Preparation of Metformin- loaded microspheres by ionic gelation method

Dr. Lama Al Haushey*
Laila Yazigi**

(Received 24 / 7 / 2021. Accepted 6 / 9 / 2021)

□ ABSTRACT □

Diabetes mellitus (DM) is a chronic metabolic disorder of elevated blood glucose level. Metformin is an effective agent with a good safety profile that is widely used as a first line treatment for type 2 diabetes. It has several drawbacks (eg. low oral bioavailability, short half-life, and gastrointestinal side effects). Modified release formulations of metformin have been developed to increase its tolerability and to improve patient compliance. The aim of this work is to prepare and to evaluate metformin- loaded microspheres using the ionic gelation method as a simple, low cost, and eco-friendly process to control the release of metformin hydrochloride. The microspheres were prepared using chitosan as the natural polymer and Sodium Tripolyphosphate (TPP) as a crosslinking agent, with studying the effect of different variables on microspheres characteristics. Theses variables are chitosan concentration%, TPP concentration %, drug concentration %, stirring speed, and stirring time. The prepared microspheres were further evaluated for particle shape and size, drug entrapment efficiency, percentage yield, equilibrium swelling index, and *in-vitro* drug release. Microscope images revealed that microspheres had spherical shape and their sizes ranged between (539-1063) µm. It was found that the F15 with (1.5% chitosan, 1.5% TPP, 1%drug, 600 rpm stirring speed, 45 min stirring time) with average particle size of (827 µm), had the highest encapsulation efficiency of (50%), percentage yield of (34%), and showed the ability to control microspheres swelling and therefore drug release in buffered gastrointestinal environment.

Keywords: Ionic gelation, Metformin Hydrochloride, Controlled drug release, Chitosan, Sodium tripolyphosphate.

* **Associate Professor, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Tishreen University, Latakia, Syria. lamaalhaushey@yahoo.fr**

** **Postgraduate Student , industrial pharmacy, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Tishreen University, Latakia, Syria. lailayzg@gmail.com**

تحضير جسيمات دقيقة حاوية على الميتفورمين باستخدام طريقة التهلم الشاردي

د. لمى الهوشي*
ليلى يازجي**

(تاريخ الإيداع 24 / 7 / 2021. قُبِل للنشر في 6 / 9 / 2021)

□ ملخّص □

داء السكري هو اضطراب استقلابي مزمن متمثل بارتفاع مستويات الغلوكوز في الدم. الميتفورمين هيدروكلورايد هو دواء فعّال وأمن يستخدم على نطاق واسع كخط أول لعلاج السكري النمط الثاني. له مشاكل عديدة (على سبيل المثال انخفاض توافره الحيوي عن طريق الفم، قصر عمره النصفي، وتسببه بآثار جانبية معدية معوية). تم تطوير صيغ مضبوطة التحرر من الميتفورمين لزيادة تحمّله وبالتالي تحسين مطاوعة المريض. الهدف من هذا البحث هو تحضير وتقييم جسيمات دقيقة حاوية على الميتفورمين باستخدام طريقة التهلّم الشاردي باعتبارها طريقة بسيطة، منخفضة التكلفة وصديقة للبيئة، بهدف ضبط تحرر الميتفورمين. تم تحضير الجسيمات الدقيقة باستخدام مادة الكيتوزان كبوليمر طبيعي وثلاثي فوسفات الصوديوم TPP كعامل للتصليب، وتبع ذلك دراسة تأثير المتغيرات المختلفة على خصائص الجسيمات الدقيقة. هذه المتغيرات هي تركيز الكيتوزان ٪، تركيز TPP ٪، تركيز الدواء ٪، سرعة التحريك، وزمن التحريك. تم تقييم الجسيمات الدقيقة المحضرة من حيث شكل وأبعاد الجسيمات، فعالية التمحفظ، المردود المئوي، مشعر الانتباج عند التوازن، وتحرر الدواء في الزجاج. أظهرت الصور تحت المجهر أن الجسيمات الدقيقة كان لها شكل كروي وتراوحت أبعادها بين (539–1063) ميكرومتر. وجد أن الصيغة رقم 15 التي تحتوي على (1.5٪ كيتوزان، 1.5٪ TPP، 1٪ دواء، بسرعة تحريك 600 دورة في الدقيقة، و زمن تحريك 45 دقيقة) تملك بعد وسطي يقدر بـ (827 ميكرومتر)، أعلى فعالية تمحفظ (50%)، ومردود مئوي يقدر بـ (34٪)، كان لهذه الصيغة القدرة على ضبط انتباج الجسيمات الدقيقة وبالتالي ضبط تحرر الدواء في وقاء يحاكي بيئة الجهاز الهضمي.

الكلمات المفتاحية: التهلم الشاردي، ميتفورمين هيدروكلورايد، تحرر الدواء المضبوط، الكيتوزان، ثلاثي فوسفات الصوديوم.

*أستاذ مساعد، قسم الصيدلانيات والتكنولوجيا الصيدلية، كلية الصيدلة، جامعة تشرين، اللاذقية، سورية. lamaalhaushey@yahoo.fr
**طالبة ماجستير، قسم الصيدلانيات والتكنولوجيا الصيدلية، كلية الصيدلة، جامعة تشرين، اللاذقية، سورية. lailayzg@gmail.com

Introduction

Microencapsulation is a process used to obtain microparticles. The Microparticles term refers to particles with a diameter of 1-1000 µm. There are two types of these particles: **microcapsules** which have a core surrounded by a material different from that of the core, and **Microspheres** which refer to spherical microparticles that are comprised of a fairly homogeneous mixture of polymer and active agent [1]. Microspheres have multiple advantages including: particle size reduction for enhancing solubility of the poorly soluble drugs, drugs protection against environmental conditions, masking the unpleasant taste and odour of drugs. Microspheres also enhance the biological half-life and improve the bioavailability; they reduce the chances of GI irritation. They provide prolonged and constant therapeutic effect, reduce the dosing frequency and therefore improve patient compliance [2, 3].

The various microencapsulation processes can be divided into: chemical, physiochemical, and mechanical processes [1]. The latter includes ionic gelation method which is used in this study. **Ionic gelation** method is based on the ability of polyelectrolytes to crosslink in the presence of counter ions to form hydrogel microspheres. This method of microencapsulation has attracted much attention because it is very simple and mild. It consists of natural polymers which reduces the utilization of expensive and toxic organic solvents, and provides an eco-friendly pharmaceutical product development process [4, 5].

The chosen polymer in this study is chitosan. It is derived from a material called chitin (second most abundant polysaccharide in nature next to cellulose) that is extracted from the shells of sea crustaceans[6]. The partially de-acetylated chitin is the chitosan, a linear polymer consisting of two units (glucosamine and N-acetyl glucoseamine) [6, 7]. Chitosan is soluble in acids such as acetic acid. Upon dissolution, the amine groups of the polymer are protonated. Chitosan salts are soluble in water. The solubility depends on the degree of deacetylation and the pH of the solution [8]. Chitosan is biocompatible and biodegradable. It has many applications in industry and it is used in the pharmaceutical industry as a binding agent and disintegrant, and as a drug carrier for sustained release preparations [9]. Tripolyphosphate (TPP) is a polyanion, which can interact with the cationic chitosan by electrostatic forces [4, 5].

In this study, metformin hydrochloride is the drug of choice to be formulated in microspheres delivery system. It is a hypoglycemic agent used in the treatment of type 2 (non-insulin dependent) diabetes mellitus. However, it has some drawbacks. For example, a high dose (1.5-2 g/day), low bioavailability (40%-60%), and a plasma elimination half-life of approximately (6.2 hours) [10, 11]. It requires repeated administration of high doses to maintain effective plasma concentration. It also causes gastrointestinal side effects. Metformin is defined as class III by the biopharmaceutic classification system (BPC) (high water solubility and low permeability) [12-14].

The low bioavailability, short half-life, and high water solubility of metformin make it a desirable candidate for controlled release formulation.

Materials and Methods

Materials

Chitosan (85% deacetylated) and Metformin Hydrochloride were received as gift samples from Balsam Pharma. Co. (Syria) and GoldenMed Pharma Ltd. (Syria) respectively, Acetic Acid 99.5% (POCH SA – Poland), Sodium Tripolyphosphate (Titan Biotech Ltd, India), Glutaraldehyde (Chem-Lab NV, Belgium), Hydrochloric acid (HCl 37%) (Scharlau Chemie S.A., Spain), Potassium chloride (Riedel-de Haen, Germany), Potassium

phosphate (POCH SA), Dipotassium phosphate, and Sodium chloride were used of analytical grade.

Equipment

Analytical balance (Radwag wagi elektroniczne – Poland), Monotherm (H+P Labortechnik AG – Germany), Ultraviolet spectrophotometer V-530 (Jasco – Japan), Optical microscope (Olympus Optical Co. Ltd, CH20BIMF200), Magnetic bead, vials, Disposable syringes 3 ml, Glass beakers (50 ml – 100 ml – 250 ml), Measuring cylinders (100 ml), Glass pipettes (1 ml – 2 ml – 10 ml), Whatman filter paper no. 4 and micro filters 0.45µm pore size.

Methods

Preparation of Chitosan Microspheres

The microspheres were prepared by ionic gelation method described by (Bodmeier R. et al. 1998) [15], with some modifications. Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) with stirring at 37º C. Metformin hydrochloride was dissolved in the prepared chitosan solution (1% w/v). 10 ml of this bubble free solution was dropped through a disposable syringe needle (25 gauge × 5/8") into a gently agitated (600 rpm) sodium tripolyphosphate solution (1% w/v). The dropping rate and falling distance were kept constant. The solution was magnetically stirred for 45 minutes. For hardening, the microspheres were treated with 1%v/v gluteraldehyde solution, and left for 90 min as incubation time, then filtrated and rinsed with distilled water. Microspheres were obtained and air dried for 24 hours followed by oven drying for two hours at 40ºC [12, 13].

Formulation variables

The study involved the investigation of the effect of independent variables in different levels. These variables were: Chitosan concentration (1 – 1.5 – 1.75 – 2) %, TPP concentration (0.75 – 1 – 1.5 – 2 – 3) %, drug concentration (0.75 – 1 - 2) %, stirring speed (600 – 900) rpm, and stirring time (45 – 60) min. 16 batches were prepared by altering the formulation variables, and they are presented in Table (1).

Table (1): Variables for preparation of cross-linked metformin-loaded chitosan microspheres

Formulation (F)	Chitosan concentration (%)	TPP concentration (%)	Drug concentration (%)	Stirring speed (rpm)	Stirring time (min)
1	1	1	1	600	45
2	1	2	1	600	45
3	1	3	1	600	45
4	1.5	1	1	600	45
5	1.5	1	2	600	45
6	1.5	2	1	600	45
7	1.5	2	2	600	45
8	1.5	3	1	600	45
9	1.5	0.75	1	600	45
10	1.5	1	1	600	60
11	1.5	1	1	900	45
12	1.75	1	1	600	45
13	2	3	1	600	45
14	1.5	1	0.75	600	45
15	1.5	1.5	1	600	45
16	1.5	1.5	2	600	45

Microspheres characterization and evaluation

After the preparation of microspheres by ionic gelation method, they were evaluated for particle shape and size, entrapment efficiency, percentage yield, equilibrium swelling index, and *in-vitro* dissolution studies.

Particle shape and size analysis

The microspheres shape and size were examined using the optical microscope at 10x magnification. Approximately 200 microspheres were measured [16].

Encapsulation efficiency (EE)

To calculate the amount of drug trapped in microspheres, 20 mg of microspheres were crushed to powder in a mortar and pestle, then digested in a vial containing 20 ml of 0.1N HCl with continuous stirring for 24 hours [10, 13]. Afterwards, the solution was filtered by Whatman filter paper no. 4, followed by using Whatman micro filters 0.45µm pore size. The absorbance of the filtrate was taken spectrophotometrically at 210 nm against a suitable blank. Each experiment was repeated six times and the average value was taken as the encapsulation efficiency, calculated using the following equation:

% Encapsulation efficiency = (Actual entrapment level / Theoretical entrapment level) ×100

Percentage yield

The yield of microspheres is indicative of the weight gained by the chitosan solution inside TPP solution in form of microspheres. After the preparation of microspheres they were collected, dried, and weighed [13]. The percentage yield was calculated by the formula:

Percentage yield (w/w) = (weight of dried microspheres / (weight of chitosan + weight of TPP + weight of metformin)) × 100

Equilibrium swelling studies

The water uptake of the chitosan microspheres and the changes in weight during the swelling was measured to evaluate the effect of gastrointestinal environment on microspheres, it also gives an indication of the drug release behaviour in the body [13, 17]. 20 mg of microspheres were put in the first buffered medium (pH=1.2) continuously stirred at 150 rpm and allowed to swell during 120 min at 37ºC. The same process was repeated in the buffered medium (pH=6.8). The swollen samples were removed periodically (5, 10, 15, 30, 60, 90, 120 min) and their weight was determined [13, 17]. Each swelling experiment was run in duplicate and the average value was taken as the swelling degree, calculated using the following equation:

% Equilibrium swelling = (weight at equilibrium - initial weight of microspheres / initial weight of microspheres) × 100

In-vitro drug release study

The in vitro release studies were performed in hydrochloric acid buffer (pH=1.2) and subsequently in saline phosphate buffer (pH=6.8) close to the physiochemical gastrointestinal conditions. 20 mg of drug loaded chitosan-TPP microspheres were incubated in 20 ml buffer of (pH 1.2) in a vial under continuous stirring (250 rpm) at 37ºC for the first 4 hours, then the microspheres were filtered and transferred into 20 ml buffer of (pH 6.8) for the next 20 hours. 3 ml sample was withdrawn to spectrophotometrically determine the released drug at (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 hours) intervals and replaced

with equal amount of fresh buffer. The cumulative drug release from the microspheres was estimated by comparing each observation with the drug actually entrapped [13].

Results and discussion

Standard curves of metformin hydrochloride

Standard curves of metformin hydrochloride in 0.1N HCl, pH=1.2 buffer, and pH=6.8 buffer are shown in Figures (1,2,3).

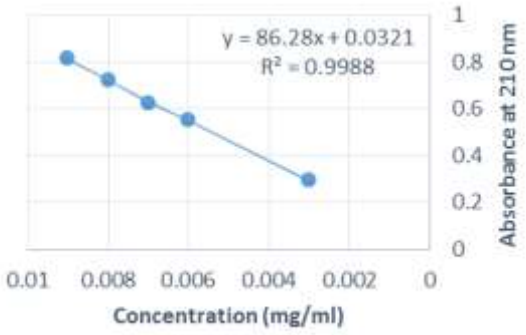


Fig (1): Standard curve of metformin hydrochloride in 0.1N HCl (used for EE determinations)

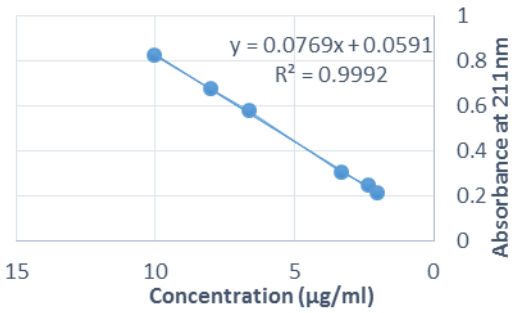


Fig (2): Standard curve of metformin hydrochloride in hydrochloric acid buffer pH=1.2 (used for *in-vitro* release study)

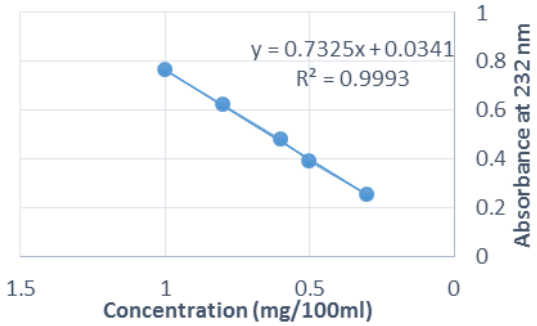


Fig (3): Standard curve of metformin hydrochloride in saline phosphate buffer pH=6.8 (used for *in-vitro* release)

Evaluation of metformin- loaded chitosan microspheres

Particle shape and size analysis

The mean particle size of all the prepared batches of microspheres ranged between (539-1063) µm. Particles tend to have a spherical form as the mixing speed decreases [18] which was best at 600 rpm (Fig.4) and it was observed that microspheres do not form at high mixing speed.

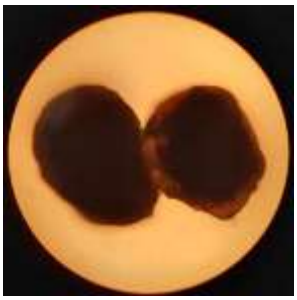
It was found out that particle size increases as chitosan concentration increases (F1, F4 $P<0.05$) [19-21] because of the fact that TPP amount proved insufficient due to the

increase in the chitosan viscosity, and the decreased crosslinking density due to its being incapable of diffusing into the particles [18].

While particle size decreases as TPP ratios increase because of increasing the crosslinking density (F4, F8 $P<0.05$) [18]. Also, as a result of increasing drug concentration, which leads to decreasing chitosan amount in the dribble of the same volume, crosslinking density decreases, and thus particle size increases (F4, F5 $P<0.05$) [18] (Table 2).

Table (2): Particle size determination of metformin- loaded chitosan microspheres

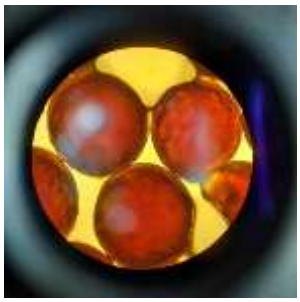
F	Average size (M ± SD) (µm)
1	644 ± 0.7
2	539 ± 0.9
3	608 ± 1.3
4	836 ± 0.8
5	899 ± 1.2
6	825 ± 0.6
7	845 ± 0.7
8	762 ± 0.3
9	830 ± 0.2
10	741 ± 0.7
11	776 ± 1.2
12	1063 ± 0.9
13	918 ± 1.3
14	842 ± 0.7
15	827 ± 0.5
16	800 ± 0.8



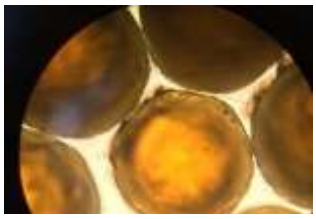
F1: 1% chitosan – 1%TPP

F2: 1% chitosan – 2% TPP

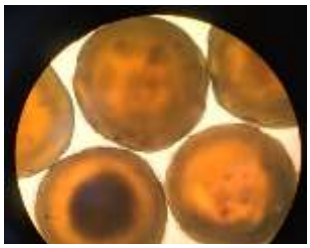
F3: 1% chitosan – 3% TPP



F4: 1.5% chitosan – 1% TPP



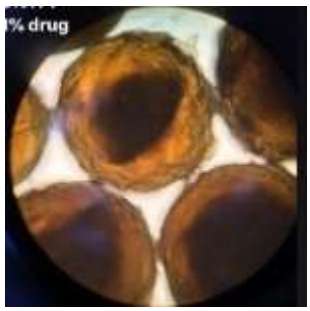
F5: 1.5% chitosan – 1% TPP- 2%drug



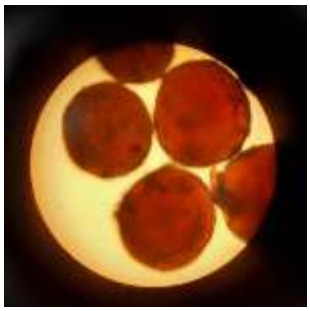
F6: 1.5% chitosan - 2% TPP

F7: 1.5% chitosan – 2% TPP – 2% drug

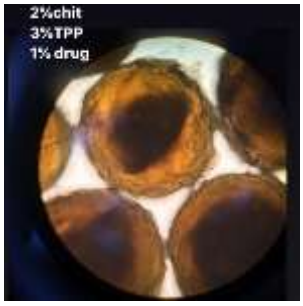
F8: 1.5% chitosan – 3% TPP



F15: 1.5% chit – 1.5% TPP- 1% drug



F16: 1.5% chitosan – 1.5% TPP- 2% drug



F13: 2% chitosan – 3% TPP

Fig (4): microscope images of metformin- loaded chitosan microspheres

Encapsulation efficiency

The maximum entrapment efficiency was obtained from F15 (1.5% chitosan 1.5% TPP and 1% drug).

Drug entrapment increases with increasing of chitosan concentration [16, 19, 22] and achieves the highest amount of nearly 50% at 1.5% chitosan, then the drug entrapment decreases with the rising chitosan concentration (F1, F4, F12 $P<0.05$) [23], because of the increased chitosan viscosity and the insufficient TPP amount that decreased the crosslinking density [18].

The encapsulation efficiency was reduced with higher TPP concentration than 1.5% (F4, F6, F15 $P<0.05$) [10], it might be explained by the positively charged amino groups of chitosan were probably converted to the unionized state at the higher pH values, this resulted in reduced ionic interactions or crosslinking with the anionic counterion TPP [15]. The TPP solution to chitosan solution ratio should be kept to a minimum for maximum drug entrapment [12, 15]. In the case of ionized drugs, the pH of the TPP phase could be adjusted to minimize drug solubility [15]. In this study, a single experiment was conducted to increase the pH of the external phase to 13.

Regarding the effects of drug concentration on drug entrapment, it was found that drug concentration of 1 % has the highest encapsulation efficiency (Table 3), and in the study of (Singh. KS et al. 2015) they found that 0.5% metformin HCl had the highest efficiency [13]. Also, in the study of (L. Alhaushey et al. 2012) higher (drug:polymer) concentration led to lower encapsulation efficiency [20], because higher drug concentration and decreased chitosan amount in a fixed volume caused decreasing crosslinking density [18]. While in other studies the encapsulation efficiency increased with higher drug concentration [10, 24].

Table (3): encapsulation efficiency of metformin- loaded chitosan microspheres

F	EE% (M ± SD)
1	1.5 ± 2
2	0.5 ± 0.02
3	1 ± 0.1
4	37 ± 0.5
5	22 ± 1.7
6	19 ± 1.7
7	2.4 ± 0.4
8	3.2 ± 0.2
9	3.9 ± 0.5
10	1.6 ± 0.1
11	0.5 ± 0.1
12	1 ± 0.08
13	19 ± 1.6
14	2 ± 0.07
15	50 ± 0.7
16	41 ± 0.4

Percentage yield

Percentage yield increased as the chitosan:TPP ratio increased, while it decreases with decreasing chitosan:TPP ratio and increasing metformin HCl concentration (F15, 16 $P<0.05$). Highest yield (84.6%) was with F9 containing 1.5% chitosan and 0.75% TPP (ratio=2). Also good percentage yield (30.9%) was shown with F15 containing 1.5% chitosan and 1.5% TPP. As described in previous studies, percentage yield varies considerably with pH and concentration of TPP solution but it doesn't change as much with different stirring time [13] (Table 4), and in the study of (H. Akel et al. 2016) the percentage yield increased as the stirring speed increased [25], and maximum yield (11.8%) was observed with 1.5% chitosan, 2% TPP, at pH=7, and 30 minutes stirring time [13]. In another study it was mentioned that maximum yield (26.5%) was obtained using 1.5% chitosan, 2% TPP, and 30 minutes stirring time [19].

Table (4): Percentage yield of metformin- loaded chitosan microspheres

F	Chitosan:TPP	Percentage Yield % (M ± SD)
1	1	15.4 ± 0.5
2	0.5	8 ± 0.1
3	0.3	6.6 ± 0.4
4	1.5	36.3 ± 0.2
5	1.5	18.2 ± 0.7
6	0.75	13.2 ± 0.2
7	0.75	12.3 ± 0.3
8	0.5	11 ± 0.03
9	2	84.6 ± 0.7
10	1.5	33.3 ± 1.1
11	1.5	26.5 ± 0.5
12	1.75	30.6 ± 0.4
13	0.6	13 ± 0.7
14	1.5	19.3 ± 0.2
15	1	30.9 ± 0.2
16	1	10.7 ± 0.3

Equilibrium swelling studies

Equilibrium swelling test was performed on formulas that showed good encapsulation efficiency (F4, F6, F13, F15, F16). The swelling of chitosan microspheres was dependent on the pH of the dissolution medium. The spheres swell extensively in (pH=1.2) [10, 12, 15], the protonation of excess amino groups of polysaccharide in the stomach pH conditions is responsible for its swelling [26]. Optimal swelling was observed in F15 (1.5% chitosan and 1.5% TPP) with 135% swelling index in pH=1.2 and 86% in pH=6.8 which indicates a balanced swelling and a sustained drug release from the microspheres while passing through the gastrointestinal tract. The more concentrated and more viscous chitosan solution led to microspheres with a higher swelling capacity [17]. It was observed that swelling index decreases when drug concentration increases (F16), due to dense structure of the membrane at high loadings, and medium could not easily diffuse into the matrix of microspheres [26] (Table 5), and according to (Singh. KS et al. 2015) optimal swelling in buffer 1.2 was observed in Fmade with 0.5% drug [13].

Table (5): Swelling index of metformin- loaded chitosan microspheres

Swelling Index % (M ± SD)					Time (min) pH=1.2
F4	F6	F13	F15	F16	
178.3 ± 3.2	2.5± 2.5	77.5± 2.3	25.5 ± 0.5	25.0 ± 1	**5**
221.5 ± 0.8	37.0 ± 5	126.3 ± 2.7	48.6 ± 0.7	45.3 ± 0.6	**10**
245.0 ± 1	55.3±3	123.8 ± 1.3	73.3 ± 1.5	87.0 ± 0.4	**15**
245.0 ± 2	87.0 ± 2.4	128.0 ± 3.5	89.5± 1.7	107.8 ± 0.6	**30**
180.5 ± 2.2	124.8 ± 0.9	38.5 ± 0.9	93.0 ± 1.5	107.8 ± 0.7	**60**
198.8± 5	148.2 ± 1.1	-29.3 ± 2.6	121.0 ± 2.6	123.5 ± 1.9	**90**
150.5 ± 2.5	167 ± 0.4	-67.5 ± 0.8	1350. ± 3.1	123.5 ± 1	**120**
					pH=6.8
21.4± 0.8	2.6 ± 0.4	-6.5 ± 0.9	6.8 ± 1	4.9± 1.2	**5**
15.7 ± 2.3	12.8 ± 0.6	-2.4± 1.3	15.9 ± 1.8	7.4 ± 0.8	**10**
12.8 ± 2.8	18.5 ± 1.3	-100.0 ± 1.1	18.6 ± 2.2	8.8 ± 0.3	**15**

8.7± 0.7	21.3 ± 1.2	-51.6 ± 0.7	27.2 ± 3.5	9.1 ± 0.9	**30**
6.0 ± 2	21.5 ± 0.6	-50.0 ± 1.4	46.6 ± 2.7	15.0 ± 1.5	**60**
5.9 ± 4.1	26.2 ± 1.5	-50.0 ± 0.6	62.5 ± 2.8	21.9 ± 1.3	**90**
3.0 ± 5.6	28.2 ± 2.4	-63.5 ± 1.5	86.2± 4	25.4 ± 0.8	**120**

Evaluation of *in-vitro* drug release

Figure (5) shows metformin dissolution diagram of the formulations that showed good encapsulation efficiency. The dissolution behavior of the chitosan-TPP microspheres was dependent on pH of the medium. The drug release from the chitosan microspheres depended on the penetration of the dissolution medium into the microspheres, the eventual swelling and dissolution of the chitosan matrix, and the dissolution and subsequent diffusion of the drug through the swollen or un-swollen chitosan matrix [12, 15]. Some of the batches showed fast release of drug in the first hours (slight dose dumping) which is due to the presence of drug entrapped on the surface of microspheres and also to the faster ingress of dissolution medium and subsequent diffusion of drug, followed by a slow drug release [10, 16, 19, 22]. Increased TPP concentration and crosslinking with the polymer reduced the degree of swelling and drug release (F4, F15 $P<0.05$) [18], but according to (Bodmeier R. et al. 1989), this is only important in the acidic media where the free amino groups in chitosan get ionized and cause hydration and swelling, whereas they do not swell in the intestinal fluid and the effect of the crosslinking agent is not significant [15].

Cumulative percentage drug release increased with the increase in drug concentration (F15, F16 $P<0.05$) in concordance with previous studies [10, 18, 20]. On the contrary of the work of (Bodmeier R.) where the cumulative drug release decreased with increasing drug content in both media [15].

Drug release was slower in batches run with stirring time (45 and 60 mins) and final drug release was even lower, this behavior might be due to increased interaction (cross-linking) between TPP and Chitosan [13]. Whereas, no significant effect of the stirring rate during processing was noticed on drug eject [27]. In this study, the drug release study was carried out until 48 hrs in some batches to monitor the continuous release of the drug after 24 hours. In the work of (Sanjukta Duarah et al. in 2015), release studies were carried out up to 220 hours and depending on the release properties; chitosan was selected as the best polymer [27, 28].

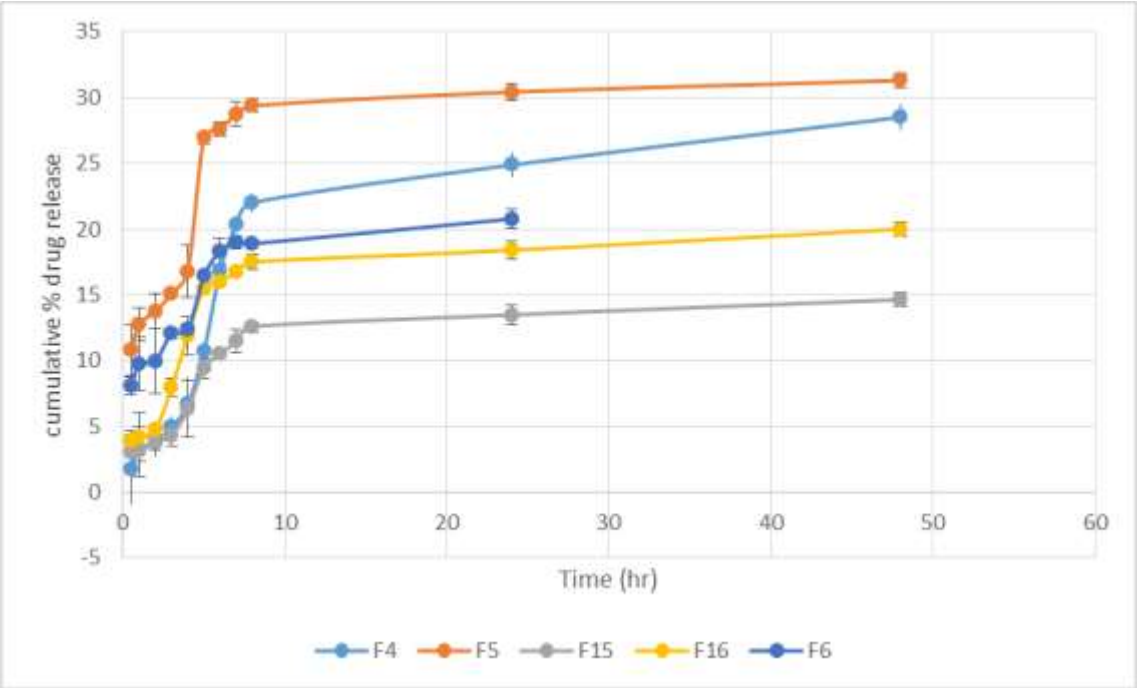


Fig (5): The drug release curve of (F4, F5, F6, F15, F16)

Conclusion

This research demonstrates that the controlled release of Metformin hydrochloride microspheres can be attained by ionic gelation technique using chitosan as the polymer. The batches were evaluated for particle size and shape, entrapment efficiency, percentage yield, swelling index and in vitro drug release. Microscopic studies revealed that the microspheres were spherical. Microspheres properties were affected by various factors like: chitosan concentration, TPP concentration, drug loading, stirring time, and stirring speed. F15 with 1.5% chitosan, 1.5% TPP, 1% drug, 600 rpm stirring speed, and 45 minutes stirring time, was considered to be superior showing particle size of (827 µm), high entrapment efficiency (50%), good percentage yield (34%), swelling index and prolonged release of drug. Consequently, it was concluded that Metformin hydrochloride loaded chitosan microspheres were found to be promising in the treatment of Diabetes in a controlled release mode.

References

1.Singh, M., et al., *Microencapsulation: A promising technique for controlled drug delivery.* Research in pharmaceutical sciences, 2010. **5**(2): p. 65.
2.Solanki, N., *Microspheres an Innovative Approach in Drug Delivery System.* MOJ Bioequiv Availab, 2018. **5**(1): p. 00083.
3.Kadam, N. and V. Suvarna, *Microsphere: a brief review.* Asian journal of biomedical and pharmaceutical sciences, 2015. **5**(47): p. 13.
4.Patil, P., D. Chavanke, and M. Wagh, *A review on ionotropic gelation method: novel approach for controlled gastroretentive gelispheres.* Int J Pharm Pharm Sci, 2012. **4**(4): p. 27-32.
5.Agnihotri, S.A., N.N. Mallikarjuna, and T.M. Aminabhavi, *Recent advances on chitosan-based micro-and nanoparticles in drug delivery.* Journal of controlled release, 2004. **100**(1): p. 5-28.
6.Panos, I., N. Acosta, and A. Heras, *New drug delivery systems based on chitosan.* Current Drug Discovery Technologies, 2008. **5**(4): p. 333-341.
7.Varshosaz, J., *The promise of chitosan microspheres in drug delivery systems.* Expert opinion on drug delivery, 2007. **4**(3): p. 263-273.
8.Ilium, L., *Chitosan and its use as a pharmaceutical excipient.* Pharmaceutical research, 1998. **15**(9): p. 1326-1331.
9.Hemant, K., M.N. Singh, and H. Shivakumar, *Chitosan/Sodium tripolyphosphate cross linked microspheres for the treatment of gastric ulcer.* Der Pharmacia Lettre, 2010. **2**(3): p. 106-116.
10.Singal, A.K. and M. Vasundhara, *Preparation of the Controlled Release Chitosan Microspheres and Optimisation of the Formulation Parameters*. 2011.
11.Depomed, I., *Glumetza ™, 500 mg (metformin hydrochloride extended release tablets) tablet, film coated, extended release*, FDA, Editor. 2006: united states
12.Nafady, M.M., K.M. Attalla, and M.A. Sayed, *Formulation and evaluation of extended release metformin hydrochloride beads.* Int J Pharm Pharm Sci, 2014; 6: 433, 2013. **441**.
13.Singh, K. and M. Vasundhara, *Optimization of formulation parameters for controlled drug delivery from Metformin Hydrochloride loaded Chitosan/TPP microspheres.* The Pharma Innovation, 2015. **4**(9, Part A): p. 38.
14.Petrovick, G.F., *Type 2 diabetes mellitus and metformin hydrochloride usage: A short review.* J Pharmacol, 2018. **2**(2): p. 5.
15.Bodmeier, R., K.-H. Oh, and Y. Pramar, *Preparation and evaluation of drug-containing chitosan beads.* Drug development and industrial pharmacy, 1989. **15**(9): p. 1475-1494.
16.Priyadarshini, M.P., S Kumar, GPS Tamizh Mani, T, *Formulation and evaluation of Microspheres encapsulating Zidovudine by ionic gelation techniques.* Int J Res Pharm Nano Sci, 2014. **3**(5): p. 461-8.
17.Bergson Fogaça de Oliveira1, Maria Helena Andrade Santana[1] and Maria Inês Ré[2], *SPRAY-DRIED CHITOSAN MICROSPHERES CROSS-LINKED WITH D,LGLYCERALDEHYDE AS A POTENTIAL DRUG DELIVERY SYSTEM: PREPARATION AND CHARACTERIZATION*. Proceedings of the 14th International Drying Symposium, 2004. **vol. B**: p. 1166-1173.
18.Tilkan, M.G.Y. and N. Özdemir, *Investigation of the parameters affecting the release of flurbiprofen from chitosan microspheres.* Brazilian Journal of Pharmaceutical Sciences, 2017. **53**(4).

19.Kalpna, M.D., Druv Shahnaz, Mohammad Parkash, Jyoti Prasad, DN, *Preparation of controlled release metformin hydrochloride loaded chitosan microspheres and evalaution of formulation parameters.* Journal of Drug Delivery and Therapeutics, 2018. **8**(5-s): p. 378-387.
20.Dr. Lama Mohammad Al haushey, D.F.N.M., *A Study of Factors Influencing the Release of Caffeine from, New Pharmaceutical Dosage Forms (microparticles).* Tishreen University Journal for Research and Scientific Studies, 2012. **34**.
21.ahmad, S., *Preparing Of Alginate Microspheres Containing Amoxicilline Using Emulsification/ Enternal Gelation Method.* Tishreen University Journal for Research and Scientific Studies, 2012. **34 (2)**.
22.Rashmi C*1, A.J.N.J.a.T.M.T., *PREPARATION AND EVALUATION OF NEVIRAPINE-LOADED CHITOSAN NANOPARTICLES BY IONIC GELATION TECHNIQUE.* INTERNATIONAL JOURNAL OF PHARMACY AND PHARMACEUTICAL, 2017. **01(02)**: p. 07-15.
23.Li, M. and S. Zhan. *Preparation of 5-fluorouracil loaded chitosan microparticle and its drug release properties*. in *BIO Web of Conferences*. 2017. EDP Sciences.
24.Semalty, A., *Preparation and Evaluation of Chitosan Microsphere of Metformin Hydrochloride and to Study the Effect of Drug to Polymer Ratio.* Int J Pharm. Chem Sci, 2014. **3**(2): p. 316-20.
25.Dr. Wehad Ibrahim, D.F.M., Hussein Akel, *A Study of Some Factors Influencing Encapsulation Yield& Efficacy When Preparing Microcapsules By Complex Coacervation.* Tishreen University Journal for Research and Scientific Studies, 2016. **38**.
26.Roy S., P.S.G., Nandy B. C., Rai V. K., Tyagi L. K., Dey S., Meena K. C., *effect of method of preparation on chitosan microspheres of mefenamic acid* international journal of pharmaceutical sciences and drug research 2009. **1(1)**: p. 36-42.
27.Singal, A.K., *Release of Drug (Metformin)-Chitosan Microsphere.* International Journal of Recent Development in Engineering and Technology, 2015. **4**(6).
28.Sanjukta Duarah, K.P., Jyotirmoy Ghosh, and Dhanalekshmi Unnikrishnan, *Formulation and Evaluation of Metformin Engineered Polymeric Nanoparticles for Biomedical Purpose.* Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2015. **6(3)**: p. 1005.