
ORIGINAL ARTICLE

**ENTRAPMENT OF ANDROGRAPHOLIDE IN CROSS-LINKED
ALGINATE PELLETS: II. PHYSICO-CHEMICAL CHARACTERIZATION
TO STUDY THE PELLETIZATION OF ANDROGRAPHOLIDE**

ARSHIA SHARIFF, MANNA PK*, PARANJOTHY KLK AND MANJULA M*****

Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore

**Department of Pharmacy, Annamalai University, Annamalainagar*

***Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bangalore*

****Central Instrumentation Facility, Department of Physics, Indian Institute of Science, Bangalore*

ABSTRACT

This paper deals with the characterization of pellets containing andrographolide in two parts. The first part deals with characterization of the pellets to ascertain the identity and integrity of andrographolide. Part two involves characterization of the pellets containing *Andrographis paniculata* extract (33.3%) prepared in the paper I for their micromeritic properties like Particle size, Particle size distribution, Sphericity measurements like Shape ratio and Aspect ratio, Tapped density, Compressibility index, Hausner's ratio and Angle of repose. In addition, our aim was also to derive information about the mechanism of gelation with entrapment of andrographolide to supplement results obtained about the release mechanisms deduced in paper I. Since this work requires use of techniques like FTIR, FTRaman, MTDSC and XRPD, it was necessary to prepare alginate micropellets using pure andrographolide (99.89%) rather than the multicomponent extract using the same procedure discussed in paper I. The integrity of the drug was maintained in the cross-linked micropellets as was seen in the MTDSC and FTIR spectra supported by the FTRaman spectra. The depolymerisation transitions, the reversing and non-reversing heat flow signals were determined using the MTDSC and interpreted to study the mechanism of pelletization. The MTDSC profiles also confirmed the integrity of the drug by exhibiting a sharp endotherm at 232^oc that is the melting point of andrographolide. The XRPD spectrum of the micropellets ascertained that the crystallinity of andrographolide was maintained. The relationship of the nature of the drug present in the micropellets related to release mechanisms is discussed. In conclusion, it can be said that andrographolide can be successfully incorporated into cross-linked micropellets of alginate without affecting its integrity or nature to deliver it as a bitterless monoherbal preparation.

Keywords: Andrographolide, ionotropic gelation, modulated temperature differential scanning spectroscopy, FT Raman spectroscopy, XRD, FTIR.

Corresponding author: Arshia Shariff, Assistant Professor, Department of Pharmaceutics, Krupanidhi College of Pharmacy, # 5, Sarjapura Road, Koramangala, Bangalore-560034 - E-mail: arshitan@yahoo.com

INTRODUCTION

Micropellets of alginate formed by cross-linking of alginate with a counter ion like calcium is best described by the "Egg-Box" model (Grant *et al.*, 1973). The specific binding of divalent cations to a polysaccharide polyelectrolyte like sodium alginate will lead to firm cohesion between the chains. The buckled chain of alginate is suggested as a two-dimensional analogue of a corrugated egg-box with interstices in which the cations may be packed and get coordinated. It was found necessary to confirm the presence and integrity of andrographolide that gets entrapped during the process of co-operative mechanism of binding of the alginate chains. Further the characterization of the physical and micromeritic properties can be utilized as in process control parameters for reproducibility of the micropellets during their manufacture. In this study, a multidisciplinary approach to pellet characterization has been employed by the use of vibrational spectroscopy (FTIR and FTRaman), XRPD and MTDSC. Raman and FTIR cannot only be used to confirm the integrity of the drug but also to study the polymers with different degrees of crystallinity. A number of bands are found with changed intensities as the degree of crystallinity changes. Regions of higher crystallinity exhibit sharpened Raman bands with lower band-width (Scranton *et al.*, 2005). XRD profiles are basically used to study crystalline compounds. Amorphous samples can also be clearly confirmed for their nature from the XRPD profiles. XRD patterns that differ in appearance need not necessarily represent different crystalline forms because these patterns are affected by sample size and sample preparation. MTDSC profiles can not only be used as a tool in drug-polymer interaction studies by identifying the characteristic melting point endotherm but can also be used to study the mechanism of pelletization or gelation of the polymers (Pillay and Fassih, 1999).

MATERIALS AND METHODS

All the materials listed under materials and methods of paper I were used. Formulations and the manufacturing procedures produced in paper I were used for the evaluation of the micromeritic properties.

Fourier transform infra-red spectroscopy (FTIR)

IR transmission spectra were obtained using a FTIR spectrophotometer (FTIR-8300, Shimadzu, JAPAN). A total of 5% (w/w) of sample, with respect to the potassium bromide (KBr) disc was mixed with dry KBr. The mixture was ground into a fine powder using an agate mortar before compressing into KBr disc under a hydraulic press at 10,000 psi. Each KBr disc was scanned at 4mm/s at a resolution of 2cm over a wave number region of 400-4500cm⁻¹. The characteristic peaks were recorded using the software Shimadzu FTIR 8000 SCS.

Fourier transform Raman spectroscopy (FTRaman)

Raman spectra were obtained using a BRUKER FTRaman spectrophotometer (model RFF100) with a resolution of 4cm⁻¹, an excitation wavelength of 1064nm, laser intensity of 100-mwatt and accumulation time of 30 minutes.

Modulated temperature differential scanning calorimetry (MTDSC)

One of the most classic applications of DSC is the compatibility study to study the interactions of the drug with the excipients used. Changes in melting endotherms are monitored which indicate either a physical or a chemical change in the drug due to an interaction. A complete suppression of thermal features of the drug indicates formation of an amorphous solid. However there may be only a few changes in the thermal features suggesting a possible interaction in the physical or chemical nature of the drug.

The samples are subjected to a constant heating rate in conventional DSC. The heat flow used to increase the sample temperature is divided into two components, one depends on the heat capacity of sample and the other depends on the thermally activated processes occurring in the sample. The first component that is related to heat capacity depends proportionally on the heating rate. This difference is exploited in MTDSC.

A small sinusoidal temperature modulation characterized by period (p) and temperature amplitude (Ta) is superimposed on the constant heating rate (q) of the furnace plate. This modulation is followed by the sample with a small lag phase. As a consequence, the heating rate of the sample is modulated which results in a sinusoidal modulation of the required heat flow to the sample.

Within a narrow temperature range, the difference in the heat flows at the average and maximum heating rate will only depend on the heat capacity of the sample.

The average heat flow depends on both the heat capacity and all thermal events in the sample that occur at that temperature.

So only a change in the heat capacity during the scan will be reflected as a change in the amplitude of the modulated heat flow, whereas all the thermal events will be visible in the average heat flow or total heat flow only.

The amplitude of the modulated heat flow is determined by Fourier transform deconvolution. The heat capacity (Cp) is calculated using the following formula (Vanwinden *et al.*, 1998):

$$C_p \text{ (J/g}^\circ\text{C)} = \text{Heat flow amplitude (J/s)/Heating rate amplitude (}^\circ\text{C/s)} \times \text{sample weight (g)}$$

The reversing heat flow (J/sg) is calculated by the following formula, Cp (J/g^oC) x average heating rate (°C/s)

The non-reversing heat flow then will be = Total heat flow – Reversing heat flow.

Sampling was performed under an environment of dry nitrogen gas to minimize attraction of water by the samples. Samples (≈ 6 mg) were punched and transferred to aluminum pans and sealed immediately. All scans were recorded with MTDSC (TA instruments, new castle, DE) equipped with a liquid nitrogen-cooling accessory. For a two-point calibration, indium and gallium were used as standard. Scans were recorded under “Heat only” condition. A ramp was performed from 20°C to 25°C at which equilibration was allowed. At this temperature (25°C) isothermal conditions were maintained for 5 mins. Modulation was set at ± 0.318 every 60 sec. A ramp was executed at 2°C/min to 350°C.

X-ray powder diffraction crystallography (XRPD)

X-Ray Powder Diffraction study was performed using a PANalytical Xpert Pro Diffractometer with a PW3830X X-Ray generator. Nickel filtered Cu-K α radiation operated at source was used. Samples were scanned between 5°-50° (θ) using a step size of 0.01°/0.6 Sec. The Andrographolide and the drug loaded pellets were grinded separately to a fine powder before the study.

Particle size measurements

Particle size measurement plays a major role during filling of the micropellets into the capsules. Since large particles show high weight variations during filling operations, attempts are made to develop formulations with smaller and uniform particle size. The particle size was measured using a MCCamera Motic BA 300 microscope. The mean particle size was calculated by measuring 100 particles each from the batches F1, F2 and F3.

Sphericity measurements

Shape parameters like the shape ratio and aspect ratio were measured using a photomicroscope. A special computer program based on obtaining the co-ordinates (x, y) of the particle boundary through digitization of the pellet image was used. The obtained co-ordinates were used to calculate the parameters (Irwin *et al.*, 2005) using the formulae given below.

The shape ratio gives information about the shape of the micropellet. For a spherical pellet, the ratio is unity and all other shapes the ratio is less than 1. The aspect ratio is also used to study the shape of the pellets. A round or square particle will have a aspect ratio equal to 1. For particles elongated in the X-direction, the ratio will be larger than 1 and particles elongated in the y- direction, the aspect ratio will be smaller than 1.

$$\text{Shape ratio} = 4\pi\{\text{area}/(\text{perimeter})^2\}$$

Aspect ratio = Horizontal maximum distance of micropellet/vertical maximum distance of the micropellet.

Tapped density

The tap density of a material is basically used to investigate the packing properties of the microspheres into capsules but may also affect a number of pharmaceutical processes like flow, mixing and tableting. The tapped density was measured by employing the conventional tapping method using 10ml measuring cylinder and the number of tapings were reduced to 100 tapings as it was sufficient to bring about a plateau condition with our micropellets. The tapped densities were calculated using the following formula,

$$\text{Tapped Density} = \text{Mass of micropellets}/\text{Volume of micropellets after 100 tapings.}$$

Compressibility index

It is an indirect measure of bulk density, size and shape, surface area, moisture content and cohesiveness of materials since all of them can influence the compressibility index.

It is also called as Carr’s index because Carr (Gregory, 1995) proposed it as a direct measure of the “potential strength that a material could build up in its arch in a hopper and also the ease with which such an arch could be broken. Compressibility index is calculated using the following formula.

$$C_i = \text{Initial volume} - \text{final volume}/\text{initial volume} \times 100.$$

Hausner’s ratio

The Hausner’s ratio is another index of the flowability of the pellets. It is calculated using the following formula, Hausner’s Ratio = $V_{\text{before}}/V_{\text{tapped}}$

A value <1.2 is preferable for a free flow. However a Hausner’s ratio closer to 1 indicates good flow properties.

Angle of repose

Angle of Repose was measured by passing beads through a funnel on the horizontal surface. The height (h) of the heap formed was measured and the radius r of the cone base was also determined. The angle of repose (θ) was calculated from,

$$\tan \theta = h/r.$$

RESULTS AND DISCUSSION

Fourier transform infra red spectroscopy (FTIR)

FTIR of Andrographolide (98.8%), Andrographolide loaded calcium alginate pellets. Physical mixture of andrographolide with sodium alginate, Sodium alginate and Blank calcium alginate pellets are shown in fig. 1 and

the resulting peaks interpreted accordingly (Coates J, 2000). The $-OH$ stretching vibration was observed at $3319-3402\text{ cm}^{-1}$ due to presence of three $-OH$ groups. The aliphatic $C-H$ stretching vibration was observed at $2848-2990\text{ cm}^{-1}$ whereas the lactone carbonyl of cyclopentane ring was observed at 1728 cm^{-1} . The $=CH_2$ was observed at 1674 cm^{-1} and $C-C$ was observed at 1031 cm^{-1} . However the $C-O$ stretching was observed at 1220.9 cm^{-1} .

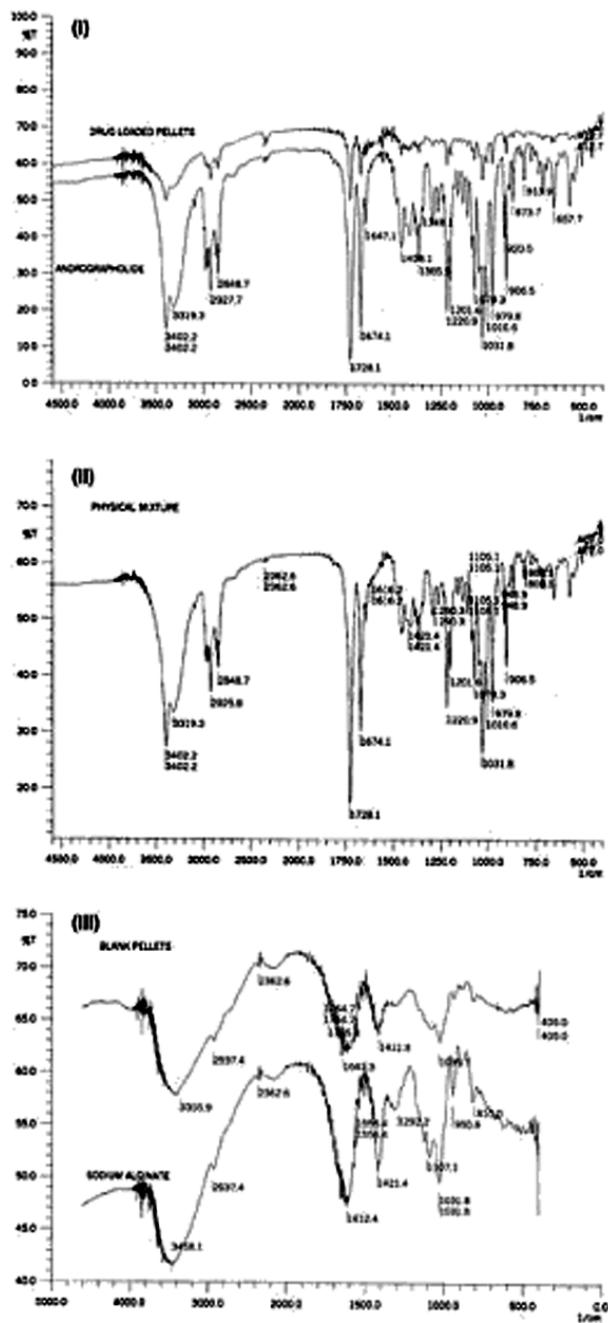
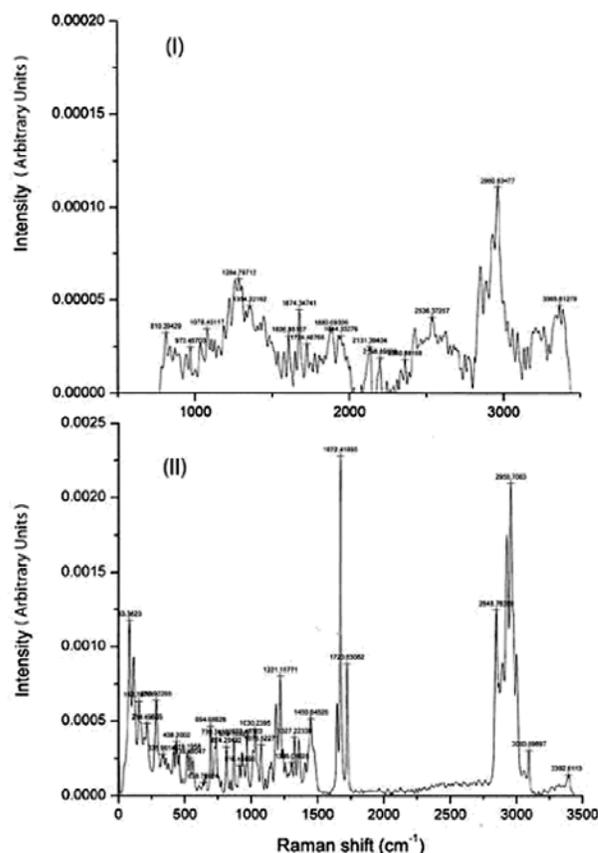


Fig. 1: FTIR profiles of (I) Drug loaded pellets and Andrographolide, (II) Physical mixture and (III) Sodium alginate and blank pellets.

The signature peaks of andrographolide were observed in the drug loaded calcium alginate pellets indicating that the integrity of andrographolide was maintained. Cross-linking of sodium alginate with Ca^{+} ions was demonstrated by an increase in the wave number of carbonyl peak from 1612 to 1641.7 cm^{-1} in fig. 2. The hydroxyl peak of sodium alginate also shifted to a higher wave number from 3355 cm^{-1} to 3458 cm^{-1} . The signature peaks of sodium alginate showed slight shifts presumably due to the cross-linking of the alginate chains.



andrographolide were reproduced in the Raman spectra of the drug-loaded pellets in fig. 2(I) however with reduced intensity possibly due to its presence along with alginate in an entrapped form.

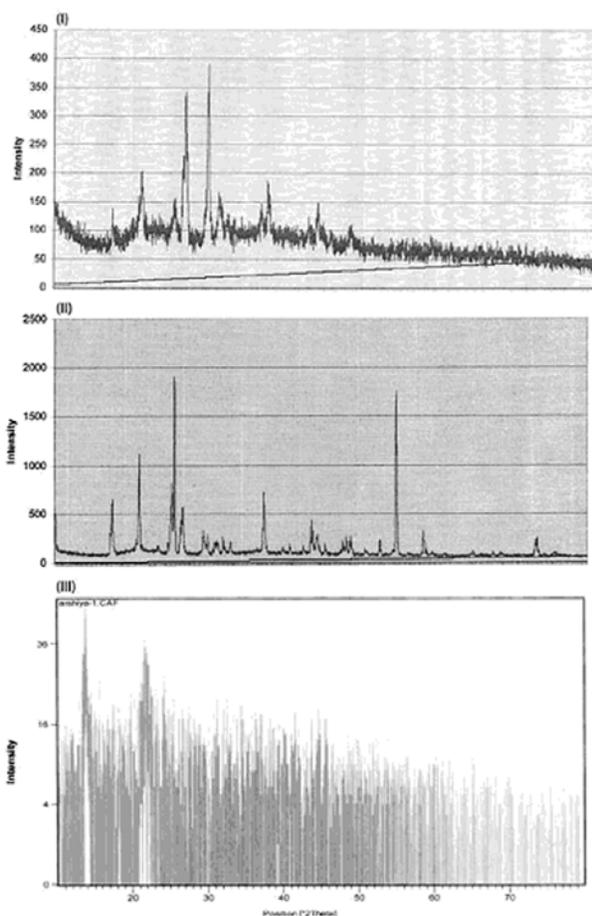


Fig. 3: XRD patterns of (I) Andrographolide loaded pellets (II) Andrographolide (III) Sodium alginate.

X-ray diffraction studies

X ray diffraction patterns of andrographolide and andrographolide loaded pellets in fig. 3 confirm the presence of crystalline structure of andrographolide in the pellets. However the profile has peaks at different positions, some peaks are missing and intensity of some peaks has decreased implying that the habit of the crystal may have undergone a change. XRD patterns that differ in appearance need not necessarily mean different crystalline forms because these patterns are greatly affected by sample size and preparation. Andrographolide being entrapped into the alginate matrix offers different preferred orientation resulting in a different pattern yet confirming its presence as a crystalline substance. The grinding of the drug loaded pellet prior to the analysis during sample preparation may also have affected its XRD pattern. Hydrogel-based polymeric systems can take

up water and retain water within their structures. The physical state of the drug in matrix systems depends on the solubility of the drug in the matrix. The drug could either be completely dissolved in the matrix or a part of it may be dissolved and the remaining may be dispersed in the matrix. This was further confirmed in our SEM results which showed crystals of drug dispersed on high magnification and the drug being slightly soluble in water, partial dissolution of the drug in the matrix may have resulted in the decrease in peak intensity of a drug. This physical state of drug in the micro pellets may have an influence on the morphology and release kinetics of the micro pellets. The diffuse XRD pattern seen only in the profile of the drug loaded pellets may possibly be that seen with polymers which exhibit both crystalline and non-crystalline patterns i.e. sharp peaks arising from evolution of latent heat on cooling from the melt and diffuse patterns characteristic of its non-crystalline fraction (Suryanarayanan, 1995).

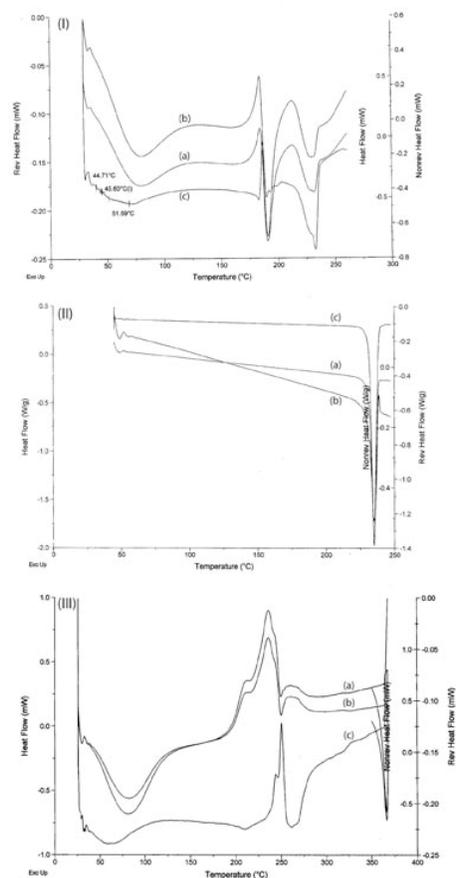


Fig. 4: MTDSC patterns of (I) Andrographolide, loaded alginate pellets (II) Andrographolide and (III) Sodium alginate in Figs. 1, 2, 3 (a) Total heat flow (W/g) (b) Non-rev heat flow (W/g) (c) Rev heat flow (W/g).

Modulated temperature differential scanning calorimetry (MTDSC) studies

Conventional DSC can just give information in the thermal behavior and interactions between materials if any. MTDSC helps in studying the effect of processing parameters on the degree of complexation. Fig. 4 shows the thermograms of the pure drug, drug loaded pellets and for sodium alginate.

As seen in the graph fig. 4 (II), Andrographolide shows a clear endothermic melting peak at 232°C. There were no interactions detected between polymer and drug as the shallow transition of the total heat flow signal seen between (50-100°C) in sodium alginate is seen in the drug-loaded pellets also. However this shallow transition signal is obtained at a slightly lower temperature due to the presence of andrographolide and the calcium ions nestled in the alginate matrix which may pose as impurity and lead to depression in the melting point of sodium alginate. The sharp endothermic signal characteristic of andrographolide at (232°C) is seen in the drug-loaded pellets confirming the integrity of the entrapped drug.

A sufficient magnification of a part of thermogram of sodium alginate displays the Tg of sodium alginate at 148.28°C (not shown in figure). Further study of the thermogram of drug loaded pellets after sufficient magnification shows the shift of Tg to 130.76°C indicating changes in the polymer presumably due to the ionotropic gelation of sodium alginate with Ca²⁺.

By deconvoluting the total heat flow signals to reversing and non-reversing components at all temperatures, changes in the molecular mobility at glass transition (Tg) could be easily characterized. This in turn provided information on the structural changes in the polymer over a range of temperatures. As seen in fig. 4(II), the initial shallow endotherm noticed between 75-100°C is seen in the reversing heat flow signal and so may be attributed to the loss of adsorbed or entrapped moisture. A similar finding was reported by (Pillay *et al.*, 1999b). The sharp endotherm at 232°C is due to the melting of the drug andrographolide. On high magnification (not shown in figure), the reversing heat flow signal clearly shows no glass transition while a superimposed endotherm is seen in the non-reversing signal.

Previous studies by (Pillay *et al.*, 1999,b) have demonstrated a jagged endothermic region for Calcium alginate pellets between (150-175°C) and attributed it to the de-polymerisation process of Calcium alginate and called such temperature transitions as T depol.

In our case the relaxation endotherm was seen as broadband between (175-200°C) indicating a higher complexed structure. This difference may be due to the fact that the pellets made in our case were of higher

drug:polymer ratio (1:2) compared to those used in the referred study (1:1).

Determination of particle size and shape

The particle size of the wet beads is determined by the size of droplets extruded from the syringe. The Size of the dried beads depends mainly on the drug content in the pellets. The average particle size of micropellets of F1, F2 and F3 were found to be 1.36mm, 1.29mm and 1.43 mm respectively. The average particle size increased with increase in the drug content (F3>F1>F2). The shape factor data obtained from the microscopical studies for the different formulations yielded values less than unity as shown in table 1. This shows that the micropellets are not perfectly spherical but have a few variations in their silhouette. The variations in the shape may be due to the variations in the manual pressure applied while dropping the alginate solution into the calcium chloride solution which sometimes gives rise to slight tailing of the micropellets. This can however be minimized by using a multiorifice piston pump which would drop the alginate solution at uniform speed, pressure and height. However the aspect ratio data obtained for the same micropellets showed values close to unity indicating regularity of the form in all the formulations.

Table 1: Determinants of sphericity measurements.

Formulation code	Shape ratio	Aspect ratio
F1	0.53	0.97
F2	0.53	0.89
F3	0.59	1.07

The results as shown in table 2 for the Carr's index (compressibility index) for all the formulations were close to 4 indicating excellent flow as per the scale given by Carr. The Hausner's ratio was less than 1.25 again indicating free flow. This was further confirmed by the angle of repose values obtained for all the three formulations that were close to 30° indicating once again the free flowing nature of the micropellets.

Table 2: Determinants related to flow properties of micropellets

Formulation code	Tapped density	Compressibility index	Hausner's ratio	Angle of Repose
F1	0.6804	3.73	1.03	29.76°
F2	0.6538	3.70	1.03	30.00°
F3	0.65	3.34	0.98	26.47°

CONCLUSION

The multidisciplinary approach towards characterization of the pellets using FTIR, FTRaman, XRPD AND MTDSC confirmed the integrity of the andrographolide

entrapped in the alginate matrix which would not have been otherwise possible with any single technique. It also gave in-depth information about the nature of the drug mechanism of gelation of alginate. In-addition, the physico-chemical characterization of the micro-meritic properties of the pellets has given us an insight into the fundamental properties governing the flow of the micropellets that may be helpful in formulation, development and validation processes in the pelletization technology. In conclusion, the micropellets of alginate can be successful in entrapping the drugs which are basically water-insoluble and this technique of cross-linking can be exploited to mask the bitterness of drugs or their extracts.

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