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Review Article

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A Review on In-Vitro Characterization of Remipril Microsphere

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Abstract: Ramipril is a second-generation antihypertensive drug useful in the treatment of hypertention mainly in myocardial infraction which has short plasma half-life of 2-4 hours in adults along with bitter taste and faint odor. Microspheres are suitable drug delivery system for such drug candidate. In the present article endeavor, ramipril microspheres were attempted with a view to reduce the frequency of dosing and to attain steady state drug levels in addition to masking the bitter taste and faint odor of drug. Different batches of ramipril microsphere formulations F1 to F10 were developed using different polymers like eudragit RS-100, eudragit RL-100 and hydroxy propyl methyl cellulose (HPMC) at various ratios. The microspheres were prepared by non-aqueous emulsion solvent evaporation technique. Based on the results of the physicochemical characterization and in vitro drug release studies of all the batches of the formulated microspheres from F1 to F10 formulation F4 was chosen as the most satisfactory formulation as it possessed all the required physicochemical characters and prolonged duration of the drug release up to 24 hours. Scanning electron microscopy studies were showed microspheres are spherical and porous in nature and the drug release was found to be diffusion controlled.

Keywords: Microspheres, Emulsion solvent evaporation technique, Ramipril.

Introduction

An ideal controlled drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically for a specified period of time. Controlled drug delivery system releases the drug according to zero order rate in which the amount of drug released to the absorption site remains reasonably constant over a prolonged period of time.

The figures given below show the difference of drug release between conventional and sustained release dosage forms.

Profile of drug level in blood:

(a) Traditional dosing of tablets

(b) controlled drug delivery dose

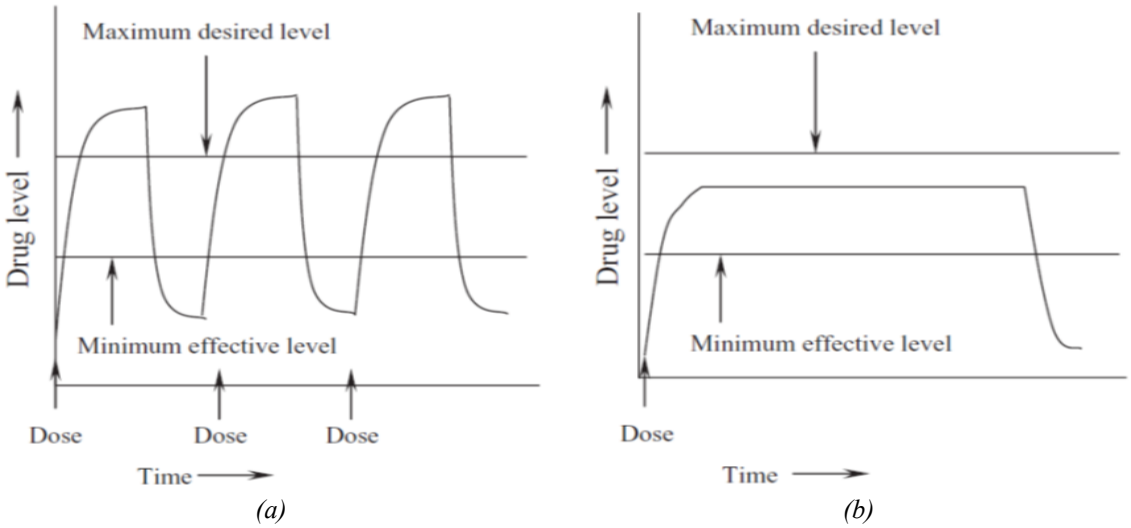

Figure 1: drug level in blood

Zero order process can be defined as the one whose rate is independent of the concentration of drug undergoing reaction. If the drug released from controlled release formulation is stable in fluids at the absorption site, has similar absorption efficiency from all absorption sites and is absorbed rapidly and completely after its release, then, its rate of appearance in plasma will be governed by its rate of release from the formulation. Thus, when the drug release follows zero order kinetics, absorption will also be a zero order process and concentration of drug in plasma at any time can be written as an equation.

$$C = C_0\text{-}K_0t$$

Where,

C_0 = concentration of the drug at time t=0

C = concentration of the drug yet to undergo reaction at time

K_0 = zero order rate constant

Microspheres as drug carriers

Microsphere based drug delivery systems have received considerable attention in recent years. The most important characteristic of microspheres is the microphase separation morphology which endows it with a controllable variability in degradation rate and also drug release.

Microspheres can be given orally as well as by injection.

Prerequisites for Ideal Micro particulate Carriers

The materials used in the preparation of microparticulate should fulfill the following prerequisites

- **Longer duration of action**
- **Control of content release**
- **Taste masking**
- **Increase of therapeutic efficiency**
- **Protection of drug**
- **Reduction of toxicity**
- **Biocompatibility & bioresorbability**
- **Sterilizability**
- **Relative stability**
- **Water solubility or dispersibility**
- **Targetability**
- **Polyvalent**

Microspheres - Description

Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000 μm. They are made up of polymeric, waxy protective substances, where the entrapped substances (the drug) are completely surrounded by a distinct wall, or the substance is dispersed throughout the microsphere matrix.

Microsphere is a structure made of a continuous phase of one or more miscible polymers in which particulate drug is dispersed, at either the macroscopic (particulates) or molecular (dissolution) level.

These polymer particles with porous inner surface and variable surface (from smooth and porous to irregular and nonporous) are produced on a micron scale, capable of releasing a preloaded drug that has been incorporated into a central reservoir and the release of the drug via the surface or bulk degradation of the polymer, with the release kinetics controlled by the type of the polymer and its properties.

The substances preferred in their formulation should be biocompatible synthetic polymers and natural products like starches, gums, proteins, fats and waxes.

Microspheres – Historical, Contextual Advent and Advantages

The concept of packaging microscopic quantities of material with in microspheres dates back to the 1930s and the work of Bungunberg de Jong and Co-workers on the entrapment of substances within coacervates; The first commercial application of encapsulation was by the national cash register company for the manufacture of carbon less copying paper. The technology and applications have advanced over the last several decades. The agricultural, food, household products, medical, graphics and cosmetic industries use this technology. The potential use of microspheres in the pharmaceutical industry has been considered since the 1960. Since then this concept has found a special relevance in the pharmaceutical formulations because of some mundane and inherent challenges associated with the present-day formulation problems.

Microspheres have emerged as one of the desired methods of drug administration since they offer a sophisticated grasp over the control aspects of drug administration. The administration parameters, which can be satisfactorily controlled, include-

- Taste and odor masking
- Conversion of oil and other liquids facilitating ease of handling
- Protection of the drugs from the environment
- Delay of volatilization
- Freedom from incompatibilities from drugs and excipients especially the buffers
- Improvement of flow properties
- Safe handling of toxic substances
- Dispersion of water insoluble substances in aqueous media
- And most finally production of sustained release, controlled release and targeted medications

Techniques for preparation of microspheres

1. Polymer phase-separation methods
2. Emulsification method (emulsification-solvent evaporation/Emulsification-solvent extraction)
3. Spray drying technique
4. Fluidized bed coating
5. Centrifugal extrusion technique
6. Rotational suspension separation
7. Melt dispersion technique

Ramipril is an angiotensin converting enzyme (ACE) inhibitor used mainly for the treatment of hypertension. It is a prodrug is hydrolysed in vivo to release the active metabolite ramiprilat. Ramipril has been classified as a class-IV substance according to Biopharmaceutics Classification System (BCS) by the Food and Drug Administration (FDA), meaning that it is low soluble and low permeable. Ramipril has short plasma half-life in adults, which is 2-4 hours with bitter taste and faint odor. The exatant of absoption of ramipril is 50% to 60% following oral administration.

Polymeric micro/nanoparticles are being increasingly investigated for controlled release effects and to achieve target drug delivery. Colloidal drug carriers are investigated in this field because their small particle size allows their permeation through biological barriers. Thus, a controlled release oral microparticle system could be an interesting and suitable way for its administration by promising to control the release and period of action of the drugs.

Formulation studies

Formulation Development of Ramipril Microspheres

Table 1: Composition of ramipril microspheres

FC	Ingredients (Ratio)			Liquid paraffin(ml)	Span-80(%)	Stirring speed (rpm)
	Ramipril	EudragitRs-100+HPMC	EudragitRL-100+HPMC			
F1	1	1	-	100	1	1000
F2	1	2	-	100	1	1000
F3	1	3	-	100	1	1000
F4	1	4	-	100	1	1000
F5	1	3	-	50	1	1000
F6	1	3	-	150	1	1000
F7	1	3	-	100	1	500
F8	1	3	-	100	1	1500
F9	1	-	3	100	1	1000
F10	1	-	4	100	1	1000

Various batches of microsphere formulations F1 to F10 for the drug ramipril were developed, as indicated in Table 3. Different batches of ramipril microsphere formulations F1 to F10 were developed using different polymers like eudragit RS-100, eudragit RL-100 and hydroxy propyl methyl cellulose (HPMC) at various ratios. The microspheres were prepared by non aqueous emulsion solvent evaporation technique using liquid paraffin as the processing medium for the purpose of choosing best polymers to prepare best batch of microspheres.

Results & Discussion

Pre-formulation studies

Drug Polymer Compatibility Studies By FTIR

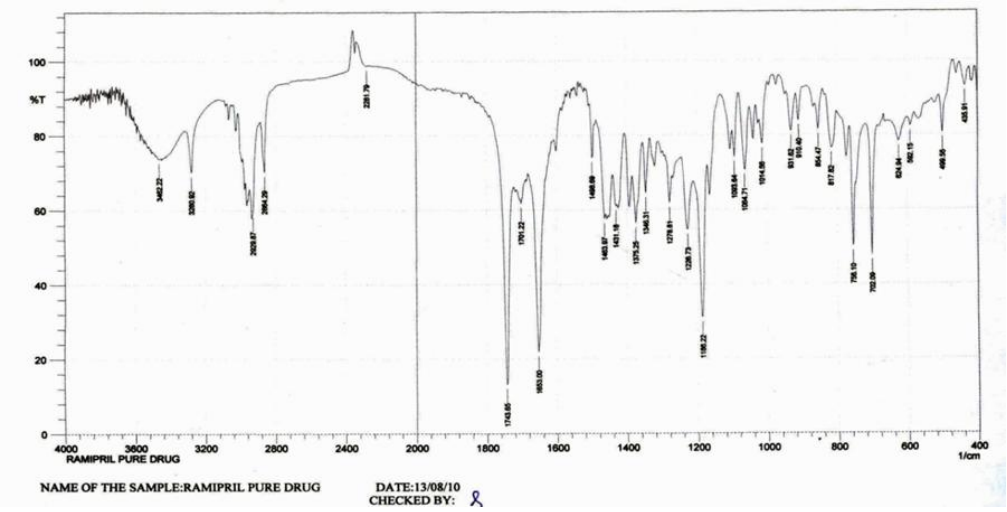

Figure 2(a): IR spectrum of pure drug sample ramipril

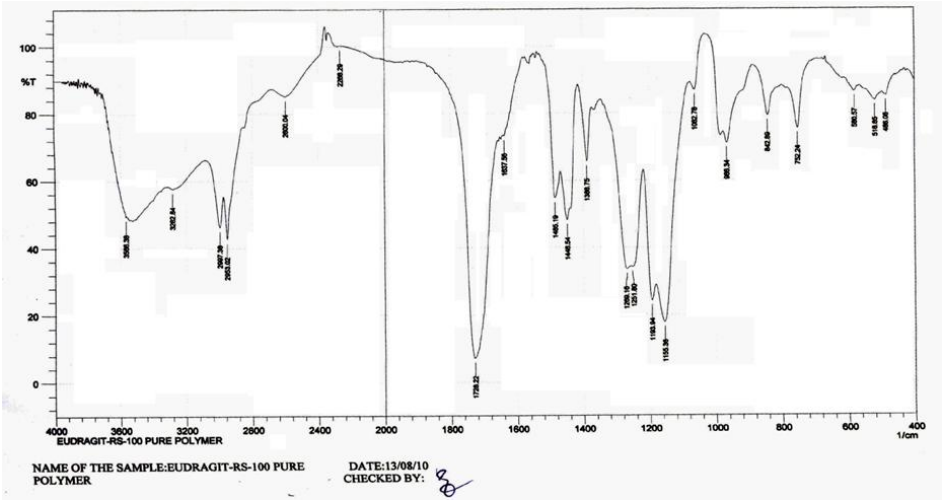

Figure 2(b): IR spectrum of pure polymer sample eudrajitRS-100

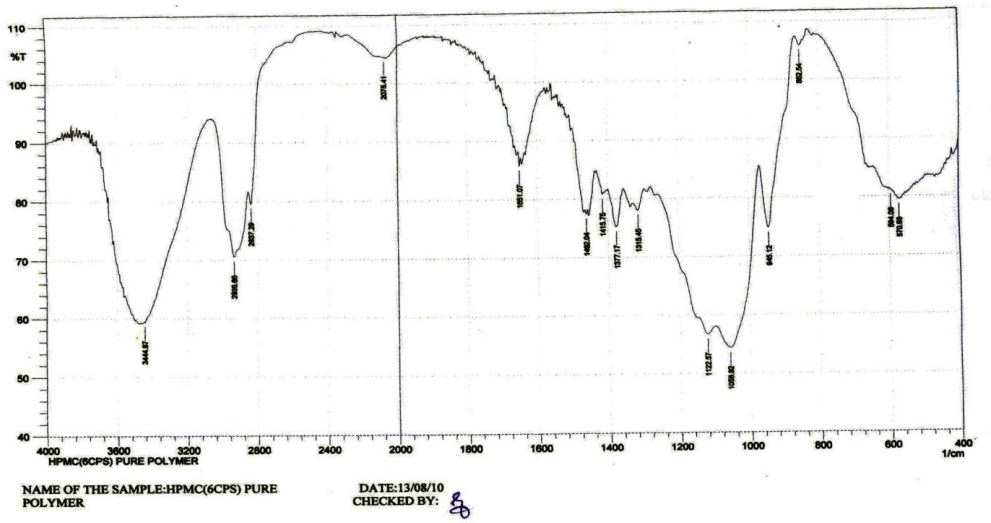

Figure 2(c): IR spectrum of pure polymer sample HPMC 6cps

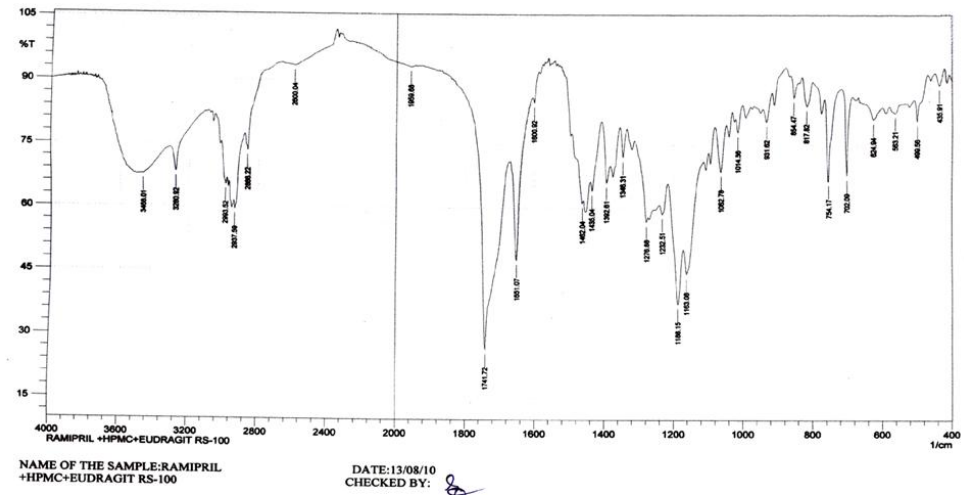

Figure 2(d): IR spectrum of physical mixture of drug ramipril and polymers eudrajitRS -100+HPMC (6cps)

Table 2: Observed IR values for physical mixture of drug and polymer

S. No.	Functional Group	Theoretical value (Cm^{-1})	Observed value (Cm^{-1})
1	CH_3	2872-2962	2866.22
2	COOH	2520-3300	2937.59
3	C-N	1266-1342	1276.88
4	C=O	1540-1870	1741.72
5	NH	3400-3500	3468.01

FT-IR studies and thermal analysis can be used to investigate and predict any physico-chemical interactions between components in a physical mixture and therefore can be applied to the selection of suitable chemically compatible excipients.

Drug Polymer Compatibility Studies By DSC

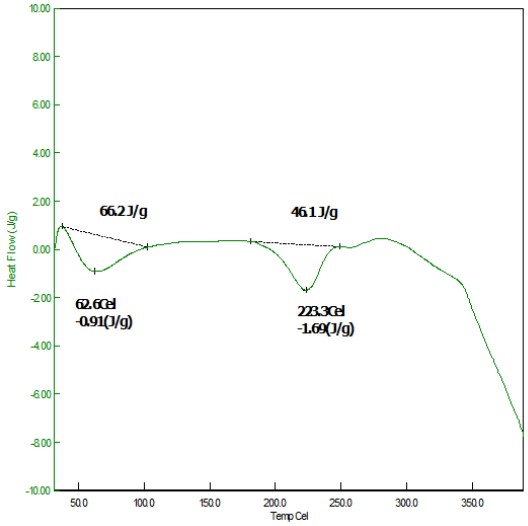

Figure 3: Drug Polymer Compatibility Studies By DSC

DSC thermogram of pure drug sample ramipril

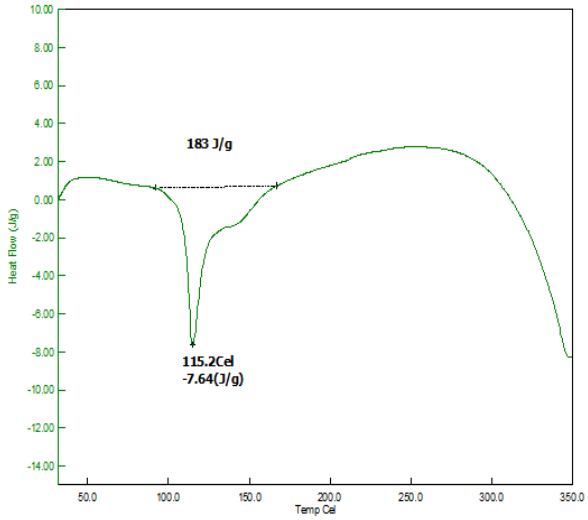

Figure 4: DSC thermogram of pure drug sample ramipril

In vitro drug release profiles of the formulated ramipril microspheres

Table 3: In vitro drug release profile of ramipril from formulations F-1toF-4

Time (hours)	Average*%drug released [(± SD) (n=6)]			
	F1	F2	F3	F4
0	0 ±0.00	0±0.00	0±0.00	0±0.00
1	17.6±1.11	8.77±0.50	5.04±082	4.56±0.65
2	27.8±0.65	17.9±1.05	14.6±0.60	12.16±1.14
3	36.5±0.52	24.5±0.51	21.6±0.23	23.7±1.12
4	45.9±0.46	31.7±1.66	32.3±0.61	29.56±0.61
5	57.8±1.05	38.5±1.05	35.2±1.94	36.7±0.4
6	67.4±0.46	46.8±0.61	41.3±0.28	38.2±0.23
7	75.7±1.05	53.7±1.006	46.3±1.00	41.1±0.65
8	83.8±0.88	56.7±±0.23	51.4±0.65	47.6±0.23
9	90.4±0.61	62.8±1.05	53.8±0.46	54.1±0.61
10	97.1±0.65	69.8±0.61	55.9±1.00	58.9±1.25
11	-	76.2±0.85	58.7±0.25	64.03±0.23
12	-	82.4±0.85	61.4±0.4	68.53±0.46
13	-	87.5±0.61	64.9±0.61	71.1±0.46
14	-	88.8±0.25	68.5±0.46	74.4±0.83
15	-	92.1±1.41	72±0.65	77.7±0.41
16	-	97.9±0.61	77.03±3.32	81.2±0.8
18	-	-	85.4±2.21	86.73±0.25
20	-	-	90.83±1.59	91.5±0.4
22	-	-	95.4±0.23	94.8±0.43
24	-	-	-	97.2±0.25

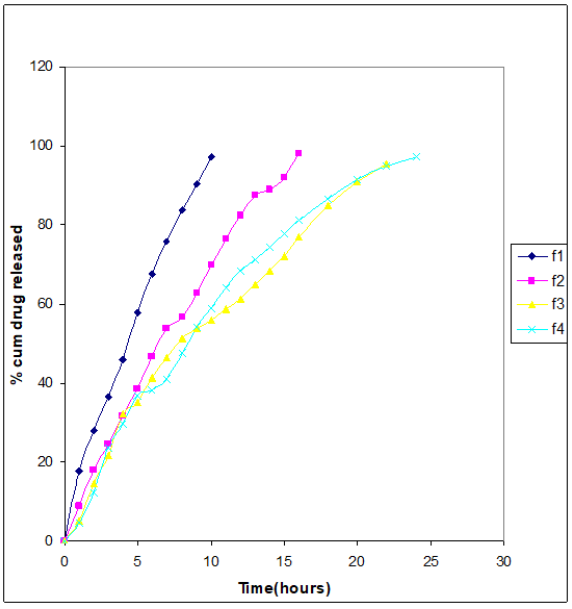

Figure 5: Comparison of the effect of polymer ratio on drug release profiles.

As illustrated in Figure 5, in formulations F1 to F4, 100% of the drug was released at the end of 10_{th} hour, 16_{th} hour, 22^{nd} hour and 24^{th} hour, respectively, when the drug-to-polymer ethyl cellulose ratio increased from 1:1, 1:2, 1:4 and

1:4 respectively. The decrease in drug release rate from 10 to 24 hrs from formulations F1 to F4 was due to increasing polymer can be explained by a decreased amount of drug present close to the surface and also by the fact the amount of uncoated drug decreases with increase in polymer concentration. This may be attributed to the higher polymer content which resulted in a larger particle size and a tightened polymer network and thus retarding drug release. In all the above formulations F1 to F4, the stirring speed was 1000rpm.

Table 4: In vitro drug release profile of ramipril from formulations F-5&F-6

Time (hours)	Average*%drug released [(± SD) (n=6)]	
	F5	F6
0	0±0.00	0±0.00
1	5.43±0.47	6.24.±0.31
2	8.73±0.76	12.3±0.90
3	13.58±0.89	15.5±0.70
4	19.9±1.68	20.6±0.78
5	26.4±1.2	29.3±1.10
6	31.4±0.85	36.4±1.13
7	39.2±0.91	46.4±1.77
8	44.5±0.65	52.6±2.1
9	48.3±1.26	57.3±1.87
10	55.1±1.25	62.8±2.45
11	61.4±0.83	67.7±1.89
12	64.4±1.30	71.7±0.45
13	71.2±0.8	75.8±0.83
14	76.8±2.31	80.8±1.41
15	83.2±2.18	88.5±0.65
16	87.7±1.67	94.5±1.05
18	93.8±0.4	98.9±0.32
20	98.4±0.80	-

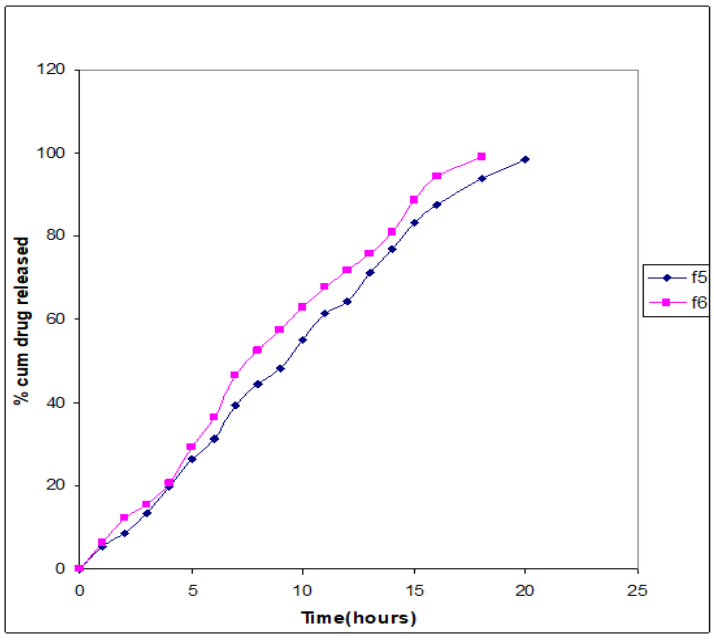

Figure 6: Comparison of the effect of volume of processing medium on drug release profiles.

As illustrated in Figure 6, in formulations F5 and F6, 100% of the drug was released at the end of 20th hour, 18th hour, , respectively. Therefore it was concluded that the duration of drug release depended on average particle size as discussed under section . The other reason could be due to the higher migration of drug to the surface of the microspheres during solvent evaporation from the freely moving emulsion droplets in large volume of processing medium.

Table 4: In vitro drug release profile of ramipril from formulations F-7&F-8

Time (hours)	Average*%drug released [(± SD) (n=6)]	
	F7	F8
0	0±0.00	0±0.00
1	5.63±0.77	7.1±1.41
2	8.5±0.83	11.6±0.80
3	12.8±0.69	19.6±0.49
4	19.9±0.66	24.8±2.14
5	25.7±0.66	28.5±0.43
6	30.5±0.85	36.5±0.45
7	38.5±0.61	46.2±0.39
8	44.9±0.45	51.5±0.45
9	48.9±0.23	57±0.23
10	55.4±0.65	62.7±0.4
11	60.8±0.61	67.4±0.38
12	65.8±0.51	70.5±0.45
13	71.3±0.8	74.7±0.83
14	76.5±0.23	78.4±0.61
15	82.7±0.36	82.5±0.32
16	87.5±0.61	88.4±0.66
18	94±0.4	96.59±0.63
20	98.9±0.4	-

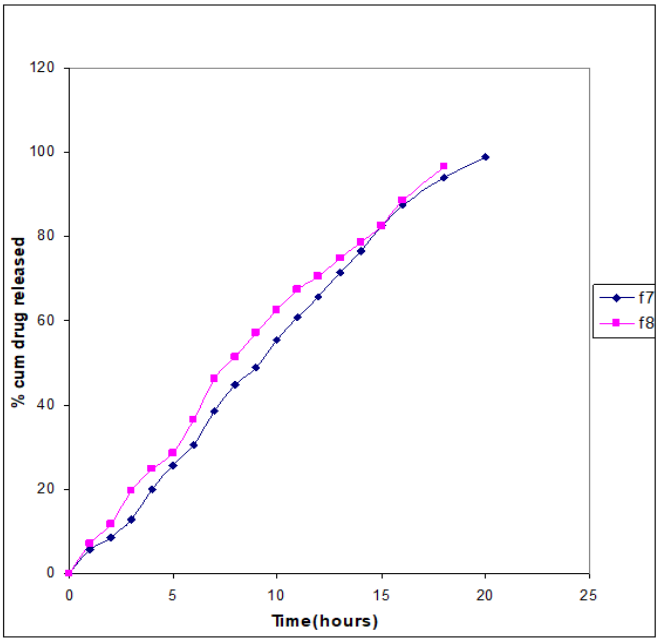

Figure 7: Comparison of the effect of stirring speed on drug release profiles.

In formulation F7 and F8, the effect of stirring speed on the drug release profile was studied and it was observed that 100% of the drug was released at the end of 20^{th} hour and 18^{th} hour, respectively, as illustrated in Figure 4; When the stirring speed was changed from 1000rpm to 500rpm and to 1500rpm, respectively. Faster drug release was observed from microspheres prepared at both the speeds, due to the formation of larger and smaller emulsion droplets, respectively, ensuring drug diffusion out of the microspheres before they harden at lower rpm as in case of formulation F7. This assumption of drug diffusion to the processing medium was supported by SEM analysis, which showed the presence of drug particles on the surface of the microspheres leading to initial burst effect. As indicated in Table 3, the particle size decreased at higher rpm as in case of formulation F8, from which the drug release was faster which could be due to larger available surface area.

All the above formulations F1 to F8 were prepared by using eudragitRS-100 and hydroxy propyl methyl cellulose (HPMC) combination, whereas formulations F9 and F10 were prepared by using eudragitRS-100 and hydroxy propyl methyl cellulose (HPMC) combination.

Table 5: In vitro drug release profile of ramipril from formulations F-9&F-10

Time (hours)	Average*%drug released [(± SD) (n=6)]	
	F9	F10
0	0±0.00	0±0.00
1	30.1±0.4	17.9±0.61
2	37.2±0.66	28±0.55
3	45.9±0.23	35.1±0.4
4	61.8±0.45	47.6±0.46
5	72±0.95	57.5±1.05
6	79±0.65	69.7±0.4
7	88.7±0.35	75.9±0.67
8	97.1±0.66	86.1±0.45
9	-	90.6±0.47
10	-	97.9±0.62

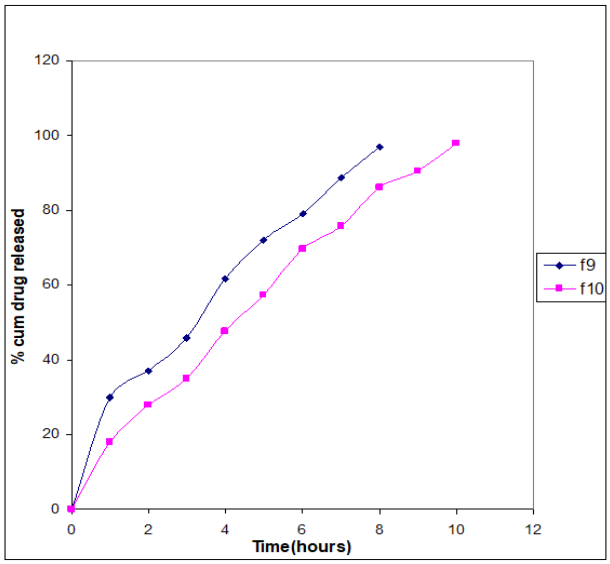

Figure 8: In vitro drug release profiles of ramipril from the formulationsF-9&F-10

In formulations F9 and F10, 100% of the drug released at the end of 8th hour, and 10th hour as illustrated in Figure 8. The duration of drug release was shorter in formulations F9 and F10 when compared to formulations F3 and F4

prepared by using eudragitRS-100 and hydroxy propyl methyl cellulose (HPMC) combination, which could be due to the difference in the content of quaternary ammonium groups, where the amount of quaternary ammonium groups of eudragitRS-100 is lower than that of eudragitRL-100, which renders eudragit RS-100 less permeable. The eudragitRL-100 highly permeable polymer increases the porosity of the matrix and, thus, accelerates the drug release.

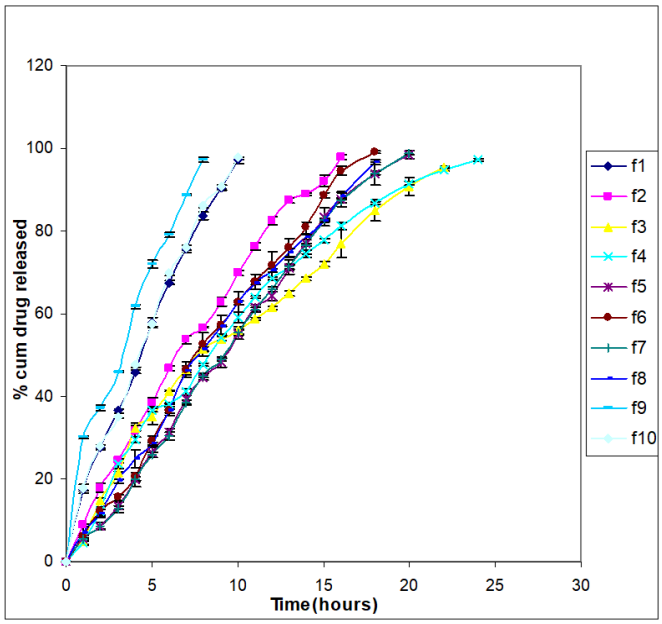

Figure 9: Comparison of drug release profiles from all the developed formulations F-1 to F-10

Based on the results of the physicochemical characterization and in vitro drug release studies on all the batches of the formulated microspheres from F1 to F10, formulation F4 was chosen as the most satisfactory formulation as it possessed all the required physicochemical characters and prolonged the duration of the drug release up to 24 hours where it released 100% of the drug.

Therefore, further studies like SEM analysis and mechanism of drug release were carried out on the formulation F4.

Release kinetics

Table 6: In vitro release kinetic parameters of formulation F-4

Formulation	Zero order (R^2)	Higuchi (R^2)	Korsemeyer-Peppas		Hixson-Crowell (R^2)
			R^2	N	
F-4	0.951	0.978	0.903		0.567

The in vitro ramipril release data from the most satisfactory formulation F-4 was fitted to various kinetic models and the mechanism of drug release was studied from the R^2 values obtained.

The data fitted with higher values in Higuchi model as well as Korsemeyer-Peppas model as indicated in Table 12. The 'n' value was found to be 0.567, which confirmed that the formulation followed Non-Fickian diffusion kinetics, i.e. the release is ruled by both diffusion of the drug and dissolution of the polymer. In this case the release mechanism shifted from initial dissolution to later extended diffusion in which both diffusion and erosion are governing the drug release.

Thus, the most satisfactory formulation F-4 satisfied the physico-chemical parameters and in vitro drug release profile requirements for a controlled release microsphere formulation of ramipril, in addition to masking the bitter taste and faint odour of the drug.

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