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SOLUBILITY ENHANCEMENT OF ANDROGRAPHOLIDE AND FORMULATION DEVELOPMENT OF HOLLOW MICROSPHERES

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ABSTRACT

Andrographolide is water insoluble and belongs to BCS Class II. It is used widely in treating diabetes, diarrhoea and viral infections. Traditionally it is also used for treating peptic ulcer. Andrographolide was obtained by extraction using methanol in soxhlet extractor from *Adrographis paniculata leaves*, Family Acanthaceae, defatted prior with Petroleum ether. Ethanol extrative value was found to be 2.92% w/w. The extract was subjected to column chromatography. Andrographolide was obtained with repeated column chromatography using chloroform and methanol as a mobile phase. The percentage purity of Andrographolide was 85.84% by densitometric analysis. The obtained Andrographolide was reconfirmed by UV-Visible spectroscopy. FT-IR and TLC. The solubility of Andrographolide was increased to about 13% by Solvent evaporation technique using PEG 6000. Hollow microspheres were developed with emulsion solvent evaporation method. The resulted formulation revealed 74.44% drug content. The formulation was characterized for Particle size, SEM, Floating lag time, Total floating time, Porosity, *In-vitro* drug release and stability studies. The *in-vitro* release was found to be 85% in 12 h revealing sustained release from the developed hollow microspheres.

KEYWORDS: Andrographolide; Hollow microspheres; Column Chromatography.

1. INTRODUCTION

Kalmegh plant is traditionally used to treat treat infections and some diseases. Kalmegh consists of the dried aerial parts, mainly stems and leaves *Andrographis paniculata* Nees. Family Acanthaceae. Kalmegh contains not less than 1.0 per cent w/w of Andrographolide, calculated on dried basis.[1-3] Some of the recent research proved clinically and non-clinically,

anti-inflammatory, aantioxidant, anti-diabetic, anti-diarrhoeal, anti-HIV activities [4-6]. Some studies are focusing on the mechanism of action for anti-malarial, anti-bacterial, anti-fungal, hepato-protective and anticancer activity.[7-9]

Novel drug delivery systems like hollow microspheres are designed to achieve a continuous delivery of drugs at probable and reproducible kinetics over an extended period of time in the circulation. The potential advantages includes minimisation of drug related side effects due to controlled therapeutic systemic levels. Novel drug delivery systems are designed to achieve continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimisation of drug related side effects due to controlled therapeutic blood levels. Hollow microspheres are empty spherical particles without core. These types of systems are based on non-effervescent approach. Hollow core is responsible for the floating behaviour of particles and which gives rise to very promising and sustained release of drug from matrix. These particles are free flowing in nature having particle size less than 200µm. The sustained release of drug from floating system increases the gastric retention and reduces the fluctuations in drug plasma concentration. The quantity of polymers, the plasticizer-polymer ratio and the solvent used for formulation modulate buoyancy and drug release from the dosage form. Research has proved that these kinds of particles can float on surfactant containing acidic for more than 12 hours.[10, 11]

Hollow microspheres can be prepared by using any one method of following[12, 13]:

- Emulsion solvent evaporation
  - Oil in water emulsion solvent evaporation
  - Water in oil emulsion solvent evaporation
- Emulsion solvent diffusion
- Spray drying

2. MATERIALS AND METHODS

2.1. Materials

Andrographolide RS was purchased from Sigma-aldrich India and polymer was obtained from Evonik, Mumbai as a gift sample. All the solvents were purchased from SRL labs. Glyceryl mono stearate was purchased from Otto Chemie Pvt. Ltd., Poly vinyl alcohol was purchased from Loba chemicals. Dialysis membrane was purchased from HiMedia.

2.2. Extraction and Separation Procedure

Kalmegh leaves were collected in the month of May from Dhule Locality, India, dried at 60°C pulverised and then passed through #120. Resulting powder was subjected to defatting by using Petroleum Ether at 40-50°C for 12 h. The remnant was subjected to reflux condensation at 40-50°C for 8 hours using methanol as a solvent. Resulting extract then concentrated and the loaded on column containing silica 60-120 mesh. The separation was carried out using gradient system of chloroform : methanol (100:0, 98:2, 95:5, 90:10, 80:20, 70:30, 50:50, 30:70, 80:20, 10:90, 5:95, 2:98, 0:100). Fractions of 100 ml were collected at a speed of 2 ml/min. The fractions were monitored by thin layer chromatography (TLC) for the constituents. Based on the TLC results, fraction no. 4 and 5 were combined, concentrated and re-loaded onto another column and separated using gradients elution using same mobile phase in the ration of 100:0, 99:1, 98:2, 97:3, 96:4, 95:5. Fractions of 10 ml were collected at a speed of 1 ml/min. TLC monitoring revealed separation of Andrographolide in the last pooling from the column. The isolated Andrographolide was characterized.[14-16]

2.3. Characterization of Isolated Andrographolide

Isolated Andrographolide and the Andrographolide RS were subjected to UV Spectroscopy and FTIR for qualitative determination. Quantitative analysis was performed using HPTLC. HPTLC analysis of extracted and maker andrographolide was done by using silica gel GF-254 and using Chloroform: Methanol (9:1) as a mobile phase.[17, 18]

2.4 Enhancement of solubility

Solubility of andrographolide was enhanced by using solvent evaporation method in which drug and Glycerol mono stearate 6000/ HPMC/ Polyvinyl acetate was dissolved in methanol in 1:1 and 1:2 proportion and kept at 40°C until all solvent gets evaporated. And after that the solubility was evaluated by saturation solubility method.

2.5 Formulation of Hollow Microspheres

Hollow microspheres containing andrographolide are prepared by emulsion solvent diffusion method. Weighed amount of andrographolide was mixed with Eudragit-S 100 in a mixture of Dichloromethane and Ethanol (1:1) at room temperature. Glycerol Mono stearate was used as an emulsifying agent. The resulting drug-polymer solution was poured gradually into water containing polyvinyl alcohol, final solution was maintained at 40° C and the preparation was stirred for 3 hours to obtain o/w emulsion. The resulting hollow microspheres were filtered, washed with water and dried in air for 24 h.[19-20] The details of formulation batches are given

In table No. 1.

2.6 Evaluation of Hollow microspheres

2.6.1 Percentage Drug Content

The drug content of hollow microsphere was estimated by UV-Visible spectroscopy in which specific amount of powder was dissolved in methanol and further dilution were made by distilled water and absorbance was taken at 231 nm. The formula used for calculation of % drug content is as follows:

% Drug Content = Practical drug present/ Actual Drug Present X 100.

2.6.2 Particle size evaluation

The particle size of andrographolide hollow microspheres was evaluated by using phase contrast microscope.

2.6.3 Floating lag time

The floating lag time was evaluated by pouring specified amount of hollow microspheres in 0.1N HCl.

2.6.4 Total Floating time

The total floating time of particle was evaluated by noting the time taken by microsphere to submerse into 0.1N HCl.

2.6.5 In-vitro drug release

The level of drug release from microspheres having diameters of between 7 and 10 micron was measured by the paddle method at 100 rpm specified in JP XIII as follows. Microspheres (74 mg) were dispersed in JP XIII No.1 solution composed of HCl and NaCl (300 ml, pH 1.2, 37 8°C). The level of the drug release was determined spectrophotometrically employing a UV detector (Perkin Elmer). The absorbance of aliquots was measured at 231nm.

2.6.6 Porosity

Sample equivalent to 25 ml was tapped 100 time using a measuring cylinder. After completion of 100 taps sample is measured by its volume in measuring cylinder. The percentage porosity was calculated using the following formula:

% Porosity = Bulk Volume – True Volume / Bulk Volume X 100.

2.6.7 Scanning Electron Microscopy (SEM)

The SEM (Quanta 200 ESEM system) of optimized microsphere batch was performed at ICON Analytical Equipment Pvt. Ltd., Mumbai.

2.6.8 Stability

Stability of Hollow Microspheres containing andrographolide was checked at different storage conditions for which a weighed amount of formulation was taken in a vial and kept at different storage condition for one month, and then evaluated for visual observation, FT-IR and for % Drug Content. Details of Temperature and humidity conditions for stability studies are as listed below,

Condition	*Temperature*	*Humidity*
1	30ºC ± 2ºC	60% ± 5%RH
2	25ºC ± 2ºC	60% ± 5%RH
3	05ºC ± 2ºC	------

3. RESULTS AND DISCUSSION

3.1. Evaluation of extracted andrographolide

The extracted andrographolide was characterized on UV spectrophotometer revealed λmax of 231.13 which was comparable to andrographolide RS as shown in fig no 1. IR fingerprinting as given in fig no. 2 revealed major peaks at 3399.71 cm-1, 1727.21 cm-1, 1454.50 cm-1 and 741.27 cm-1 which shows presence of –OH group, Carbonyl group, C=C stretch and C-C stretch, respectively.

The HPTLC chromatograms as shown in fig No 3. revealed 85.84% purity of the isolated andrographolide which was calculated from AUC of andrographolide RS

3.2. Evaluation of Solid dispersion

The solid dispersion for the solubility enhancement were evaluated which revealed maximum increase in the solubility of andrographolide with PEG 6000 (1:1 ratio) as shown in table 2.

3.3. Evaluation of Hollow microspheres containing Andrographolide

The formulation F2 prepared with Solid dispersion of Drug: PEG 6000 was evaluated for the size and shape by Phase contrast microscopy and SEM imaging revealed spherical and hollow natured hollow microspheres with the particle size of 7 um as shown in Fig no. 4. & 5.

Drug content of batch F2 was found to 72.84% as shown in table no 3. The less entrapment of the solid dispersion may be due to increased solubility but this could resulted in sustaining the release from the developed formulation.

The time required by Hollow microspheres to reach surface of media is found be 30 seconds. Buoyancy and drug release from microspheres containing andrographolide drug exhibited sustained release in 0.1 N HCl as illustrated in Fig. 5. The Hollow microspheres were in floating state till end of 6 hours and 30 minutes.

3.3. Stability Studies

The hollow microspheres are evaluated after storing the hollow microspheres for one month at different storage conditions and found no significant difference in its visual characteristics and FT-IR spectra. The % drug content was found to be 70.78%, 67.25% and 71.25% at 30ºC±2ºC- 60%±5%, 25ºC±2ºC-60%±5% and at 05ºC±2ºC respectively.

Figure and Table legends

Fig. 1. Comparison of extracted andrographolide and andrographolide RS by UV-Vis. Spectroscopy.

Fig. 2. Comparison of FT-IR spectrum of extracted andrographolide and andrographolide RS

Fig. 3. HPTLC chromatogram for A) extracted andrographolide and B) andrographolide RS

Fig. 4. Contrast phase imaging of Hollow Microspheres containing Andrographolide

Fig. 5. SEM images of Hollow Microspheres containing Andrographolide

Fig. 6. Release pattern of Hollow microsphere (F2) and pure drug

Table 1. The details of formulation batches

Table 2. Evaluation of Solubility Enhancement

Table 3. Percentage drug content of respective batches

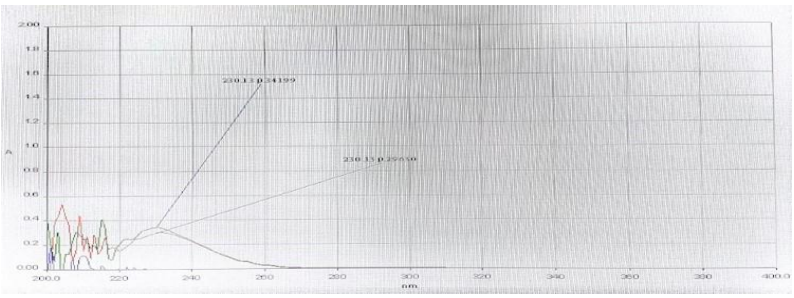


Fig. 1. Comparison of extracted andrographolide and andrographolide RS by UV-Vis. Spectroscopy.

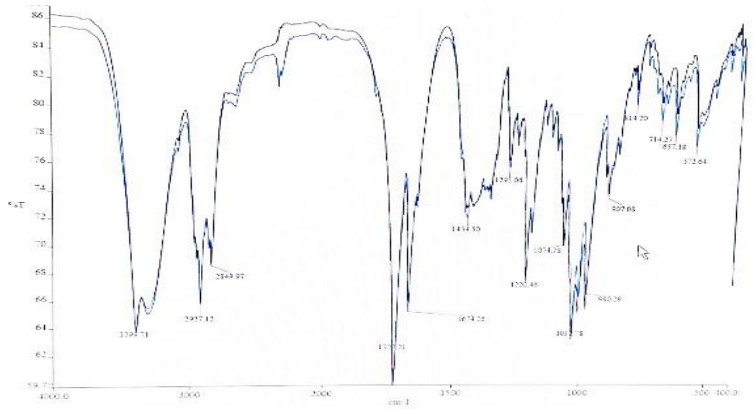
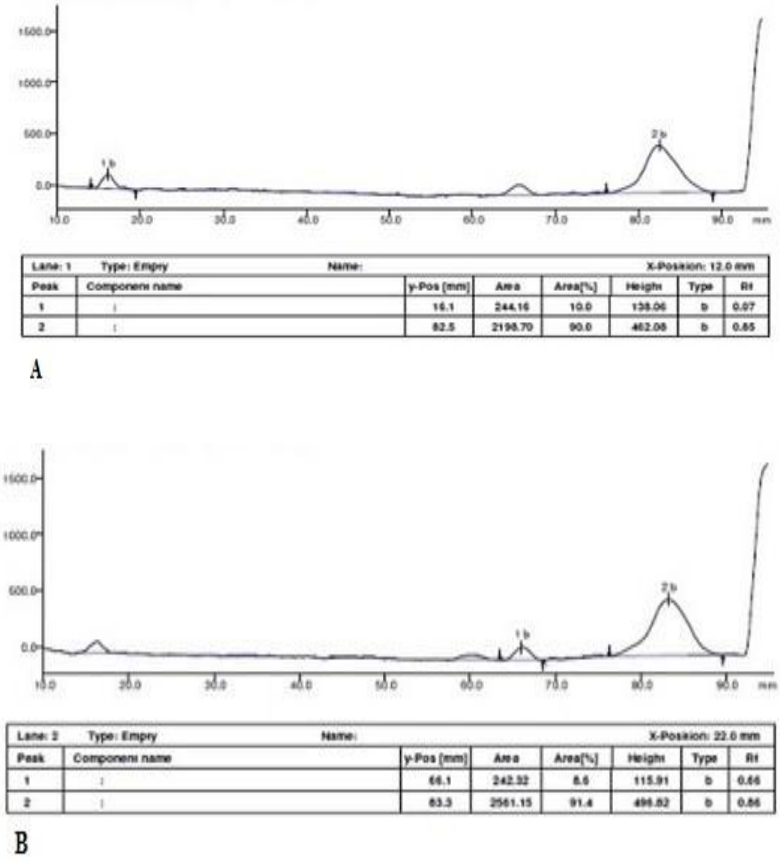


Fig. 2. Comparison of FT-IR spectrum of extracted andrographolide and andrographolide RS.



Lane: 1 Type: Empty Name: X-Position: 12.0 mm

Peak	Component name	y-Pos [mm]	Area	Area[%]	Height	Type	Rf
1		16.1	244.16	10.0	138.06	b	0.07
2		82.5	2198.70	90.0	462.06	b	0.85

A

Lane: 2 Type: Empty Name: X-Position: 22.0 mm

Peak	Component name	y-Pos [mm]	Area	Area[%]	Height	Type	Rf
1		66.1	242.32	8.6	115.91	b	0.66
2		83.3	2561.15	91.4	496.82	b	0.86

B

Fig. 3. HPTLC chromatogram for A) extracted andrographolide and B) andrographolide RS.

Fig. 4. Contrast phase imaging of Hollow Microspheres containing Andrographolide

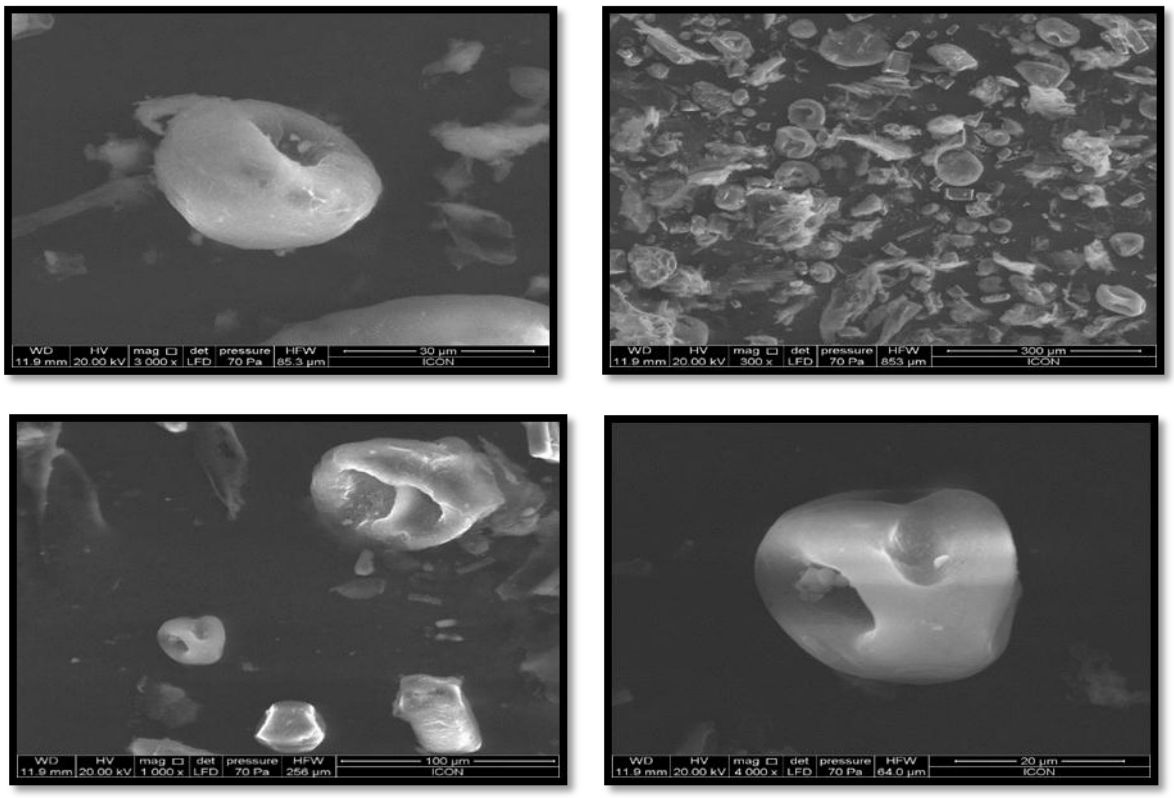


Fig. 5. SEM images of Hollow Microspheres containing Andrographolide

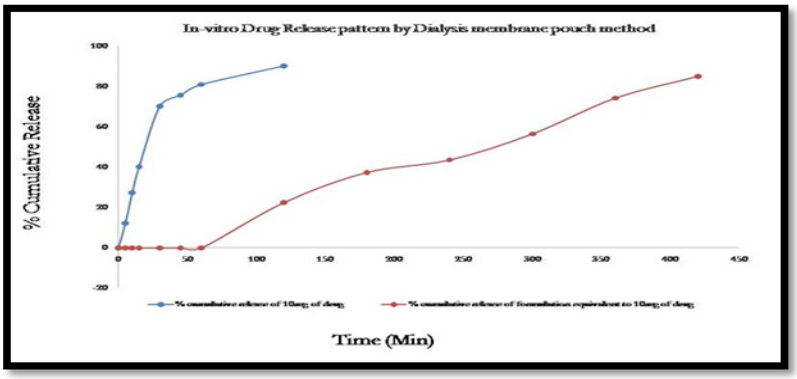


Fig. 6. Release pattern of Hollow microsphere (F2) and pure drug

Table 1 The details of formulation batches

Batch Excipients	*F 1*	*F 2*	*F 3*	*F 4*	*F 5*	*F 6*	*F 7*	*F 8*	*F 9*
Andrographolide	10	10	10	10	10	10	10	10	10
Water (ml)	2	2	2	2	2	2	2	2	2
PVA (mg)	20	20	20	20	20	20	20	20	20
DCM (ml)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Ethanol (ml)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
GMS (mg)	5	5	5	5	5	5	5	5	5
Eudragit RS-100 (mg)	10	10	10	20	20	20	30	30	30
RPM	500	1000	1500	500	1000	1500	500	1000	1500

Table 2 Evaluation of Solubility Enhancement

Sample	*Ratio*	*Absorbance*	*Solubility (µg/ml)*
Pure Drug	--	0.075	33.24
Drug: PEG 6000	1:1	0. 1435	**46.94**
Drug: PEG 6000	1:2	0. 1460	47.44
Drug: HPMC	1:1	0.0982	37.88
Drug: HPMC	1:2	0. 1230	42.84
Drug: Polyvinyl Acetate	1:1	0. 1047	39. 18
Drug: Polyvinyl Acetate	1:2	0.1257	43.38

Table 3 Percentage drug content of respective batches

Batch	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*
% Drug content	54.82	72.84	61.04	50.82	56.78	62.84	49.24	60.28	64.28

4. CONCLUSION

The Andrographolide was isolated with the significant purity and then it was converted into a formulation that is hollow microspheres. The Andrographolide Hollow microspheres a floating system is a promising approach for targeting the drug to gastric diseases or disorder (peptic ulcer) for sustained drug delivery. Further *In-vitro* and *In-vivo* studies are required to check the efficacy of hollow microspheres and for its applicability as an anti-microbial agent. Stability studies of one month has been performed but study for 3 months and 6 months are also necessary to be performed.

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