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**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.583707>Available online at: <http://www.iajps.com>**Research Article****IMPROVED ORAL DELIVERY OF AGOMELATIN FROM
MALTODEXTRIN BASED PROLIPOSOMES POWDERS**Pradeep Kumar Y^{1*}, Raju J², Subba Rao D³¹Department of Pharmaceutics, Jangaon institute of pharmaceutical sciences, Warangal, Telangana, India.² Telangana Drugs Control Administration, Karimnagar, Telangana, India.³Department of Chemical Engineering, JNTU ananthapur, Ananthapuramu, Andhra Pradesh, India.**Abstract:**

The aim of the present study was to develop proliposomal formulations to enhance the oral bioavailability of agomelatin by improving solubility, dissolution and/or intestinal permeability. Proliposomal powder formulations were prepared using different ratios of agomelatin, phospholipon 90H and cholesterol by solvent evaporation method. The effect of phospholipid composition and drug:lipid ratio on in vitro performance of proliposomes was studied. Proliposomes were characterized for their particle size distribution, zeta potential, micromeritics, and entrapment efficiency. Further, the formulated proliposomes were subjected to in vitro drug release performance in both simulated gastric and intestinal fluid demonstrate improved dissolution characteristics compared to pure drug. Multimedia dissolution profiles were carried out to demonstrate enhanced dissolution characteristics compared to pure drug. Proliposomes provided enhanced agomelatin dissolution due to incorporation into the phospholipid bilayers and change in the physical state from crystalline to amorphous. These proliposomal formulations of agomelatin could provide improved oral bioavailability due to enhanced solubility, permeability and enhanced absorption.

Keywords: Proliposomes, agomelatin, perfusion, bioavailability, Dissolution, Permeability**Corresponding Author:**

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INTRODUCTION:

The carriers used to carry the drug to the site of action include immunoglobulins, serum proteins, synthetic polymers, lipid vesicles (liposomes), microspheres, erythrocytes, reverse micelles, niosomes, pharmacosomes etc [1,2]. Liposome is a micro vesicle in which an aqueous volume is entrapped within the lipoidal membrane. Drug molecules can be entrapped within the lipid bilayer or in the aqueous space. Since the shelf life of liposomal suspensions can be limited, it would be useful to have a method of producing liposomes quickly, at the point of use and without excessive manipulation. These needs are met by the "proliposome" method [3]. Proliposomes (PLs) are covered with liposomal membrane when they are hydrated they produce liposomes. The concept of proliposomes was first introduced by Payne et al., in 1875; they described proliposomes as dry free flowing granular product that forms liposomal dispersion on hydration or on contact with biological fluids in the body the stability problems of liposomes can be overcome by PLs without affecting their intrinsic characteristics. PLs are composed of phospholipid and water soluble porous powder [4] [5].

Agomelatine is a melatonin receptor agonist MT1 and MT2 and a 5-HT_{2C} receptor antagonist. Agomelatine resynchronises circadian rhythms in animal models of delayed sleep phase syndrome. By antagonizing 5-HT_{2C} receptors, it increases noradrenaline and dopamine release specifically in the frontal cortex. Therefore, it is sometimes classified as a norepinephrine-dopamine disinhibitor. It has no influence on the extracellular levels of serotonin. It also have positive phase shifting properties; it induces a phase advance of sleep, body temperature decline and melatonin onset. Low <5% Absolute bioavailability at the therapeutic oral dose and the inter individual variability is substantial. The chemical structure of agomelatine is very similar to that of melatonin. Where melatonin has an NH group, agomelatine has an HC=CH group. Thus melatonin contains an indole part, whereas agomelatine has a naphthalene bioisostere instead [6],[7].

According to BCS classification, the class II drugs have low solubility-high permeability and their absorption is dissolution rate limited. Several researchers are adopted for enhancing the dissolution behavior of BCS class II drugs by particle size reduction, crystal engineering, salt formation, solid dispersion, use of surfactant, complexation, increasing the surface area by micronization or nanonization, spray drying and microencapsulation [8]. Due to its low aqueous solubility and poor absolute bioavailability as it fall under BCS-II. Several strategies have been reported to enhance the dissolution properties and bioavailability of agomelatine by formulating it into different dosage forms. However, not yet reported relevant studies for oral administration of agomelatine proliposomal powders.

MATERIALS & METHODS:**Materials:**

Agomelatine was gifted from MSN Laboratories, India, Maltodextrin was gifted by Sigma Aldrich, Hyderabad, Cholesterol was purchased from SD Fine chemicals, Hyderabad Phospholipon 90H (highly purified hydrogenated soyphosphatidylcholine, 90% purity, HSPC) was a kind gift sample from Lipoid, Germany. Methanol and Chloroform were purchased from Merck Specialties Pvt. Ltd, Mumbai. All other chemicals used were of analytical grade and solvents were of HPLC grade. Freshly collected double distilled water was used all throughout the experiments.

Methods:**Preparation of calibration curve:**

From prepared working standard stock solution of agomelatine (100µg/mL), the final concentrations of 1µg/mL to 10µg/mL were prepared. The absorbance was measured at 236nm by using UV-Visible spectrophotometer and calibration curve was plotted as absorbance vs. Concentration.

HPLC analysis of agomelatine

The samples were assayed for agomelatine by using a stability-indicating HPLC method reported earlier [9]. The analysis of the drug was carried out on waters instrument with PDA waters 2998 detector with Empower software and hamilton syringe with 20µL, and a Lichrospher C18 column (150×4.6mm, 5µm). Isocratic elution was carried out at a flow rate 1mL/min (UV detection- 236 nm). The mobile phase consisted of acetonitrile: methanol: water (55:25:20, v/v/v). The retention time was found to be 4.2 min. Aliquots of 20 µL of each sample were spiked onto the column. The assay was linear ($r^2 = 0.9988$), in the concentration range of 1–10µg/mL with the lowest detection.

Preparation of agomelatine-loaded proliposome

The thin film deposition method was adopted for formulation of proniosome powders reported in literature [10]. Table1 represented that the different proniosomal formulations compositions. accurately weighed quantities of lipid mixture 100 µM of Phospholipon 90H, and cholesterol at various ratios and drug (25mg) were dissolved in 20mL of solvent mixture containing chloroform and methanol (2:1). The resultant solution was transferred into a 250mL RB flask and maltodextrin was added to form slurry. The flask was attached to a rotary flash evaporator (Laborota 4000, Heidolph, Germany) and the organic solvent was evaporated under reduced pressure at a temperature of 45±2°C. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature so as to obtain dry, free-flowing product. The obtained proliposome powders were sieved with a # 60 mesh screen (250µm). The obtained proniosome powders were stored in a tightly closed container at 2-8°C for further evaluation and characterization. The composition of different proniosomal formulations is represented in (Table1).

Table 1: Formulation codes of agomelatine loaded proliposomes

Formulation code	Phospholipon 90H: cholesterol	Agomelatine (mg)	Phospholipon 90H (mg)	Cholesterol (mg)	Maltodextrin (mg)
APL1	1:2	25	31.8	77.2	150
APL2	1:1.5	25	31.8	57.9	150
APL3	1:1	25	31.8	38.6	150
APL4	1.5:1	25	47.7	38.6	150
APL5	2:1	25	63.6	38.6	150

Physico-chemical characterization of proliposome powders:

Formation of liposomes from proliposome powders

The optical microscopy was employed to visualize the derivation of liposomes upon hydration of proliposome powder. A cavity glass slide was taken and few mg of proliposomes powder was spread over it and followed by the addition of few μL of water along the side of the cover slip. The liposome formation on hydration of solid particle was monitored and photographed (Coslabs micro, India) and photograph was taken (Fig -2).

Transmission Electron Microscope

The proliposome powder was hydrated with distilled water and agitated manually for two minutes and the transmission electron microscope observations were performed [11]. A thin film was made on a carbon-coated copper grid by placing a drop of liposome dispersion. Before the film dried on the grid, it was negatively stained with sodium phosphotungstate solution (0.2%w/v); any excess solution was drained off with a filter paper. The grid was allowed to air dry, and samples were viewed under a transmission electron microscope (Tecnai G2, Jeol-100CX-II, and Netherlands).

Micromeritics of proliposome powders and number of vesicles per mm^3

The measurement of the flow properties of proliposome powders was evaluated by determining the angle of repose, Carr's compressibility index and Hausner's ratio. The fixed funnel method was used for determination of angle of repose and Carr's compressibility index and Hausner's ratio were calculated from the bulk density and tapped density of the proliposome powders [12]. The number of vesicles formed after hydration of proliposome powder were counted by optical microscope using a haemocytometer and the number of vesicles per cubic mm was calculated by using the following formula [13].

$$\text{Total no. of liposomes per mm}^3 = \frac{\text{Total no. of liposomes counted} \times \text{dilution factor} \times 4000}{\text{Total number of squares counted}}$$

Vesicle size and zeta potential

The liposome dispersion was prepared by manually agitating proliposome powders with distilled water for few minutes. The mean size and polydispersity index of

liposomes was determined by photon correlation spectroscopy using zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK). Each sample was diluted to a suitable concentration with filtered distilled water and analysis was performed at 25°C with an angle of detection of 90°C . Zeta potential was also measured using Zetasizer Nano ZS90 (Malvern Instruments, UK) [14].

Drug content and entrapment efficiency

The drug content was determined by dissolving the proliposome powder (25 mg) in 50 mL of methanol. An aliquot of sample was taken in microcentrifuge tubes and followed by centrifugation at 10,000 rpm for 15 min. The supernatant was separated, suitably diluted with mobile phase and 20 μL of the sample was injected onto HPLC and quantified[15].

percentage entrapment of agomelatine in liposomes was calculated from the following equation:

$$\% \text{ DE} = (\text{Total amount of drug added} - \text{Unentrapped drug}) / \text{Total amount of drug added} \times 100$$

In vitro dissolution study

In vitro dissolution study of proliposomal powders and pure drug was performed using USP type II (paddle) apparatus (Electrolab, TD L8, Mumbai, India) in multimedia dissolution media of pH 1.2, pH 4.5 and pH 6.8 to maintain sink conditions. The volume of dissolution medium used was 900 mL and maintained at a temperature of $37 \pm 0.5^\circ\text{C}$ with 50 rpm of paddle speed set at throughout the experiment. 5 mL of aliquot of was collected at predetermined time intervals at 15, 30, 60min and replaced with fresh dissolution medium to maintain constant volume and sink condition [16]. The samples were filtered by passing through 0.45 μm PVDF membrane filter (Millipore) and analyzed for agomelatine by HPLC at 236 nm. Cumulative % of drug released was calculated and plotted against time (t) [17].

Solid state characterization

Scanning Electron Microscopy (SEM)

The surface morphology of the pure drug, maltodextrin and optimized proliposome powder (APL4) was investigated by scanning electron microscope (SEM) (S-4100, Hitachi, Japan). Samples were fixed on a brass stub using double sided adhesive tape and were made electrically conductive by coating with a thin layer of gold

and SEM images were recorded at 15 keV accelerating voltage.

Differential Scanning Calorimetry (DSC)

The physical state of the drug, unloaded proliposomes and optimized proliposome formulation was evaluated by performing DSC analysis of pure drug, unloaded proliposomes and optimized proliposome powder. The DSC curves of the samples were obtained by a differential scanning calorimeter (Mettler DSC 823e, Mettler-Toledo, Germany). Average sample weight of 5 ± 2 mg were heated in hermetically sealed aluminum pan over a temperature range of 30°C to 230°C under a constant nitrogen gas flow of 70 mL/min at a heating rate of $10^\circ\text{C}/\text{min}$. The instrument was calibrated with indium (calibration standard, purity $>99.9\%$) for melting point and heat of fusion.

Fourier Transform Infrared (FT-IR) spectroscopy

Infrared spectra of agomelatin, physical mixture and optimized proliposome powder formulation (APL4) were obtained using FT-IR spectrophotometer (Paragon 1000, Perkin Elmer, USA) by the conventional KBr pellet method. The scanning range was $4000\text{--}500\text{ cm}^{-1}$ and the resolution was 4 cm^{-1} .

Powder X-ray Diffractometry (PXRD)

The PXRD patterns of agomelatin, maltodextrin and optimized proliposome powder formulation (APL4) were obtained using X-ray diffractometer (X'Pert PROPANalytical, Netherlands). The measuring conditions were as follows: $\text{CuK}\alpha$ radiation, nickel filtered; graphite monochromator; 45 kV voltage; and 40 mA current with X'celerator detector. All samples were run at $1^\circ (2\theta)$ min $^{-1}$ from 3° to $45^\circ (2\theta)$.

Stability Studies

The formulations stored in clear glass vials with stopper, seal and were kept at room temperature and in refrigerator ($2 \pm 8^\circ\text{C}$) for a period of 180 days. At definite time intervals (0, 30, 60, and 90, 180 days), samples were withdrawn and hydrated with phosphate buffered saline pH (7.4) and observed for any sign of drug crystallization under optical microscope. Further the samples were also evaluated for % retention of agomelatin.

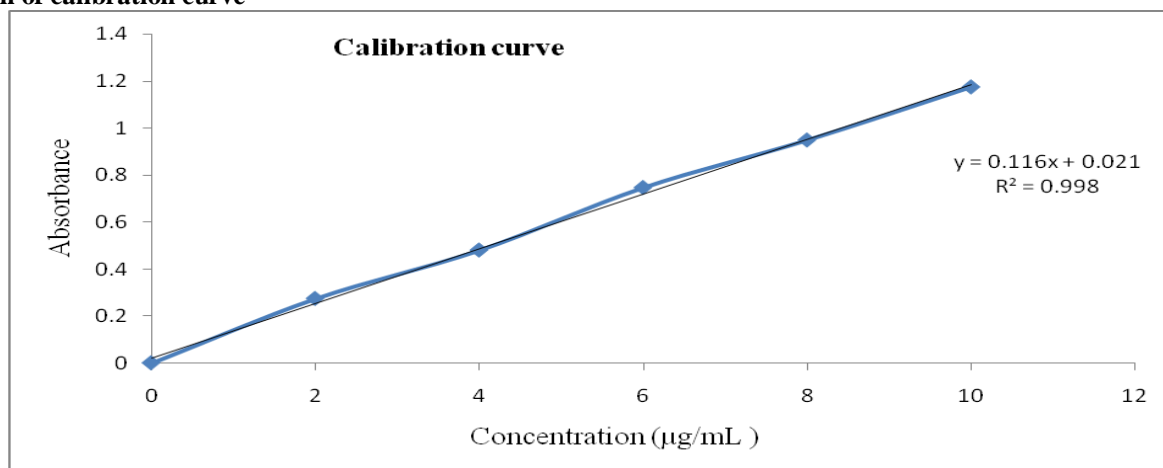
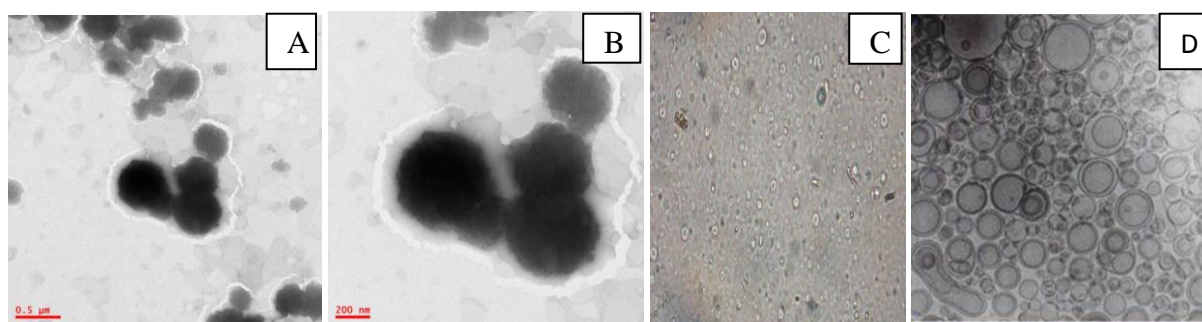
RESULTS AND DISCUSSION:

Preparation and physico-chemical evaluation of proliposomes

The proliposomes proved to be the efficient carriers for improved oral delivery of lipophilic and amphiphilic drugs. In this study, the proliposomes have been prepared and evaluated their potential in improving the oral delivery of agomelatin. Several methods have been reported for the formulation of proliposomes which include crystal-film method [18], film deposition on carrier method [19], freezing and drying method [20], powder bed grinding method [21], fluidized-bed method [22] and spray drying method [23],[24]. According to the feasibility in our laboratory, we have employed film deposition on carrier technique for the preparation of agomelatin containing proliposomes.

The formation of liposomes after reconstitution depends on the ease of dispersibility of the carrier in aqueous fluids. Among the different carriers which include neusilin US2, sorbitol, microcrystalline cellulose, etc. we preferred to use maltodextrin which confer porous structure and high surface area and enables the formulator for easy adjustment of amount of carrier required to support the lipid and also to prepare proliposomes with high surfactant to carrier mass ratios [25].

The selection of phospholipid is important because it dictates the stability of the liposomes formed. Since the risk of oxidation is high in phosphatidylcholine due to the presence of unsaturated bonds in the fatty acid tails [26], hydrogenated soyphosphatidylcholine which is in powder form was used in the formulations. The high phase transition temperature and solid state renders more stability in GI fluids and augment the flow characteristics of the proliposomes respectively which is an important prerequisite for solid dosage forms. Apart from this, lipid to carrier load can be increased so that lipophilic drugs with high dose can be incorporated without any hindrance to the flow properties [27]. The proliposome concept has resolved many stability issues pertaining to the aqueous liposome dispersions. The maximum benefit of proliposomes can be achieved when it forms stable vesicles with high entrapment efficiency after hydration in the gastric fluids. In this perspective the structural lipid, cholesterol was used which is known to increase the stability of the bilayer with high amounts of drug entrapment. However the formation and stability of the formed liposomes is by and large dependent on the composition of phospholipid to cholesterol ratio because any alteration in their composition results in leakage of drug before the drug diffusion and fusion of vesicles with the GI membrane [28]. Therefore the effect of cholesterol was investigated by varying the HSPC to cholesterol ratio.

Construction of calibration curve**Fig No 1 : Standard Graph of Agomelatin****Fig. 2. Microphotographs showing A) proliposome powder B) formation of vesicles on maltodextrin after hydration with distilled water C & D) TEM image of liposome dispersion from reconstituted proliposome powder upon manual agitation.****Morphological evaluation of prepared proniosome powders by optical microscope**

The proliposomes upon hydration derive the formation of liposomes and was spontaneous suggesting a rapid conversion to liposomes on contact with physiological fluids in the body (Fig. 2A&2B). It is evident from the figure that in initial stages upon contact with water the lipids tend to form tubular structures and upon manual agitation they have deformed into small multilamellar vesicles acquiring spherical shape. Further the TEM analysis confirms the shape of vesicles formed after hydration of proliposomes (Fig. 2C & 2D).

The micromeritics of the proliposome powders is vital in handling and processing operations because the dose uniformity and ease of filling into container is dictated by the powder flow properties. In general, three types of flow measurements can be used to evaluate the nature of powder flow i.e. angle of repose; Carr's index and Hausner's ratio and the results were depicted in Table 2. The smaller the value of angle of repose, lesser the internal friction or cohesion between the particles and greater the flow characteristics and vice-versa. It is apparent from the results that small angle of repose ($<20^\circ$) assure good flow properties for proliposome powder formulations. In addition to angle of repose, Carr's index and Hausner's

ratio were also less than 17 and 1.25 respectively ensuring acceptable flow for proliposome powder formulations (Table 2) [43]. The distinctive advantage of proliposome formulations can be speculated only when abundant numbers of vesicles are derived from hydration of proliposome powders in the gastrointestinal tract. Among all the formulations, the proliposome formulation (APL4) containing equimolar ratio of HSPC and cholesterol (1:1) demonstrate good number of vesicles (Table 3). This is in correlation with our earlier reports [44].

One of the important parameter for the vesicular systems is vesicle size and size distribution [45]. The mean size of the vesicles was in the range of 192 ± 14 to 292 ± 19 (Table 3). The size of the vesicles seems to be dependent on the cholesterol concentration. The PI used as a measure of a unimodal size distribution was within the acceptable limits for all the proliposomal formulations (Table 3). The zeta potential of the proliposome formulations (APL1 to APL5) was between 6.8 ± 2.7 to 7.9 ± 1.6 mV (Table 3). Earlier reports suggest that the surface charge has a significant influence on the drug uptake by biological membrane due to electrostatic attraction or improved vesicle enterocyte interactions [46]. The classical liposomes carry a feeble charge and considered to be neutral and zeta potential (7.9 ± 1.6 mV) of liposomes formed from optimized formulation (APL4).

Table 2: Flow properties of agomelatin loaded proliposome powder formulations.

Formulation	Angle of repose (θ)	Compressibility index	Hausner's ratio
APL1	16.8 \pm 0.21	10.8 \pm 0.08	1.09 \pm 0.15
APL2	19.2 \pm 0.23	12.4 \pm 0.06	1.18 \pm 0.19
APL3	19.8 \pm 0.32	15.5 \pm 0.25	1.22 \pm 0.13
APL4	18.1 \pm 0.35	16.2 \pm 0.37	1.17 \pm 0.21
APL5	19.3 \pm 0.27	15.7 \pm 0.28	1.25 \pm 0.12

Table 3: Physico-chemical characterization of agomelatin loaded proliposome formulations

Formulation	Size (nm)	P.I	Zeta potential (mV)	Entrapment efficiency (%)	No. of vesicles per mm ³ x 10 ³
APL1	192 \pm 14	0.209	7.2 \pm 1.9	72.7 \pm 2.2	3.02
APL2	209 \pm 13	0.198	7.6 \pm 2.3	82.2 \pm 1.6	3.81
APL3	229 \pm 10	0.181	7.3 \pm 1.5	86.5 \pm 1.1	3.21
APL4	256 \pm 12	0.192	7.9 \pm 1.6	95.9 \pm 1.6	4.14
APL5	292 \pm 19	0.244	6.8 \pm 2.7	79.2 \pm 1.5	3.23

Among different methods used for determination of entrapment efficiency, we have employed ultra-filtration technique because no dilution step is involved unlike dialysis and column chromatography. The entrapment efficiency of proliposome formulations was between 73 to 96 % (Table 3). Our results envisage that the entrapment efficiency of agomelatin is dependent on the composition of liposomes. This can be owed to the decreased leakage of the drug because of high compactness and hydrophobic interactions making the bilayer more stable. Further the effective intercalation of hydrophobic drug, agomelatin within the hydrophobic core of the bilayer may enhance drug pay load [47]. Interestingly, the same findings we could not extrapolate with proliposome formulation (APL4) and in converse the entrapment value reduced (Table 3). The perturbation of the linear structure of bilayer and decreased packing space available for drug

molecules might have resulted in expulsion of drug molecules [19].

***In-vitro* dissolution study**

The dissolution profiles of proliposomes were shown in figure 3. The amount of agomelatin released from proliposomes was ranging between 65 to 87% and was higher compared to control (31 %). The dissolution efficiency of insoluble drug agomelatin has been significantly improved when encapsulated in proliposomes. This might be due to the enhanced solubility of agomelatin by phospholipid molecules or transformation of the crystalline state of the drug to amorphous state [31]. Further these results were consistent with the DSC and PXRD studies. However, we could not notice any remarkable change in the dissolution behavior with different proliposome formulations .

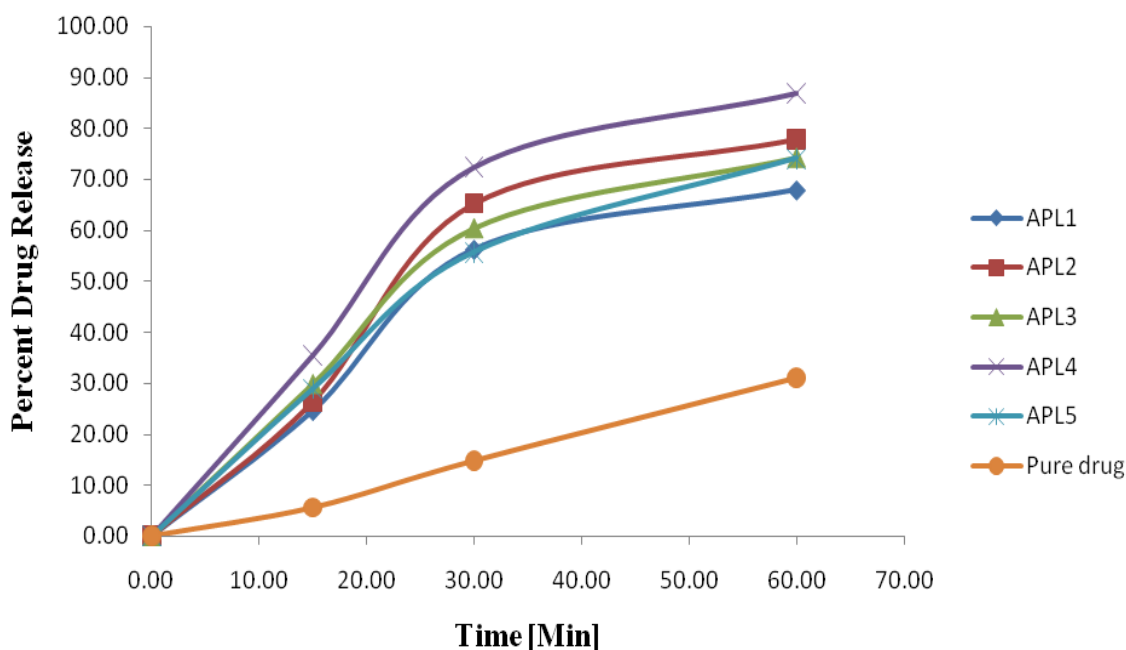


Fig No.3: Dissolution profile of agomelatin released from proliposome powder formulations

Solid State Characterization

The surface morphology of the pure drug, maltodextrin and proliposome powders were examined by SEM and the images are represented in figure 4. The absence of typical crystalline structures of agomelatin in proliposome formulation indicates the transformation of drug to amorphous or molecular state. Further, the porous structure of maltodextrin as evident in figure was illegible in proliposome powders because of the deposition of phospholipids on the surface of maltodextrin.

The thermotropic behavior and the physical state of the drug in proliposome powders were evaluated by performing DSC analysis. The DSC thermograms of agomelatin, unloaded proniosomes, loaded proniosomes optimized formulation (APL4) were recorded Fig. 5A-C. The unloaded proniosomes exhibited a difused peak at 73.9°C, The loaded proniosomes exhibited a difused peak at 68.0°C corresponding to its melting point. The absence of conspicuous peak in proliposome formulation over the melting range of agomelatin unravels the transformation of the physical state of the drug (crystalline to amorphous) which was further confirmed by PXRD analysis.

The PXRD patterns of agomelatin, maltodextrin and proliposome powder were represented in figure 6A-C. The pure drug showed numerous characteristic high intensity diffraction peaks at 2θ of 18.6, 19.7, 20.9, 24.1 and 44.1 demonstrating the crystalline nature of the drug. The absence or reduced intensities of characteristic agomelatin peaks in proliposome formulation suggest the change in physical state i.e. amorphization of drug.

Figure 7 A-C illustrates the FT-IR spectra of agomelatin, physical mixture and proliposome formulation (APL4). The pure drug agomelatin exhibit characteristic peaks bands at 1542.56 (N-H Bend) 1211.32, 1026.05 (C-N Stretch (alkyl)), 1297.46, 1250.36 (C-N Stretch (aryl)), 1026.05, 1211.32, 1250.36, 1297.46 (C-O-C Stretch), 3652.48, 3546.03 (O-H Stretch), 1645.45, 1697.93 (C = C Stretch), 3446.03 (N-H Stretch), 1211.32, 1026.05 (C-O Stretch). The peaks at 1542.56 (N-H Bend), 1211.32, 1026.05 (C-N Stretch (alkyl)), 1297.46, 1250.36 (C-N Stretch (aryl)), 1026.05, 1211.32, 1250.36, 1297.46 (C-O-C Stretch), 1645.45, 1697.93 (C = C Stretch), 1211.32, 1026.05 (C-O Stretch), were disappeared in proliposome formulation and the intensity of peaks at 3478.87, 3535.43 (O-H Stretch), 3478.87 (N-H Stretch) was reduced in proliposome formulation. The peaks 1645.45, 1697.93 (C = C Stretch), 1297.46, 1250.36 (C-N Stretch (aryl)) and 1542.56 (N-H Bend), were disappeared in proliposome formulation and the intensity of peaks at 1148.02, 1078.70 (C-N Stretch (alkyl)), 1078.70, 1148.02 (C-O-C Stretch), 3547.02, 3439.12 (O-H Stretch), 3439.12, 3337.42 (N-H Stretch), 1078.70, 1148.02 (C-O Stretch) was reduced in proliposome formulation. However no additional peaks in proliposome formulation indicate the absence of chemical interaction between the drug and formulation ingredients.

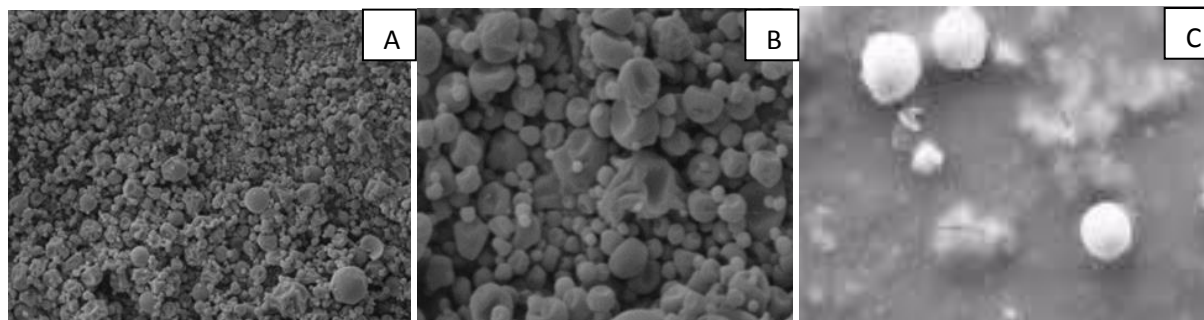


Fig.4. SEM images of (A) Agomelatin (B) Maltodextrin (C) Proniosome powder (APL4).

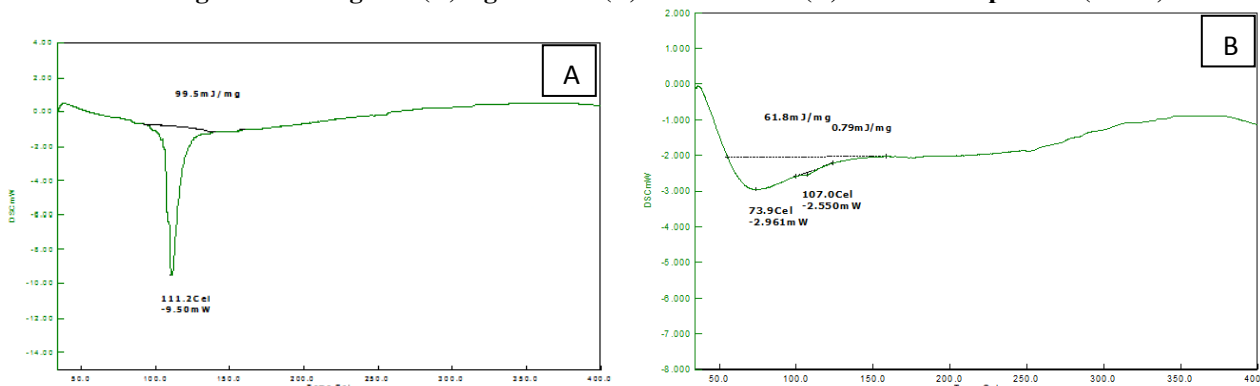


Fig. 5A. DSC thermogram of Agomelatin Fig.5B. DSC thermogram of unloaded proliposomes

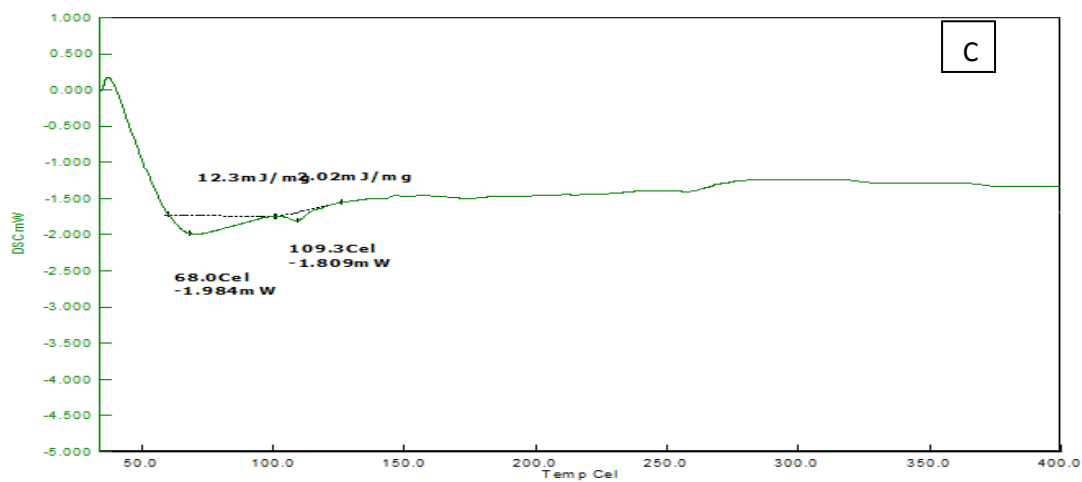


Fig. 5C. DSC thermogram of optimized proliposomes APL4

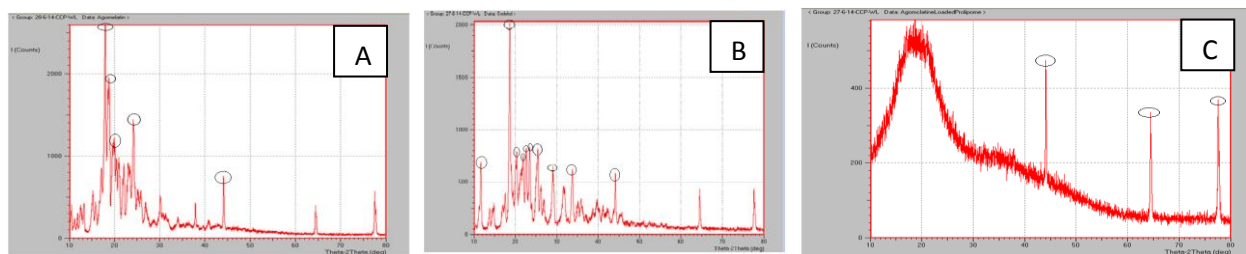


Fig.6. [A]Powder X-ray diffraction pattern of Agomelatin, [B]Maltodextrin, [C]optimized Proliposome formulation APL4

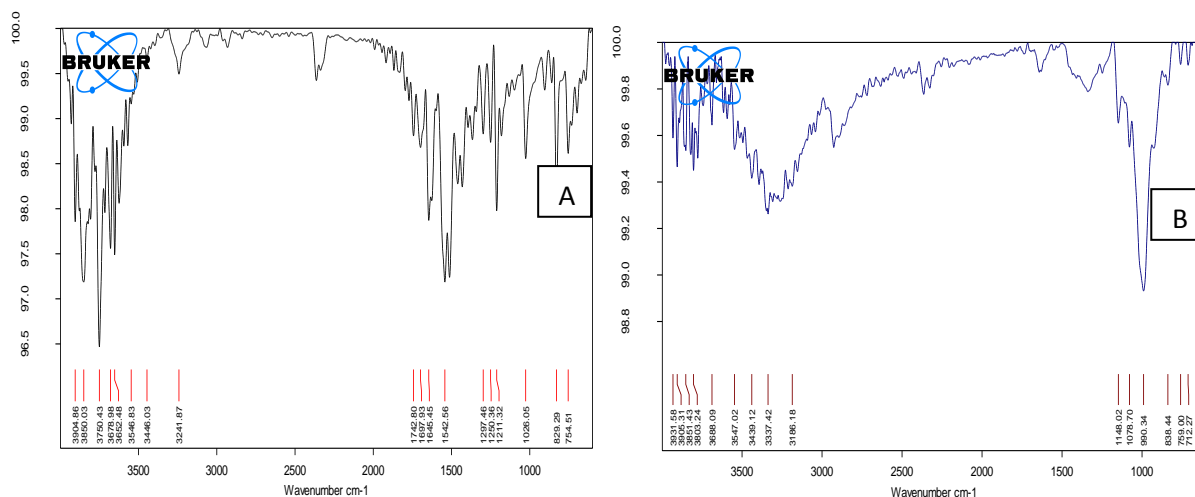
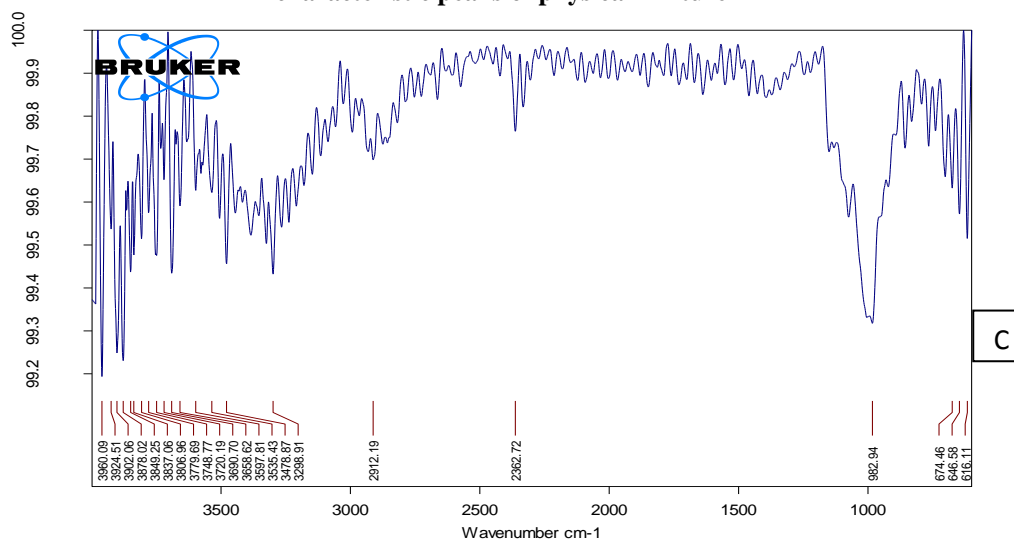


Fig No.7. [A] FT-IR characteristic peaks of pure Agomelatine, FigNo.7. [B] FT-IR characteristic peaks of physical mixture



FigNo.7. [C] FT-IR characteristic peaks of optimized Proliposome formulation [APL4]

Stability study

The physical appearance % retention of agomelatine was monitored for the optimized proliposome powder formulation (APL4) upon storage at refrigerated and ambient room temperature for a period of 180 days. At definite time intervals, the proliposome powder was hydrated to form liposomes and we could observe the formation of vesicles without any signs of drug crystallization. The entrapment efficiency was also

monitored and the results indicate that there was no appreciable change in the % retention of agomelatine when stored at refrigerated temperature (Fig. 8) (Table No: 4). In addition, the formulation was destabilized at room temperature resulting in drug leakage with decreased % retention. The stability studies suggest that the proliposome formulation was comparatively less stable when stored at room temperature compared to refrigerated conditions.

Table 4: Percentage potency of agomelatine in proliposome powder formulation (APL4) upon storage in 2-8° and at room temperature.

Time (Days)	Potency (%) at 2-8°C	Potency (%) at 25°C
0	99.8	99.8
30	99.5	91.50
60	98.4	86.20
90	98.2	80.60
180	98.2	75.20

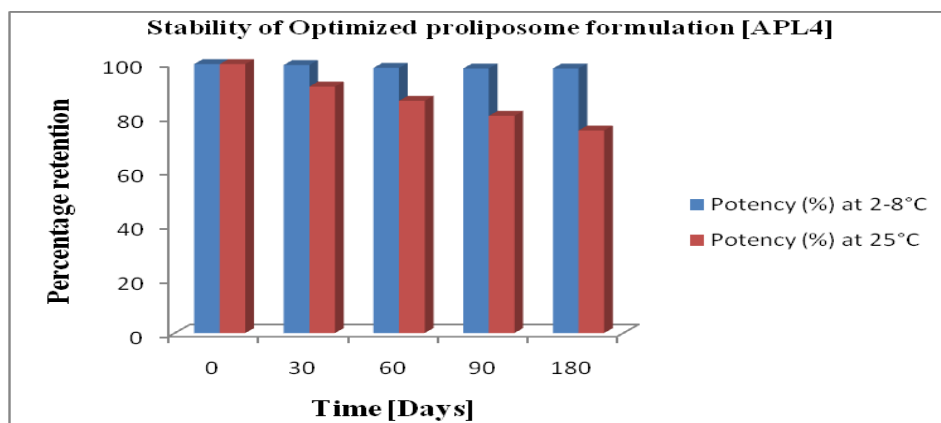


Fig No.8. Percentage retention of agomelatin in proliposome powder formulation (APL4) upon storage in refrigerator and at room temperature

CONCLUSION:

The agomelatin proliposomes were prepared by film deposition method using maltodextrin as carrier at varying ratios of HSPC and cholesterol. The formulation containing equimolar ratio of HSPC and cholesterol exhibited low size, high surface charge and entrapment efficiency. The formulations possess good flow properties and the *in vitro* dissolution behavior was improved compared to control. The solid state characterization results suggest that the native crystalline state of the drug has been transformed to amorphous and molecular state. In conclusion, the improved oral delivery of agomelatin proves the potential of proliposomes as suitable carriers for poorly soluble drugs. However, further *in vivo* studies need to be conducted to prove the feasibility of proliposome carriers for enhanced bioavailability of agomelatin.

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