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Preparation and *In- vitro* Evaluation of Baclofen as an Oral Microsponge Tablets

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Abstract

Baclofen (BFN) is a skeletal muscle relaxant used for the treatment of spastic muscle disorder; BFN suffers from short plasma half-life and narrow absorption window. The aim of the present study was to formulate floating effervescent microsponge tablets of BFN for controlling drug release and thereby decrease the side effects of the drug. The microsponges of BFN were prepared by non-aqueous emulsion solvent diffusion method (oil in oil emulsion 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 were characterized for production yield (PY), loading efficiency (LD), particle size, surface morphology, and *in vitro* drug release. The selected microsponge formula was incorporated into the floating effervescent gastro-retentive tablets. The prepared floating microsponge tablets were evaluated for their hardness, friability, swelling in addition to *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 hr.) when compared with other formulas and pure BFN. Therefore, the non-aqueous emulsion solvent diffusion method is a promising method to produce baclofen microsponges.

Keywords: Baclofen, Microsponge, Floating Tablet .

تحضيروتقييم خارج الجسم للحبوب المايكروأسفنجية الفموية للباكلوفين

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الخلاصه

الباكلوفين هو دواء مرخي للعضلات ويعاني الباكلوفين من نصف عمر قليل في البلازما ومن نافذة امتصاص ضيقة. الهدف من هذه الدراسة هو صياغة قرص أسفنجي ميكروي عائم لعقار الباكلوفين للتحكم في إطلاق الدواء وبالتالي تقليل التأثيرات الجانبية للدواء. أعدت الاسفنجيات الميكروية بواسطة طريقة نشر المذيب المستحلب الغير مائي (طريقة مستحلب زيت في زيت). تم بحث تأثير نسبة الدواء: البوليمر ، زمن التحريك ونوع البوليمر يودراجيت على الخصائص الفيزيائية و تم توصيف الاسفنجيات الميكروية المحضرة من حيث الانتاجية ، وكفاءة التحميل ، وحجم الجسيمات ، والشكل السطحي ، وتحرير الدواء خارج الجسم. وقد تم دمج الاسفنجيات المايكروية المختارة على شكل حبوب فوارة عائمة محتجزة في الجهاز الهضمي. تم تقييم الحبوب المحضرة من حيث الصلابة, الهشاشة بالاضافة الى تحرير الدواء خارج الجسم .أظهرت النتائج أن تركيبة الاسفنجيات الميكروية مع يودراجيت آر أس ١٠٠ كانت لها خواص فيزيائية مثالية وانتاجية عالية تساوي ٩٧% وكفاءة تحميل للعلاج تساوي ٨١% وتحكم في اطلاق الدواء (٧٥٪ من إطلاق الدواء في ٨ ساعات) بالمقارنة مع الصيغ الأخرى والباكلوفين النقي.لذلك يمكن الاستنتاج ان طريقة نشر المذيب المستحلب غير المائي هي طريقة واعدة لانتاج الاسفنجيات الميكروية.

الكلمات المفتاحية : الباكلوفين ، الاسفنجيات الميكروية ، حبوب عائمة .

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 [(1)]. Gastroretentive 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 gastrointestinal tract [(2)]

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There are two types to design a floating-dose model. These are single-unit systems and multiple unit systems [3]. One of the new ways of a gastro-retentive dosage form is the floating microsponge. It significantly increases the residence time of medication in stomach, improve bioavailability, improve patient compliance by reducing frequency doses, reduce drug waste [4]. 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 [5]. BFN belongs to class III according to biopharmaceutical classification system, has a pKa1 = 3.85; pKa2 = 9.25, molecular weight is equal 213.7 g/mol and log p is equal to (-1) [6]. The solubility of BFN decreases with increase pH, having maximum solubility at pH 1.2 which equal to 26mg/ml. It has short plasma half-life which is about 2–4 hr. [7]. Baclofen has a narrow absorption window in the small intestine because on arrival to colon the absorption becomes low or nonexistent [8]. It is stable and well absorbed within pH range 1-4 [9].

The primary objective of the present investigation was to develop and optimize the BFN microsponge formula that to control the release rate of the drug and subsequent evaluation of different variables affecting it, then selection of the optimum microsponge formula to be incorporated into gastroretentive effervescent floating tablets to determine the effect of varying polymer type and concentration on the release profile of the tablets.

Materials and Methods

Materials

Baclofen (Hyperchem company, China), Eudragit polymers RS100 and RL100 (Rhom pharma, Germany), liquid paraffin (Solvochem company, United Kingdom), n-hexane (Chem-lab, Belgium), carbopol 934 (Himedia, India), sodium bicarbonate and citric acid (AGC chemicals, Japan). All other materials used in this study were of analytical grade.

Methods

Determination of BFN λmax

A diluted solution of BFN in 0.1 N (pH 1.2) was prepared. The solution was scanned by UV visible spectrophotometer from 200-400 nm, and the λmax of the drug was detected.

BFN calibration curve

A stock solution (0.1mg/1ml) was prepared by dissolving 10 mg of drug in 100 ml of 0.1N HCl (pH 1.2), then preparing serial dilution of different concentration of (0.002, 0.004, 0.006, 0.008, 0.01, 0.013, 0.015 and 0.017 mg /ml) from stock solution. The absorbance was then measured at the λmax of the drug. The measured absorbance plotted against the concentrations.

Preparation of BFN microsponges

The BFN microsponge formulas were prepared by oil in oil emulsion solvent diffusion technique. The internal phase consisted of polymer that was dissolved in organic solvent. Once a clear solution was obtained, BFN was added gradually to the internal phase with addition of magnesium stearate and the whole mixture was kept 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 of magnetic stirrer. The oil in oil emulsion formed was stirred for 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 [10]. The microsponge formulas are illustrated in table 1.

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 [11].

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

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 (%) [12].

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

Particle size measurement

The particle size of microsponge was determined using 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 [13].

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

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

Table (1) Formulas of BFN microsponges

Formula no. / Ingredient	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8	BF9	BF10	BF11	BF12	BF13	BF14	BF15	BF16	BF17
Baclofen (g.)	0.5	1	1.5	0.5	1	1.5	2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	1
Eudragit RS100(g.)	0.5	0.5	0.5	/	/	/	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Eudragit RL100(g.)	/	/	/	0.5	0.5	0.5	/	/	/	/	/	/	/	/	/	/	/
Acetone (ml)	5	5	5	5	5	5	5	/	/	5	5	5	5	7.5	10	7.5	10
Ethanol (ml)	/	/	/	/	/	/	/	5	/	/	/	/	/	/	/	/	/
Dichloromethane (ml)	/	/	/	/	/	/	/	/	5	/	/	/	/	/	/	/	/
Paraffin (ml)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Magnesium Stearate (w/v %)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Stirring rate (rpm)	1500	1500	1500	1500	1500	1500	1500	1500	1500	1000	500	1500	1500	1500	1500	1500	1500
Rotation time (hr.)	1	1	1	1	1	1	1	1	1	1	1	0.5	2	1	1	1	1

Scanning electron microscope (SEM) study

The surfaces morphology of 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 [14].

In-vitro drug release studies of microsponge formulations

Baclofen microsponge formulas were subjected to an *in-vitro* drug release study by using dissolution testing apparatus type II (paddle). The dissolution test was carried out utilizing 900 ml of 0.1N HCl (pH1.2). A specified weight of microsponge corresponding to 15 mg of BFN was taken and by using semipermeable membrane rotated at 50rpm at 37 $\pm$ 0.5 ^{0}C. A sample of 5 ml was collected every hour for 10hrs 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 0.45µm filter syringe .The absorbance of the filtrate was measured by a UV spectrophotometer at the corresponding λ_{max} of BFN [15,16]. 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 [17].

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. Samples were submitted to DSC analysis using a differential scanning calorimeter (Schimadzu, model TA-50 WSI, Kyoto, Japan). Thermogram obtained at a scanning rate of 10°C/min using a dry nitrogen flow of 25 ml/min. Each sample was scanned between 0°C and 400°C [18].

Powder x-ray diffraction (PXRD) analysis

The X-ray diffractometry was used to study the molecular structure of crystalline chemicals such as BFN and additives. Samples were submitted to x-ray analysis using XRD-6000 (Shimadzu, Japan). The results were recorded over a range of 10–80°(2θ). The operating conditions were: voltage 40 kV, current 30 mA, scanning speed 1/min., and this test was done for drug, physical mixture of drug with polymer and for selected formula [19].

Formulation of BFN floating microsponge tablets by effervescent technique

Different formulas of BFN microsponge floating tablets were prepared as shown in table 2. Direct compression method was used to prepare tablets. Homogeneously mixing of previously weighed components (BFN microsponge equivalent to 15mg BFN, polymer, gas generating agent sodium bicarbonate with citric acid and diluent) in a mortar for 15 minute to obtain powder blend, this blend was mixed with a calculated amount of magnesium stearate and talc powder for 3 minutes [20] and compressed by using Erweka tablet machine to get a tablet with total weight of 200 mg.

Table (2) Composition of BFN floating effervescent microsponge tablets

Formula no. / Ingredients (mg)	FMT1	FMT2	FMT3	FMT4	FMT5	FMT6	FMT7
The selected microsponge formula	*	*	*	*	*	*	*
Carbopol 934P				60	40	60	80
Sodium carboxy methylcellulose (NaCMC)			60				
Sodium alginate		60					
HPMC E5	60						
PVP K30	5	5	5	5	5	5	5
Sodium bicarbonate	35	35	35	35	35	20	35
Citric acid	15	15	15	15	15	15	15
Lactose fine powder	38	38	38	38	58	53	18
Talc	5	5	5	5	5	5	5
Magnesium stearate	5	5	5	5	5	5	5
Total tablet weight	200	200	200	200	200	200	200

(*): amount of the microsponge equivalent to 15mg BFN

Evaluation of the prepared floating microsponge tablets

The standard evaluation of the prepared tablets was done according to USP specification [21].

Weight variation

Weight variation was done by weighed individually twenty tablets and the average weight was calculated. Then each individual tablet was compared to the average, it should no more than two tablets differed from average weight. Then measure a percentage of deviation by using equation:

$$\%\ \boldsymbol{Deviation} = \frac{(\boldsymbol{Individual\ weight} - \boldsymbol{Average\ weight})}{\boldsymbol{Average\ weight}} \boldsymbol{x}\ \mathbf{100}$$

Hardness test

Three tablets of each batch were selected for measuring the force required to break the tablet by using YD1 hardness (Beijing, China) tester in which the scale of the tester was adjusted to zero and the tablet placed diametrically and then gradually increase of the load until the tablet break. The hardness was expressed as a force in kg/cm^2.

Friability

The friability test was done for all batches using CS-2 Friabilator (Tianjin Guoming Medicinal, China) for 4 min at 25 rpm by weighing 20 tablets all together then placing them inside the tester. After their revolution, they were de-dusted and weight again. The friability was estimated as the percent weight loss and calculated using equation, Friability value should be not more than 1%.

$$\boldsymbol{F}\ \% = \frac{(\boldsymbol{W\ initial} - \boldsymbol{W\ final})}{\boldsymbol{W\ initial}} \boldsymbol{x}\ \mathbf{100}$$

Where, F % = percentage of friability, W initial=weight of tablet before the revolution, W final= weight of tablets after revolution.

Drug content

Five tablets were weighed individually, then placed in a mortar and powdered. An amount equivalent to 15 mg drug was extracted with 100 ml of 0.1N HCl (pH 1.2) and sonicated for 15 minutes. The solution was filtered and properly diluted with 0.1 N HCl (pH 1.2), and then the drug content was measured by using UV-visible spectrophotometer at λmax of drug [7].

In-Vitro Buoyance Studies

Buoyancy study was done by placing the tablet in 100 ml beaker of 0.1N HCl and determine the time needed for tablet to float (FLT) and the total time that remained buoyant (TFT), the method was described by Rosa et al [22].

Swelling index

The swelling index was done by putting previously weighed tablet in a beaker containing 0.1N HCl and the tablet removed at predetermined time interval (after 1, 2, 3 , 4, 5 and 6 hr.), the excess water removed by tissue and re-weighed [23]. The swelling index is calculated by the equation

$$\boldsymbol{Swelling\ index} = \frac{(\boldsymbol{Weight\ of\ wet\ tablet} - \boldsymbol{Weight\ of\ dry\ tablet})}{\boldsymbol{Weight\ of\ dry\ tablet}} \times \mathbf{100}$$

In-vitro dissolution test

The procedure for dissolution was the same as that mentioned for the drug release study from microsponge.

In-vitro drug release kinetic studies

The data obtained from in vitro dissolution profile of BFN floating microsponge tablets versus time were plotted at different mathematical expressions to describe the kinetic and mechanism of release. The kinetic models used were zero order kinetic (percentage drug release vs. time), first order kinetic (Log percentage drug release vs. time), Higuchi model (percentage drug release vs. √T), Hixon-Crowell model (cube root of percentage drug release vs. time), and Korsmeyer - Peppas (Log of percentage drug release vs. log time). The best fitted model was that with the highest correlation coefficient.

Statistical analysis

The results of the experiments were given as a mean of three samples ± 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 Discussions

Determination of BFN λmax

Baclofen solution in 0.1 N HCl (pH 1.2) gave the highest peak of absorbance at 220 nm as shown in figure 1; this result was with an agreement with that previously recorded [24] [25].

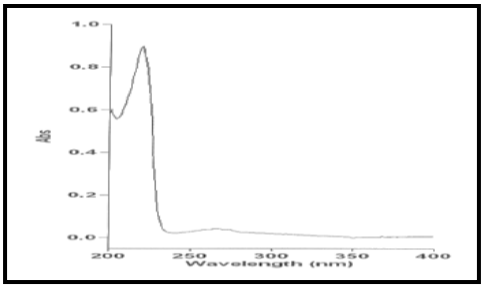


Figure (1) UV spectrum of BFN in 0.1 N HCl (pH 1.2)

Calibration curve of BFN

Calibration curve of BFN in 0.1 N HCl (pH 1.2) is shown in figure 2. A straight line was obtained with R^2 value (0.998) indicated that calibration curve obeys Beers law within the used concentrations.

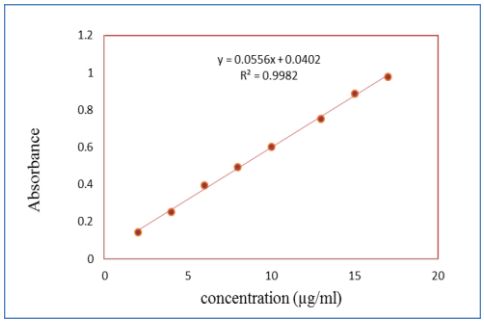


Figure (2) Calibration curve of BFN in 0.1 N HCl (pH 1.2) at λmax 220 nm

Preparation of BFN microsponges

Baclofen loaded microsponges were prepared by oil in oil emulsion solvent diffusion method in which high viscosity paraffin employed as external phase with either one of acrylic polymer (Eudragit RL100 or Eudragit RS100), these polymers are water-insoluble polymers have been shown to be pH independent which are suitable for the sustained release applications [26]. The use of paraffin which is non-polar as external phase instead of water to prevent the escape of hydrophilic drug into the external phase and provide better encapsulation efficiency for these drugs [27]. BFN has poor lipid solubility. Eudragit RS100, RL100 are very slightly soluble in liquid paraffin [28]. Microsponges will be formed by the diffusion of solvent into the external phase, the instant mixing of the solvent and paraffin at the interface of the droplets induced precipitation of the polymer, thus forming a shell encloses the solvent and the drug, the evaporation of solvent lead to the formation of pores.

Effect of drug: polymer ratio on the microsponges

The drug-polymer ratio had considerable effect on the nature of microsponges as shown in table 3. 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.

Table (3) 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

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 [29].

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 [30]. It was detected that formulation of microsponge using Eudragit RS100 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 [31]. Statistically this increment in the PY, LD and mean particle size with 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 increasing volume of acetone to 10ml [32].

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 increase the LD [33] associated with increasing the volume of internal phase solvent from 5 ml to 7.5 ml [34]. 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 in to the external paraffin phase. The effect of acetone volume on the particle size was shown in figure 3.

The increase solvent volume from 5 ml to 7.5 ml showed significant effect on LD and mean particle size as p value <0.05 when using the one-way ANOVA.

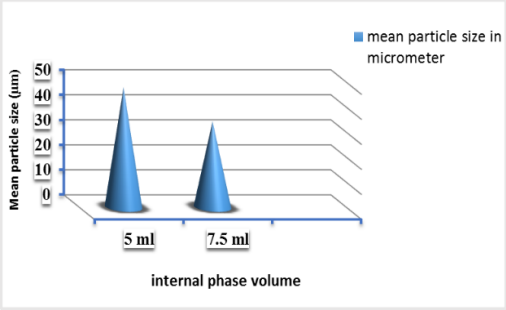


Figure (2) Histogram showing effect of internal phase volume on mean particle size

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 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.[35].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 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 4. 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, at 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. stirring duration was associated with lower PY% and LD%. This finding was similar to previously reported by Roaa et al [36] Increase stirring duration from 0.5 hr. to 1 hr. produced significant effect on PY% and LD% as P value < 0.05 when use the one-way ANOVA test.

Table (4) Effect of stirring duration on physical prosperities of microsponges

formula	drug: polymer ratio	type of polymer	acetone(ml)	paraffin(ml)	stirring speed(rpm)	stirring duration(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**

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 external paraffin medium, resulted in slow precipitation of polymer matrix, and subsequent separation of a microsphere with a spongy structure [31]. Microsponge preparation by using acetone gave higher PY%, LD% than that of ethanol as illustrated in figure 3.

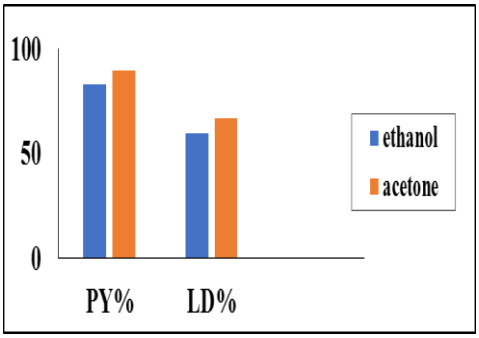


Figure (3) Effect of solvent type on physical properties of microsponges

***In- vitro* drug release study of microsponges**

Dissolution was done for BF14, BF16, and pure BFN as illustrated in figure 4. 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 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.

BF14was 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.

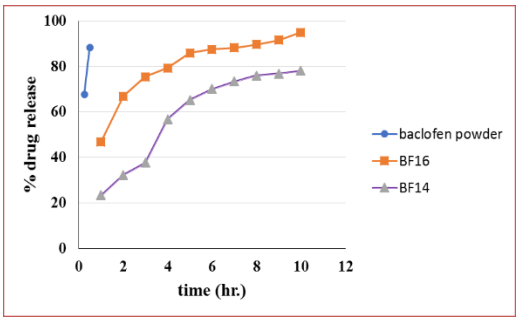
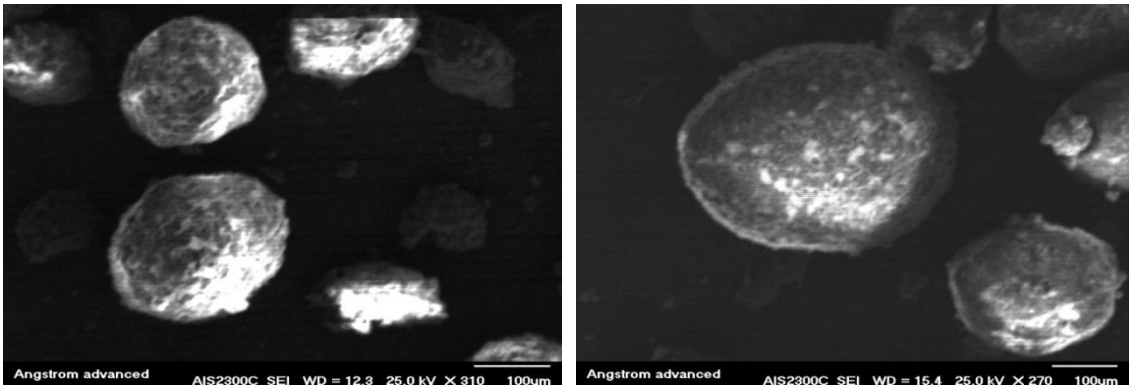


Figure (4) Dissolution profile of BFN from different microsponge formula in 0.1N HCl (pH1.2) at 37ºC .

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

The SEM result of the selected microsponge formula showed (figure 5) a spherical nature of the microsponge, uniform size with sufficient pores that loaded with drug.



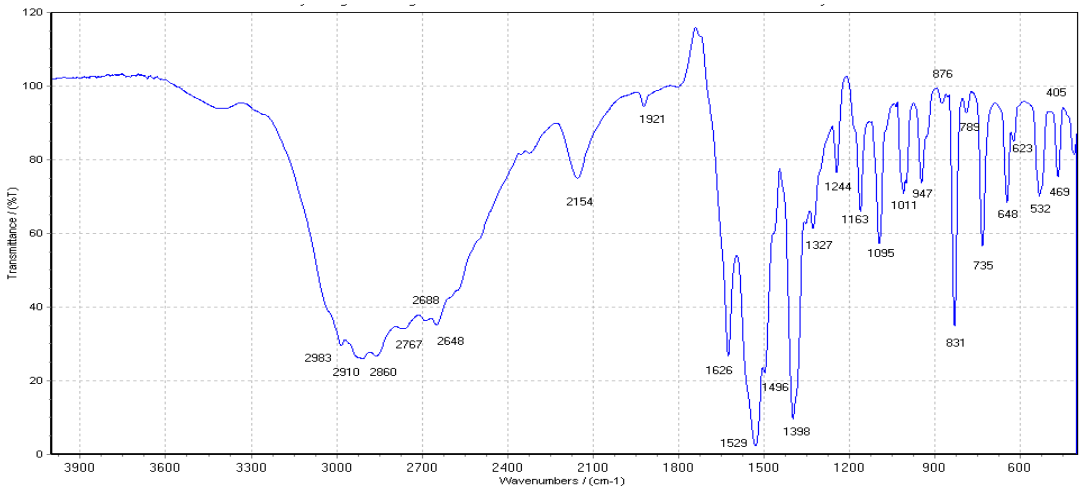
(A) **(B)**

Figure (5) SEM of the selected microsponge formula BF14 at (A) 310 X magnification and (B) 270 X magnification

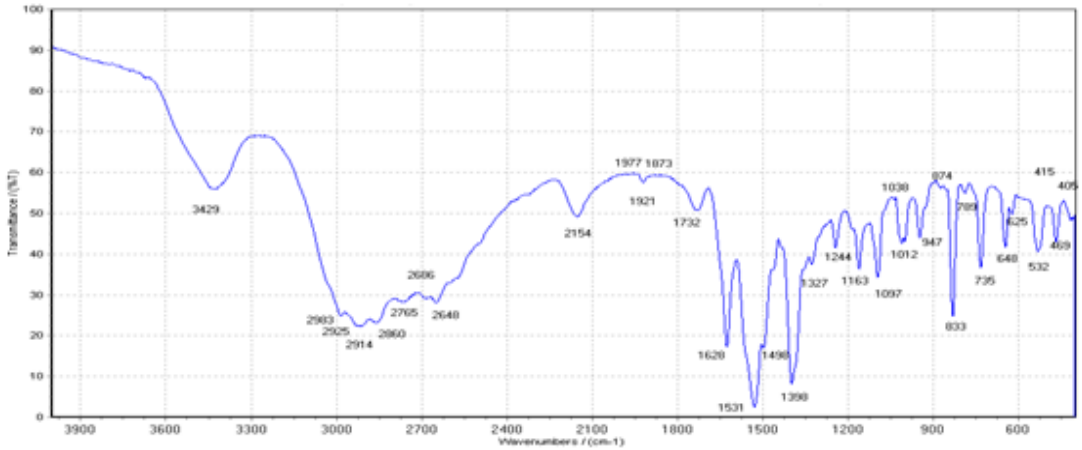
Fourier transforms infrared spectroscopy

The FTIR spectrum of pure BFN, physical mixtures of drug and Eudragit RS100, and the selected microsponge formula (BF14) were given in figure (6:A-C).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 finger print of BFN, the FTIR of BFN also showed broad peak at 2590 and extend up to 3100cm^{-1}(O-H of alcohol and carboxylic acid stretching). The spectrum of physical mixture

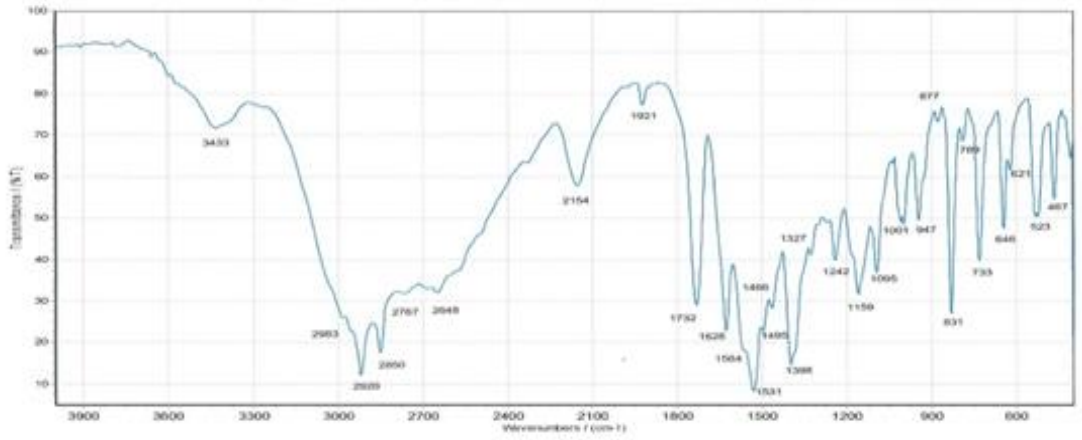
was as that of the drug, indicating no chemical interaction or complexation. The spectrum of the selected formula BF14 exhibited similar peaks, no appearance or disappearance of peaks and/or shift of their positions and therefore BFN was apparently stable in the microsponges



(A)



(B)



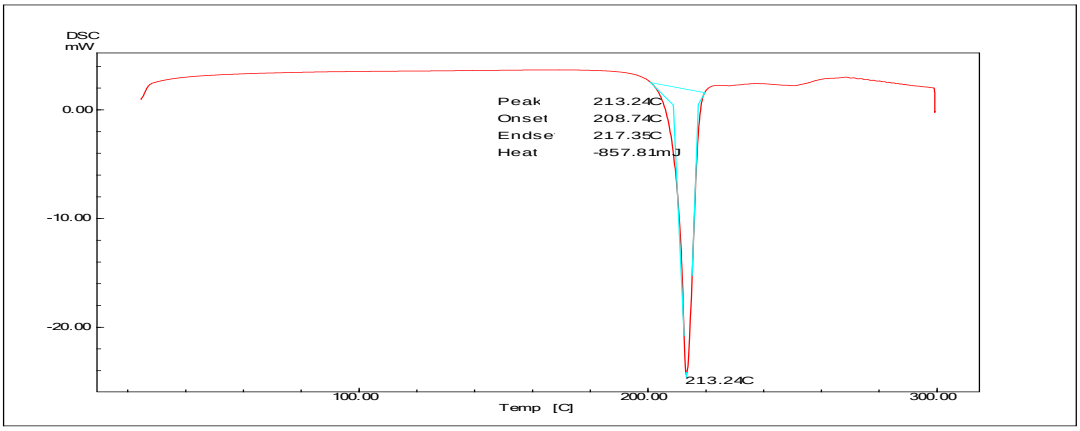
(C)

Figure (6) FTIR spectrum (A) Pure BFN, (B) Physical mixture ratio1:1 of BFN and Eudragit RS100, (C) BF 14

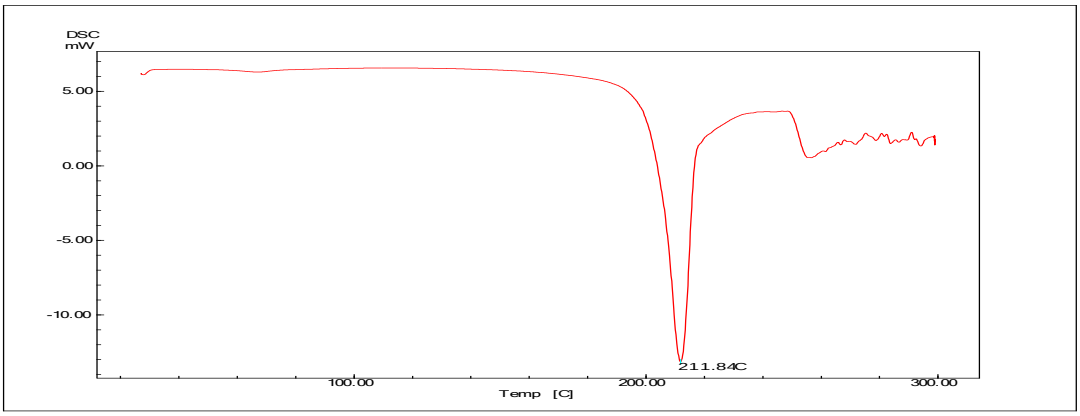
Differential scanning electron microscopy (DSC)

The thermal behavior of pure BFN showed a sharp endothermic peak at 213.24°C corresponding to BFN melting temperature with onset of peak at 208.74°C and end set at 217.35°C as shown in figure 7A this indicates that the drug was in pure crystalline state.

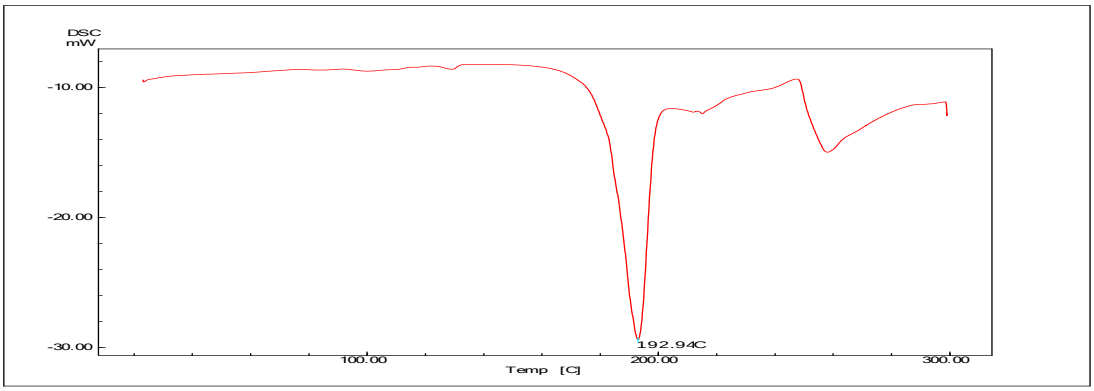
The thermogram of the physical mixture of BFN and Eudragit RS100 at equal ratio (1:1), and the selected BFN microsponge formula (BF14) exhibited endothermic peaks at 211.84 and 192.94 °C respectively, the slight decrease in the endothermic peak of the microsponge formula might be due to the alteration in the form of the material from crystal to amorphous especially there was no broadening or appearance of new peak and no other thermal event occurred. So, one can be concluded that the excipients and drug were compatible with each other as such case there was no incompatibility [37].



(A)



(B)



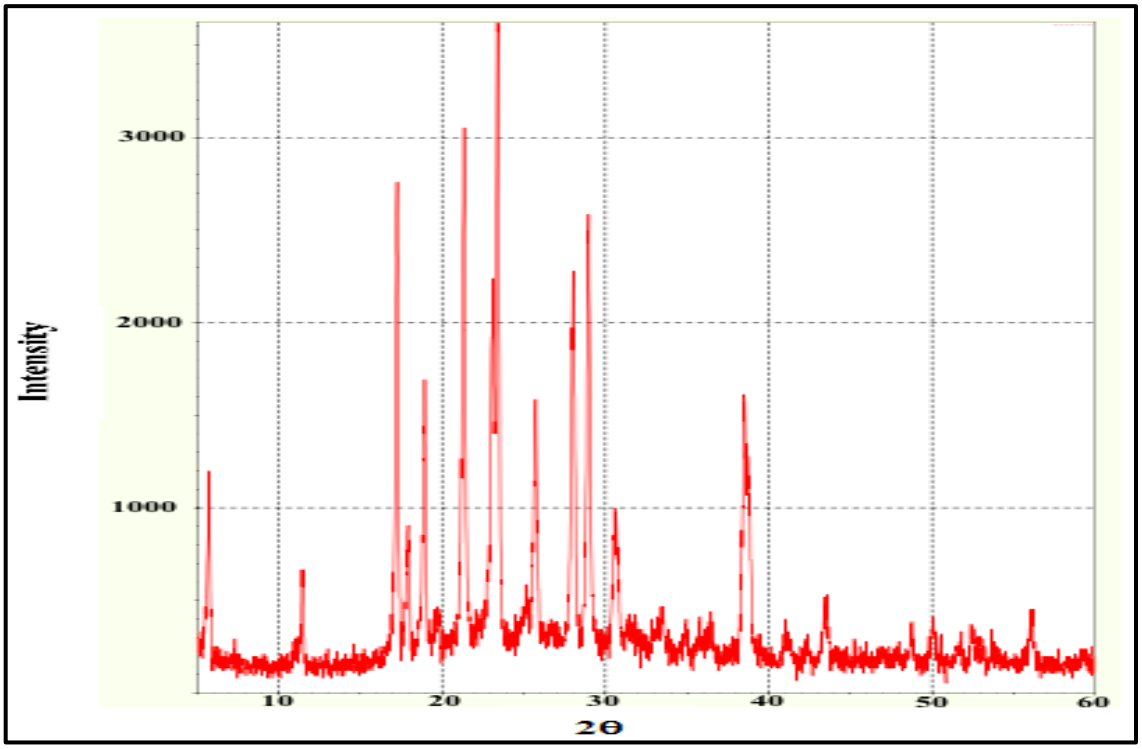
(C)

Figure (7)DSC Thermograms of (A) Pure BFN, (B) Physical mixture ratio1:1 of BFN and Eudragit RS100, (C) BF 14

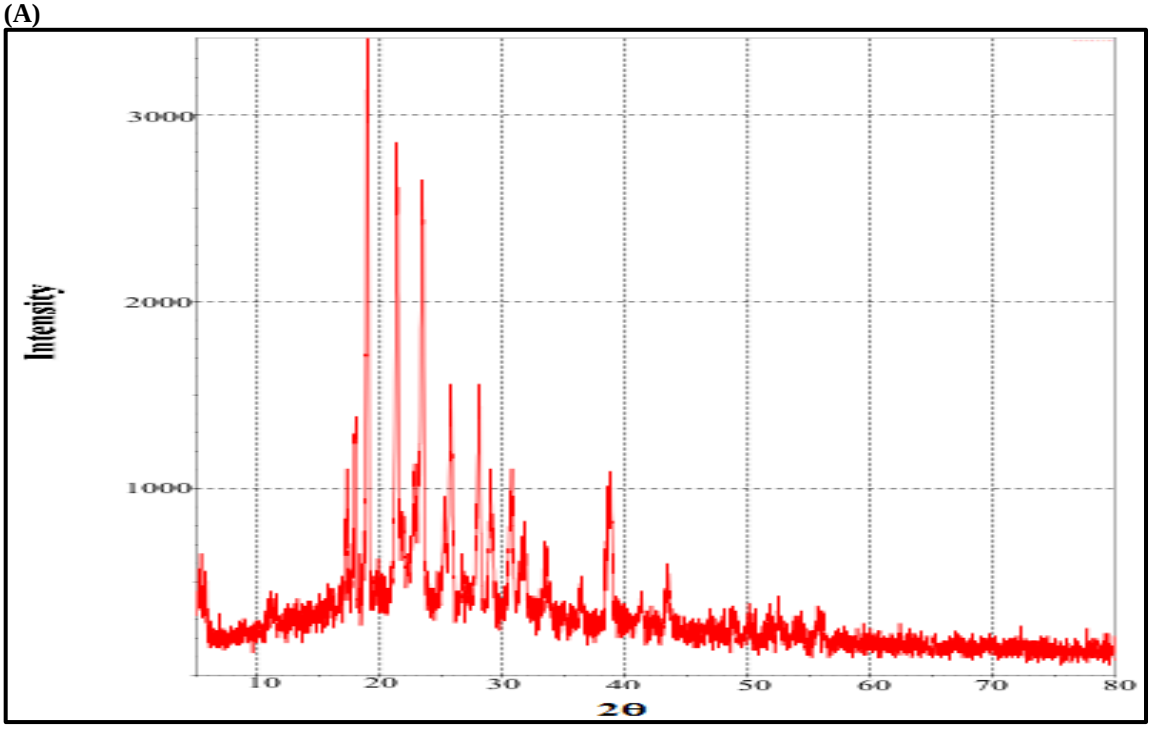
Powder X-ray diffraction

The x-ray is a tool for study of the crystal lattice of the drug and for indicating if possible interaction between the drug and excipients. The X-Ray of BFN showed strongest sharp distinct peaks at different angle (2θ) of 21.3˚, 23.4˚ and 28.9˚ which indicated that pure BFN was in crystalline nature as shown in figure 8.

These results were in agreement with the previous study [38]. The PXRD pattern of the selected BFN microsponge formula showed the main peaks of pure BFN but with lower intensity which might be due to conversion of BFN to amorphous form [39]. The obtained result is with agreement with that obtained by DSC.



(A)



(B)

Figure (8) X-ray diffractions of (A) Pure BFN, (B) BF 14.

Formulation of BFN floating microsponge tablets by effervescent technique

The development of floating effervescent microsponge tablets needs ideal polymer that allows water to permeate at fast enough rate for immediately activate the effervescent reaction and float and thereby preventing the individual unit for transit to the small intestine and at the same time should be firm and tight for resistance to rupture under high forces. Different polymers used as showed previously in table 2 and selected the best one as discussed later.

Evaluation of BFN floating microsponge tablets

The weight variation of the BFN floating microsponge tablets was found in the range of 196.35±0.69 to 199.87±0.8 for all the formulations as shown in table 5. These results were within the USP requirements limits. This referred to good flow properties that caused uniform fill of the tablet die and finally limit the weight variable. The hardness for formulas was in the range of 4.7 to 6.1±0.79 kg/cm^2 as shown in table 5. These ranges of tablet hardness indicated good mechanical strength [40].

Table (5) Physical parameters of BFN floating microsponge tablets

Formula	Weight variation(mg)±SD	Hardness (kg/cm^2) ±SD	Friability (w/w)	BFN content (%)±SD
FMT1	198±0.63	5.5±1.01	0.45	97.03±1.5
FMT2	196.43±1.81	5.46±0.1	0.78	98.2±2
FMT3	198±0.61	4.7±0.62	0.69	96.04±2.1
FMT4	197.8±1.14	5.5±1.1	0.56	98.7±1.6
FMT5	196.35±0.69	6.1±0.79	0.81	96.7±2.7
FMT6	199.87±0.8	5.82±1.7	0.73	99±0.61
FMT7	198.9±.91	5.82±1.0	0.49	98.89±0.9

In vitro buoyance studies

The prepared floating tablets exhibited different FLT and TFT depending on the polymer type and sodium bicarbonate concentration. The FLT and TFT of the prepared formulas are listed in table 6.

Table (6) The FLT and TFT of the prepared formulas

Formula	FLT (sec.) ±SD	TFT (hr.) ±SD
FMT1	23.15±2.12	Not float
FMT2	Not float	Not float
FMT3	Not float	Not float
FMT4	7.18 ±1.53	>24
FMT5	10.825±1.93	Not float
FMT6	25±0.32	2 ±0.79
FMT7	6.54±1.27	>24

FMT1 which was prepared by using 30 % HPMC E5 exhibited FLT of 23.15 sec. but the tablet disintegrated immediately in the dissolution media. HPMC E5 which is a low viscosity grade polymer, gels with low swelling ability and thus didn't trapped carbon dioxide therefore the tablet ruptured after floating immediately [41].

Sodium alginate that used in FMT2 failed in achieving floating tablet. This was due to its pH sensitive nature which made it soluble in 0.1N HCl (pH 1.2) [42].

(NaCMC) at 30 % of tablet weight (FMT3) failed in maintaining integrity of the tablet and tablet disintegrated immediately in the 0.1N HCl (pH 1.2). Easy solubility in water and rapid erosion of NaCMC matrix tablet were some of the limitations to make it an ideal tablet matrix material [43].

FMT4 which was formulated by using 30 % carbopol 934 p showed good FLT 7.18 sec. and excellent TFT (>24 hr.) and maintained the integrity of the tablet due to crosslink structure of carbopol polymer and there was larger region of low micro viscosity. FMT6 prepared with the same carbopol 934p concentration as that in FMT4 but with less concentration of gas generating agent (sodium bicarbonate). This formula exhibited longer FLT and TFT of 2 hr. as showed in table 6.

The gas generating agent concentration must be optimized within suitable limits for maintaining the generated gas bubbles within the floating tablet for longer period and thereby achieving longer TFT for tablet and this explained the shorter TFT of FMT6 at low concentration of sodium bicarbonate [44].

FMT5, FMT4 and FMT7 were formulated with 20 %, 30 % and 40 % carbopol 934 p polymer respectively but with same sodium bicarbonate concentration. These formulas showed different FLT which was 10.825±1.93 sec., 7.18 sec. ±1.53sec. and 6.54 sec.±1.27 sec. respectively. The reverse relationship between FLT and carbopol concentration as shown in figure 9 could be attributed to the high water swallability and hydrocolloid forming tendency of carbopol that lead to rapid wetting ability and floated immediately. Therefore, shorter FLT was obtained with increase carbopol concentration [45].

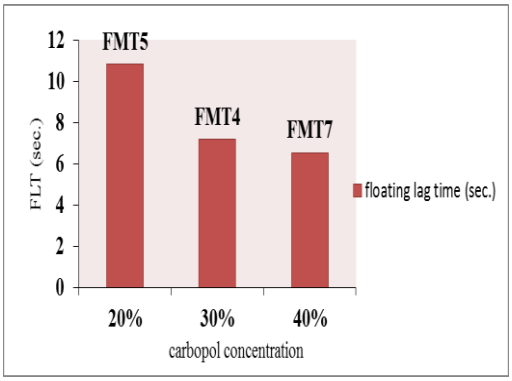
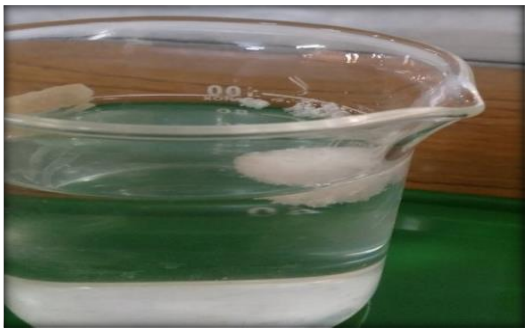


Figure (9) Histogram showing the effect of carbopol 934p concentration on FLT

The low level of polymer in FMT5 caused the poor strength of the gel layer and hence could not sustain the amount of gas generated as shown in figure 10. Therefore, BFN floating microsponge tablets formulated with 30 % and 40 % carbopol 934 p polymer showed an acceptable tablet shape with excellent FLT and TFT and good gel strength layer that maintained integrity of the tablets.

(A)



(B)

Figure (10) Tablets with different carbopol 934 p concentrations (A) 20%, (B) 40 %.

Effect of carbopol 934 p concentration on in vitro drug release

The effect of carbopol 934p concentration on the *in vitro* drug release was studied and illustrated in figure 11, as the concentration of the carbopol 934 p increase, the drug release decreased, this due to increase the concentration of carbopol would decrease the interstitial space between particles in the tablet. However, the higher swelling was associated with higher carbopol concentration would cause increase in dimension of the tablet and hence increased diffusion pathway that prevented the passage of the drug molecules and produced retardation of drug release [46].

FMT7 showed sustained drug release for 10 hr. with 81.529±0.79 % drug release in 7 hr. while for FMT4; the percentage of drug release was 95.478± 1.07 in 7 hr. Therefore, FMT7 was selected as the best formula for the preparation of BFN floating microsponge tablets by using the effervescent technique that at the same time exhibited short FLT (6.54 sec.±1.27) and TFT > 24hr. and resulted tablets with maximum swelling.

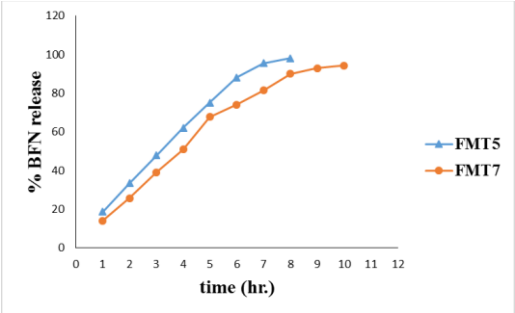


Figure (11) Effect of carbopol 934 p concentration on the % drug release in 0.1 N HCl (pH 1.2) at 37ºC

Kinetic analysis of BFN release data from FMT 7 floating microsponge tablets

The release of BFN from floating microsponge tablet FMT7 obeyed Higuchi-release as their R^2 values gave higher results. It was found that the mechanism of drug release was non -Fickian diffusion as the release exponent "n" value of FMT7 was more than 0.5 and less than 1 which was the standard value for declaring non -Fickian anomalous diffusion as shown in table7 [47].

Table (7) Kinetic analysis of BFN floating microsponge tablet release data (FMT7)

Formula	Mathematical model for drug release kinetics				Korsmeyer-Peppas	
	Zero order	First order	Higuchi	Hixson-Crowell		
FMT7	R^2	R^2	R^2	R^2	R^2	N
	0.955	0.905	0.9933	0.8839	0.987	0.735

Conclusion

Microsponges of BFN were successfully formed by the non-aqueous emulsion solvent diffusion method. BFN microsponge tablets prepared using 40 % (w/w) carbopol 934p as a gastroretentive polymer and 17.5 % (w/w) sodium bicarbonate maintained the tablet integrity and achieved tablets with maximum swelling and shorter FLT with controlled drug release.

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