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FORMULATION DEVELOPMENT AND EVALUATION OF MICROSPHERES CONTAINING QUERCETIN EXTRACTED FROM *Phoenix sylvestris* ROOTS

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Abstract:

Phoenix sylvestris is generally known as Indian date and is native to India. It is traditionally important and known for its nutritional values throughout the world. It is a rich source of carbohydrate, phenols, amino acids, flavonoids, tannins, alkaloids, terpenoids, dietary fibers, essential vitamins and minerals. In the present study roots of Phoenix sylvestris was extracted by Soxhlet extraction method and the quercetin was isolated from the extract by preparative TLC method. Isolated compounds were characterized by spectroscopic techniques like Mass, IR, NMR, etc. Pharmacological investigation includes anti-cancer effect of methanolic extract of root. Isolated quercetin was used as drug to formulate microspheres. The microspheres were prepared by solvent evaporation method using Eudragit LR, 100 as polymer. Different batches (F1, F2, F3, F4, F5 and F6) were prepared with different concentration of polymer (Eudragit). The fabricated microspheres were subjected for different evaluation Parameters. Flow properties such as angle of repose, bulk density, car's index, Hausner'sratio and it were found that the microsphere shows the good follow properties. Particle size analysis was performed and it shows that increase in polymer concentration increases the particle size. All formulations were subjected for in vitro dissolution study and it was observed that the formulation F6 shows the highest release (93.79%). The release kinetic study has shown that drug release from microspheres follows the Higuchi model as the drug release occurs by diffusion. After performing Stability study for three month of the selected formulation shows no any changes in physical properties as well as in release pattern.

Key words: *Microspheres, Quercetin, Eudragit, Phoenix sylvestris*

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INTRODUCTION:

Natural products can be good remedies because they are inexpensive and easy to access [1]. Many plants, including some of the commonly consumed herbs and spices in our daily food, can be safely and effectively used to prevent and/or treat some health concerns. For example, caffeine the active ingredient found in coffee beans (*Coffea*), shows biological activity in the treatment of the central nervous system (CNS) disorders indole-3-carbinol, and 3,3′-diindolylmethane are both broccoli (*Brassica oleracea*) derived phytochemicals with potential anti-cancer activity [2]. Plant, Phoenix sylvestris, is widely known as Wild date palm. The synonyms of P. sylvestris are Date-sugar palm, Indian wild date, Indianwine palm, Silver date palm, Sugar date palm, and Sugar palm. P. sylvestris has been considered as a traditional medicine to cure various ailments like abdominal complaints, fevers, loss of consciousness, constipation and in heart complaints [3]. Date pits are rich in protein (5.1 g/100 g), fat (9.0 g/100 g), dietary fiber (73.1 g/100 g), phenolics (3942 mg/100 g), and antioxidants (80,400 µmol/100 g), and may be of use in enhancing the nutritional value of incorporated food products [4]. Flavonoids have been referred to as nature's biological compound because of their inherent ability to modify the reaction taking place in the body due to allergies, virus and carcinogens [5]. The flavonolquercetin (3, 3', 4', 5, 7- pentahydroxyflavone) a phytoalexin, is one of themost potent biomedical agents known. Several typesof diseases are inhibited by this biocompound such ascataract, coronary heart disease, diabetes and cancer, especially prostate cancer [6].

One of the most challenging areas of research in pharmaceuticals is the development of novel delivery systems for the controlled release of drugs and their delivery at the targeted site in the body to minimize the side effects and enhance the therapeutic efficacy of drugs [7]. Microsphere, as carrier for drug is one such approach which can be used in a sustained controlled release fashion [8]. Microspheres are used as carriers of drug and used to the controlled release of drug, vaccines, antibiotics, and hormones. Microspheres are defined as "therapeutic agent distributed throughout the matrix either as a molecular dispersion of particle. There is small spherical free flowing particle with a diameter in a range of 1 µm to 1000 µm. Microspheres are manufactured by using natural and synthetic materials polymer and waxes. Stability, solubility, and drug release depend upon the type of polymer used for the preparation of microspheres. Glass microspheres, polymer microspheres and ceramic microspheres are available. Glass microspheres are used as fillers and volumizer for weight reduction, retroreflector for high safety, additives for cosmetics and adhesives in medical technology. Polyethylene, polystyrene and expandable microspheres are the most common types of polymeric microspheres. Ceramic microspheres are used primarily as grinding media. Microspheres are manufactured in solid and hollow form. Hollow microspheres are used as additives to lower the density of a material. Microsphere-based topical formulations have gained wider importance in the treatment of psoriasis due to their ability for controlled drug delivery and enhanced therapeutic effectiveness for prolonged periods of time. Microparticulate drug delivery systems have recent trends that are especially suitable for achieving controlled or delayed release oral formulations with low risk of dose dumping, a flexibility of blending to attain different release patterns as well as reproducible and short gastric residence time. The stability and biological activity of drug should not affect during the microencapsulation process, yield and drug encapsulation efficiency should be high, microspheres quality and drug release profile should be reproducible within specified limits [9].

2. MATERIALS AND METHOD:

Quercetin was isolated from the methanolic extract of *Phoenix sylvestris* roots and it was used as active pharmaceutical ingredient for formulation of microspheres. Eudragit RL 100, polyvinyl alcohol, potassium hydrogen phosphate, sodium lauryl sulphate was obtained from Loba Chemicals, (Mumbai).

2.1. Preparation of microspheres

Suitable amounts of polymer (Eudragit RL 100) were added to a methanolic solution of the drug (Quercetin isolated from *Phoenix sylvestris* root extract). The aqueous phase was prepared by dispersing 0.2% PVA (polyvinyl alcohol) in water. The drug polymer solution was added to the aqueous phase with constant mixing. The mixture was stirred with a propeller at 500 rpm for 3 hours at 25 °C for complete removal of chloroform. The mixture was filtered to collect the microspheres, which were then washed with deionized water. These microspheres were dried at room temperature for 24 hours [10]. The compositions of the different formulations are shown in Table 01.

Table 1: Formulation design

Sr. No.	Formulation Code	Drug Polymer Ratio
1	**F1**	1:1
2	**F2**	1:2
3	**F3**	1:3
4	**F4**	1:4
5	**F5**	1:5
6	**F6**	1:6

2.2. EVALUATION OF MICROSPHERES

2.2.1. Micromeritic studies

A). Angle of repose

The fixed funnel method was used for estimating the angle of repose for different formulations. (n ¼ 3)

$$\Theta = \tan^{-1} (h/r)$$

Where q is angle of repose, r is the radius, and h is the height [11].

B). Bulk density and tapped density

Microspheres (5 g) were added into a 5-mL graduated cylinder and the final volume was noted down to calculate bulk density (Db). The cylinder was then tapped mechanically 100 times to obtain the tapped volume for computing the tapped density (Dt) [12, 13].

C). Carr's index. Carr index and Hausner ratio

The Carr Compressibility Index and Hausner Ratio are two measures which can be used to predict the propensity of a given powder sample to be compressed, and which are understood to reflect the importance of interparticulate interactions. These interactions are generally less significant for a free-flowing powder, for which the bulk and tapped densities will be relatively close in magnitude. Poorer flowing materials are characterized by the existence of larger interparticle interactions, so a greater difference between bulk and tapped densities is observed.

The two indices are calculated using the following relations [14, 15].

$$\text{Carr index} = (Dt - Db) \times 100/D_t$$

$$\text{Hausner ratio} = D_t/D_b$$

D). Particle size and shape

The sizes of the microspheres were determined using an optical microscope, fitted with an ocular micrometer. The ocular micrometer was calibrated with a stage micrometer. A total of 100 microspheres were evaluated and the mean diameter was reported.

2.2.2. Drug Entrapment Efficiency

The 10 mg of prepared microspheres were crushed in a glass mortar and the powdered microspheres were suspended in a 10 ml of methanol after 24 hours, the solution was filtered and the filtrate was analyzed after suitable dilutions using UV spectrophotometer (V- 630, Jasco, Japan) at λ max 268 nm. The amount of drug entrapped in the microspheres was calculated by the following formula:

Drug entrapment efficiency =Practical drug content / Theoretical drug content X 100

Samples of the formulations were crushed with potassium bromide, and pellets were formed. The spectra of quercetin, Eudragit, placebo microspheres, and drug-loaded microspheres were recorded in the range of 4000–400 cm−1 using IR spectrophotometer (FT/IR 4100, Jasco, Japan).

2.2.3. SCANNING ELECTRON MICROSCOPY

SEM (Scanning Electron Microscopy) allows investigations of the microspheres surfaces and after particles are cross-sectioned, it can also be used for the investigation of double walled systems. Scanning electron microscopy (SEM) was used for determining the surface morphology of microspheres with drug (JEOL Model JSM-6390LV SAIF, Kochi, Kerala).

2.2.4. IN VITRO DISSOLUTION STUDY

In vitro drug release study of microspheres was carried out using USP paddle type apparatus at 37 ± 1◦C and at 100 rpm using 900mL 0.1 N HCl (pH 1.2). Cumulative % release was calculated for all the formulation and compared.

2.2.5. DRUG RELEASE KINETICS

The zero-order rate describes systems where drug release is independent of its concentration and this is applicable to the dosage forms like transdermal system, coated forms, osmotic system, as well as matrix tablets with low soluble drugs. The first-order equation describes systems in which the release is dependent on its concentration (generally seen for water-soluble drugs in porous matrix). Higuchi‖ developed an for the release of a drug from a homogeneous polymer matrix type deliver system that indicates the amount of drug release is proportional to the square root of time. If the release of drug from the matrix, when plotted against square root of time, shows a straight line, it indicates that the release pattern is obeying Higuchi‘s kinetics.

Zero order release equation

$$Qt = k0\ t$$

First order release equation

$$\text{In } Qt = \text{In } Qo - k1\ t$$

Higuchi‘s square root of time equation

$$Qt = kH\ t^{1/2}$$

Korsmeyer and Peppa's equation

$$F = (Mt/M) = Km\ t^{n}$$

Hixon—Crowell equation

$$Wo^{1/3} - Wt^{1/3} = K_s t$$

Where Qt is the amount of drug released at time t; Qo is the initial amount of the drug in the formulation; k0, k1, kH, and km are release rate constants for zero-order, first-order, Higuchi and korsmeyer model rate constant of equations respectively.

Where,
Wo is the initial of drug in the microsphere,
Wt is the remaining amount of drug in microspheres at time t and Ks is a constant incorporating the surface-volume relation.

2.2.6. DIFFERENTIAL SCANNING CALORIMETRY

Thermal behavior of the drug and polymer and the Data obtained by performing angle of repose are as follows.

behavior of the drug in the fabricated microspheres was determined employing differential scanning calorimetry (DSC). Samples were placed into aluminum containers and heated in a temperature range of 20–400°C under nitrogen gas flow by applying minimum pressure, an empty aluminum pan was used as a reference.

2.2.7. Stability studies of optimized formulation

The optimized formulations sealed in aluminum packaging and kept in the humidity chamber maintained at 40 ± 2 ºC and 75% ± 5 RH for three months. At the end of each interval, samples were analyzed for the physical observation, drug content and *in vitro* release profile.

3.RESULT AND DISCUSSION

3.1. Micromeritic studies

Different Micromeritic studies were performed as mentioned below.

3.1.1. Angle of repose

Table 02: Angle of repose

Sr. No.	Formulation Code	Angle of repose (°)
1	F1	25.23
2	F2	22.19
3	F3	23.43
4	F4	20.19
5	F5	23.22
6	F6	19.33

It was observed that all the formulations were free flowing as indicated by the angle of repose value less than 30.

3.1.2. Bulk density and tapped density

The values of bulk and tapped densities have shown good packing ability. Results achieved are as under.

Table 03: Bulk density and tapped density of microspheres

Sr. No.	Formulation Code	Bulk density (g/mL)	Tapped density (g/mL)
1	F1	0.55	0.60
2	F2	0.52	0.54
3	F3	0.49	0.68
4	F4	0.48	0.61
5	F5	0.33	0.43
6	F6	0.52	0.51

3.1.3. Carr's index Carr index and Hausner ratio

The values of Carr indices were 13.22 to 19.55% with the lowest Ci value for F5, indicating its excellent compressibility. The Hausner ratio for the formulations was in the range of 1.17 to 1.23, showing their good flow properties. After considering various micromeritic parameters, it can be inferred that F6 was the best formulation having the best flow properties with low angle of repose value (19.33), Carr index (17.52%), and Hausner ratio (1.19).

Table 04: Carr's index Carr index and Hausner ratio of microspheres

Sr. No.	Formulation Code	Car's index	Hausner ratio
1	F1	15.25	1.23
2	F2	13.22	1.17
3	F3	19.55	1.19
4	F4	16.45	1.22
5	F5	12.59	1.15
6	F6	17.52	1.19

3.1.4. Particle size and shape:

Particle-size distribution is affected by the interaction between the dispersed phase and the dispersion medium. In this study, the mean particle size increases with the increase in polymer concentration due to increase in relative viscosity. Increase in polymer content increases particle size, which subsequently decreases the effective surface area. In addition, the path length traveled by the drug molecule is also increased. The average particle size for each formulation is presented in Table below.

Table 05: Particle size analysis of microspheres

Sr. No.	Formulation Code	Particle Size (µm)
1	F1	754.9
2	F2	772.3
3	F3	781.1
4	F4	707.9
5	F5	732.4
6	F6	629.9

2.3. Drug Entrapment Efficiency

Drug entrapment efficiency of all batches was determined. The results indicated the polymer concentration plays a major role in drug entrapment efficiency. As the polymer concentration were entrapment efficiency increased respectively. The data obtained are mentioned as follows.

Table 06: Percentage yield, percentage loading, and encapsulation efficiency

Sr. No.	Formulation Code	Drug Polymer Ratio	Theoretical Loading (%)	Actual drug Loading (%)	Encapsulation Efficiency (%)	Yield (%)
1	F1	1:1	51.21	28.9	58.6	76.3
2	F2	1:2	32.33	22.8	64.50	74.8
3	F3	1:3	23.00	17.84	73.96	80.3
4	F4	1:4	50.33	31.89	66.52	81.4
5	F5	1:5	33.33	25.18	76.26	87.3
6	F6	1:6	26.1	22.64	90.56	92.5

3.3. **Infrared Spectroscopy**

Formulations F6 were subjected for infrared spectroscopy as this batch shows the highest yield as well as good follow properties. From the FTIR spectra, one can confirm the chemical stability of the drug in a formulation, here, the IR spectra of quercetin exhibited a broadened phenolic −OH band at 3407/cm, −CO stretching at 1670/cm, an aromatic bending and stretching about at 1100 and 1600/cm, and a − OH phenolic bending about at 1200 and 1400/cm. IR spectra of Eudragit RL100 showed a characteristic peak at 1731/cm. A small peak of quercetin −CO stretching at 1670/cm can be observed around the characteristic peak of Eudragit around 1730/cm thus concluding with the FTIR analysis, it can be inferred that the main characteristic peaks of quercetin were extant in the entire microspheres which established its presence without any interaction with the excipients.

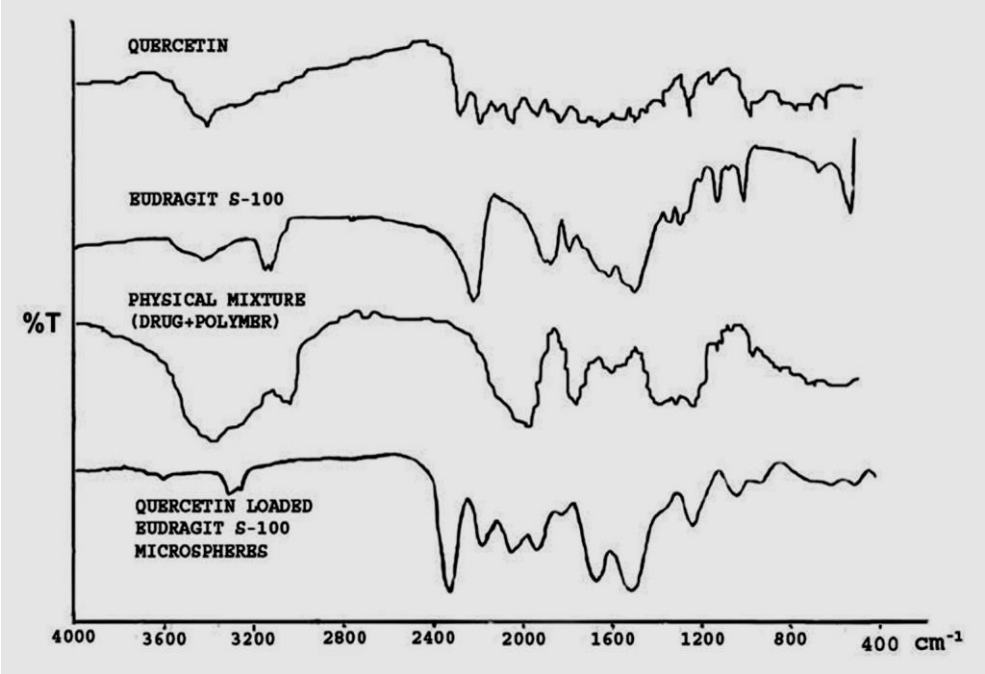

Figure01: Fourier transform infrared spectrum of quercetin, Eudragit RL100, physical mixture of drug with polymer and drug-loaded microspheres.

2.4. SCANNING ELECTRON MICROSCOPY

The prepared microspheres were found to be rough surface but spherical and good appearance. SEM photomicrographs of microspheres are shown in FigureNo.02.

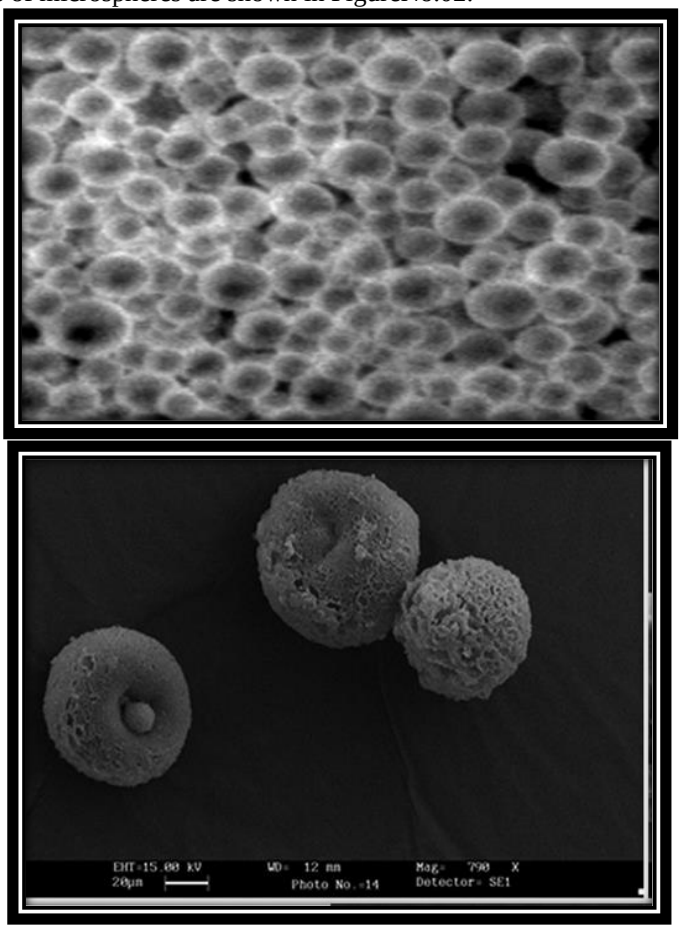

Figure 02. Scanning electron microscopy images of quercetin-loaded Eudragit microspheres

***2.5: IN VITRO* DISSOLUTION STUDY**

All formulations were subjected for in vitro drug release by using USP paddle type apparatus at 37 ± 1◦C and at 100 rpm using 900mL 0.1 N HCl (pH 1.2). Cumulative % release was calculated for all the formulation and compared. F6 formulation showed the maximum release in constant manner for 24 hours. Results obtained from dissolution study are stated as below.

Table 07: *in vitro* dissolution study of microspheres

Sr.No	Time (H)	% CDR (F1)	% CDR (F2)	% CDR (F3)	% CDR (F4)	% CDR (F5)	% CDR (F6)
1	0	0.00	0.00	0.00	0.00	0.00	0.00
2	1	1.07	4.82	7.31	5.21	4.55	5.21
3	2	4.82	6.89	12.52	7.33	6.89	7.15
4	3	5.31	9.63	17.89	11.25	12.18	10.89
5	4	7.65	12.55	23.13	15.56	17.49	14.78
6	5	10.71	15.07	27.88	19.22	21.55	18.08
7	6	13.52	16.22	32.44	22.07	25.78	22.52
8	7	15.36	18.63	35.21	25.87	28.79	27.14
9	8	16.12	21.77	37.54	28.12	33.12	31.25
10	9	18.22	23.89	41.32	31.41	38.22	35.85
11	10	21.33	27.55	46.87	33.78	42.55	39.28
12	11	22.89	29.93	49.25	37.25	47.21	42.89
13	12	24.12	34.48	51.22	41.09	50.83	46.11
14	13	28.45	36.88	55.32	44.52	55.52	50.22
15	14	29.94	39.55	58.52	48.77	59.87	55.60
16	15	32.44	40.89	60.44	51.33	64.25	60.21
17	16	33.51	43.78	63.55	56.25	67.88	64.93
18	17	34.87	46.52	66.85	60.18	72.01	68.09
19	18	36.55	49.03	68.45	64.82	76.55	73.52
20	19	38.27	51.23	70.79	68.77	79.32	77.02
21	20	40.41	53.44	72.66	73.51	81.11	81.43
22	21	43.22	55.98	74.52	78.22	83.45	85.23
23	22	45.35	59.55	75.12	82.05	85.12	89.14
24	23	46.63	61.22	77.85	85.32	86.99	91.85
25	24	48.92	62.08	77.85	87.12	90.22	93.79

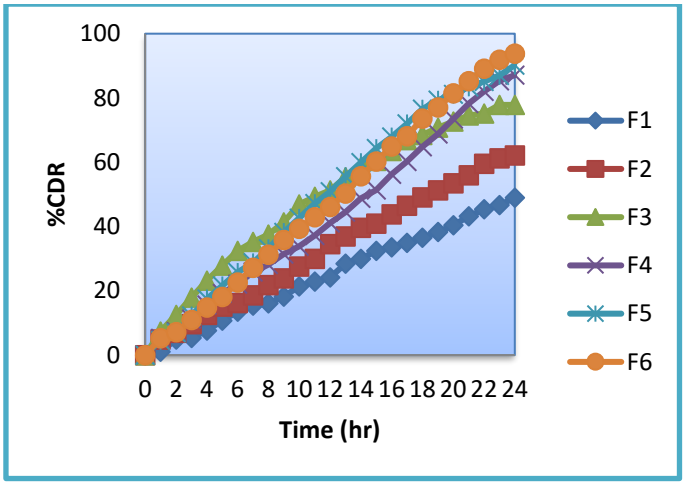

Figure 03: cumulative drug release from microspheres

2.5. DRUG RELEASE KINETICS

The drug-release mechanism was studied by comparing the respective correlation coefficients for different release models. It was observed that the drug release was diffusion controlled.

Table 08: Drug release kinetics for microspheres

Formulation Code	Zero Order (R^2)	First Order (R^2)	Higuchi Model (R^2)	Korsmeyerpeppas Model (R^2)
F1	0.9835	0.8364	0.9863	0.9217
F2	0.9615	0.9709	0.9794	0.9133
F3	0.8626	0.9877	0.9356	0.9505
F4	0.9098	0.9892	0.9907	0.8991
F5	0.919	0.9849	0.9918	0.9481
F6	0.9576	0.9813	0.9953	0.9721

2.6. DIFFERENTIAL SCANNING CALORIMETRY

Quercetin as a pure drug presented a sharp endothermic peak at 318°C and a broad endothermic peak for dehydration from 101 to 108°C. Eudragit RL100 exhibited two endothermic peaks at 81.79°C and 230°C whereas the physical mixture of the drug and polymer showed a reduced peak intensity of quercetin in the mix from 318°C to 310°C. Quercetin loaded Eudragit RL-100 microspheres showed a characteristic melting peak for Eudragit at 93°C, possibly due to the dilution effect of the amorphous polymer, the peak for the drug was not bulging due to the dispersion of the drug in the microspheres indicative of the absence of any crystalline form of the drug.

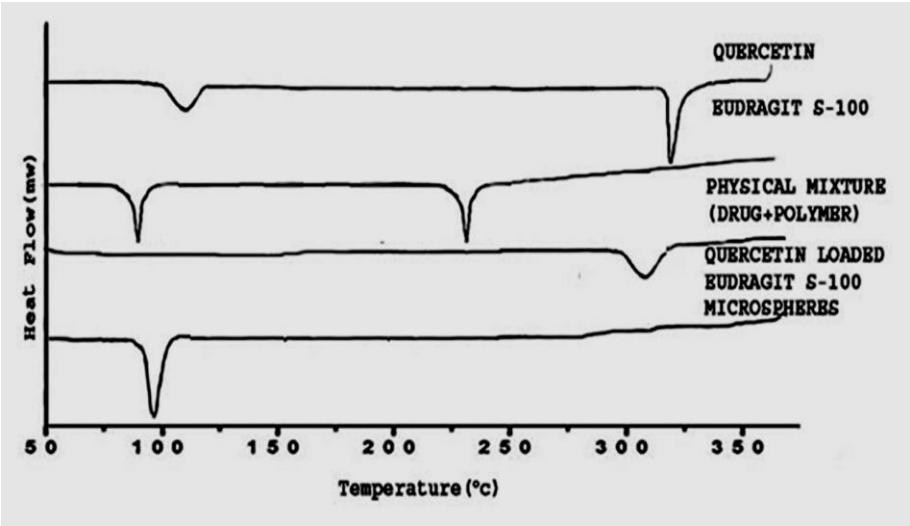

Figure 04: Differential scanning calorimetry thermogram of quercetin, Eudragit RL100, physical mixture of drug with polymer and drug-loaded microspheres

2.7. Stability studies of optimized formulation

For stability study the formulation F6 was selected and after performing the stability test for three month it was observed that the formulation does not shows any changes in physical properties as well as in drug release. The data obtained is shown in bellow table.

Table 09: Stability study of microspheres

Parameters	Temperature 40 ºC / 75% RH			
Time	**Initial**	**1st month**	**2nd month**	**3rd month**
Physical appearance	-	No Change	No Change	No Change
In-vitro drug release (%)	93.16	92.92	94.00	93.10

3.CONCLUSION:

Quercetin is the plant lavonol from flavonoid group of polyphenols. It is found in many plants including *Phoenix sylvestris* which is from Indian origin. Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half-life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. For obtain maximum therapeutic efficacy and minimum side effects it necessary to deliver the agent to the target tissue in the optimal amount. Microspheres are the one of the approaches to increase the bioavailability. On the basis of present study Eudragit microspheres containing quercetin showed the excellent results and it can be increasing the oral bioavailability of quercetin for the treatment of number of diseases such as arthritis, bladder infection and cancer.

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