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FORMULATION, EVALUATION AND CHARACTERIZATION OF NARINIGN LOADED MICROPARTICLES USING ACACIA NILOTICA GUM

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ABSTRACT

The objective of the work was to purify natural polymer (*Acacia nilotica* gum) and formulate Naringin loaded microspheres using sodium alginate and *Acacia nilotica* gum. The microspheres were prepared by ionic gelation method using fixed concentration of sodium alginate and varying amounts of Acacia nilotica gum. The average percentage yield of all batches ranged from 59.4 to 78.4% suggesting that ionic gelation technique is suitable for microspheres preparation. The average particle size of the formulations ranged from 26.4 ± 16.025 µm to 30.58 ± 10.724 µm. The drug content of the microsphere formulations ranged from 63.50 to 86.17%. The drug release ranged from 42.18 to 98.77% from the microsphere formulations at the end of 6 hours of the study. From the overall results obtained for drug content, particles size and micromeritic properties it could be concluded that the formulation F4 containing sodium alginate and *Acacia nilotica* gum in the ratio 1:1.25 was the best formulation.

KEYWORDS: Naringin, Microsphere, Ionic gelation, Sodium alginate, *Acacia nilotica* gum.

INTRODUCTION

Of the several possible routes of introducing controlled release medication into the body, the oral administration of single-dose medicine is one of the simplest and safest because it does not pose the sterility problem, and risk of damage at the site of administration is also minimal.[1] Although it does possesses issues related to frequent dosing of drugs owing to first pass metabolism of drugs leading to a reduced bioavailability. In order to maintain the therapeutic level of the drug for longer periods and to decrease its toxic levels, many efforts have been made to use polymers as membrane devices.[2] Microspheres are composed of

artificial or natural polymers that can be modified to speed up or slow down the degradation of the polymer reservoirs (and, therefore, modify drug release kinetics). These can be used to improve the bioavailability of drugs encapsulated in them thereby enabling reduced dosing.[3] Hence it is often considered to be a very good approach for extended release of drug molecules especially those with a shorter half life. An increase in the release duration increases the inherent efficacy of the drug molecule and also reduces the overall cost of the regimen due to a decreased dosing frequency.

Naringin is a natural antioxidant isolated for *Grapefruit* and is known to possess a large number of pharmacological actions. The antioxidant potential of the molecule is mainly responsible for almost all of its effect on the human body.[4] The half life of the molecule is though low (3.5h) which limits the use of the molecule in therapy.

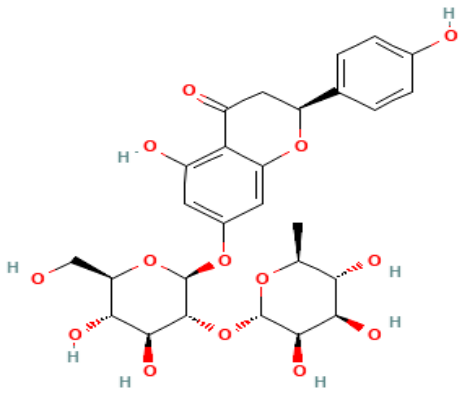

Figure 1: Chemical structure of naringin.

A few reports have mentioned the use of naringin microspheres for improvement of bioavailability as well as to improve the half life of narinign.[5,6] It was therefore envisioned to encapsulate Naringin in microparticles and increase the half life of the molecule thereby its antioxidant potential.

MATERIAL AND METHODS

Naringin was procured from Yarrow Pharmaceuticals, Mumbai; Acacia nilotica gum was purchased from online e-commerce store (Amazon India) and sodium alginate was purchased from Oxford Fine Chemicals, Mumbai. All other reagents as well as chemicals were of analytical grade. UV Visible spectrophotometer (Labtronics, LT-2201) was used for measuring the absorbance were ever required.

Purification of *acacia nilotica* gum

The purchased gum was subjected to purification process as reported in previous study.[7,8] Briefly, 100 g of the procured crude gum was oaked in warm water for 4 h, boiled for 2 h and kept aside for 2 h in order to release the gum in water. The material was then squeezed using muslin cloth to separate the mark and the filtrate. Equal volume of ethyl alcohol was added to the filtrate for separating the gum. After separation, the gum was dried in the oven at 45ºC, powdered and passed through sieve #80. The powdered gum was stored in the desiccator until further use.

Preformulation studies of naringin

The procured Naringin was observed its color; the taste and odor were observed using tasting and smelling the drug. Solubility of Naringin was determined in water, methanol, ethanol, ethylacetate, n-butanol and petroleum ether. The melting point of Naringin was determined by open capillary method.

Standard curve of naringin

The maximum absorption of Naringin in ethanol was observed at 295 nm. The calibration curve was obtained using different concentrations of the drug at the above wave length.[9]

The stock solution was freshly prepared by dissolving 5 mg of Naringin in 50 ml of ethanol in a 10 ml volumetric flask and then made up the solution upto the mark using the same buffer for obtaining the solution of strength 100 µg/mL (stock I). 5 mL stock solution was taken and volume made up to 50 mL by using ethanol to obtain 10 µg/ml. From this solution with draw 2, 4, 6, 8, 10 ml of solution in to the 10 ml volumetric flask and volume made up to 10 ml by using ethanol to get the solutions of 2, 4, 6, 8, 10 µg/ml. The absorbance of each dilution was observed at 295 nm using UV spectrophotometer and a calibration curve was plotted.

Formulation of microspheres

The Naringin loaded microspheres were formulated using the ionic gelation method. Sodium alginate was dissolved in sufficient amount of water by heating at temperature between 40–50ºC. The specified quantity of polymer (*Acacia nilotica* gum) was then added to the above solution (Table 1). When the polymer dissolved, the drug (Naringin) was added and dispersed in the polymeric solution under continuous stirring for 2 h. Meanwhile a 5% calcium chloride solution in water was prepared as a cross-linking agent and placed on the magnetic stirrer.

The drug and polymer mixture was filled into the syringe and drop wise added into the calcium chloride solution using needle #24 under continuous stirring at 50 rpm. The prepared microspheres were allowed to stand in the calcium chloride solution for 2 h for curing. After that the prepared microspheres were filtered by using Whatman filter paper and dried using hot air oven at 50ºC temperature and stored carefully.

Table 1: Composition of naringin microspheres.

Ingredient	Formulation					
	F1	F2	F3	F4	F5	F6
Sodium alginate (g)	1.0	1.0	1.0	1.0	1.0	1.0
Acacia nilotica gum (g)	0.5	0.75	1.0	1.25	1.50	1.75
Naringin (mg)	100	100	100	100	100	100
$CaCl_2$ (%)	5	5	5	5	5	5
Distilled Water (mL)	30	30	30	30	30	30

Evaluation of naringin loaded microspheres

Determination of yield

The microspheres were collected and weighed accurately. The percentage yield was then calculated using formulae given below:

$$\% \text{ yield} = \frac{\text{Mass of the dried microspheres obtained} * 100}{\text{Total Weight of drug and polymer}}$$

Determination of particle size of microspheres

The particle size of the microspheres was determined by using an Magnus microscope, employing the calibrated eye piece and stage micrometer method. A small amount of the dry microspheres were dispersed in 10 mL hexane and a drop of the dispersion was placed on a glass slide. The size of 50 particles of each formulation was measured using calibrated eye piece.

Micromeritic properties

The angle of repose was determined by fixed funnel technique, bulk density and tapped density were determined using tapped density apparatus and the flow indicators (Carr's Index and Hausner's ratio) were calculated using formula.[1]

Drug content

The drug content of the prepared microspheres was determined by the method of extraction of drug present in microspheres. Drug loaded microspheres (100 mg) were powdered and extracted in 100 ml Phosphate buffer 6.8 PH for 24 h. Then the resultant dispersion of microspheres was sonicated for 30 minutes for complete mixing and filtered through a Whatman filter paper. The concentration of drug present in filtrate was determined spectrophotometrically at 295 nm using phosphate buffer pH 6.8 as blank. The drug content of prepared microsphere was calculated by the formula

% Drug content = Drug content found X 100 /Total weight of microspheres

***In vitro* release of naringin**

In vitro release of Naringin from the microspheres was determined in Phosphate buffer at pH 6.8 using paddle type dissolution test apparatus. Microspheres equivalent to 100 mg of drug were taken and packed in capsule and suspended in dissolution medium maintained at 50 rpm at 37 ± 0.5°C. 5 mL of sample was withdrawn periodically at different intervals for the next 6 h, replacing same volume of fresh medium. The samples were filtered through Whatman filter paper and analyzed at 295 nm by UV spectrophotometer for amount of drug released.

RESULTS AND DISCUSSION

Purification of *acacia nilotica* gum

The yield of the purified gum was found to be 77% (77 g) of dry weight of raw gum and the gum was white in color with no odor and had a sweet taste.

Physical characterization of naringin

Naringin was found to be a pale yellow, amorphous powder with a bitter taste and no odor. It exhibited good solubility in water, methanol and ethanol while was not very soluble in ethyl acetate, n-butanol and petroleum ether. The melting temperature range of naringin was observed to be 231-240°C. Previous study has also revealed the solubility order of Naringin to be methanol > ethyl acetate > n-butanol > isopropanol > petroleum ether > hexane.[10]

The Calibration curve of Naringin was constructed by plotting absorbance versus concentration (µg/ml) at 295 nm (Figure 2). The regression equation was used to calculate the concentration of Naringin in the microspheres as well as in the release study.

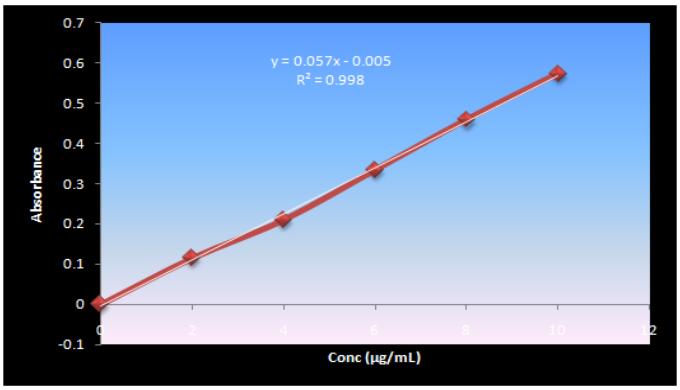

Figure 2: Calibration curve of naringin in ethanol.

Formulation of naringin microspheres

The microspheres were prepared by ionic gelation method using fixed concentration of sodium alginate and varying amounts of Acacia nilotica gum. A 5% concentration of calcium chloride was used as the counter ion solution for cross-linking the particles. A total of six microspheric formulations were prepared.

Evaluation of microspheres

The yield of the microspheres was calculated by weighing the dry mass of the microspheres obtained and utilizing the prescribed formula (Table 2). The average percentage yield of all batches was higher than 50%. This shows that ionic gelation technique is suitable for microspheres preparation. The average particle size of the formulations ranged from 26.4 ± 16.025 µm to 30.58 ± 10.724 µm. It was observed that on increasing the concentration of *Acacia nilotica* gum, the particle decreased perhaps owing to dense packing of the particles due to the gum acacia. The lowest particle size was obtained when the ratio of alginate and acacia gum was 1:1.25. On increasing the concentration of the gum to more than 1.25 times of the alginate, the particle size started to increase. The drug content of the microsphere formulations ranged from 63.50 to 86.17 %. The drug entrapment was highest in F4 and lowest in F6.

Table 2: Yield, Size and Drug content in formulated microspheres.

Formulation	Yield %)	Particle size (µm)	Drug content (%)
F1	59.4	29.92 ± 15.242	71.24
F2	63.8	29.48 ± 13.778	75.51
F3	78.2	27.06 ± 15.747	79.43
F4	78.4	26.4 ± 16.025	86.17
F5	77.9	30.58 ± 10.724	76.33
F6	78.1	31.24 ± 14.463	63.50

Micromeritic properties

The angle of repose was found to be in the range of 21°32' to 27°54'. The bulk density value ranged from 0.350 to 0.444 g/cm^3. The tapped density value of various formulations of the microspheres was found to be in the range from 0.370 to 0.487 g/cm^3. The formulations prepared in the present work were found to have the Carr's Index between 5.405 to 12.258 % and Hausner's ratio from 1.057 to 1.139 (Table 3).

Table 3: Micromeritic characters of formulated microspheres.

Batch Code	Angle of Repose	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Hausner's Ratio	Carr's Index
F1	24.33	0.444	0.487	1.096	8.829
F2	21.32	0.408	0.465	1.139	12.258
F3	27.54	0.416	0.455	1.093	8.571
F4	24.32	0.35	0.37	1.057	5.405
F5	24.18	0.422	0.451	1.068	6.430
F6	26.19	0.418	0.469	1.122	10.874

***In vitro* drug release from microspheres**

As the half life of Naringin is around 3.5 h, it was considered that a drug release for upto 6 h from the microspheres would be justifiable to as improvement in bioavailability of the drug (Figure 3).

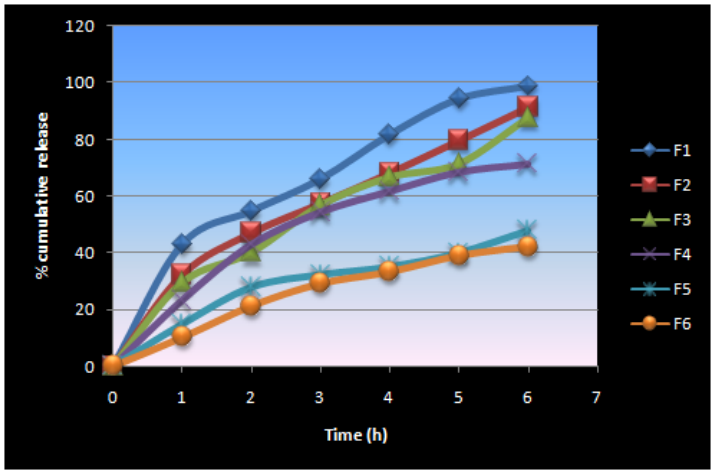

Figure 3: Cumulative drug release graph of naringin loaded microspheres.

It was pretty evident from the release profile that around 90 to 100 % of Naringin was released from formulations F1 to F3 during the 6 hours of the study suggesting that lower ratios of *Acacia nilotica* gum were not able to prepare the matrix required for entrapping the drug for longer durations and eroded fast as evident from the burst release during the first hour. On the other hand F4 exhibited a release of around 70 % at the end of the 6th hour. The

formulations F5 and F6 were not able to release even 50% of the drug during the study duration. The drug release ranged from 42.18 to 98.77 % from the microsphere formulations at the end of 6 hours of the study.

From the overall results obtained for drug content, particles size and micromeritic properties it could be concluded that the formulation F4 containing sodium alginate and *Acacia nilotica* gum in the ratio 1:1.25 was the best formulation.

CONCLUSION

In the present study, microspheres loaded with Naringin were prepared using ionic gelation using sodium alginate and *Acacia nilotica* gum as the natural polymers and 5% calcium chloride as the crosslinking counter ion. The results obtained showed that this methodology was able to produce reproducible microspheres and for sustained release of drug from the formulations. From the overall results obtained for drug content, particles size and micromeritic properties it could be concluded that the formulation F4 containing sodium alginate and *Acacia nilotica* gum in the ratio 1:1.25 was the best formulation.

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