Bull. Pharm. Sci., Assiut University, Vol. 32, Part 1, 2009, pp. 45-64.

FORMULATION AND EVALUATION OF A BUCCOADHESIVE CAPTOPRIL TABLETS

Mohammed H. El-Shabouri, Hamdy M. Abd El-Aleem, Osama A. Soliman and Marwa S. El-Dahhan

Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

تناول هذا البحث تحضير أقراص لها خاصية الالتصاق بالتجويف الفمى محتوية على الكابتوبريل بطريقة الكبس المباشر وذلك باستخدام بوليمرات مختلفة لها خاصية الالتصاق الحيوى مثل الكاربوبول ٩٣٤ ، الأيدراجت أر أس ١٠٠، الكيتوزان، الهيدروكسى بروبيل ميثيل سيليلوز ٤٠٠٠ وعديد فينيل بيروليدون ك ٣٠ بنسب مختلفة. تم تقييم الاقراص المصاغة من حيث تجانس محتواها للعقار، تجانس الوزن، الهشاشة ، الصلابة ، معامل الانتفاخ ، الاس الهيدروجينى السطحى للأقراص، قوة الالتصاق الحيوى وكذلك الانطلاق المعملى للعقار. كما تمت دراسة التوافر الحيوى للعقار من صواغين مختلفين تم إختيارهما فى ضوء الخواص الفيزيائية ومعدل الانطلاق المعملى للعقار من حيث امتداد المفعول وهما خليط العقار مع الكاربوبول ٩٣٤ مع هيدروكسى بروبيل ميثيل السيليلوز ٤٠٠٠ بنسبة ٤:٦ والكيتوزان مع هيدروكسى بروبيل ميثيل السيليلوز ٤٠٠٠ بنسبة ٤:٦.

وقد أظهرت النتائج أن قوة الالتصاق الحيوى وخواص الانطلاق المعملى للعقار تعتمد على نوع البوليمر المستخدم ونسبته وأيضا مكونات الأقراص. كما بينت النتائج أن زيادة نسبة الكاربوبول ٩٣٤ والكيتوزان فى الأقراص يؤدى إلى زيادة معامل الانتفاخ وقوة الالتصاق الحيوى. بالإضافة إلى التوافر الحيوى لأقراص الكابتوبريل المحتوية على الكاربوبول ٩٣٤ مع هيدروكسى بروبيل ميثيل السيليلوز ٤٠٠٠ بنسبة ٤:٦ كانت أفضل من الكيتوزان مع هيدروكسى بروبيل ميثيل السيليلوز ٤٠٠٠ بنسبة ٤:٦ والأقراص الحاكمة وذلك فى ضوء زيادة تركيز العقار فى بلازما الدم (C_{max}) ، الوقت اللازم لوصول أعلى تركيز فى الدم (T_{max}) بالإضافة إلى المساحة تحت منحنى الوقت - تركيز العقار فى بلازما الدم (AUC_{0-8}).

Buccoadhesive tablets of captopril were prepared by direct compression of the drug with different polymers; Carbopol 934 (CP 934), Eudragit RS 100 (EU RS 100), Chitosan (Ch), Hydroxpropyl methylcellulose (HPMC) and Polyvinylpyrrolidone K_{30} (PVP K_{30}) either singly or in blends of different ratios. The tablets were evaluated for their weight variation, drug content uniformity, friability, hardness, swelling index, surface pH, in-vitro

Received in 25/11/2008, Received in revised form in 2/3/2009 & Accepted in 3/3/2009

bioadhesive strength and release characteristics. The bioavailability and the pharmacokinetics parameters of captopril from two selected formulations (CP 934:HPMC 6:4 and Ch:HPMC 6:4) were evaluated.

The in-vitro bioadhesive strength and release characteristics were found to be a function of the type of polymer and ratio of polymer blends. Swelling and bioadhesive characteristics were determined for both plain and medicated tablets. The high concentration of carbopol and chitosan containing formulations showed the greatest adhesive strength. The mean pharmacokinetic parameters of captopril after buccoadhesive tablet administration were: C_{max} 506.9 ng/ml, T_{max} 4 hr, AUC_{0-8} 2359.5 ng.hr/ml for CP 934: HPMC (6:4), while C_{max} 429.02 ng/ml, T_{max} 2.67 hr, AUC_{0-8} 1637.43 ng.hr/ml for Chitosan: HPMC (6:4). In comparison, in case of oral administration of control tablet the C_{max} 591.28 ng/ml, T_{max} 1.5 hr, AUC_{0-8} 1869.29 ng.hr/ml.

INTRODUCTION

Captopril, (1-[(2S)-3-mercapto-2-methyl propionyl]-L-proline), an orally active inhibitor of angiotensin-converting enzyme (ACE)[1&2], has been widely used for the treatment of hypertension and congestive heart failure. The drug is considered as a drug of choice in antihypertensive therapy due to its effectiveness and low toxicity[3-6].

Captopril is freely water soluble and has a relatively short elimination half life (1.7 hr) in plasma after an oral dose[7]. Food may decrease oral absorption of captopril by up to 25–40%[8]. Captopril is usually prescribed to patients who are chronically ill and require long-term treatment for its therapeutic benefit. Development of a once daily captopril oral formulation would be a significant advantage for patient compliance which is accompanied by minimization of the drug side effects as a result of reduction of the drug blood concentration fluctuations especially in long-term therapy[9-11].

Mucoadhesive drug delivery system is a new system of drug delivery and has recently gained great concern in pharmaceutics[12]. The most important goals in mucoadhesion consist of drug targeting, controlled and sustained releasing, increasing of residence time, decreasing of adverse effects and minimizing of the first-pass effect and long-term drug delivery[13&14]. The buccal mucosa has been investigated for local and systemic delivery of therapeutic peptides and other drugs that subjected to first-pass metabolism or are unstable within the rest of the gastrointestinal tract[15].

Mucosal-adhesive polymers are hydrophilic macromolecules contain-

ing numerous hydrogen bond-forming groups[16]. Bioadhesive polymers not only cause the adhesion effects, but also control the release rate of drug[17]. Also, different blends of two or more adhesive polymers may be used as mucoadhesive systems[18].

Water soluble drugs are considered difficult to deliver in the form of sustained or controlled release preparation due to their susceptibility to "dose dumping phenomenon". Attempts have been made to regulate their release process by using of mucoadhesive polymers in order to achieve a once-a-day dose treatment[19].

The short half-life and sever first pass metabolism of captopril makes it suitable for administration via a buccal delivery system that provides controlled drug delivery, by passing first-pass effect. Successful buccal delivery requires at least three of the following: (a) a bioadhesive to retain the drug in the oral cavity and maximize the intimacy of contact with the mucosa; (b) a vehicle that releases the drugs at an appropriate rate under the conditions prevailing in the mouth; and (c) strategies for overcoming the low permeability of the oral mucosa[20].

The objective of the present study was to formulate captopril buccoadhesive tablets. The prepared tablets were evaluated for their physical properties and *in-vitro* release characteristics. The *in-vitro* bioadhesive strength, swelling behavior and surface pH of the tablets were also considered. In addition, the bioavailability and the pharmacokinetic parameters of captopril from two selected formulations on the basis of good physical properties regarding bioadhesive strength and acceptable sustained-release profiles were studied.

EXPERIMENTAL

Material

Captopril (CP), was obtained from Chang Zhou Pharmaceutical Company (Chang Zhou, China). Hydroxpropylmethyl cellulose 4000 cp (HPMC) was provided by Colorcon Company (Shanghai, China). Polyvinylpyrrolidone K_{30} (PVP K_{30}) was obtained from BASF (Ludwigshafen, Germany). Chitosan (Ch), high molecular weight was supplied from Sigma-Aldrich chemie GmbH Chemical Co. Germany. Carbopol 934 (CP 934) was obtained from BF Goodrich, Hounslow, U. K. Eudragit RS 100 (Eu RS 100) and spray-dried lactose (Zeparoxe), were kindly provided by Röhm Pharma (Darmstadt, Germany). Magnesium stearate was supplied from European Egyptian Pharmaceutical Industries, Egypt. D(-) mannitol, potassium dihydrogen orthophosphate, EDTA, acetic acid and sodium dibasic phosphate were kindly supplied by Adwic, El Nasr Pharmaceutical Chemicals, Egypt. P-bromophenacyl bromide (p-BPB) and Diazepam were supplied from Sigma Chemical Company, St Louis, USA. Acetonitrile for HPLC was supplied from Sigma-Aldrich Chemie GmbH Chemical Co., Germany. Double

distilled water has been used in HPLC assay. All other chemicals were of an analytical grade and were used without further purification.

Apparatus

Single punch tablet machine, tablet hardness tester, Roche friabilitor, and tablet disintegration tester (Erweka-Apparatus, GmbH, E. K. O., Germany). Six jars dissolution apparatus, DA-6D, India. Ultra violet-visible spectrophotometer, Jasco, V-530, Japan. Millipore filter adaptor with 10 mm diameter and membrane filter with 0.45 µm pore size, Berlin, Germany. High performance liquid chromatography (HPLC) consisted of series 200 LC pump, side winder column heater, series 200 vacuum degasser, 600 series link and series 200 UV/visible detector, Perkin Elmer, U.S.A. Reversed-phase column containing Linchrosorb Rp 18-5, dimensions 4.6 x 250 mm and 5 µm particle size, Charomatographie Technik GmbH., VDS Optilab, Berlin, Germany.

Methods

Preparation of buccoadhesive tablets

Captopril bioadhesive tablets (160 mg) were prepared by mixing the drug (50 mg) with 100 mg of each of the selected polymers or their mixtures as mentioned in Table 1. Each tablet contains 1% magnesium stearate, 1% mannitol and spray dried lactose and then compressed directly on a 9-mm flat-faced punches and die using the single punch tablet press. Control tablets (160 mg) containing 50 mg drug, 1% magnesium stearate, 1% mannitol and spray dried lactose were prepared in a similar manner as mentioned above.

Evaluation of tablets

Physical characteristics

The prepared tablets were evaluated for their uniformity of weight, thickness, hardness, friability and drug content uniformity (USP XXIII).

***In-vitro* drug release**

In-vitro release studies of captopril were performed in 500 ml of phosphate buffer solution (pH 6.6) at 37±0.5°C using the USP XXVII dissolution apparatus 2 (paddle). The rotational speed of the paddles was set at 50 rpm throughout the dissolution studies. Each tablet was inserted in a metal die having a central hole which was sealed at the lower end with paraffin wax, so that, the drug could be released from one surface of tablet[21]. Samples of 1 ml were withdrawn at different time intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5 and 6 hrs), diluted with 0.1 N sodium hydroxide and then analyzed spectrophotometrically at 230 nm. The same volume of buffer solution of pH 6.6 was added to the dissolution medium after each sampling. Blank experiments were carried out using non-medicated tablets and served as control. The obtained data are the mean of the release of three tablets.

Table 1: Compositions of prepared buccoadhesive captapril tablet formulae.

Formulae	Formulae Code	The amunt of ingredients in each tablet (mg)									
		Drug	CP 934	Chitosan	Eu RS 100	PVP K_{30}	HPMC	Mg stearate 1%	Spray dried Lactose	Mannitol 1%	Tatal weight
Control	CT	50						1.6	106.8	1.6	160
HPMC	H	50					100	1.6	6.8	1.6	160
CP 934	C	50	100					1.6	6.8	1.6	160
Chitosan	K	50		100				1.6	6.8	1.6	160
Eu RS 100	E	50			100			1.6	6.8	1.6	160
PVP K_{30}	P	50				100		1.6	6.8	1.6	160
CP 934:HPMC (2:8)	CH 1	50	20				80	1.6	6.8	1.6	160
CP 934:HPMC (4:6)	CH 2	50	40				60	1.6	6.8	1.6	160
CP 934:HPMC (6:4)	CH 3	50	60				40	1.6	6.8	1.6	160
CP 934:HPMC (8:2)	CH 4	50	80				20	1.6	6.8	1.6	160
Chitosan:HPMC (2:8)	KH 1	50		20			80	1.6	6.8	1.6	160
Chitosan:HPMC (4:6)	KH 2	50		40			60	1.6	6.8	1.6	160
Chitosan:HPMC (6:4)	KH 3	50		60			40	1.6	6.8	1.6	160
Chitosan:HPMC (8:2)	KH 4	50		80			20	1.6	6.8	1.6	160
Eu RS 100:HPMC (2:8)	EH 1	50			20		80	1.6	6.8	1.6	160
Eu RS 100:HPMC (4:6)	EH 2	50			40		60	1.6	6.8	1.6	160
Eu RS 100:HPMC (6:4)	EH 3	50			60		40	1.6	6.8	1.6	160
Eu RS 100:HPMC (8:2)	EH 4	50			80		20	1.6	6.8	1.6	160
PVP K_{30}: HPMC (2:8)	PH 1	50				20	80	1.6	6.8	1.6	160
PVP K_{30}: HPMC (4:6)	PH 2	50				40	60	1.6	6.8	1.6	160
PVP K_{30}: HPMC (6:4)	PH 3	50				60	40	1.6	6.8	1.6	160
PVP K_{30}: HPMC (2:8)	PH 4	50				80	20	1.6	6.8	1.6	160

Swelling studies of buccoadhesive tablets

Three tablets of each formulation were separately placed in a series of baskets made of stainless steel mesh. Each set (the tablet and the basket) was accurately weighed before being vertically placed in a beaker containing 40 ml phosphate buffer of pH 6.6 at 37°C. At time intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 hrs), excess fluids were removed; the set containing the swollen tablet was weighed. The weight of the swollen tablet was calculated. The swelling index (S.I.) was determined from the following relation[22]:

$$S.I. = (W_t - W_o)/ W_o$$

Where,

W_t The weight of swollen tablet at each time interval t

W_o The initial weight of each tablet.

Surface pH of the tablets

The surface pH of tablets was determined to evaluate the possible irritative effects of the formulation on the buccal mucosa. The tablets were left to swell for 2 hrs in 2 ml of distilled water; after this time the surface pH was measured by placing the electrode in contact with the surface of tablets[23].

***In-vitro* bioadhesion test**

The tensile strength required to detach the bioadhesive tablet from the mucosal surface, obtained from rabbit intestine, was applied as a measure for the bioadhesive performance[24&25]. The apparatus is locally assembled and is composed mainly of a modified two-arm balance (Fig. 1). A piece of 0.636 cm^2 surface area of rabbit intestinal mucosa was fixed to the stainless steel piece with cynoacrylate adhesive and then placed in a beaker. Saline solution was added into the beaker up to the upper surface of intestinal mucosa to maintain intestinal mucosal viability during the experiments.

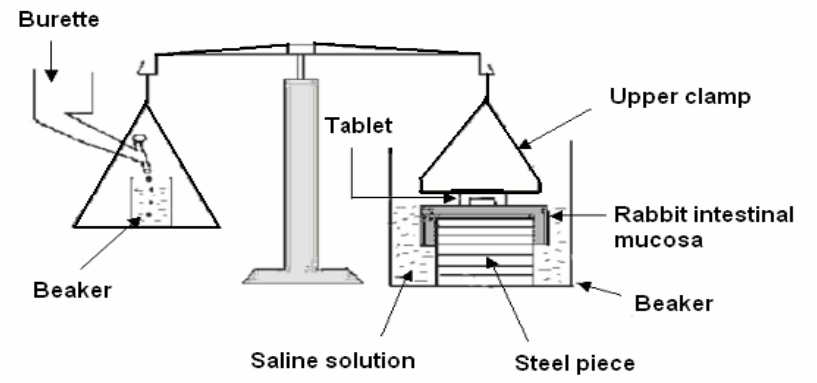

Fig. 1: Modified apparatus for *in-vitro* bioadhesion test.

The tablet was attached to the upper clamp of the apparatus and then the beaker was raised slowly until contact between rabbit intestinal mucosa and tablet was established. A preload of 50 gm was placed on the clamp for 5 min. (preload time) to establish adhesion bonding between tablet and rabbit intestinal mucosa. The preload and preload time were kept constant for all the formulations. After completion of the preload time, preload was removed from the clamp, and water was then added into the beaker from the burette at a constant rate. The addition of water was stopped when tablet was detached from either rabbit intestinal mucosa. The weight of water required to detach tablet from intestinal mucosa was measured and expressed as weight (gm). This experiment was repeated, with fresh mucosa, three times and the mean was considered[26].

The bioadhesion force was calculated per unit area of the tablet as follows:

$$F = (W.\ g)/A$$

where,

F: is the bioadhesion force ($dyne.cm^{-2}$)

W: is the minimum weight required to break the bioadhesive bond (gm)

g: is the acceleration due to gravity ($cm.sec^{-2}$) = 981 $cm.sec^{-2}$

A: is the surface area of the tablet (cm^2) = ($\pi\ r^2$) =22/7 x $(0.45)^2$ = 0.636 cm^2

Determination of the *in-vitro* residence time

The *in-vitro* residence time was determined using a locally modified USP disintegration apparatus (Fig. 2). The disintegration medium was composed of 800 ml phosphate buffer solution (pH 6.6) maintained at 37±0.5°C. A segment of rabbit intestinal mucosa, 5 cm long, was glued to the surface of a glass slab, vertically attached to the apparatus. The buccoadhesive tablet was hydrated from one surface using 15 µl buffer and then the hydrated surface was brought into contact with mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that it was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the tablet from the mucosal surface was recorded. This experiment was repeated, with fresh mucosa, three times and the mean was considered[22].

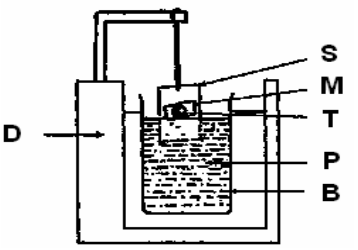

Fig. 2: Schematic diagram of the apparatus used for the *in-vitro* determination of residence time.

where,

(S) glass slab

(D) disintegration apparatus

(B) glass beaker

(M) mucosal membrane

(T) mucoadhesive tablet

(P) phosphate buffer pH 6.6

Bioavailability study

For this study, two formulations were selected in comparison with the control. The two selected formulae are CH 3, KH 3 (Table 1) on the basis of their acceptable physical characteristics, good bioadhesive force and sustained release profiles.

In this study, three groups (6 each) of albino rabbits weighing 2.0-2.5 kg were selected. The rabbits were fasted for 24 hr before drug administration and anaesthetized with secobarbital (25 mg/kg). A buccal adhesive tablet containing 25 mg captopril was attached on the cheek pouch of each rabbit. For control tablet, the tablet was taken orally by a stomach tube. At different time intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hrs after drug administration), blood samples were taken from the ear vein. Venous blood samples (2 ml) were withdrawn to the tubes containing 0.05 ml of EDTA (0.1 M) and 0.05 ml of ascorbic acid (0.1 M), The blood samples were centrifuged for 10 min. at 3000 rpm and 0.5 ml of plasma was added immediately (time <15 min) to the screw-cap glass tube containing 0.03 ml of p-BPB (1 mg/m1) in acetonitrile and 0.05 ml of sodium hydroxide (0.1 M).The sample was shaken for 15 min and allowed to stand to 30 min. to essentially complete the process of derivatization. Resulting captopril-adduct was stabilized by adding 0.075 ml of HCl (1 M). The obtained mixture was stored in the frozen state for subsequent assays[27].

Analysis of the plasma samples

The plasma concentration of captopril was determined according to the HPLC method reported by Jankowski *et al.*[27], with a slight modification. To 1 ml of plasma, the internal standard, diazepam (1 µg/ml) and 0.15 ml of acetate buffer, pH 4.0 (0.2 M) were added. Extraction was made by 4.0 ml of benzene. After centrifugation (10 min at 4000 rpm), the organic phase was evaporated to dryness under a stream of nitrogen and reconstituted in 0.5 ml of mobile phase, then filtered through millipore filter (0.45 µm). An aliquot of 20 µ1 was injected into the column. Separation was performed on reversed phase column C18 (5 µm particle size and 4.6 x 250 mm dimensions). The mobile phase used was a mixture of acetonitrile and 1% acetic acid (60:40% v/v) and was pumped at a flow rate 0.8 ml/min. The column temperature was maintained at ambient temperature. The U.V. detector was adjusted at 260 nm. Triplicate 20 µL injections of the filtrate were made for each standard sample to see the reproducibility of the detector at each concentration. A standard curve was plotted by analysis of drug-free plasma samples spiked with different amounts of captopril ranging from 25 to 2000 ng/ml. The peak height ratio of captopril to diazepam (as internal standard) was constructed against the concentration of captopril in plasma to obtain the standard calibration curve.

Pharmacokinetic parameters

The maximum plasma concentration (C_{max}) and the time required to reach maximum plasma concentration (T_{max}) after tablet administration were directly determined from the plasma concentration-time curves. Also, the area under the plasma concentration-time curve from 0 to 8 hr (AUC_{0-8} hr) was calculated. Data are expressed as mean ± S.D. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test[28&29]. Statistical calculations were carried out using Instate - 2 computer program (Graphpad software Inc., V2. 04 San Diego, CA, USA).

RESULTS AND DISCUSSION

Tables 2 & 3 summarize the physical, mechanical and bioadhesive characteristics of the prepared tablets. The results revealed that, physical and mechanical properties of the prepared tablets were within the acceptable pharmacopoeal limits.

All the prepared tablets containing CP 934 and HPMC swelled but not eroded within 6 hrs. In case of tablets containing Ch: HPMC and Eu RS 100: HPMC swelled except their tablets in ratio of (6:4 and 8:2) were eroded. Also all tablets containing PVP K_{30} in different ratios were eroded.

Table 2: Physical characteristics of buccoadhesive captopril tablets formulated with a single polymer.

Physical characteristics	Formulae containing				
	HPMC	Carbopol 934	Chitosan	Eudragit RS 100	PVP K_{30}
1- Tablet weight (mg) mean ± SD	160 ± 2.2	159 ± 1.99	161 ± 2.35	158 ± 1.8	159 ± 2.05
2- Tablet thickness (mm) mean ± SD	2.1 ± 0.09	2.2 ± 0.05	2.1 ± 0.04	1.9 ± 0.05	2 ± 0.08
3- Drug content (%) mean ± SD	99.5 ± 1.9	98.7 ± 2.2	97.6 ± 2.0	98.1 ± 2.4	97.3 ± 2.3
4- Friability % mean ± SD	0.23 ± 0.06	0.35 ± 0.009	0.56 ± 0.023	1 ± 0.08	0.72 ±0.06
5- Hardness (kg) mean ± SD	12 ± 2	9 ± 1.5	9 ± 1.9	2.5 ± 1.1	5 ± 1.6
6- Swelling index (S.I.) (after 6 hr) mean ± SD	0.618 ± 0.005	0.984 ± 0.006	0.482 ± 0.008	_____	0.005± 0.004
7- Surface pH mean ± SD	6.7 ± 0.15	3.5 ± 0.09	7 ± 0.3	5.5 ± 0.9	6 ± 0.52
8- Bioadhesion force x 10^3 ($dyne.cm^{-2}$) mean ± SD	30.83 ± 3.56	154.14 ± 3.51	47.78 ± 2.37	0.154 ±0.07	15.41± 2.866
9- *In-vitro* residence Time (hr) mean ± SD	5.5 ± 1.3	> 6 ± 1.25	6 ± 1.33	_____	< 30 min ± 3.2

Table 3: Physical characteristics of buccoadhesive captopril tablets formulated with polymer blends.

Physical characteristics	Formulae																
	CT	CH 1	CH 2	CH 3	CH 4	KH 1	KH 2	KH 3	KH 4	PH 1	PH 2	PH 3	PH 4	EH 1	EH 2	EH 3	EH 4
1- Tablet weight (mg) mean ± SD	158± 2.2	158± 2.12	159± 3.44	161± 2.32	160± 2.77	159± 1.95	158± 1.35	159± 1.1	159± 1.34	158± 1.52	157± 2.10	156± 1.66	157± 1.8	156± 2.86	158± 1.77	157± 2.36	158± 2.35
2- Tablet thickness (mm) mean ± SD	2.1± 0.01	2.18± 0.08	2.02± 0.05	2.00± 0.05	2.10± 0.02	2.15± 0.05	2.07± 0.03	2.10± 0.05	1.99± 0.04	2.10± 0.07	2.05± 0.08	2.18± 0.05	2.18± 0.02	2.08± 0.05	2.10± 0.04	1.98± 0.06	2.18± 0.08
3- Drug content (%) mean ± SD	99 ± 1.8	98.84 ±1.75	101.9 ±1.94	97.90 ±1.78	99.10 ±2.01	98.90 ±1.52	99.20 ±1.73	98.50 ±1.7	99.10 ±2.1	100.2 ±1.98	99.30 ±2.3	98.80 ±2.1	96.80 ±1.79	100.6 ±1.9	97.80 ±2.3	96.34 ±2.1	93.40 ±3.01
4- Friability % mean ± SD	0.6 ± 0.02	0.17± 0.002	0.14± 0.001	0.13± 0.02	0.17± 0.005	0.57± 0.001	0.55± 0.03	0.66± 0.01	0.49± 0.006	0.33± 0.005	0.35± 0.01	0.28± 0.003	0.43± 0.008	0.87± 0.012	0.75± 0.05	0.91± 0.007	1.00± 0.09
5- Hardness (Kg) mean ± SD	3.5 ± 1.5	8 ± 1.17	7.5 ± 1.5	7.5 ± 1.9	6.5 ± 1.21	6.75 ± 1.32	6.0 ± 1.1	6.5 ± 1.35	5.5 ± 1.51	5.5 ± 1.77	5.00 ±1.32	5.0 ± 1.11	4.5± 1.51	4.0± 1.23	3.5± 1.7	3.5± 1.61	2.00± 1.98
6- Swelling index (S.I.) after 6 hr mean ± SD	----	0.66± 0.006	0.71± 0.006	0.75± 0.012	0.89± 0.025	0.65± 0.005	0.71± 0.006	0.73± 0.01	0.48± 0.03	0.53± 0.01	0.57± 0.011	0.14± 0.006	-----	0.56± 0.002	0.53± 0.004	0.19± 0.07	------
7- Surface pH mean ± SD	4± 0.12	6.3± 0.23	5.7± 0.17	5.6± 0.24	5.2± 0.32	5.9± 0.41	6.2± 0.13	6.6± 0.35	6.7± 0.12	6± 0.62	6.2± 0.22	5.5± 0.32	5± 0.12	5.8± 0.32	5.6± 0.41	5.4± 0.32	5 ± 0.23
8- Biadhesion force x 10^3 (dyne.cm^{-2}) mean ± SD	----	30.828 ±2.1	53.949 ±2.7	86.319 ± 3.01	107.89 ± 3.9	29.306 ± 2.5	37.018 ± 2.9	43.188 ± 3.3	49.36 ± 3.5	27.76 ± 4.1	21.59 ± 3.6	16.59 ± 3.2	12.33 ± 2.5	24.66 ± 3.2	18.49 ± 3.6	12.33 ± 2.1	3.082 ± 0.5
9- *In-vitro* residence time (hr) mean ± SD	-----	6 ± 1.3	> 6 ± 1.5	> 6 ± 1.57	> 6 ± 1.11	6 ± 1.35	6 ± 1.24	4 ± 0.95	3 ± 0.58	5 ± 1.24	4 ± 0.87	2 ± 0.56	30min ± 3.5	5 ± 1.2	3 ± 0.54	30min ± 4.45	< 10min ± 1.32

The surface pH of the tablets varied according to the polymer nature and their ratios; tablet containing HPMC, Ch and PVP K_{30} alone showed surface pH from 5 to 7, so, no irritation is expected from these polymers when applied to the buccal mucosa. On the other hand, CP 934 being acidic has a surface pH of 3.5. The acidity surface may induce high mucosal irritation. In case of combinations of CP 934 with HPMC, the values of pH were increased with reduced percentage of CP 934 in the formulation, so no mucosal irritation could be observed.

Figure 3 shows the swelling profiles of plain mucoadhesive tablets containing polymers. Tablets containing CP 934 alone showed a maximum swelling behavior at all time intervals. While, tablets containing HPMC showed comparatively limited water uptake; swelling index varied from 0.172 after 15 min to 0.618 after 6 hrs. Mortazavi and Smart[30] suggested that, the limited hydration of HPMC was responsible for its prolonged duration of adhesion despite its relatively weak mucoadhesive strength. Chitosan tablets showed low swelling behavior at all time intervals. This could be explained by its poor water solubility at buccal pH. Hydrphobicity and weak gel forming capacity at neutral and alkaline pH were reported to be responsible for the weak swelling characteristics of chitosan[31]. Poly-vinylpyrrolidone K_{30} tablets has minimum swelling behavior, swelling index= 0.05 after 15 min and swelling index= 0.217 after 1 hr, after which the polymer started to erode slowly in the medium. Eudragit RS 100 tablets show no swelling behavior. Figure 4 shows the different swelling behavior after 6 hrs for different tablet formulations containing different polymer combinations.

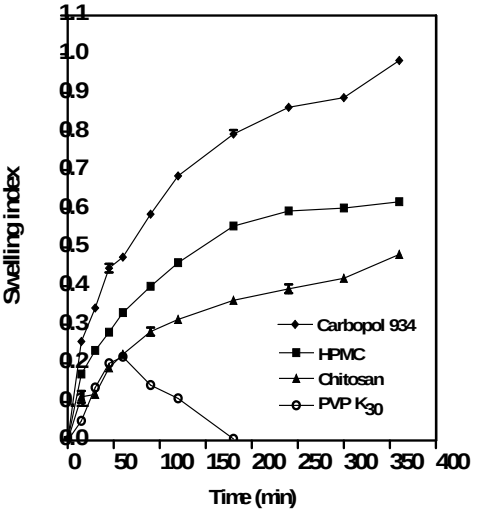

Fig. 3: Swelling profiles of buccoadhesive tablets containing captopril and respective polymers.

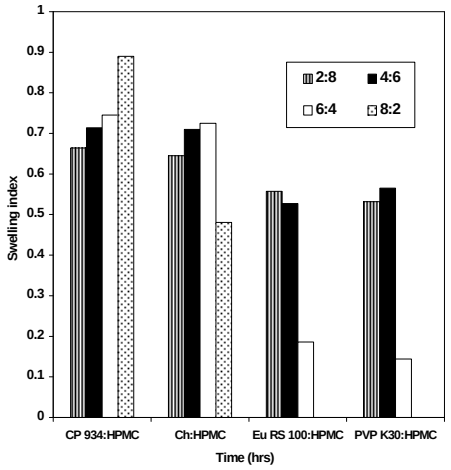

Fig. 4: Swelling index of captopril tablets containing different polymers in phosphate buffer (pH 6.6) after 6 hr.

The values of the bioadhesion force (Table 2), for tablets containing the polymer alone, show the superiority of the anionic polymer (F for tablet containing CP 934 alone = 154.14 x 10^3 dyne.cm^{-2}), over the cationic one (F for tablet containing Ch alone = 47.78 x 10^3 dyne.cm^{-2}), and then non-ionic polymer (F for tablet containing HPMC alone = 30.83 x 10^3 dyne.cm^{-2}). PVP K_{30} has low bioadhesion force (F = 15.414 x 10^3 dyne.cm^{-2}) compared with other polymers. Negligible bioadhesion force was observed for Eu RS 100 containing tablets (F = 0.154 x 10^3 dyne.cm^{-2}).

The physicochemical interactions at the adhesive substrate interface were studied by Jacques and Buri[32]. The authers have suggested that, mucoadhesion of cellulose derivatives resulted mainly from the pressure developed by their swollen gels against the mucin, whereas that, of CP 934, is a polyacrylic acid, was driven by attractive interactions at the polymer-mucin interface. The comparatively weak bioadhesion force of non-ionic polymer may be attributed to the presence of only hydroxyl groups and absence of a proton-donating carboxyl group. So, it could form a weak hydrogen bond with mucus layer[33].

The bioadhesion force of the cationic polymer (Ch) was less than that of anionic one CP 934. Chitosan is a promising bioadhesive material at neutral or alkalin pH, which was found to be advantageous for adsorption on the mucosal surface[34]. They have suggested that, at this pH, chitosan has numerous amine and hydroxyl groups as well as a number of amino groups that may increase the interaction with negative charged mucin so, cause a strengthening of the mucoadhesive interface[35].

Polyvinylpyrrolidone K_{30} has a high water solubility that critically limits its application as an effective mucoadhesive polymer, because after hydration, the formed gel starts to disintegrate due to dissolution[33].

From the results of bioadhesion force of different formulations containing different polymers, it is found that, the addition of HPMC to CP 934 or Ch, reduce the bioadhesion force. On the other hand, the addition of HPMC to PVP K_{30} or Eu RS 100 can enhance the adhesive properties of them.

In general, the swelling behavior of the mucoadhesive polymer was

extensively related to its bioadhesive performance. According to Jacques and Buri[32], materials have highest initial rate of hydration reach the highest mucoadhesive strength.

Values of the *in-vitro* residence time are shown in Tables 2 & 3. From the tablet containing individual polymer evaluation, it can be concluded that, CP 934 was characterized by very strong mucoadhesive force and so prolonged residence time between the tablet and mucosal membrane (> 6 hrs), followed by Ch (6 hrs) and then HPMC (5.5 hrs). On the other hand, the residence time of PVP K_{30} (< 30 min), while, Eu RS 100 has negligible residence time. The addition of HPMC to different polymers can improve the residence time of different polymers. In case of Ch and CP 934, the addition of HPMC decreases the residence time and facilitate erosion and promote tablet disintegration. But the addition of HPMC to PVP K_{30} and Eu RS 100 increases the residence time of these polymers.

The release profiles of the drug from different buccoadhesive tablets are shown in Figures 5-8. The percent release of the drug from different formulations varied with the polymer types and the ratios of polymer in each formulation. On the other hand, complete release of the drug after 30 min. in case of control tablet.

The release of the drug from buccoadhesive tablets containing CP 934 and HPMC, varied according to the ratio of these polymers. The increase in percent of CP 934 was found to increase the drug release. This may be due to the ionization of CP 934 at pH 6.6, a pH environment higher than its ionization constant (pKa = 6). Ionization of CP 934 leads to development of negative charges along the backbone of the polymer. Repulsion of like charges (polymer and mucin) leading to slightly higher uptake of water that might have contributed to higher drug release from the polymer matrix system[36]. The release of the drug from these formulations can be arranged as follows according to the ratio of two polymers: CH 4>CH 3 >CH 2>CH 1. These results are in agreement with results obtained by Çelebi and Kişlal[37], who have reported that, increase in the ratio of CP 934 causes an increase in the amount of propranolol hydrochloride release.

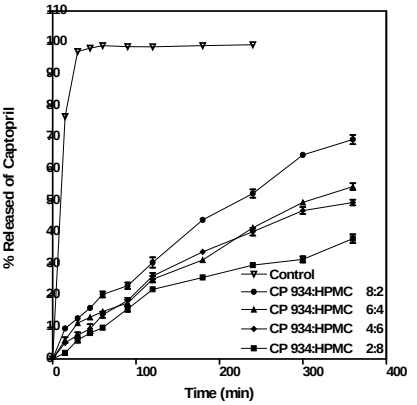

Fig. 5: *In-vitro* release profiles of captopril from buccoadhesive tablets containing Carpobol 934: HPMC.

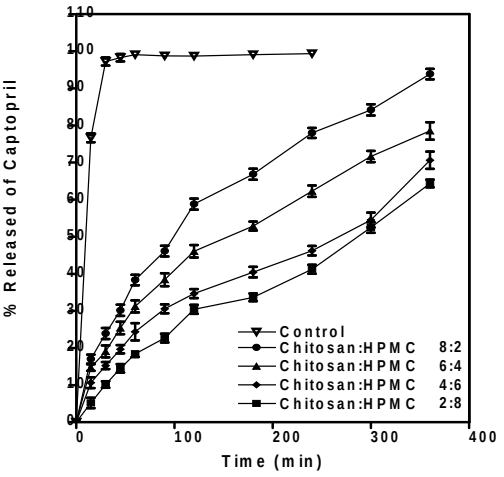

Fig. 6: *In-vitro* release profiles of captopril from buccoadhesive tablets containing Chitosan: HPMC.

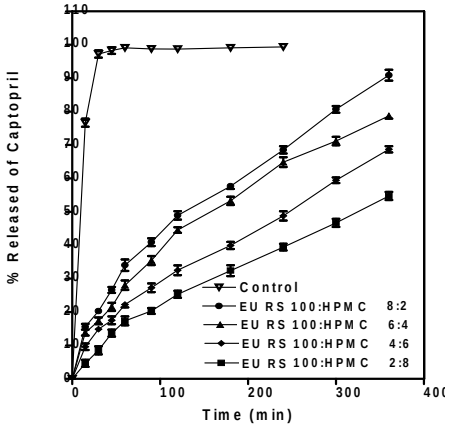

Fig. 7: *In-vitro* release profiles of captopril from buccoadhesive tablets containing Eudragit RS 100:HPMC.

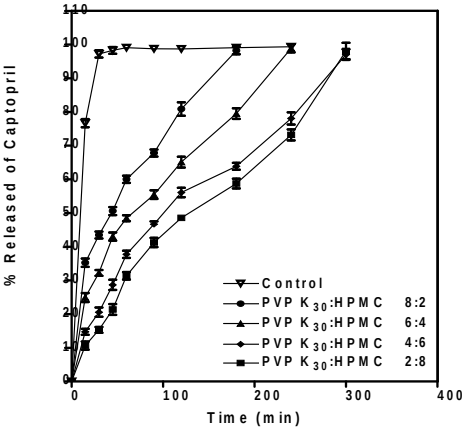

Fig. 8: *In-vitro* release profiles of captopril from buccoadhesive tablets containing PVP K_{30}: HPMC.

The release of the drug from buccoadhesive tablets containing Ch and HPMC, varied according to the ratio of these polymers. The release of the drug from these formulations can be arranged as follows: KH 4> KH 3> KH 2> KH 1.

The release of the drug from buccoadhesive tablets containing Eu RS 100 and HPMC, can be arranged as follows: EH 4>EH 3>EH 2>EH 1.

Also, the release of the drug from buccoadhesive tablets containing PVP K_{30} and HPMC, can be arranged as follows: PH 4>PH 3>PH 2>PH 1. The initial drug release from these formulations was 10.6, 15, 25 and 35% for formula PH 1, PH 2, PH 3 and PH 4, respectively, after 15 min. Complete release was observed after 300 min. for formulation PH 1 and PH 2, 240 min. for formula PH 3 and

180 min. for formula PH 4. This variation in the release can be attributed to ratio of PVP $K_{30.}$ As PVP K_{30} water soluble polymer and so rapid erosion of the resultant gel layer upon increasing the polymer concentration and so increase the drug release.

From the previous results, increase the percent of HPMC to those formulations lead to a decrease in the release of the drug and so give sustained release pattern of the drug.

Data of the *in-vitro* release of captopril were fitted to different equations and kinetic models to explain the release kinetics of the drug from formulated buccoadhesive tablets. The kinetic models used were, zero-order equation[38], first order, Higuchi model[39&40]. The best fit with the highest correlation coefficient "r^2" was shown with Higuchi model (r^2:0.9708-0.9944) for formulae containing (Ch & HPMC) and (r^2:0.9568-0.9930) for formula containing (PVP K_{30} & HPMC) in different ratio. While, in case of formula containing (CP 934 & HPMC) and (Eu RS 100 & HPMC) the best fit with the highest correlation coefficient "r^2" was shown with first-order equation (r^2:0.9699-0.9925 and 0.9839-0.9942, respectively). To explore the kinetics behavior further, the release data were analyzed using Korsmeyer and Peppas equation[40]:

$$M_t/M_\infty = K.t^n$$

where,

$M_{\infty:}$ amount of drug in the matrix;

M_t: amount of drug released at time t.;

M_t/M_∞: fraction of drug released at time t;

K: Kinetic constant incorporating the structural & geometric characteristics of the drug/polymer system and

n: The release exponent, is a parameter which depends on the release mechanism and is thus used to characterize it.

For non-Fickian release (an anomalous), the value of n falls between 0.5 and 1 ($0.5 < n < 1$), n equals 0.5 corresponds to a Fickian diffusion release (case I diffusion), n= 1 corresponds to a zero-order release (case II transport) and for n>1 corresponds to super case II transport[26]. The kinetic parameters, n and K, were calculated by plotting log M_t/M_∞ versus log t where log K is the intercept and n is the slope of the straight line. K is a characteristic constant of the tablet and n is an indicative of release order. Hence, as the K value increases, the release of drug occurs faster[41]. It is obvious that the values of n for all these formulae fall between 0.5 and 1 (except KH 2, KH 3, KH 4, and EH 4 where n value nearly 0.5 so, indicating Fickian transport) indicating non-Fickian release behavior controlled by a combination of diffusion and chain relaxation mechanism[37]. This is in agreement with Desai and Kumar[26], who have reported that the swellable matrices such as CP 934 exhibit anomalous non-Fickian release kinetic. Also, these results are in agreement with the results obtained

by Yaziksiz-iscan *et al.*[42] which indicated that, the release behavior of the system containing captopril and the two polymers, CP 934 and HPMC, at different ratios found to be non-Fickian.

Bioavailability study

The mean plasma concentration as a function of time of tested formulations is illustrated in Figure 9 and the pharmacokinetic parameters are listed in Table 4. It could be observed that, there is a difference between the mean plasma concentrations as a function of time for captopril after oral administration of control formula and the other buccoadhesive formulations at all time intervals. The pharmacokinetic parameters of captopril represented by the value of C_{max} (ng/ml), T_{max} (hr) and AUC $_{0-8}$ hr (ng.hr.ml^{-1}).

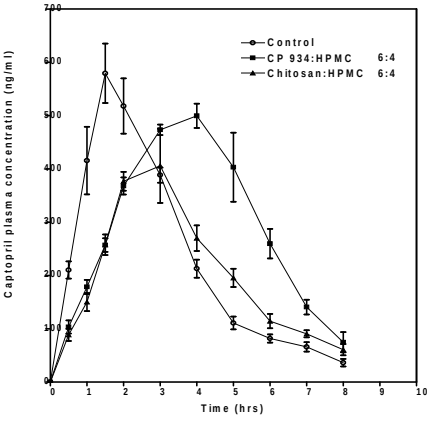

Fig. 9: Plasma concentration-time profiles of captopril after oral dministration of control and buccoadhesive tablets in rabbits.

From these results it was evident that, the absorption of captopril from control tablets was rapid and reached its peak plasma concentration in 1.5±0.316 hr whereas, following buccoadhesive administration of the developed tested formulations, CH 3 and KH 3, the mean T_{max} was 4±0.632 hrs and 2.67±0.516 hrs, respectively. These result indicats the sustained release effect of both tested formulae compared to the control tablets.

The mean plasma concentration (C_{max}) is 506.9 ± 11.365 (ng/ml) for CH 3 and 429.02±39.554 (ng/ml) for KH 3 compared to 591.28 ± 37.489 (ng/ml) for the control tablets. While the mean AUC $_{0-8}$ is found to be 2359.5 ± 113.94 (ng.hr.ml^{-1}) for CH 3 and 1637.43 ± 81.35 (ng.hr.ml^{-1}) for KH 3 compared to 1869.29 ± 64.1 (ng.hr.ml^{-1}) for the control tablet. These results indicated the more suitability of both CH 3 and KH 3 as sustained-release systems than the control tablets. However, the more suitability of CH 3 as a sustained-release system than KH 3 was also observed. These findings assure the goal of sustained release concept which has been estimated in reducing high peak plasma concentration (C_{max}), prolong the time required to reach maximum plasma concentration (T_{max}), increasing the total amount of captopril present in the blood for longer time as indicated by the high values of AUC_{0-8} values and finally increasing the bioavailability of the drug. These results are in agreement with the results obtained by Yaziksiz-

iscan *et al.*[42], who have indicated that, the formulation of captopril as buccoadhesive tablet containing Carbopol 934 and Hydroxpropyl methylcellulose only at different ratios produced sustained-release systems.

The results listed in Table 5, represents the statistical analysis for the pharmacokinetic parameters of captopril from the three tested formulations. From these results it could be observed that, the pharmacokinetic parameters of captopril in buccoadhesive tablets containing CP 934: HPMC in ratio of 6:4 are significantly different from the pharmacokinetic parameters of captopril in buccoadhesive tablets containing Chitosan: HPMC in ratio of 6:4. The pharmacokinetic parameters of both formulae are significantly different from those of captopril in case of the control tablet using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.

Table 4: The mean pharmacokinetic parameters of captopril from the control tablets and buccoadhesive tablets.

The mean pharmacokinetic parameters	Formulae		
	CT	CH 3	KH 3
C_{max} (ng/ml)	591.28± 37.49	506.9± 11.37	429.02 ± 39.55
T_{max} (hrs)	1.50 ± 0.3160	4.00± 0.6320	2.670 ± 0.516
AUC_{0-8} (ng.hr.ml^{-1})	1869.29± 64.1	2359.5± 113.94	1637.43 ± 81.35

Table 5: Statistical analysis of the pharmacokinetic parameters of captopril from control and buccoadhesive tablets.

Formulae	The mean pharmacokinetic parameters		
	C_{max} (ng/ml)	T_{max} (hrs)	AUC_{0-8} (ng.hr.ml^{-1})
CH 3 vs KH 3	** at P < 0.01	** at P < 0.01	* at P < 0.05
CH 3 vs CT	** at P < 0.01	* at P <0.05	* at P < 0.05
KH 3 vs CT	* at P < 0.05	** at P < 0.01	* at P < 0.05

where

* Significantly different ** Highly significant difference

Conclusion

A bucco-adhesive system for the release of captopril was developed using different polymers; CP 934, Ch, Eu RS 100, PVP K_{30} and HPMC in various ratios. The non-Fickian release behavior obtained, suggests that, the release of the drug is controlled by a diffusion mechanism. The adhesion characteristics were significantly affected by the various ratios of bioadhesive polymers in each tablet. Tablets containing CP 934/HPMC and Ch/HPMC showed good bioadhesive characteristics in contact with rabbit intestinal mucosa, good swelling characteristics and good residence time in the buccal cavity. *In-vivo* study of captopril from two selected formulations (CP 934/HPMC and Ch/HPMC) produced extended drug release when compared with control tablet taken orally. The formula containing CP 934/HPMC gives more sustained effect than the other one Ch/HPMC. Thus, our results suggest that the captopril buccoadhesive tablet would be useful to deliver the drug in a sustained release manner.

REFRENCES

1- R. K. Ferguson, H. R. Brunner, G. A. Turini, H. Gavras and D. N. McKinstry, Lancet, 1, 775 (1977).
2- M. A. Ondetti, B. Rubin and D. W. Cushman, Science, 196, 441 (1977).
3- H. Gavras, H. R. Brunner, G. A. Turini, G. R. Kershaw, C. P. Tifft, S. Guttelod, I. Gavras, R. A. Vukovish and D. N. McKinstry, New Engl. J. Med., 298, 991 (1978).
4- E. L. Bravo and R. C. Tarazi, Hypertension, 1, 39 (1979).
5- H. R. Brunner, H. Gavras, B. Waebar, G. R. Kershaw, G. A. Turini, R. A. Vukovish and D. N. McKinstry, Ann. Intern. Med., 90, 19 (1979).
6- M. A. Testa, R. B. Anderson, J. F. Nackley and N. K. Hollenberg, New Engl. J. Med., 328, 907 (1993).
7- K. L. Düchin, S. M. Singhvi, D. A. Willard, B. H. Migdalof and D. N. McKinstry, Clin. Pharmacol. Ther., 31, 452 (1982).
8- K. P. Ohman, B. Kagedal, R. Larsson and B. E. Kalberg, J. Cardiovasc. Pharmacol., 7, S20 (1985).
9- J. Singh and D. H. Robinson, Drug Dev. Ind. Pharm., 14, 545 (1988).
10- Y. Seta, F. Higuchi, Y. Kawahara, K. Nishimura and R. Okada, Int. J. Pharm., 41, 245 (1988a).
11- I. R. Wilding, S. S. Davis, M. Bakhshaee, H. N. Stevens and R. A. Sparrow, Pharm. Res., 9, 654 (1992).
12- N. R. Jimenez-Castellanos, H. Zia and C. T. Rhodes, Drug Dev. Ind. Pharm., 19, 143 (1993).
13- A. Ahuja, R. K. Khar and J. Ali, ibid., 23, 489 (1997).
14- C. Eouani, P. H. Piccerelle, P. P. Rinderre, E. Bourret and C.

Joachim, Eur. J. Pharm. Biopharm., 52, 45 (2001).
15- J. D. Smart, Adv. Drug Del. Rev., 11, 221 (1993).
16- A. G. Mikos and N. A. Peppas, STP. Pharma. Sci., 2, 705 (1986).
17- D. Duchene, F. Touchard and N. A. Peppas, Drug Dev. Ind. Pharm., 14, 283 (1988).
18- G. Ponchel, F. Touchard, D. Duchene and N. A. Peppas, J. Control. Rel., 5, 129 (1987).
19- M. Z. I. Khan, Drug Dev. Ind. Pharm., 21, 1037 (1995).
20- D. E. Chickering III and E. Mathiowitz, "Definitions, mechanisms, and theories of bioadhesion" in: "Bioadhesive Drug Delivery Systems, Fundamentals, Noval Approaches, and Development", E. Mathiowitz, D. E. Chickering and C. M. Leher (Eds.), Marcel Dekker Inc., New York, NY, 1999, pp. 1-2.
21- B. Taylan, Y. Capan, O. Guven, S. Kes and A. A. Hincal, J. Control. Rel., 38, 11 (1996).
22- N. A. Nafee, A. Ismail, N. A. Boraie and L. M. Mortada, Drug Dev. Ind. Pharm., 30, 985 (2004).
23- G. E. El-Gindy, A. M. El-Said, A. Abdel- Magied and G. M. Abdel-Aal, Bull. Pharm. Sci., Assiut University, 25, 125 (2002).
24- N. Langoth, J. Kalbe and A. B. Schmùrch, Int. J. Pharm., 252, 141 (2003).
25- H. H. Alur, S. I. Pather, A. K. Mitra and T. P. Johnston, ibid., 188, 1 (1999).
26- K. G. H. Desai and T. M. P. Kumar, AAPS Pharm. Sci. Tech, 5, 1 (2004).
27- A. Jankowski, A. Skorek, K. Krzy'sko, P. K. Zarzycki, R. J. Ochocka and H. Lamparczyk, J. Pharm. and Biomed. Anal., 13, 655 (1995).
28- A. Petrie, Lecture notes on "Medical Statistics", 2nd Ed., Blackwell scientific publications, Oxford, U.K. (1987).
29- S. C. Gad and C. S. Weil, "Statistics for Toxicologists", in: "Principles and Methods of Toxicology", 2nd Ed., Hayes, A. W., ed., Raven press Ltd., New York, 1989, pp. 435-483.
30- S. A. Mortazavi and J. D. Smart, Int. J. Pharm., 116, 223 (1995).
31- L. Illeum, Pharm. Res., 15, 1326 (1998).
32- Y. Jacques and P. Buri, Pharm. Acta. Helv., 72, 225 (1997).
33- E. Karavas, E. Georgarakis and D. Bikiaris, Eur. J. Pharm. Biopharm., 64, 115 (2006).
34- I. Henriksen, K. L. Green, J. D. Smart, G. Smistad and J. Karlsen, Int. J. Pharm., 145, 231 (1996).
35- S. Rossi, F. Ferrari, M. C. Bonferni and C. Caramella, Eur. J. Pharm. Sci., 12, 479 (2001).
36- V. Agarwal and B. Mishra, Drug Dev. Ind. Pharm., 25, 701 (1999).
37- N. Çelebi and Ö. Kişlal, Pharmazie, 50, 470 (1995).

38- S. K. Braveja, K. V. RangaRao and K. P. Devi, Int. J. Pharm., 39, 39 (1987).
39- T. Higuchi, J. Pharm. Sci., 52, 1145 (1963).
40- R.W. Korsmeyer, R. Gurny, E. M. Doelker, P. Buri and N. A. Peppas, Int. J. Pharm., 15, 25 (1983).
41- H. -G. Choi, J. -H. Jung, C. S. Yong, C. -D. Rhee, M. -K. Lee, J. -H. Han, K. -M. Park and C. K. Kim, J. Control. Rel., 68, 405 (2000).
42- Y. Yaziksiz-iscan, Y. Capan, S. Senel, S. Sahin and A. A. Hincal, STP. Pharma. Sci., 8, 357 (1998).