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Original Article

EFFECT OF BIODEGRADABLE CO-POLYMERS AND DIVALENT CATIONS ON THE SUSTAINED RELEASE ABILITY OF PROPRANOLOL HYDROCHLORIDE LOADED BIOMATERIAL MICROSPHERES

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ABSTRACT

Objective: Propranolol Hydrochloride (PHCL) is used for the treatment of hypertension and angina pectoris; however it has two major problems; short biological half-life and low bioavailability, so the aim of the present work was to develop PHCL mucoadhesive microsphere to prolong the residence time at the absorption site, therefore, increase the bioavailability.

Methods: PHCL microspheres were prepared by ionotropic gelation method using nature polymers. Factorial design (3^3) was used to develop PHCL mucoadhesive microspheres, the independent factors used were polymer type (Sodium carboxymethyl cellulose (Na CMC), and Hydroxyl propyl methyl cellulose (HPMC), Carpobol 940), cross-linking type (calcium chloride, zinc chloride and barium chloride) and the concentration of Chitosan (0.5, 1, 1.5 %w/v). The developed microspheres were physicochemical characterized. The selected formula was selected for mucoadhesive test and *in vivo* study on human volunteers.

Results: The results revealed that the PHCL mucoadhesive microspheres have good flowability, the mean particle sizes ranged from 541 to 815 µm and the entrapment efficiency ranged from 35.6% to 69.53%. The selected PHCL microspheres showed spherical particles with a rough surface and exhibited a slow release over 8h. The pharmacokinetic data of selected PHCL microspheres showed prolonged T_{max}, decreased C_{max} and $AUC_{0-\infty}$ value of 926.21±40.74ng. h/ml indicating improved relative bioavailability by144.93% compared with marketed tablets.

Conclusion: PHCL microspheres were successfully prepared by ionic gelatin method that retards the release and enhances the oral bioavailability.

Keywords: Propranolol HCL, Microspheres, Ionic gelation method, Chitosan, Relative bioavailability

INTRODUCTION

PHCL is a non-selective β-adrenergic blocking agent used in the treatment of hypertension, angina pectoris and many other cardiovascular disorders [1]. A drug with short elimination half-life are most suitable for prolonged release dosage design, and it can be achieved by microspheres formulation which provides a larger surface area. It's also limiting fluctuate within a therapeutic range, reduction in side effects and dose frequency, hence improved patient compliance [2]. Conventional form available is administered orally in doses of 40 mg to 160 mg twice a day [3], but due to raising problems with the conventional tablet resulting in fluctuations of drug plasma levels and also the drug has short biological half-life (3-4 h) and first pass metabolism makes it a potential candidate for the design of sustained release dosage forms [4].

Alginates, an anionic, biodegradable and biocompatible natural polymer which are naturally occurring substances, found in brown algae have received much interest as a vehicle for sustained drug delivery [5]. Alginates can form hydrogels in the presence of divalent cations like Ca^{2+}through ionically cross-linked by the addition of divalent cations in aqueous solution[6]. Chemical and/or physical cross-linking of hydrophilic polymers are typical approaches to form hydrogels, and their physicochemical properties are highly dependent on the cross-linking type and cross-linking density, in addition to the molecular weight and chemical composition of the polymers. Here, we summarize various approaches to cross-link alginate chains to prepare gels, and how these approaches alter the hydrogel properties relevant to biomedical applications.

Chitosan, a polysaccharide derived from chitin by alkaline deacetylation, has been used to strengthen alginate microspheres based on the electrostatic interaction between carboxylate groups in alginate and ammonium groups in Chitosan. Chitosan–alginate complex erode slowly in phosphate buffer, and this behavior leads to inhibition of the initial release of drugs[7]. In this work, nature of the biodegradable polymers (Chitosan, Na CMC, Carpobol 940, HPMC and Sodium alginate) were used to prepare mucoadhesive microspheres.

MATERIALS AND METHODS

Materials

PHCL was generously gifted from Astra-Zeneca, Egypt. Sodium alginate, NaCMC, Carpobol 940 and HPMC (Sigma Chemical Co., St. Louis). Chitosan: Group corneal laboratories company. Calcium chloride, zinc chloride, barium chloride, and glacial acetic acid. All other chemicals were of analytical grade.

Method

Experimental design

Propranolol loaded microspheres were prepared using a (3^3) full factorial design. The design was applied to investigate the effect of the independent variables; chitosan concentration (X1), the type of crosslinking agent (X2), and polymer type (X3) on the physicochemical properties of the prepared microspheres (table 1).

Preparation of PHCL loaded microspheres

Drug-loaded microspheres were prepared using a modified ionic gelation method [8]. A 5% (w/v) sodium alginate solution was prepared in distilled water by properly mixing with slight heating. This concentration of sodium alginate remains constant in all formulations. To the alginate solution, an accurately weighed (100 mg) propranolol was dispersed and mixed. A 3% w/v aqueous solution of NaCMC, Carpobol 940 or HPMC was then added and uniformly mixed under magnetic stirrer to form

a smooth, viscous dispersion. The previous solution was then added dropwise using a hypodermic syringe (21 gauge needle) to the continuously stirred glacial acetic acid solution of chitosan and the divalent cross-linking agent. The microspheres were cured for 15 min and then collected, washed twice with acetone, and dried at room temperature (25±0.5 °C) for 24hours (fig. 1). The matrix of the 27 formulations design including responses such as yield percentage %, particle size (µm), entrapment efficiency, and percentage drug released after 8 h is shown in table 2.

Table 1: Composition of different coded values in 3^3 full factorial designs.

Independent variable	Code value -1 0+1		
Chitosan concentration w/v % (X1)	0.5	1	1.5
Type of cross-linking agent (X2)	Calcium chloride	barium chloride	zinc chloride
Polymer type (X3)	*HPMC	*Na CMC	Carpobol 940

*Na CMC (Sodium carboxymethyl cellulose), HPMC (Hydroxyl propyl methyl cellulose)

Characterization of the prepared microspheres

Percentage yield

The percentage yield was determined for the dried microspheres using the following equation[9]:

$$\%\text{Yield} = \frac{\text{Actual weight of microsphere}}{\text{Total weight of excipient and drug}} \times 100 \quad \text{(Equation1)}$$

Micrometric properties evaluation

Particle sizes analysis

The particle size of the Propranolol loaded microspheres was determined by optical microscopy fitted with a stage calibrated micrometer. About 50 particles from each formulation were examined, and the mean diameter was calculated.

Flow properties

Both bulk density and tapped density were determined. A quantity of 5 g of powder from each formula was introduced in 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall onto a hard surface. The tapping was continued until no further change in volume was observed [10].

The bulk density of the microspheres was calculated by using the following equation:

$$\text{bulk density} = \text{weight of powder/volume of packing} \quad \text{(Equation2)}$$

The tapped density was calculated by using the following equation:

$$\text{bulk density} = \text{weight of powder/tapped volume of packing} \quad \text{(Equation3)}$$

Hausner ratio and Carr's index were estimated using the below-mentioned equations;

$$\boldsymbol{Hausner\ ratio} = (\boldsymbol{tapped\ density/bulk\ density}) \quad \text{(Equation4)}$$

$$\text{Carr's inde} = (\text{tapped density} - \text{bulk density})/\text{tapped density} \times 100 \quad \text{(Equation5)}$$

The angle of repose of different formulations was measured according to fixed funnel flow method using the following equation[4].

$$\theta = \tan - 1\ \text{height/radius} \quad \text{(Equation6)}$$

Drug entrapment efficiency

The prepared drug-loaded microspheres were checked for their drug content. Accurately weighed 100 mg of the dried microspheres were powdered and dissolved in 100 ml phosphate buffer (pH 7.4). The solution was kept overnight and then filtered using 0.45 µm cellulose acetate syringe filter. The filtered solution was analyzed spectrophotometrically at 290 nm using UV-visible Spectrophotometer (UV-1800 Shimadzu, Japan). The entrapment efficiency was determined [11] using the following equation:

$$\text{Drug entrapment}\ (\%) = \frac{\text{actual drug concentration}}{\text{theoretical drug concentration}} \times 100 \quad \text{(Eqe... 7)}$$

***In vitro* drug release**

The *in vitro* release was determined in 900 ml 0.1N HCL (pH 1.2) for 2 h and then in 900 ml phosphate buffer (pH 7.4) using USP dissolution tester (apparatus I) at 50 rpm and a temperature of 37±0.5 °C. Capsules filled with microspheres containing an equivalent to 40 mg PHCL were tested, samples were taken over a period of 8 h and analyzed spectrophotometry at 290 nm [12].

Kinetic analysis of the release data

In order to analyzes the release mechanism, the obtained *in vitro* release data was fitted to various mathematical models such as zero order, First order, Higuchi diffusion, and Korsmeyer-Peppas model [13].

***In vitro* muco adhesivity**

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test [14]. A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (∽50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At hourly intervals up to 8hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following equation:

$$\%\ \text{Mucoadhesion} = (\text{no of microspheres remains/no of applied microspheres}) \times 100 \quad \text{(Equation 8)}$$

Fourier transform infra-red spectroscopy (FTIR)

FTIR of the selected best formula was studied to check possible drug-polymer incompatibilities. The FTIR spectra for propranolol, various polymers, and drug-loaded microspheres (F20) were obtained at room temperature in KBr pellets using FTIR spectrophotometer (Alpha-Bruker, Germany) in the transmission mode range 500-4000 cm^1 [15].

Scanning electron microsphere and morphological studies

The surface morphology of the microspheres (F20) was studied using scanning electron microscopy (SEM; S-4100, Hitachi, Japan). The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to on aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. Finally, samples were randomly scanned for particle size and surface morphology [14].

***In vivo* study**

A double-blind, randomized, cross-over study was carried out to compare the pharmacokinetic parameters of PHCL from the chosen microsphere formulation (F20) and the market product, Inderal ®, tablet (AstraZeneca, Egypt). A single dose containing an equivalent amount to 40 mg of PHCL was given to the volunteers, who were fastened overnight. The washout period was one week. The protocol of the study was conducted according to the requirements of the ethical committee of the Faculty of pharmacy, Beni-Suef University, Egypt.

Subject population

Six healthy Egyptian male volunteers aged between 25 and 35 y (median weight: 75±6.5 kg and median height: 173±6.9 cm) were chosen. The health status was confirmed by complete physical examination and laboratory analysis. No drugs were allowed to be taken one week before and during the study.

Sample collection

Blood samples (5 ml) were collected in heparin rinsed tubes at different time intervals: 0.5, 1, 1.5, 2, 4, 6, 10, 12, 18 and 24 h after oral administration. The samples were then centrifuged at 3000 rpm and the plasma was stored at-20 ˚C.

Chromatographic conditions

A modified method [16] was conducted for the analysis of PHCL in plasma using high-performance liquid chromatography (HPLC; Shimadzu, Koyoto, Japan). The mobile phase consisted of acetonitrile: aqueous acetic acid (1%containing 0.2% trimethylamine) (35:65, v/v), the pH adjusted at 3.6 with 0.1 N sulphoric acid. The flow rate was 1.5 ml min^{-1}, and the fluorescence detector was adjusted at an excitation wavelength of 230 nm and an emission wavelength of 340 nm.

Plasma analysis

1 ml of acetonitrile was added to 200 µl of plasma followed by vortex shaking and centrifugation at 2000 rpm. The clear supernatant was evaporated under a stream of nitrogen at 50-60 ˚C until dryness. The residue was dissolved in 100 µl of methanol and then injected into the HPLC for PHCL quantitation.

Pharmacokinetic study

The pharmacokinetic parameters from plasma data following the oral administration of the two formulae were estimated using WinNonlin, version 1.5 (Scientific Consulting, Inc., Cary, NCT). Maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), elimination half-life ($t_{½}$) and area under the plasma concentration-time curve (AUC) were determined. The level of absorption from selected microspheres formulation relative to the reference was calculated as the relative bioavailability by using the equation [17]:

$$\text{The relative bioavailability} = (\text{AUC test}/\text{AUC reference X100}) \quad \text{(Equation 9)}$$

T-test has been used to verify the differences in drug bioavailability between the selected formula and the reference. The level of statistical significance was chosen at $p<0.05$.

Statistical analysis

Statistical analysis was performed using three-way analysis of variance (ANOVA) test followed by Tukey-Kramer multiple comparisons test, by applying statistical package for the social sciences (SPSS; version 19.0) computer software program (SPSS Inc., Chicago, IL, USA), with the value of $p<0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Preparation and characterization of PHCL loaded microspheres

The chosen concentration of the alginate, polymers, and the cross-linking agent were optimized by trial and error method. Ramalingam Nethaji *et al.* formulated PHCL microspheres by ionic gelation, PHCL were mixed with NaCMC and different ratios of sodium alginate, using calcium chloride 5% as cross-linking agent [2]. Nazia Khanam *et al.* formulated PHCL microspheres by ionic gelation method using various ratios of sodium alginate and Eudragit RS100 and using calcium chloride 5% as cross-linking agent [8].

In this research, PHCL microspheres were prepared using three different divalent cations, calcium, barium and zinc as cross-linking agents, with chitosan added to them. Chemical cross-linking of hydrophilic polymers is a typical approach to form microspheres, and the physicochemical properties of the prepared microspheres are highly dependent on the cross-linking type, as well as the molecular weight and chemical composition of the used polymers. fig. 1, illustrated an example of the prepared microspheres before and after the drying process.

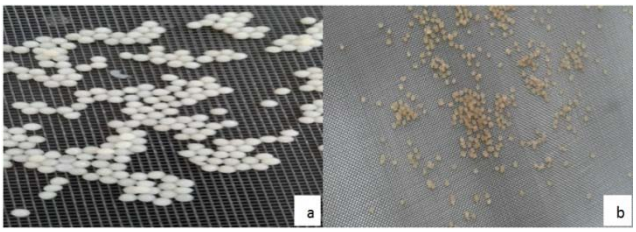

Fig. 1: The prepared microspheres before drying (a) and after drying (b)

The percentage yield of PHCL microspheres was found to range from 46% to 65%. There was no significant difference at $p<0.05$ in the percentage yield between the prepared formulations and hence no effect of the independent variables on this parameter.

The particle sizes of microspheres were illustrated in table 2 and ranged from 541 µm to 815 µm. Statistical analysis of the particle size data (table 4), showed that the variation of microspheres size may be attributed to the change in the co-polymer type. There was a significant increase in the particle size of the PHCL spheres prepared with Na CMC. Regarding the type of cross-linking agent, the use of calcium chloride resulted in a significant decrease in particle size. Calcium chloride is one of the most frequently used materials for alginate crosslinking. However, it could lead to rapid and poorly controlled gelation due to its high solubility in aqueous solutions and hence small size microspheres in comparison with other cross-linking agents [18]. The increase in the size of the microspheres was obviously significant above 0.5% chitosan concentration which may be attributed to the increase in the viscosity of the gelation medium [19].

The bulk and tapped densities were measured to evaluate the packability of microspheres. Meanwhile, Hausner's ratio and Carr's index give indication about flowability. Carr's index greater than 25 indicating poor flowability and below15 indicating good flowability. All formulation exhibited Carr's index below 15 which is considered as an indication of good flow properties angle of repose ranged from 26.65 to 34.95 as shown in table 3.

The percentage drug entrapment efficiency of the prepared microspheres was found to be in the range of 35.6% to 69.53% as shown in table 2. The co-polymers could be arranged according to their ability to encapsulate the drug in the following order: Carpobol 940>HPMC>Na CMC (table 4). These results may be attributed to decreased diffusion of the drug from the microspheres by increased viscosity of the polymer used [20].

The statistical analysis of the encapsulation efficiency data revealed that there was the insignificant effect on this parameter by the change of the type of cross-linking agent as well as the increase in the concentration of chitosan (table 4).

Regarding the drug release from the prepared microspheres, alginate is able to form complex with divalent ions like Zn^{+2}, Ba^{+2}, Ca^{+2} and the association is stronger with calcium than other ions [21]. The formulations containing Ca+2 showed a slower release of PHCL as calcium is more densely cross-linked with alginate than Zn^{+2}, Ba^{+2}. This could be attributed to the increased interaction between Na+present in alginate and Ca^{+2}, forming closer network which decreased the diffusion of drug outwards the alginate microspheres [15]. However, there was insignificant effect between the three cations and the concentration of chitosan on the total amount released after 8 h as shown in (fig. 2).

Formulations containing Na CMC showed faster release of the drug than HPMC and Carpobol 940, which may be due to high swelling action and increased solubility of Na CMC in the dissolution medium that may lead to increase the transport of the drug outside the matrix [14].

Table 2: 3^3 full factorial design of PHCL loaded microspheres with responses

Formulation code	X1	X2	X3	Percentage yield (±SD)	Particle size (µm) (±SD)	Percentage drug entrapment Efficiency (±SD)	Percentage drug release after 8 h (±SD)
M1	-1	-1	-1	64.01±0.17	560±0.05	63.80±0.20	83.14±0.02
M2	-1	0	-1	52.21±0.11	630±0.14	59.30±0.50	85.25±0.03
M3	-1	+1	-1	46.02±0.01	655±0.10	48.93±0.06	91±0.06
M4	0	-1	-1	59.62±0.03	584±0.04	52.50±0.13	81.33±0.03
M5	0	0	-1	61.15±0.17	659±0.08	48.90±0.30	90±0.05
M6	0	+1	-1	62.41±0.05	701±0.05	50.00±0.26	96±0.03
M7	+1	-1	-1	64.01±0.11	591±0.03	52.14±0.03	85.23±0.02
M8	+1	0	-1	61.97±0.29	679±0.04	48.48±0.03	95.78±0.05
M9	+1	+1	-1	62.55±0.47	720±0.07	40.6±0.09	88±0.05
M10	-1	-1	0	55.36±0.27	640±0.04	50.83±0.04	90±0.02
M11	-1	0	0	56.45±0.23	710±0.01	45.03±0.05	92.25±0.06
M12	-1	+1	0	53.97±0.12	740±0.03	35.6±0.02	98.67±0.06
M13	0	-1	0	50.21±0.23	735±0.06	37.84±0.02	95.33±0.04
M14	0	0	0	52.88±0.24	780±0.08	38.96±0.03	91.2±0.02
M15	0	+1	0	65.32±0.19	803±0.09	37.49±0.03	97.2±0.03
M16	+1	-1	0	59.92±0.26	730±0.12	50.00±0.02	93.82±0.02
M17	+1	0	0	58.1±0.18	780±0.06	45.90±0.03	95.78±0.03
M18	+1	+1	0	53.45±0.20	815±0.02	45.50±0.02	94±0.09
M19	-1	-1	+1	64.01±0.14	541±0.09	69.53±0.02	72.33±0.03
M20	-1	0	+1	60.43±0.29	595±0.09	66.12±0.02	61.34±0.04
M21	-1	+1	+1	48,09±0.05	602±0.07	59.42±0.03	79.93±0.03
M22	0	-1	+1	56.29±0.22	590±0.06	57.25±0.03	80.43±0.03
M23	0	0	+1	59.97±0.19	622±0.02	43.40±0.01	66.34±0.04
M24	0	+1	+1	56.53±0.40	640±0.09	42.30±0.02	81.57±0.05
M25	+1	-1	+1	64.28±0.27	612±0.12	65.80±0.02	79.89±0.04
M26	+1	0	+1	55.37±0.09	685±0.04	54.50±0.02	81.09±0.05
M27	+1	+1	+1	56.29±0.26	705±0.06	50.80±0.02	83.94±0.04

*(mean±SD, n=3)

Table 3: The micrometric properties of the prepared PHCL microspheres

Formulation code	Bulk density (±SD*)	Tapped Density (±SD*)	Carr's Index (%)	Hausner's ratio	Angle of repose
M1	0.734±0.12	0.801±0.05	8.36	1.09	27.95±2.11
M2	0.721±0.11	0.812±0.03	11.20	1.12	30.45±1.97
M3	0.792±0.03	0.887±0.08	10. 71	1.13	29.36±3.01
M4	0.751±0.09	0.850±0.10	11.64	1.13	31.75±2.67
M5	0.742±0.79	0.866±0.09	14.34	1.16	33.84±1.18
M6	0.722±0.11	0.852±0.08	15.25	1.18	34.95±3.64
M7	0.733±0.13	0.821±0.12	10.71	1.12	29.46±2.54
M8	0.698±0.21	0.800±0.09	12.75	1.14	31.82±3.17
M9	0.870±0.06	0.997±0.13	12.73	1.14	26.81±2.58
M10	0.725±0.03	0.789±0.04	8.11	1.08	23.52±1.77
M11	0.756±0.11	0.862±0.06	12.29	1.14	27.92±3.05
M12	0.820±0.15	0.932±0.08	12.01	1.13	33.61±2.66
M13	0.744±0.13	0.825±0.13	9.81	1.10	27.51±1.45
M14	0.724±0.17	0.867±0.11	16.49	1.19	35.76±4.21
M15	0.755±0.07	0.888±0.06	14.97	1.17	33.99±1.79
M16	0.734±0.12	0.845±0.03	13.13	1.15	26.98±1.98
M17	0.777±0.25	0.856±0.11	9.22	1.10	27.85±2.47
M18	0.754±0.09	0.844±0.05	10.66	1.11	29.40±1.69
M19	0.775±0.13	0.850±0.08	8.8	1.09	28.10±2.07
M20	0.740±0.05	0.821±0.06	9.86	1.10	26.65±1.21
M21	0.735±0.14	0.833±0.03	11.76	1.13	30.78±1.58
M22	0.723±0.05	0.802±0.11	9.85	1.10	27.33±3.66
M23	0.775±0.12	0.887±0.12	12.62	1.14	32.43±2.90
M24	0.730±0.08	0.845±0.09	13.60	1.15	33.25±1.55
M25	0.744±0.09	0.821±0.07	9.37	1.10	28.71±4.22
M26	0.740±0.13	0.823±0.06	9.82	1.11	27.99±3.62
M27	0.723±0.12	0.845±0.13	13.37	1.16	27.31±2.08

Each sample was analyzed in triplicate (n = 3) *mean±SD

Table 4: Statistical analysis for the effect of independent variables on the microspheres characteristics

Factors Independent variables		Percentage yield%	Particle size	% Drug entrapment efficiency	Release% over 6 h
Polymer type	HPMC	59.32±2.04	642.11±18.34$^{(ab)}$	51.62±2.22	88.31±1.74$^{(ac)}$
	Na CMC	56.32±2.04	748.11±1.79$^{(abc)}$	43.01±1.88$^{(bc)}$	94.25±0.93$^{(bc)}$
	Carpobol 940	57.91±1.66	621.33±16.66$^{(bc)}$	56.56±3.26$^{(bc)}$	76.31±2.61$^{(abc)}$
Cross linking type	Ca^{+2}	58.49±1.48	637.44±24.56$^{(def)}$	53.98±3.66	83.76±3.79
	Ba^{+2}	56.42±2.15	698.33±21.88$^{(de)}$	46.95±2.55	86.60±3.35
	Zn^{+2}	58.51±1.61	675.77±27.80$^{(df)}$	50.27±2.8	88.61±2.12
Chitosan concentration	0.5	59.74±1.65	620.33±23.17$^{(ghi)}$	55.52±3.24	87.11±2.6
	1	57.61±1.19	682.22±21.86$^{(gh)}$	50.06±2.23	85.05±3.7
	1.5	56.07±2.18	709.00±23.76$^{(gi)}$	45.62±2.5	86.80±3.2

Each value represents the mean of each factor±standard error of the mean (SE), Statistical analysis was determined using ANOVA test followed by Tukey-Kramer multiple comparisons test. [a]HPMC Significantly different at $p<0.05$, [b]CMC Significantly different at $p<0.05$, [c]Carpobol 940 Significantly different at $p<0.05$, Ca^{+2} Significantly different at $p<0.05$, [e]Ba^{+2} Significantly different at $p<0.05$, [f]Zn^{+2} Significantly different at $p<0.05$, [g]chitosan o.5% Significantly different at $p<0.05$, [h]chitosan o.5% Significantly different at $p<0.05$, [i]chitosan o.5% Significantly different at $p<0.05$.

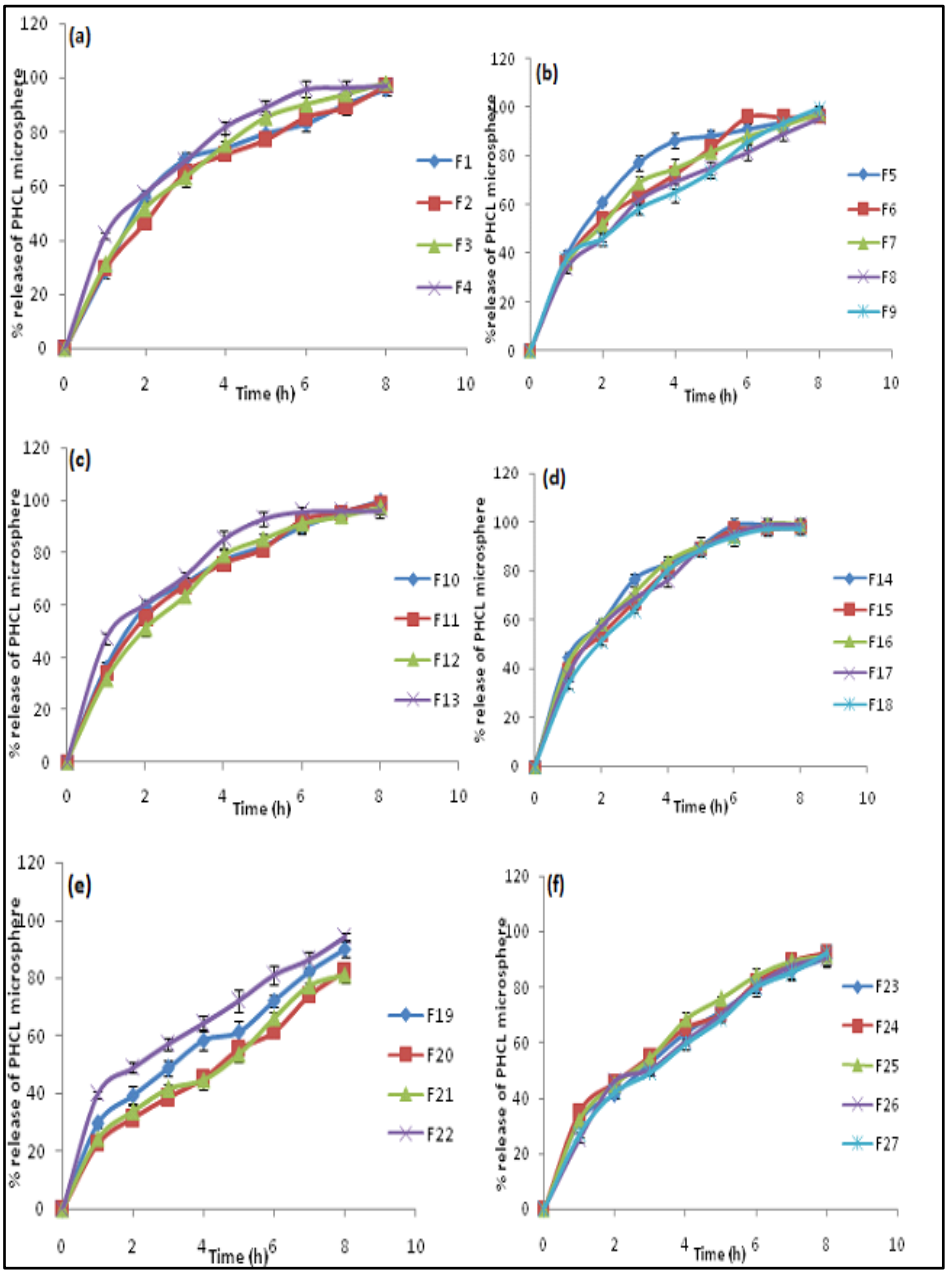

Fig. 2: Comparative *in vitro drug* release of PHCL microspheres where, (a) and (b) are formula from F1 to F9 containing HPMC, (C) and (d) are formula from F10 to F18 containing Na CMC and (e) and (f) are formula from F19 to F27 containing Carpobol 940

The coefficient of variation (R^2) was used as an indicator of the best fitting for each model. The release pattern followed Higuchi diffusion with R^2 = 0.988; this could be attributed to the high charge density of chitosan at the dissolution medium resulting in the formation of the much stronger ionic network [22]. Formulation F20 containing; Carpobol 940, $CaCl_2$, and 1% Chitosan showed the highest sustained release and was chosen for further investigations.

FTIR studies

The FTIR spectrum of pure PHCL, pure polymers, and formulations are shown in (fig. 3). The spectra showed band assignments at the same wavelength ranges indicating no interaction between the drug and the polymers.

***In vitro* mucoadhesivity**

The selected formula (F20) exhibited good mucoadhesive property in the *in vitro* mucoadhesion test. It exhibited % mucoadhesion 70 % over 8 h.

Scanning electron microsphere and morphological studies

The morphology and surface of PHCL loaded microspheres were studied by SEM. A SEM photograph of the formulation (F20), a single microsphere taken at 45X magnification, was shown in Fig. 4, the drug loaded alginate microspheres were almost of spherical in shape and have a rough surface. Microsphere taken at 800X magnification was shown spherical in shape with porous.

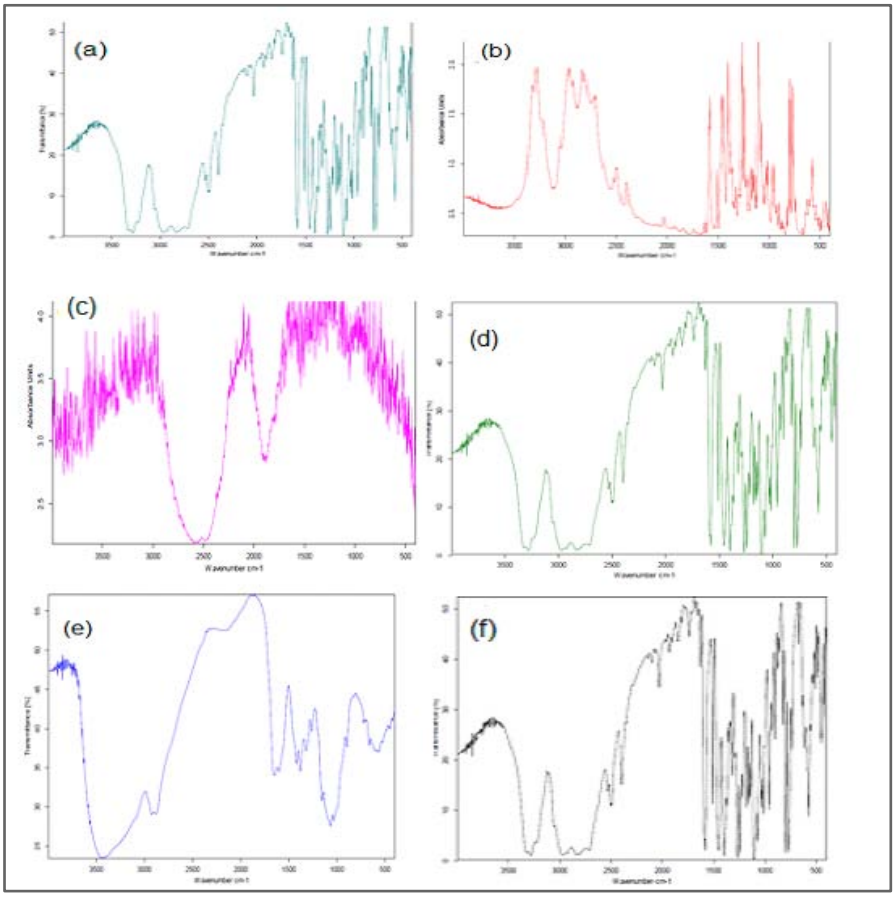

Fig. 3: FT-IR spectra of a) pure drug, b) Carpobol 940, c) $cacl_2$, d) Chitosan, e) sodium alginate, f) PHCL microspheres (F20)

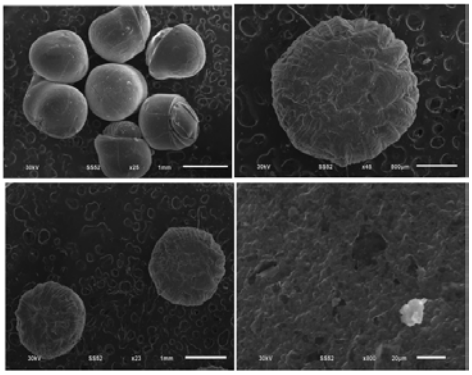

Fig. 4: Scanning electron microscopy (SEM), of (PHCL) microspheres for selected formula (F20) at different magnifications

***In vivo* study on human volunteers**

From the physiochemical characterizations and statistical analysesF20 was selected for the bioavailability compared with commercial tablets (Inderal® AstraZeneca). The pharmacokinetics parameters studied (Inderal® tablet and PHCL microspheres) show t_{max} of the prepared microspheres 4.333 h was significant (p<0.05) high comparing with1.75h of standard, which could be attributed to the delay of absorption from PHCL microspheres through release PHCL microsphere on control manner that increase mean residence time and delay T_{max}.

The plasma concentration of PHCL microspheres and Inderal® is shown in table 5. The mean pharmacokinetic parameters, C_{max}, T_{max}, and $AUC_{0-\infty}$ is show in (table 5). The value of $AUC_{0-\infty}$ is 634.66±18.91 (ng. h/ml) and C_{max} 75.635±2.33 (ng/ml) of the Inderal® while the PHCL microspheres have $Auc_{0-\infty}$ 926.21±40.74 (ng. h/ml) and C_{max} 61.94±1.83 (ng/ml). Differences between group means were considered significant at p<0.05.

Table 5: Pharmacokinetics parameters of Inderal® tablets and selected PHCL microsphere

Pharmacokinetic parameters	Formula	
	Inderal® tablet	Microspheres (F20)
C_{max} (ng/ml)	75.635±2.33	61.94±1.83[a]
T_{max}(h)	1.75±0.11	4.33±0.33[a]
AUC_{0-24}(ng. h/ml)	634.93±18.98	920.26±38.93[a]
$Auc_{0-\infty}$(ng. h/ml)	634.66±18.91	926.21±40.74[a]
%Relative Bioavailability	144.93%	
$t_{½}$(h)	6.5±0.5	7.99±0.28[a]
MRT_{0-24}(h)	8.80±0.39	13.24±0.22[a]
$MRT_{0-\infty}$(h)	8.79±0.39	13.37±0.192[a]

Each value represents the mean of 6 human±standard error of the mean. Statistical analysis was determined using by T test followed by Tukey-Kramer multiple comparisons test.

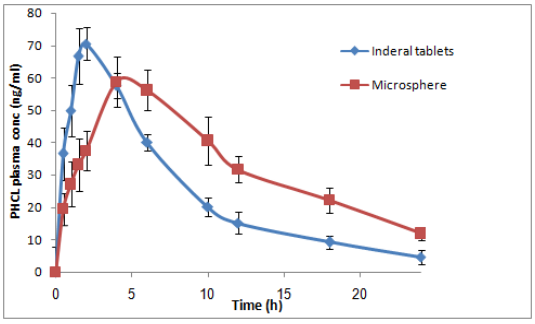

Fig. 5: Plasma concentration of PHCL following the oral administration of 40 mg of Inderal® tablets and PHCL microspheres equivalent to 40 mg to human volunteers

CONCLUSION

This study reveals that the sustained release of PHCL can be successfully achieved by ionotropic gelation technique using sodium alginate as a polymer. Prepared microspheres shown higher drug entrapment efficiency and prolonged release characteristics. PHCL from microspheres was influenced by different alginate concentrations. F20 was estimated as best formulation because this formulation drug release was observed that drug was released in a controlled manner.

CONFLICT OF INTERESTS

Declared none

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