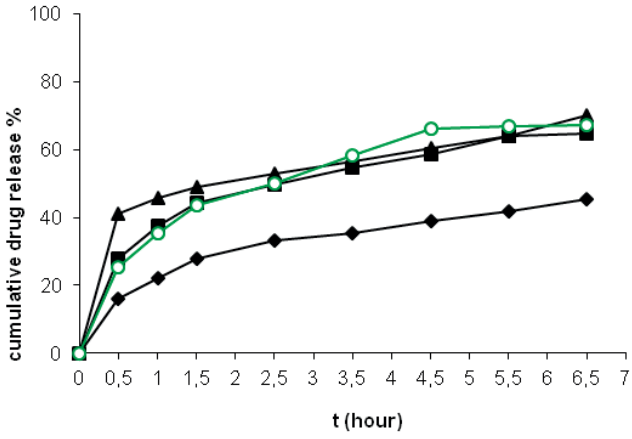

Figure 5. Drug release from formulations prepared by 10, 20 and 30% PEG 20000 additions

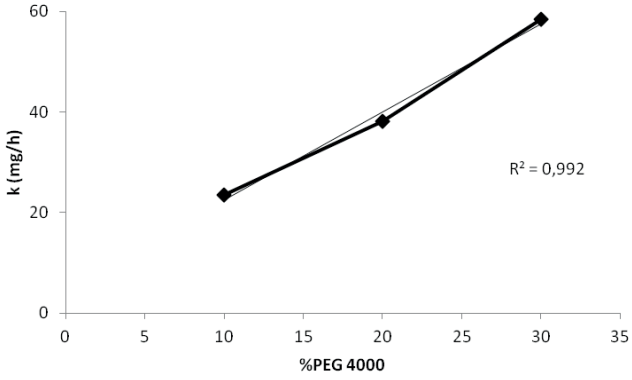

Figure 6. PEG 4000 amounts in the formulations and relation between Q→t kinetics and release rate constants

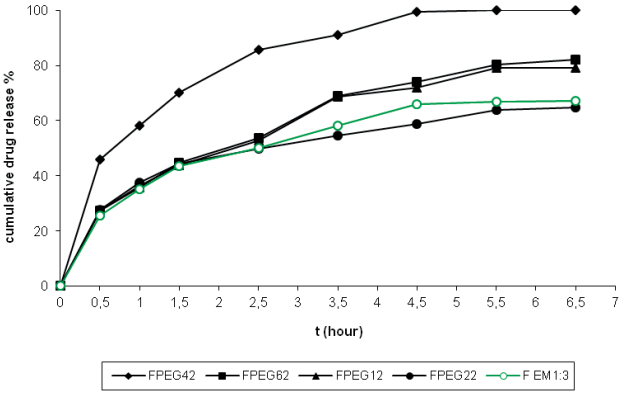

Figure 7. Effect of PEG molecular weight on drug release

of PEGs added to the formulations by 20% with varying molecular weights increased, drug release was observed to significantly decrease from FPEG42 (the one containing PEG 4000) towards FPEG22 (the one containing PEG 20000). It was observed that drug release follows Q→t kinetics, and the data collected showed that water soluble PEGs with low molecular weights lead to the formation of canaliculus and the diffusion from matrix material, as well as diffusion from these canaliculus, increased drug release. In addition, because PEGs were mixed with the drug substance before being added to the formulations, it was presumed that some solid dispersion occurred in the structure, which might have increased the drug solubility (16). From this point of view, β-CD solid dispersions of drug were prepared to increase drug solubility, thus drug release (17,18). Solid dispersion formations were identified by means of DSC analysis and IR measurements. In addition to the solid dispersions prepared, FEM/CD 1:3, FEM/CD 1:4, FEM/CD 1:5 formulations were prepared with drug:β-CD ratios 1:3, 1:4, 1:5, respectively. Drug release from formulations did not demonstrate any increase within the first 6 hours, whereas increase was observed in long-term release (Figure 8 a,b). The reason for this is presumed to be the enlargement of β-CD and molecular structure, the decrease in the diffusion behavior of the drug, and the decrease of the drug solubility, which was 748.4 mg/mL in the lipid material, to 692.3 mg/mL in the drug-β-CD complex. Nevertheless, an analysis of long term dissolution rate data (Figure 8b) revealed a link between increase in drug release to some degree, and drug dissolution and release from capillary that develops due to β-CD's absorption of water by the structure (19).

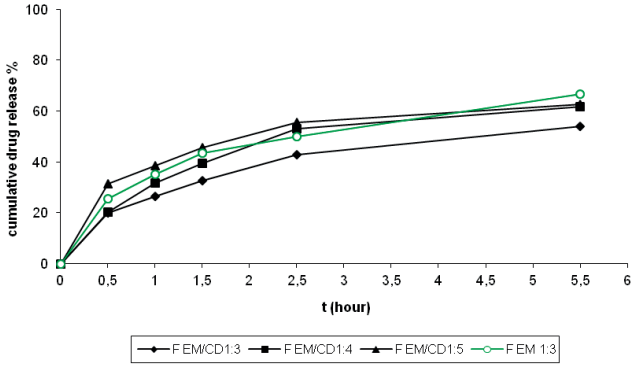

Figure 8a. Release profiles of formulations containing drug/β-CD solid dispersions within 6 hours

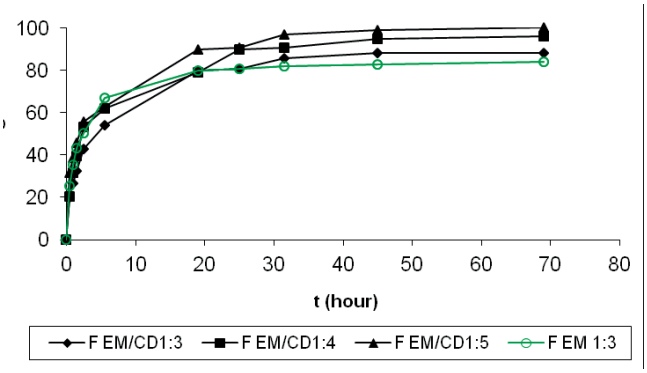

Figure 8b. Release profiles of formulations containing drug/β-CD solid dispersions within 70 hours

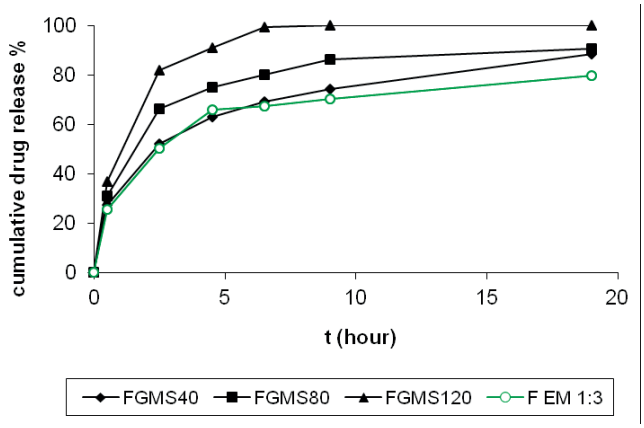

Figure 9. Drug release from formulations prepared by 10%, 20% and 30% GMS addition

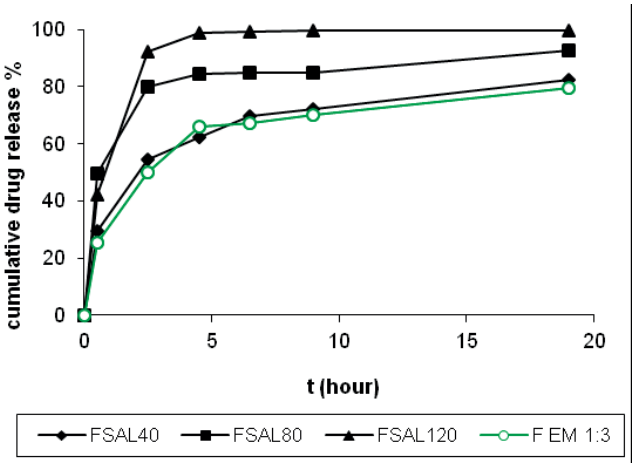

Figure 10. Drug release from formulations prepared by 10%, 20% and 30% SAL addition

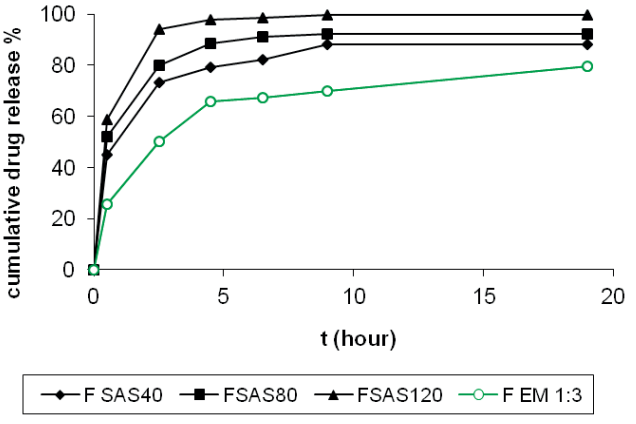

Figure 11. Drug release from formulations prepared by 10%, 20% and 30% SAS addition

Effect of oil soluble auxiliary substance on drug release

As can be seen in Figure 9-11, all oil soluble excipients increased drug release in proportion with their increasing amounts in the formulations, and the highest increase took place with GMS addition. These oil soluble substances which increased drug diffusion behavior in lipid materials have also water absorption capability, thus, when compared with the standard formulation FEM 1:3, significantly increased drug release (Figure 9-11) (20). An analysis of drug release from these preparations as to their consistency to various kinetics pointed to the existence of Q→t kinetics, which explains release from matrix through diffusion (Table 2).

CONCLUSIONS

In conclusion, the results of the study indicate the following; for oil soluble drugs, congealable hydrophobic disperse phase encapsulation method is the optimum method in lipid matrix material use and microsphere preparation to obtain congealable microspheres with high loading efficiency (90%-100%); it is appropriate to use lipophilic matrix

materials in making preparations of drugs with low water solubility that produce controlled release, the solubility of drug in water will not be effective during release; diffusion controlled release could be obtained rather than partition controlled release; the release rate and mechanism can be altered by changing the type and amount of auxiliary substances; water soluble auxiliary substances are more effective than oil soluble auxiliary substances in increasing drug release from such systems.

REFERENCES

1. Garbacz G, Klein S. Dissolution testing of oral modified-release dosage forms. *J Pharm Pharmacol* 64: 944-968, 2012.
2. Halliday AJ, Moulton SE, Wallace GG, Cook MJ. Novel methods of antiepileptic drug delivery-polymer-based implants. *Adv Drug Deliver Rev* 64: 953-964, 2012.
3. Sachs CJ. Oral analgesics for acute nonspecific pain. *Am Fam Physician* 71: 913-918, 2005.
4. Argoff CE. Recent developments in the treatment of osteoarthritis with NSAIDs. *Curr Med Res Opin* 27: 1315-1327, 2011.
5. Dalmoro A, Barba AA, Lamberti G, D'amore M. Intensifying the microencapsulation process: Ultrasonic atomization as an innovative approach. *Eur J Pharm Biopharm* 80: 471-477, 2012.
6. Wei Y-M, Zhao L. In vitro and in vivo evaluation of ranitidine hydrochloride loaded hollow microspheres in rabbits. *Arch Pharm Res* 31: 1369-1377, 2008.
7. Dixit M, Kini AG, Kulkarni PK. Enhancing solubility and dissolution of celecoxib by spray drying using Pluronic F 127. *Indian J Pharm Educ* 45: 346-352, 2011.
8. Ma N, Xu L, Wang Q, Zhang X, Zhang W, Li Y, Jin L, Li S. Development and evaluation of new sustained-release floating microspheres. *Int J Pharm* 358: 82-90, 2008.
9. Adeyeye CM, Price JC. Development and evaluation of sustained-release ibuprofen-wax microspheres. I. effect of formulation variables on physical characteristics. *Pharm Res* 8: 1377-1383, 1991.
10. Yüksel N, Aydınlı A, Özalp Y, Özdemir N. Evaluation and preparation of controlled release lipid microspheres of sulphamethizole by a congeable dispersee phase encapsulation method. *Acta Pol Pharm-Drug Res* 57: 187-192, 2000.
11. Benita S. Microencapsulation methods and industrial applications. Marcell Dekker, Inc., 1996.
12. Yan C, Resau JH, Hewetson J, West M, Rill WL, Kende M. Characterization and morphological analysis of protein-loaded poly(lactide-co-glycolide) microparticles prepared by water-in-oil-in-water emulsion technique. *J Cont Rel* 32: 231-241, 1994.
13. Vilivalam VD, Adeyeye CM. Development and evaluation of controlled-release diclofenac microspheres and tabletted microspheres. *J Microencapsul* 11: 455-470, 1994.
14. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. *Eur J Pharm Biopharm* 50: 47-60, 2000.
15. Piao MG, Kim J-H, Kim JO, Lyoo WS, Lee MH, Yong CS, Choi H-G. Enhanced oral bioavailability of piroxicam in rats by hyaluronate microspheres. *Drug Dev Ind Pharm* 33: 485-491, 2007.
16. Phuong H-LT, Thao T-DT, Park JB, Lee BJ. Controlled release systems containing solid dispersions: strategies and mechanisms. *Pharm Res* 28: 2353-2378, 2011.
17. Özdemir N, Erkin J. Enhancement of dissolution rate and bioavailability of sulfamethoxazole by complexation with *β*-cyclodextrin. *Drug Dev Ind Pharm* 38: 331–340, 2012.
18. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: An updated review. *AAPS Pharm Sci Tech* 6: 329-350, 2005.
19. Loftsson T, Duchene D. Cyclodextrins and their pharmaceutical applications *Int J Pharm* 329: 1-11, 2007.
20. Adeyeye CM, Price JC. Development and evaluation of sustained-release ibuprofen-wax microspheres. 2. in-vitro dissolution. *Pharm Res* 11: 575-579, 1994.