Department of Pharmaceutics[1], Department of Analytical Chemistry[2], Faculty of Pharmacy, Cairo University, Cairo, Egypt

Formulation and evaluation of sustained release floating capsules of nicardipine hydrochloride

N. M. Moursy[1], N. N. Afifi[1], D. M. Ghorab[1], Y. El-Saharty[2]

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Dr. Nagia Nagib Afifi, Asst. Prof. of Pharmaceutics, Pharmaceutics Department, Faculty of Pharmacy, Cairo University, Kasr El-Eini 11562, Cairo, Egypt

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Nicardipine hydrochloride, a calcium channel blocker with significant vasodilating and antihypertensive activities, was formulated in this work as sustained release floating capsules. A hydrocolloid of high viscosity grade was used for the floating systems. The inclusion of sodium bicarbonate to allow evolution of CO_2 to aid buoyancy was studied. Polymers that retard drug release were included as coprecipitates with the drug and/or as additives in the formulated capsules. Both simple powder mixing of the ingredients and granule preparation via wet granulation were used. Seven capsule formulae were prepared. The prepared capsules were evaluated *in vitro* by testing drug dissolution, floating time and the kinetics of drug release. *In vitro* evaluation of a commercially available conventional 20 mg capsule of nicardipine hydrochloride, "Micard", was carried out for comparison. The hydrocolloid used succeeded in effecting capsule buoyancy. Floating time increased with increasing the proportion of the hydrocolloid. Inclusion of sodium bicarbonate increased buoyancy. All of the seven floating capsule formulae prepared proved efficient in controlling drug release. The sustained release floating capsule formulation of choice was evaluated *in vivo* in comparison to "Micard" capsules using rabbits. Reversed phase HPLC with UV detection was used for drug determination in rabbit plasma. Plasma concentration time curves revealed a longer drug duration for administration in the sustained release formula than the conventional "Micard" capsule being 16 h in the former versus 8 h for the latter.

1. Introduction

The calcium channel blocker nicardipine hydrochloride is a dihydropyridine derivative [1, 2] with significant vasodilating and antihypertensive activities [3, 4]. It was first marketed in the United States in 1989 [5] for treatment of angina pectoris [6]. It is rapidly absorbed from the gastrointestinal tract mainly from the jejunum and ileum [7]. Being subjected to hepatic recycling, it has a biphasic plasma half-life, an early one of 2–4 h and a terminal one of 8.6 h. It is extensively metabolized in the liver and is excreted in urine and faeces mainly as inactive metabolites [8–10]. This necessitates a thrice-daily dosage regimen to maintain a uniform hypotensive effect. The aim of this work is to sustain drug release in order to reduce dosing frequency. This leads to decreased fluctuations in drug plasma level with the associated side effects besides increasing patient compliance.

A sustained release formulation that is retained in the stomach and releases the active ingredient over an extended period of time in the gastric fluids is quite suitable for drugs such as nicardipine hydrochloride. This is due to the fact that the drug is readily soluble in gastric juice and slightly soluble in intestinal fluid [11] being a weakly basic drug of pKa 7.2 [12]. In this way, the drug reaches the jejunum and ilieum, its optimum absorption sites [7], over a sustained period of time and in a soluble form ready for absorption. The controlled release properties of such a formulation can maximize the blood level profile and consequently the pharmacological response in a unique fashion that is not possible by conventional controlled release technology.

To prolong the retention time of the drug in the stomach, floating capsules were prepared in this work, based on a hydrodynamically balanced controlled release delivery system (H B S). Upon contact with gastric fluid, the capsule acquires a bulk density of less than one which is less than the specific gravity of gastric fluid, reported as 1.004–1.01 [7]. Accordingly, the capsule or its contents remain buoyant on the gastric fluid with a resultant prolonged residence time in the stomach. The buoyancy and release characteristics of the formulated capsules are achieved by the use of specific excipients which play a significant role in the product design. The drug is gradually and uniformly released from the capsule as the stomach fluid slowly permeates the matrix.

2. Investigations, results and discussion

A dissolution study over a pH gradient was carried out, starting with pH 1.2 for the first 3 h, which proved long enough to cover the floating time of all the capsules, ranging from 155 to 178 min. Figs. 1 and 2 show the average release ± S.D. of nicardipine hydrochloride from the tested capsules at gradient pH. Formula I showed an aver-

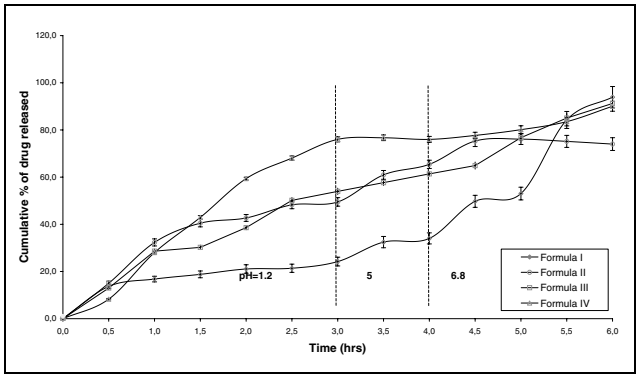

Fig. 1: Release profile of nicardipine hydrochloride from different capsule formulae at gradient pH (values are expressed as mean ± S.D.)

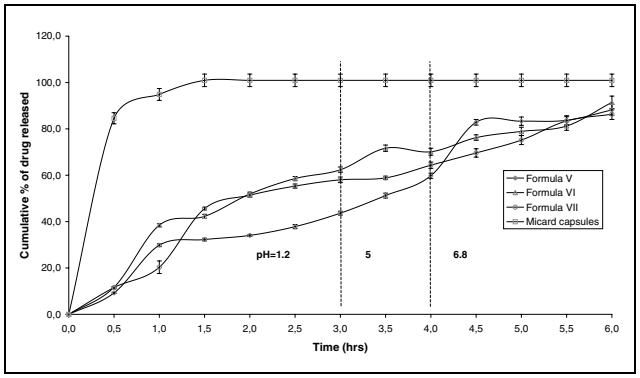

Fig. 2: Release profile of nicardipine hydrochloride from different capsule formulae at gradient pH (values are expressed as mean ± S.D.)

age release of 24.2 ± 1.8% during the first 3 h reaching 93.9 ± 4.5% at the end of 6 h.

Formula II showed a release of 49.5 ± 1.8% at pH 1.2 after 3 h. The release increased to 65.4 ± 1.8% 1 h later at the end of pH 5 until finally 74 ± 2.7% was released at pH 6.8 after 2 h more. Drug release at pH 1.2 was higher in formula II than I (nearly double) although in both the drug is present in the same form i.e. a (1 : 4) drug: Eudragit L_{100} coprecipitate. This is probably due to the presence of ethyl cellulose as an additional retarding polymer besides Eudragit L_{100} in formula I (Table 1). The rise in drug release at pH 1.2 caused an overall uniform release pattern. That was also observed with all the formulae II–VII.

Formulae III and IV (Table 1) contain the same ratio of nicardipine hydrochloride to Eudragit L_{100} but the proportion of the hydrocolloid polymer, HPMC 4000, used is higher in formula IV. This increased the floating time, being 163 min for formula III versus 175 min for formula IV, as well as the percentage drug release at pH 1.2 (Fig. 1). The high level of release from formula IV at pH 1.2, reaching 76 ± 1.2%, was followed by a slowly increasing release at pHs 5 and 6.8, reaching 90 ± 2.1%. The moderate release from formula III at pH 1.2 reaching 54 ± 1.5%, was followed by a gradual increase at pHs 5 and 6.8, finally reaching 91.2 ± 2.4% with a uniform release pattern.

Similar observations were found for formulae V and VI regarding the effect of the proportion of HPMC 4000 on floating time (165 and 178 min respectively) and drug release at pH 1.2 (Fig. 2). The proportion of Eudragit L_{100} is higher in formulae V and VI than in formulae III and IV resulting in lower drug release from the former at pH 1.2. This was followed by gradual increases in drug release at pHs 5 and 6.8 with a tendency to level off during the terminal 1.5 h in the case of formula V. Release reached 88.2 ± 2.4% from formula V and 91.5 ± 2.7% from formula VI.

The increase in floating time obtained by increasing the proportion of the hydrocolloid polymer is in agreement with the results found by Mohammed et al. [13]. This is expected since this polymer is responsible for the floating properties of the dosage form [14] as it has a lower density than the gastric fluid and therefore remains buoyant on the stomach contents [15, 16]. The associated increase in drug release may be caused by the increased swelling due to the absorbed water. This allows for increased wettability of the drug leading to higher dissolution.

Formula VII showed comparable drug release to formula III (Figs. 1 and 2) as well as comparable floating time (160 and 163 min respectively). This is because they contain a comparable proportion of HPMC 4000 and nearly the same drug: retarding polymer ratio (Table 1). The retarding polymer in formula III is Eudragit L_{100} white in formula VII it is cellulose acetate phathalate. Although both retarding polymers proved efficient, the Eudragit L_{100} in formula III caused somewhat more retardation in drug release at pH 1.2 where the drug is initially soluble. Time required to release about 50% of the dose was 2.5 h in formula III versus 2 h in formula VII. Release after the 3 h period at pH 1.2 was 54 ± 1.5% in formula III versus 58 ± 1.2% in formula VII (Fig. 2).

The commercially available nicardipine hydrochloride capsule, "Micard", showed rapid drug release reaching 100% after 1.5 h at pH 1.2 (Fig. 2). This demonstrates the suc-

Table 1: Contents of the prepared nicardipine hydrochloride floating capsule formulae I–VII*

Ingredient	Weight percent in each of the prepared capsule formulae						
	I	II	III	IV	V	VI	VII
Nicardipine hydrochloride**	1 : 4 w/w nicardipine HCl: Eudragit L100 solid dispersion was included at weight percent of 20 and 33.3, respectively		6.7	6.7	4	3.3	8
Eudragit L100			26.7	26.7	24	20	–
Cellulose acetate phthalate (CAP)	–	–	–	–	–	–	32
Ethyl Cellulose	5	–	–	–	–	–	–
Hydroxypropyl methyl cellulose (HPMC) 4000	30	33.3	33.3	50	56	70	40
Methylcellulose (MC)	30	–	–	–	–	–	–
Tragacanth	15	–	–	–	–	–	–
Sodium bicarbonate	–	3	3	3	3	3	3
Magnesium stearate	–	1	1	1	1	1	1
Lactose	–	29.3	29.3	12.7	12	2.7	16

* Under Egyptian patent application number 2000010007.
** A fixed dose of 40 mg. Nicardipine hydrochloride is used in all formulae.

Table 2: Kinetics of release of nicardipine hydrochloride from different capsule formulae at pH 1.2

Formula	Zero order		First order		Higuchi diffusion equation		Order of release	A-intercept	B-slope	K	$T^{1/2}$
	R_z	CV_z	R_f	CV_f	R_H	CV_H					
Formula I	0.98355	3.905604	−0.98512	0.194648	0.96330	2.87366	First order	1.94329	−0.001	0.0049	141.36
Formula II	0.92488	14.36139	−0.94972	1.651437	0.99387	10.1377	First order	1.93456	−0.001	0.00543	127.73
Formula III	0.98066	9.269459	−0.98395	1.149373	0.98297	8.72049	First order	1.98822	−0.002	0.00464	149.46
Formula IV	0.98662	9.961977	0.905628	10.02371	0.99800	3.85843	Diffusion	−39.1668	8.7262	8.72625	104.41
Formula V	0.89894	18.50669	0.811594	10.99337	0.93514	10.1827	Diffusion	−5.80819	3.7370	3.73703	223.02
Formula VI	0.94021	15.83305	−0.97501	1.924831	0.97052	11.2057	First order	1.97330	−0.002	0.00414	167.33
Formula VII	0.93697	18.93977	−0.9562	4.260900	0.96066	14.9604	First order	1.99931	−0.002	0.00409	169.1
"Micard"	0.8049	4.5369	0.7996	1.0511	0.8709	3.75828	First order	1.9402	0.0004	0.0623	11.12

– R_z, R_f and R_H are the correlation coefficients of zero-order, first-order and Higuchi model of kinetics respectively, while CV_z, CV_f and CV_H are their corresponding coefficients of variation.
– K is the release rate constant in ($mg \cdot min^{-1}$) in zero-order, (min^{-1}) in first order and ($mg \cdot cm^{-2} \cdot min^{-1/2}$) in Higuchi model.
– $T^{1/2}$ in min.
– (+) intercept (A) indicates flush release of the same value.

cess of all experimental formulae I–VII in controlling drug release.

Drug release from all the formulae studied (Tables 3 and 4) followed first order kinetics except for formula III at pH 6.8 and formulae IV and V at pH 1.2 where release was via diffusion. Drug release from formula III at pH 6.8 by diffusion may be due to the presence of the retarding polymer, Eudragit L_{100}, at a critical drug: polymer ratio allowing the formation of a diffusion layer around the drug particles. Although formula IV contains the same drug: Eudragit L_{100} ratio, its higher content of the hydrophilic polymer, HPMC 4000, (Table 1) may have interfered with the performance of the retarding polymer at pH 6.8.

On the other hand, drug release via diffusion from formulae IV and V at pH 1.2 may be due to the presence of the hydrophilic polymer, HPMC 4000, at a critical proportion as this polymer exists at a comparable weight percent in both formulae (Table 1). Such a critical proportion allows the formation of a hydrocolloidal diffusion layer at pH 1.2. This is in accordance with Talukdar et al. [17] and Mosguera et al. [18] who reported that caffeine came out of a hydrogel matrix by diffusion. By the time pH 6.8 was reached, most of the nicardipine hydrochloride included in the dose may have left the hydrocolloid and started to dissolve depending on its concentration. This may explain the tendency of drug release to level off, observed in both formulae after the decrease of the remaining drug to about 20%.

Formula I had the shortest floating time (155 min) although it contains three hydrophilic polymers, HPMC 4000, MC and tragacanth. Formulae II–VII contain sodium bicarbonate and only one hydrophilic polymer, but proved more efficient in causing buoyancy of the formulae besides being more economic.

Although all the experimental formulae I–VII proved efficient in controlling drug release in comparison to the marketed "Micard" capsules as regards the *in vitro* performance, formulae III, VI and VII gave the best release patterns (Figs. 1 and 2). Drug release increased uniformly with satisfactory retardation. Formula III was most economic as it had the least amount of chemicals and it showed the best retardation of drug release at pH 1.2 where the drug is originally soluble. Accordingly, formula III was chosen for further evaluation by investigating its in-vivo performance in comparison to "Micard" capsules.

The drug content of "Micard" capsules checked by HPLC and spectrophotometric assays showed an average ($n = 5$) percent recovery of 98.1 ± 0.3 and 98.3 ± 0.3 and variance values of 0.063 and 0.096% respectively. There is no significant difference between the two assay methods regarding accuracy and precision at $p \leq 0.05$ using both F and t-tests.

The validity of the method adopted for *in vivo* assay using HPLC was demonstrated by evaluating the mean percent recovery of nicardipine hydrochloride from spiked plasma samples, which was found to be 99.1 ± 2.9. The low coefficient of variation ($\leq 5\%$) provides evidence of the reproducibility of the method. The validity of the method was further checked using the standard addition technique [19]

Table 3: Kinetics of release of nicardipine hydrochloride from different floating capsule formulae at pH 6.8

Formula	Zero order		First order		Higuchi diffusion equation		Order of release	A-intercept	B-slope	K	$T^{1/2}$
	R_z	CV_z	R_f	CV_f	R_H	CV_H					
Formula I	0.95399	22.46489	−0.95545	11.76766	0.93713	26.1484	First order	2.14449	−0.011	0.00908	76.357
Formula II	−0.72347	7.492152	0.718463	0.913760	−0.65293	8.21996	First order	1.37439	0.0002	0.01121	61.822
Formula III	0.98961	11.09416	0.921089	17.31208	0.99898	1.09662	Diffusion	−22.508	4.8261	4.82606	75.094
Formula IV	0.96812	27.66918	−0.94527	5.037957	0.94872	30.3348	First order	1.49883	−0.004	0.00241	287.66
Formula V	0.85458	6.346523	−0.84338	4.194157	0.80722	7.21342	First order	1.30874	−0.002	0.02842	24.384
Formula VI	0.93192	25.01226	−0.8901	9.13919	0.90972	30.8159	First order	1.57226	−0.005	0.00759	91.359
Formula VII	0.98536	11.16222	−0.989	2.19497	0.98859	9.85794	First order	1.61184	−0.004	0.00534	129.77

– R_z,R_f and R_H are the correlation coefficients of zero-order, first-order and Higuchi model of kinetics respictively, while CV_z, CV_f and CV_H are their corresponding coefficients of variation.
– K is the release rate constant in ($mg \cdot min^{-1}$) in zero-order, (min^{-1}) in first order and ($mg \cdot cm^{-2} \cdot min^{-1/2}$) in Higuchi model.
– $T^{1/2}$ in min.
– (+) intercept (A) indicates flush release of the same value.

Table 4: Data of HPLC analysis of rabbit plasma samples containing nicardipine hydrochloride following administration of the introduced formula III and "Micard" capsules

Time (h)	"Micard"			Formula III		
	Peak Area Ratio	Concn. (ng/ml)	S.D	Peak Area Ratio	Concn. (ng/ml)	S.D
0	0	0	0	0	0	0
0.5	0.59759	97.118	±3.785	0.13329	21.6429	±1.039
1	0.77451	125.87	±5.163	0.21972	35.73299	±1.507
2	0.73694	119.76	±4.548	0.32852	53.38988	±2.349
3	0.51582	83.829	±3.404	0.32561	52.9163	±2.169
4	0.22685	36.866	±1.194	0.28068	45.61526	±1.825
6	0.13717	22.292	±0.881	0.26537	43.1281	±2.156
8	0	0	–	0.2335	37.9476	±1.518
10	0	0	–	0.14431	23.4527	±1.173
12	0	0	–	0.09992	16.2386	±0.812
16	0	0	–	0.005	0.8125	±0.041
24	0	0	–	0	0	–

to the drug content of "Micard" capsules where the percentage recovery of the added drug was found to be 100.5 ± 0.3. The results of the method used emphasized the absence of interference due to excipients.

No components were eluted with nicardipine nor with amitriptyline used as internal standard [20]. To achieve such a clean chromatogram, it was necessary to perform acid-base partitioning which was included in the extraction procedure prior to the HPLC analysis. In addition, it was found that the 2 M NaOH used in the procedure must be washed once with acetonitrile and once with ether to guard against potentially interfering components [21].

Good separation of nicardipine hydrochloride and the internal standard from each other and from other components was obtained. An acidic mobile phase (pH 4.8) with a relatively high buffer concentration (0.02 M KH_2PO_4) was used. Such a mobile phase causes nicardipine hydrochloride and the internal standard to exist as the corresponding protonated species during chromatographic separation, leading to improved peak shapes and reduced retention times. The high buffer concentration was found to be necessary to ensure good separation between the drug and the internal standard. At a lower buffer concentration (0.01 M KH_2PO_4), the separation of these compounds and even their order of elution was found to be varied.

Calibration curves for the HPLC assay of nicardipine hydrochloride alone in ethanolic solution and extracted from spiked plasma in the presence of amitriptyline as an internal standard showed good linearity with correlation coefficients of 0.9996 and 0.9989 respectively.

Fig. 3 and Table 4 show the time course of plasma concentrations of nicardipine hydrochloride after the administration of "Micard" capsules and also the experimental controlled release formula III to rabbits at a dose level equivalent to a human dose of 40 mg. Drug duration after the administration of the controlled release formula III significantly exceeded that of the "Micard" capsule. In the latter case, the drug was traced up to 8 h compared to 16 h in the former. Accordingly, formula III successfully retarded and sustained drug release.

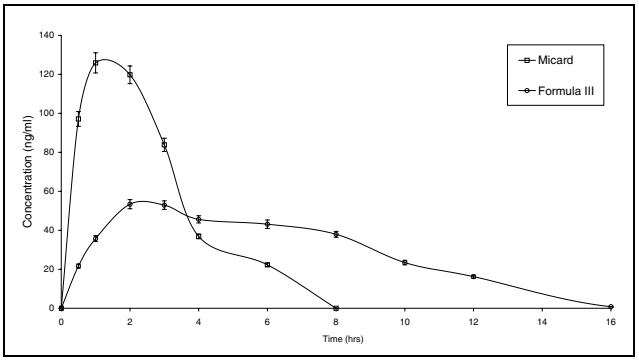

Fig. 3: Nicardipine hydrochloride average plasma concentration change by time following the administration of the prepared formula III and "Micard" capsules to rabbits (values are expressed as mean ± S.D.)

3. Experimental

3.1. Materials

Nicardipine hydrochloride batch no. 188 (kindly supplied by Misr Company, Cairo, Egypt). Eudragit L_{100} (Röhm Pharma GmbH Darmstadt, Germany). Cellulose acetate phthalate, ethyl cellulose 200, methyl cellulose low viscosity, hydroxypropyl methyl cellulose 4000 (kindly supplied by Cairo company, Cairo, Egypt). Absolute ethanol, acetone and isopropanol of pure analytical grades (Adwic, Egypt). Sodium chloride, sodium bicarbonate, tragacanth and lactose (El Nasr Company, Cairo, Egypt). Magnesium stearate (Alba Chemicals Company, USA). Hard gelatine capsules of various size numbers (kindly supplied by Egyptian International Pharmaceutical Industries Company (E.I.P.I.Co), Egypt). Commercially available nicardipine hydrochloride, Micard capsules batch no. 803037 (Misr Company, Cairo, Egypt). Acetonitrile, ethanol, methanol and ether of HPLC grade (Merck, Darmstadt, Germany). Hydrochloric acid and potassium dihydrogen phosphate (Prolabo, France). Sodium acetate (Merck, Germany). Sodium hydroxide (BDH, UK). Amitriptyline (kindly supplied by El kahira Company, Cairo, Egypt).

3.2. Equipment

USA standard testing sieve set (Sieves 1250–1000 μm). Modified USP dissolution tester (Pharma test type PTW, Germany). UV spectrophotometer (Shimadzu UV-240, Japan). Electric balance (Sartorious GmbH, Göttingen, Germany). Thermostatically controlled magnetic stirrer (Sybronthermolyne Nova 7 stir-plate). The HPLC system consisted of a Shimadzu LC-10 AD HPLC pump and a model SPD-10A Shimadzu UV-Visible detector connected to a Shimadzu C-R6A chromatographic integrator. The analytical column was a Jones ODS (250 mm × 4.9 mm I.D., particle size 5 μm) from Jones Chromatography. The system was operated at ambient temperature, and the detector sensitivity was set at 0.002 AUFS. The flow rate was isocretic at 1.5 ml/min (2000 psi).

3.3. Prepared solutions

Modified simulated gastric fluid of pH 1.2 was prepared by dissolving 2 g of NaCl in distilled H_2O, adding 7 ml of concentrated HCL then making the volume up to 1 L. A concentrated alkaline solution of 1 M KH_2PO4 containing 16.72% (w/v) NaOH was used to change the pH. The mobile phase was prepared by mixing acetonitrile with 0.02 M KH_2PO_4 (pH 4.8) (53 : 47 v/v). An aqueous 2 M NaOH solution was prepared and was washed once with an equal volume of CH_3CN and once with an equal volume of ether before use. A 0.1 M solution of HCl was prepared. Acid buffer was prepared by mixing equal volumes of 0.1 M sodium acetate solution and 0.1 M HCl. Stock solution of amitriptyline in ethanol was prepared at a concentration of 1 mg/ml. This was diluted with 2% ethanolic acid buffer to contain 100 ng/500 μl. Stock solution of nicardipine hydrochloride in C_2H_5OH at a concentration of 1 mg/ml was prepared. Spiking solutions containing 5, 10, 20, 50, 100 and 150 ng of nicardipine hydrochloride/500 μl of 4% ethanolic acid buffer, were prepared by serial dilution of the stock solution.

3.4. Animals

Adult male albino rabbits, weighing 2.2–2.5 kg, were used in this study.

3.5. Procedure

3.5.1. Preparation of solid dispersion

Coprecipitation was used, in which an ethanolic solution of the drug and Eudragit L_{100} at (1 : 4) weight ratio was evaporated under vacuum at 50 °C using a Rotavapor. The prepared coprecipitate was left in a dessicator for 24 h then pulverized. Particles which passed sieve number 60 and were retained on sieve 80 (177–250 μm) were used. Accurately weighed amounts corresponding to 40 mg drug were incorporated in the prepared formulae I and II.

3.5.2. Preparation of the floating capsule formulae

The contents of the prepared capsules are shown in Table 1. The ingredients of formula I were in powder form. It was prepared simply by mixing the accurately weighed powders until a homogenous mix was obtained then filled into the capsules.
The contents of formulae II–VII were in granulated form. The calculated amounts of powdered ingredients were mixed homogeneously and kneaded with absolute C_2H_5OH until a dough was obtained. The wet mass was granulated through a sieve of mesh size 1250 μm. The granules obtained were dried for 2 h in an oven at 50 °C and kept in a dessicator for 24 h then passed through a 1000 μm mesh size screen.

3.5.3. Testing uniformity of drug content

From the well-mixed dried granule or powder mixes, three different samples A, B and C each weighing 150 mg were tested for drug uniformity. Granules were powdered. The powder was extracted with successive small portions of absolute C_2H_5OH until 50 ml were collected by filtration in a volumetric flask. Extraction of plain granules or powder mix containing no drug was also done. The three samples were tested for drug content by measuring the solutions A, B and C spectrophotometrically at 237 nm [22] using the plain powder extract as blank.
No significant difference was found among the three tested samples indicating uniformity of drug content. Average drug content was defined. Based upon the drug content obtained, accurately weighed amounts of the prepared powder (formula I) or granules (formulae II–VII) containing 40 mg of nicardipine hydrochloride were filled into the capsules.

3.5.4. Dissolution study of nicardipine hydrochloride from the prepared floating capsules

This was determined using the USP rotating paddle method. Applying the pH shift technique [23], the dissolution media consisted of 900 ml modified simulated gastric fluid (pH 1.2) for the first 3 h followed by the addition of 4.5–7 ml of the prepared strongly alkaline solution to change the pH to 5 for 1 h then 2.5–4 ml for a further pH increase to 6.8 for 2 h. The temperature was maintained at 37 ± 0.5 °C and the paddles were rotated at 45 rpm. The dissolution test for each formula was repeated three times. The blank was made by subjecting one capsule with the same ingredients as the test sample but without nicardipine hydrochloride to the dissolution test.
Every 30 min, a 5 ml sample was withdrawn and filtered off. Each of the clear samples obtained was measured spectrophotometrically at 237 nm [22] against the corresponding blank. The dissolution volume was kept constant throughout the dissolution period, by compensation of the volume withdrawn. Mean results were tabulated and graphically illustrated.

3.5.5. Floating time determination

The USP dissolution apparatus was used in this test after removing the paddles. Each of the six glass vessels of the apparatus was filled with 900 ml of modified simulated gastric fluid of pH 1.2 maintained at 37 ± 0.5 °C. Each capsule formula was tested separately by placing it in the solution. The time for the capsule contents to remain buoyant in the solution was determined and taken as the floating time [24].

3.5.6. Bioavailability studies of nicardipine hydrochloride floating capsule formula of choice in comparison to the marketed "Micard" capsule

3.5.6.1. Construction of a calibration curve for HPLC assay of nicardipine hydrochloride in ethanolic solutions

Aliquot portions (50–1500 μl) of the nicardipine hydrochloride 1 mg/ml stock solution were transferred into 10 ml volumetric flasks. C_2H_5OH was added to volume to give final concentrations ranging from 5–150 μg/ml. A 20 μl aliquot was analyzed by HPLC and the corresponding area was determined. A curve was constructed relating concentration to area.

3.5.6.2. Determination of "Micard" drug content

The contents of 20 capsules of Micard were thoroughly mixed. The amount of powder equivalent to 20 mg of nicardipine hydrochloride (the content of one capsule) was extracted with successive small portions of C_2H_5OH and filtered in to a 100 ml volumetric flask. A 20 μl aliquot was analyzed by HPLC. For comparison, drug content was also analyzed spectrophotometrically at 237 nm [22].

3.5.6.3. Checking additive interference in "Micard" capsules by HPLC

This was done by the standard addition technique [19]. The contents of five Micard capsules were separately extracted in 100 ml volumetric flasks as previously described. Aliquots of 2, 4, 6 and 8 ml of the stock solution of nicardipine hydrochloride (1 mg/ml) were diluted by ethanol to 100 ml. 100 μls of the dilutions obtained were added to a series of volumetric flasks each containing 10 μl. of the Micard capsule extract. One of the flasks contained only 10 μl. Micard extract without any drug addition. Volume was completed to 100 mls. in all flasks. Total drug content was analyzed by HPLC. Drug content found for the sample containing only Micard extract was subtracted from the total drug content found for the other samples. Percentage recovery of the added drug was calculated.

3.5.6.4. Administration of the drug formulae to rabbits

The content of the prepared capsule formula III was administered orally to the rabbits by tube feeding, at a dose of 58.5 mg/kg body weight [25] (equivalent to 40 mg human dose). An amount of Micard capsule content equivalent to the same dose was similarly administered. The animals were divided into two groups each group containing 3 animals and each group received one of the two formulae tested. After one week the two groups exchanged the formulae in a cross over manner.

3.5.6.5. Blood sampling

Samples of blood were withdrawn from the rabbits' ear veins after 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h following drug administration.
The blood samples were collected in tubes rinsed with dilute heparin solution. After centrifugation, the clear plasma was extracted and analyzed.

3.5.6.6. Plasma sample extraction for HPLC determination of nicardipine hydrochloride

This was done according to Wu et al. [21]. One ml of rabbit plasma was transferred into a 15 ml culture tube (16 × 125 mm) fitted with a polyteflon-lined screw cap. One ml of 4% ethanolic acid buffer was added. A volume of 500 μl of the internal standard solution of amitriptyline [20] (100 ng/500 μl) and 500 μl of 2.0 M NaOH were added to the sample. The mixture was agitated on a vortex mixer for 15 s. This was followed by extraction with 4 ml ether by shaking on a mechanical shaker for 5 min then centrifugation for 5 min at 5000 rpm.
The organic layer was transferred to another 15 ml tube. A volume of 2 ml of 0.1 N HCl was added to the organic phase and the mixture was shaken for 5 min. The mixture was centrifuged, and the organic layer was removed by using a vacuum aspirator.
The aqueous fraction was alkalinized by the addition of 500 μl of 2.0 M NaoH. The mixture was extracted with 4 ml ether as described previously. After centrifugation, the organic layer was transferred to another 15 ml culture tube, and the solution was evaporated to dryness at 45 °C.
The residue was immediately dissolved in 70 μl of the HPLC mobile phase. An aliquot of 20 μl was injected into the liquid chromatograph. Spectrophotometric detection at 254 nm [21] was interpreted in the form of peak areas.

3.5.7. Preparation of a calibration curve for HPLC determination of nicardipine hydrochloride in rabbit plasma

1 ml samples of blank plasma spiked with nicardipine hydrochloride at levels of 5, 10, 20, 50, 100 and 150 ng/ml were used. The samples of spiked plasma were processed as described above. The calibration curve was obtained by plotting the ratio of the peak area of the analyte to that of the internal standard against the amount of analyte added.
Unknown concentrations of nicardipine hydrochloride in the experimental plasma samples were calculated with reference to the prepared calibration curve. Plasma levels against time were plotted.

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