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Formulation and *In-Vitro* Evaluation of Pulsatile Drug Delivery of Melatonin Using Pulsincap Technology

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ABSTRACT

The aim of the present study is to prepare a single unit pulsatile capsule of melatonin (MT) to control sleeping problems of travelers and night workers. This is achieved by preparing MT microsponges utilizing different proportions of ethyl cellulose (EC) and polyvinyl alcohol (PVA) using quasi-emulsion solvent evaporation method. Then, MT microsponges are incorporated into pulsincaps where the lag time is controlled by a polymer plug. The main constituent of the capsule is gelatin and formaldehyde. Formaldehyde is used to retard the dissolution rate of the capsule body. Microsonge formulation M2 showed optimum loading efficiency and smallest particle size. Scanning electron microscopy (SEM) images showed that the microsponges are porous, spherical in shape with smooth surface. P4 pulsincap system prepared from microsponge formulae (M2) and plugged by sodium carboxymethylcellulose (NaCMC 40 %) showed 100 % release of MT after 12 hr.

KEYWORDS : Melatonin, Microsponge, Pulsincap and Polyvinyl alcohol.

1. INTRODUCTION

Pulsatile systems are gaining a lot of interest as they deliver the drug at the right site of action at the right time thus providing spatial and temporal delivery and increasing patient compliance. These systems are designed according to the circadian rhythm of the body [1]. Circadian rhythm regulates many body functions in humans, viz., metabolism, physiology, behavior, sleep patterns, hormone production, etc. It has been reported that more shocks and heart attacks occur during morning hours. The patients suffering from diabetes are reported to have high blood sugar levels after meals compared to other timings.

Pulsatile Drug Delivery System (PDDS) are basically time –controlled drug delivery systems in which the system controls the lag time independent of environmental factors like pH, enzymes, gastro-intestinal motility, ect. These systems are designed according rhythm of the body. The principle rational for the use of pulsatile release of the drugs is where a constant drug release is not desired. A pulse has to be designed in such way that a complete and rapid drug release achieved after lag time [2]

Pulsatile delivery is desirable for drugs acting locally or having an absorption window in the gastro-intestinal tract or for drugs with an extensive first pass metabolism, for e.g. β-blockers or for drugs, which develop biological acceptance, where the constant presence of the drug at the site of action diminishes the pharmacological effect, or for drugs with special pharmacokinetic characteristics designed according to the circadian rhythm of human.

Presently, the concept of chronotherapeutics [3] has come out, where a therapeutic agent is released at a rhythm that ideally matches the biological conditions of a given disease therapy. In other words, it delivers the drug in higher concentration in the time of greatest need and lower concentrations when the need is less. Several biological processes and disease states have been shown to be influenced by circadian rhythm [4].

Microsponge delivery system also known as solid phase porous-microsphere is a patented micro-particulate system, comprising highly cross-linked, polymeric porous microspheres having numerous interconnected voids of particle size range 5-300 µm in the particle, loaded with active agent within a collapsible structure with large porous surface to entrap wide range of active agents with varying pharmacological activities administered in different doses that can be released at the desired site of absorption [5-8]. The pores in the micro-particle form a continuous arrangement open to the exterior surface of particles which permits the outward diffusion of the entrapped drug molecule at a controlled rate depending on the pore size [9]. Depending upon the size the total pore length may range up to 10 ft and pore volume up to 1 ml/g.

MT (N-acetyl-5-methoxy tryptamine) is a hormone made by the pineal gland camera.gif, a small gland in the brain. MT helps control sleep and wake cycles. Very small amounts of it are found in foods such as meats, grains, fruits, and vegetables. You can also buy it as a supplement. Normally, MT levels begin to rise in the mid- to late evening, remain high for most of the night, and then drop in the early morning hours. During the shorter days of the winter months, our body may produce melatonin either earlier or later in the day than usual. This change can lead to symptoms of seasonal affective disorder (SAD), or winter depression.

MT supplements are sometimes used to treat traveller jet lag (desynchronosis) or sleep problems (insomnia). Scientists are also looking at other good uses for MT, such as: treating seasonal affective disorder (SAD), helping to control sleep patterns for people who work night shifts, preventing or reducing problems with sleeping and confusion after surgery and reducing chronic cluster headaches [10 and 11].

2. MATERIALS AND METHODOLOGY

2.1. Materials

MT was kindly provided by Sigma Chemical Company (Saint Louis, MO), Dichloromethane (Goodrich Chemicals Co., Cleveland, USA), Lactose, Polyethylene glycol 600, EC, NaCMC, Hard gelatin capsule (size 0) and Formaldehyde were from El-Nile pharmaceutical company, Cairo, Egypt. PVA and Hydrochloric acid were purchased from Al-Nasr Company for chemicals and pharmaceuticals, Cairo, Egypt.

2.2. Methodology

2.2.1. Preparation of MT microsponges

Three batches of microsponges coded by M_1, M_2, M_3 utilizing different

proportions of EC and PVA were prepared by Quasi-emulsion solvent diffusion method. Briefly, in this method, the dispersion phase consists of MT and requisite quantity of EC dissolved in 20 ml of dichloromethane was slowly added to a definite amount of PVA in 150 ml of aqueous continuous phase as shown in *Table No. 1*. The mixture was continuously stirred at 1000 rpm for two hours on a mechanical stirrer in order to evaporate solvent. The microsponges were collected by filtration and dried in oven at 40 ˚C for 24 hours. The dried microsponges were stored in desiccators to ensure the complete removal of residual content [12].

Table (1). Composition of different batches of MT microsponges

Formula No.	MT (mg)	EC (mg)	PVA (mg)	Dichloromethane (ml)	Polyethylene glycol 600 (ml)	Distilled water (ml)
M_1	5	10	15	20	1	150
M_2	5	15	15	20	1	150
M_3	5	15	10	20	1	150

2.2.2. Compatibility studies

2.2.2.1. Fourier transforms infrared spectroscopy (FTIR) [13]

The FTIR spectroscopic studies were carried out for the standard MT, EC, PVA and a physical mixture of MT-EC, MT-PVA and MT microsponges formulation by KBr pellet technique using FT-IR spectrophotometer Genesis II, Mattson, England). The discs were scanned over a wave number range (4000-400cm).

2.2.2.2. Differential scanning calorimetry (DSC)

The DSC studies were performed for the drug, the polymers, the drug-polymer physical mixtures in the ratio 1:1 and drug microsponges. The samples (3-4 mg) were inserted in aluminum pan and heated in the rate of 10 ˚C/ min, to a temperature of 200 ˚C using a differential scanning calorimeter [TA-501; shimadzu corporation, Japan].

2.2.3. Characterization of MT microsponges

2.2.3.1. Determination of loading efficiency [5]

A sample of dried microsponges equivalent to 5 mg MT was taken into mortar and pestle and adds little amount of phosphate buffer of pH 6.8 and allowed to stand for 24 hours. Then transfer content in to 100 ml volumetric flask and make up volume to 100 ml with phosphate buffer of pH 6.8. The solution was filtered through whatmann's filter paper. The filtrate was sufficiently diluted with phosphate buffer of pH 6.8. Drug content was determined by UV spectrophotometer at 223 nm. The loading efficiency (%) of the microsponges can be calculated according to the following equation:

Loading efficiency = (Actual drug in microsponge / Theoretical drug concentration) X 100

2.2.3.2. Production yield

The production yield of the microsponges can be determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponge obtained.

Production yield = (Practical mass / The original mass) X 100

2.2.3.3. Particle size of microsponges

The mean diameter of 100 dried microsponges was determined by optical microscopy (Metzer, India). The optical microscope was fitted with a stage micrometer by which the size of microsponges could be determined.

2.2.3.4. Scanning electron microscopy (SEM) [5]

For morphology and surface topography, prepared microsponges can be coated with platinum–palladium alloy under vacuum and then the surface morphology of the microsponges can be studied by SEM. SEM of a fractured microsponge particle can also be taken to illustrate its ultra structure.

2.2.4. Formulation MT pulsincap systems

2.2.4.1. Preparation method used for cross-linking gelatin capsules [14-16]

Formalin treatment has been employed to modify the solubility of gelatin capsules. Exposure to formalin vapours results in an unpredictable decreases in the solubility of gelatin owing to the cross-linkage of the amino group in the gelatin molecular chain aldehyde group of formaldehyde by Schiff's base condensation.

First, formalin vapours were generated by adding a pinch of potassium permanganate in desiccators containing 25 ml of 15% (v/v) formaldehyde solution. Then the mesh containing about 50 empty bodies of hard gelatin capsules were exposed to these generated vapours of formaldehydes while the caps were left untreated in order to keep them water-soluble. Then this desiccator was tightly closed with a lid. The desiccator was kept aside for a period of 12 hours. After which the capsule bodies were taken out from the desiccators and dried at 50 ˚C for 30 minutes to ensure completion of reaction between gelatin and formaldehyde vapours. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated caps and stored in an air tight container.

2.2.4.2. Evaluation tests for capsules

2.2.4.2.1. Testing of formaldehyde exposed empty capsule bodies

These capsules bodies after exposing to formaldehyde were tested for various characteristics such as changes in the dimension or any type of physical defects.

2.2.4.2.2. Disintegration studies for capsules [17]

First 10 empty capsules containing both untreated caps and treated bodies were randomly selected. Then the study was carried out by dipping and stirring a single capsule in different buffers of pH 1.2, 6.8 and 7.4 filled in disintegration apparatus. This testing was carried out at room temperature for a period of 24 hours.

2.2.4.3. Preparation of polymer plugs [18]

The polymer plugs which were used for plugging the opening of capsule bodies were prepared using direct compression technique Erweka tableting machine , by using compressing polymer (NaCMC) and lactose as shown in *Table No. 2*. The polymer plugs were then considered for further study.

Table (2). Composition of polymer plug

Formula No.	Polymer (NaCMC)	Lactose
1	60	40
2	40	60

2.2.4.4. Evaluation of polymer plug [19]

The prepared polymer plugs were evaluated for hardness, thickness and weight variation.

2.2.4.5. Development of MT pulsincap systems

The microsponges equivalent to 5 mg of drug were filled into the formaldehyde treated body of empty capsule shell. The opening of capsule bodies containing microsponges was then closed by plugging it with different formulated plug. Further, small amount of 5% ethanol solution of ethyl cellulose was used to seal the joint between the capsules body and soluble cap. Formula for different batches of pulsincap dosage forms were shown in *Table No. 3*.

Table (3). Formula for MT pulsincap systems

Formula No.	Microsponges formulae equivalent to 5mg Melatonin	Polymer used as a plug	Weight of polymer plug used (mg)	
			Polymer	Lactose
P_1	M_1	Na CMC	60	40
P_2	M_1	Na CMC	40	60
P_3	M_2	Na CMC	60	40
P_4	M_2	Na CMC	40	60
P_5	M_3	Na CMC	60	40
P_6	M_3	Na CMC	40	60

2.2.5. Evaluation tests for MT pulsincap systems

2.2.5.1. *In-vitro* release profiles of MT pulsincap systems

Dissolution studies were carried out by using USP I dissolution test apparatus (Basket) method. Capsules were placed in a basket so

that the capsule should be immersed completely in dissolution media but do not float. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 hours (since the average gastric emptying time is 2 hours) then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 hours (average small intestinal transit time is 3 hours) the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hours. 900 ml of the dissolution medium was used at each time. Rotation speed was 50 rpm and temperature was maintained at 37±0.50 °C. Five milliliters of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 277 nm, 221 nm and 223 nm for 1.2, 7.4 and 6.8 pH buffers respectively by UV spectrophotometer.

2.2.5.2. Drug Release kinetics data

The release kinetics of MT from pulsincap was evaluated by employing the Korsmeyer peppa's equation: $M_t/M_\infty = k\,t^n$, where M_t is the amount of the drug released at time t, M_∞ is the amount of the drug released after infinite time, k is the kinetic constant and n is the diffusional exponent indicative of the mechanism of drug release. When n is ≤ 0.5, the drug is released from the polymer with a fickian diffusion mechanism. If $0.5 < n < 1$ this indicates anomalous or non-fickian release, while if n= 1 this indicates Case II transport. Lastly, when n is > 1.0, Super Case II transport is apparent [22]. Kinetic studies were performed by adjusting the release profiles to Higuchi, First and Zero order equations. The kinetic parameters and correlation coefficient were calculated for the *in-vitro* release of all MT prepared pulsincap systems.

3. RESULTS AND DISCUSSION

We have mentioned in the introduction, that our aim in this study was to target MT as per the circadian rhythm for controlling sleeping problem especially for travelers or night working people. So we aimed to design a novel pulsincap formulation that should release MT after a lag time of minimum 5 hours and maximum portion of the drug should be released between 6 to 8 hours. This target release profile is based on the assumption that if the patient takes our prepared formulation at 8.00 p.m. in the night, then the drug starts releasing from 1.00 a.m. and maximum portion of the drug will be available from 7.00 to 9.00 a.m. (the required time for sleeping).

The device was formulated in three steps: Firstly, MT was prepared as microsponges by quasi-emulsion solvent evaporation method; Secondly, MT microsponges were filled in a non-disintegrating capsule body and its opening was closed by plugging it with polymer plug; Thirdly, the non-disintegrating capsule body joint with soluble cap by sealing.

3.1. Preparation of Melatonin Microsponges

For the evaluation of the effect of drug: polymer: emulsifying agent ratio on the physical characteristics of microsponges, different ratios of drug to EC to PVA (1:2:3, 1:3:3 and 1:3:2) were tried. In all these formulations the volume of organic solvent and the volume of aqueous phase were kept constant. Polyethylene glycol was added to all formulations in order to facilitate plasticity.

3.2. Compatibility studies

3.1.1. Fourier transforms infrared spectroscopy (FTIR)

Spectra of pure drug MT, polymers, physical mixture and microsponge was recorded between 400 to 4000 cm^{-1}. The FTIR spectral analysis showed that all the characteristic peaks remain intact in its original form without any modification when formulated as microsponges and also shows that there was no significant interaction between drug and polymers used. Hence, the results were shown in *Figure (1).*

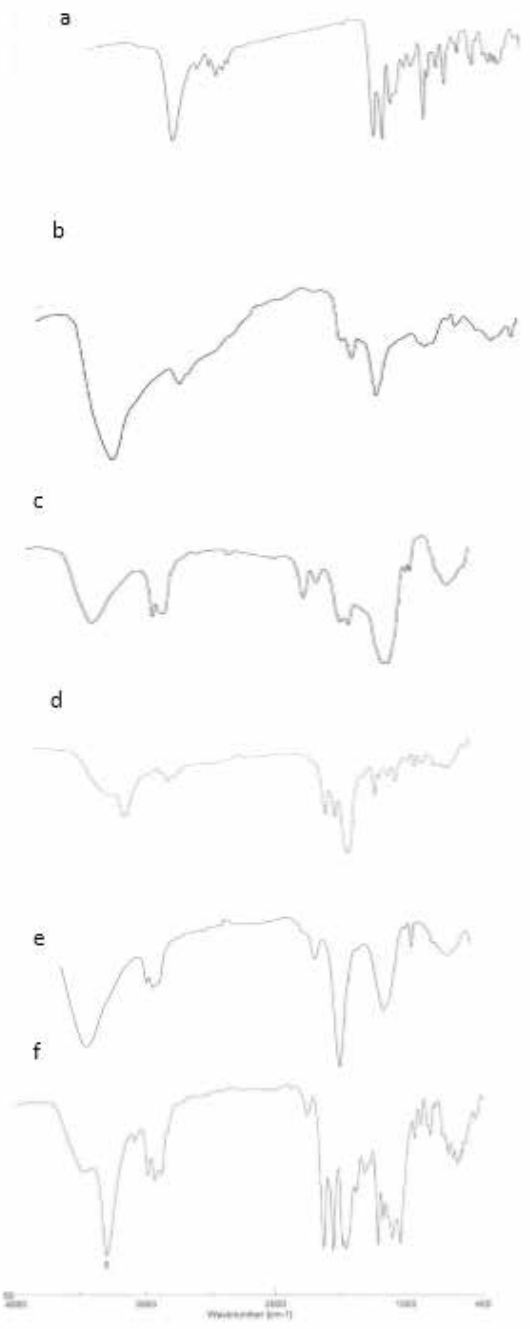


Figure (1): Shows FT-IR Spectra: (a) MT (b) PVA (c) EC (d) MT: PVA physical mixture (e) MT: EC physical mixture (f) MT microsponge.

3.1.2. Differential Scanning Calorimetry (DSC)

During the present work in addition to FTIR spectra of the drug and polymers, DSC thermo gram analysis was also used for studying physical characteristics. DSC thermo gram of MT due to its melting process shown a sharp endothermic peak at 115 °C. The peak for physical mixture of MT and EC was found at 113.67 °C. Whereas, the peaks for physical mixture of MT and PVA were found at 114.02 °C. Finally, the peak for MT microsponges formulation was found at 113.88 °C. Hence, the same range of pure drug melting peak is present even in the physical mixture with polymers or in the microsponges formulation. Thus, it indicates the absence of chemical interaction between the drug and polymer. Results were shown in *Figure (2).*

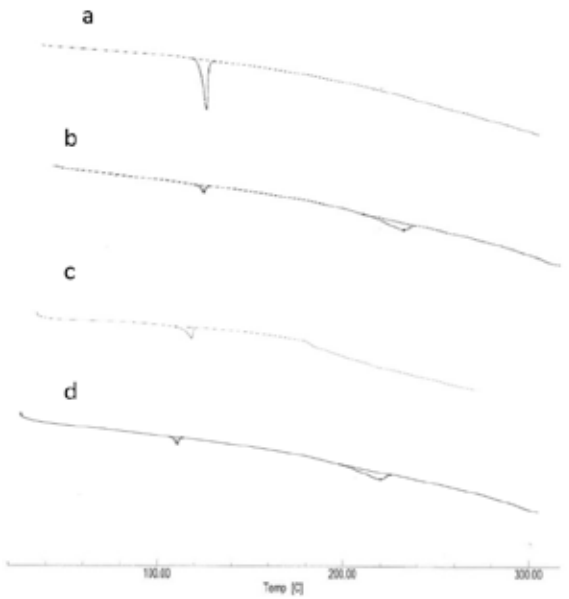


Figure (2): Shows DSC: (a) MT (b) MT: PVA physical mixture (c) MT: EC physical mixture (d) MT microsponce.

3.2. Characterization of MT microsponges

3.2.1. Determination of loading efficiency

The loading efficiency of MT microsponge formulations are given in *Table (3)* and represented in *Figure (3)*. The loading efficiency calculated for all microsponges and they were 85.25, 92.90 and 88.45 for M_1, M_2 and M_3 respectively. Loading efficiency was varying by changing the proportions of drug, polymer and emulsifier. Higher loading efficiency is observed with microsponge formulation (M_2), which consists of drug: polymer: emulsifier ratio 1: 3:3.

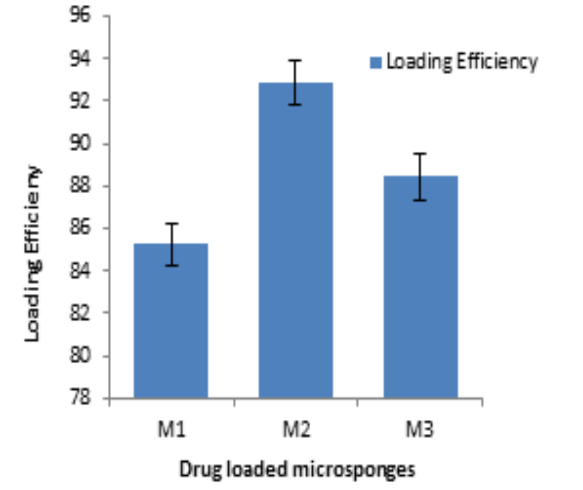


Figure (3): Comparison of loading efficiency of different MT loaded microsponge formulations

3.2.2. Particle Size of microsponges

Particle size of microsponges is varied along with the change in the ratio of polymer (EC) and emulsifier (PVA). The significant formulation factor that affects particle size include drug: polymer ratio. It was observed that increase in the drug: polymer ratio in microsponge formulations M_2 and M_3, results in small particles than M_1. On the other hand, by keeping polymer concentration constant, it was noticed that M_2 has smaller sized microsponges than M_3, and this may be due to the fact that increasing the concentration of emulsifying agent leads to formation of small-sized microsponges [20].The average values were given in *Table (4)* and represented in *Figure (4)*.

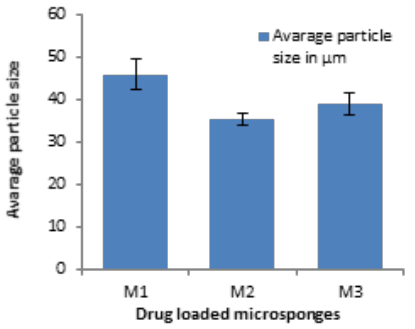


Figure (4): Comparison of average particle size of different MT loaded microsponge formulations

3.2.3. Production Yield

The production yields of MT microsponge formulations are given in *Table (4)* and represented in *Figure (5)*. Production yield determined for all microspongs ranged from 68.30 – 88.80 %. From the production yield of MT microsponge formulations it was indicated that increasing the drug: polymer ratio increased the production yield.

Table (4). Characteristics of MT microsponge formulations

Formula No.	Loading efficiency (%±SD)	Production yield (%± SD)	Mean particle size (µm± SD)
M_1	85.25 ± 0.99	68.30 ± 1.06	46.00 ± 3.53
M_2	92.90 ± 1.02	75.35 ± 0.89	35.50 ± 1.41
M_3	± 1.88.45	88.80 ± 1.13	39.00 ± 2.75

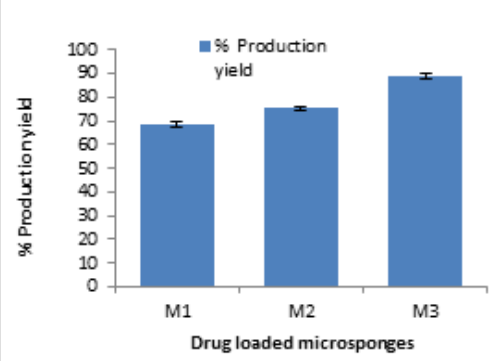
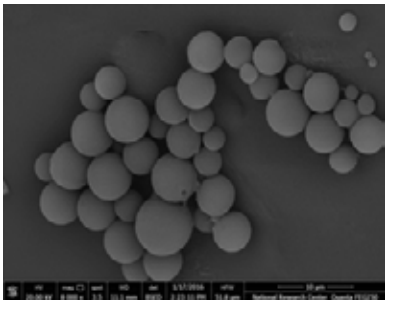
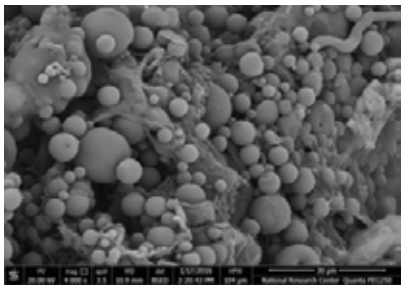


Figure (5): Comparison of production yield of different MT loaded microsponge formulations

3.2.4. Scanning electron microscopy

Microsponge formulation with least particle size and optimum loading efficiency was investigated by SEM to find out surface morphology. The representative SEM photographs of the microsponge were shown in *Figure (6)*. SEM images showed that the formulated microsponges are porous, spherical in shape with smooth surface.





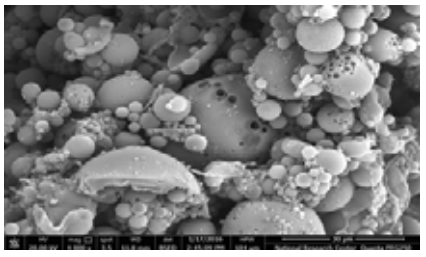


Figure (6): SEM photographs of MT loaded microspong es

3.3. Evaluation tests for capsules

3.3.1. Testing of formaldehyde exposed empty capsule bodies

When 50 capsules were taken for formaldehyde treatment, out of them some capsules were found to be shrunk or distorted. The vari ation in dimensions was also studied by measuring the formaldehyde treated and untreated capsules. It was found that the length and di mension of capsule bodies showed little decrease after formaldehyde treatment.

3.3.2. Disintegration studies for capsules

When the capsule were subjected to disintegration studies in dif ferent buffers, the untreated caps disintegrated within 9 minutes in all media whereas the treated bodies remained intact for about 24 hours, this is due to the reason that when formaldehyde reacts with gelatin it forming an irreversible complex, that decrease solubility of gelatin and at the same time, the primary amine group present in gel atin reacts with formaldehyde making it irreversibly bound [21].

3.4. Evaluation of polymer plug

Table (5): Evaluation of polymer plugs

Formula No.	Polymer plug used	Average weight (mg) ± SD	Thickness (mm) ± SD	Hardness (kg) ± SD
1	Na CMC (60 mg)	100.02 ± 0.25	2.50 ± 0.017	3.98 ± 0.055
2	Na CMC (40 mg)	100.50 ± 0.54	2.57 ± 0.010	3.85 ± 0.017

In order to have a tight fitting at the opening of the impermeable capsule body and in order to prevent the penetration of buffer into the capsule various polymer plugs were designed. We used same thickness, weight and hardness for all the polymer plugs, in order to evaluate the effect of increased concentration of polymer in the plugs. Various physico-chemical properties were evaluated. The re sults are shown in *Table (5)*.

3.5. *In-vitro* release profiles of MT pulsincap systems

Our aim in this work is to formulate a single unit pulsatile cap sules of melatonin which releases the drug after a definite lag time and provides required concentration of drug at regular in tervals of time [23].

The prepared pulsincap containing different microsponge formula tions and plugged with different polymer concentration were subject ed to dissolution testing. Our main aim in this dissolution testing was to identify an optimum microsponge formulation and the most suit able concentration of polymer plug. With all formulations, there was no drug release in pH 1.2, thus indicating the efficiency of treatment of gelatin with formaldehyde vapors, that prevent the release of MT in the stomach, further polymer plug prevent the release of MT in the small intestine, i.e., pH 7.4 phosphate buffer, only in the colon medi um i.e., pH 6.8 MT started to release after a lag time of minimum 5 hours.

The release of the drug from the pulsincap preparations was found to be dependent upon the different batches of microsponges pre pared from drug: polymer: emulsifier ratio and concentration of polymer plug used. It was observed that *in-vitro* release of MT from pulsincap influenced by drug: polymer ratio in microsponge formulation, as the drug: polymer ratio increase the release of drug increase. Also, it was found that when the polymer concen tration in the plug increased it resulted in decreased drug release. It is due to the reason that the plugged polymer after absorption the surrounding dissolution medium has completely swelled and formed a swollen matrix and after wetting became a soft mass and came out of the capsule body. Thus, it resulted in the release MT loaded microsponges. Only in the initial 2 hours the release will be pH dependent due to the treatment of gelatin with for maldehyde vapour but in further remaining hours it becomes time dependent due to hydrophilic polymer plug present at the open ing of the capsule body. The two pH buffer system pH 7.4 and pH 6.8 were used only to maintain the condition of the small intestine and colon and they did not have any effect on the drug release mechanism *Figure (7)*.

When formulation P_1, P_3 and P_5 prepared with NaCMC in 60 % con centration as a polymer plug were subjected to dissolution studies, it was found that the release of MT after 12 hours was 91.45, 99.90 and 96.45 respectively.

When formulation P_2, P_4 and P_6 prepared with NaCMC 40 % concen tration as a polymer plug were subjected to dissolution studies, it was found that the release of MT after 12 hours was 85.75, 100.05 and 97.20 respectively.

In all these formulations, pulsincap formulae (P_4) prepared from mi crosponge formulation (M_2) and plugged by NaCMC in 40 % concen tration was considered to be the optimum formulation.

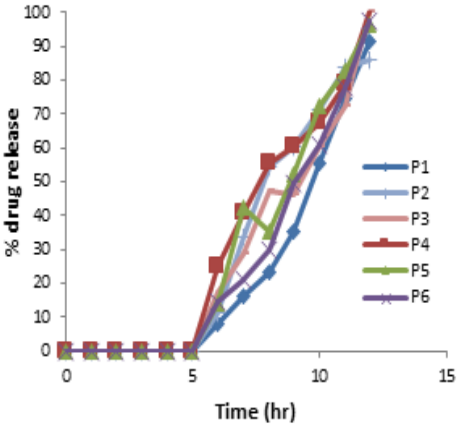


Figure (7): *In-vitro* dissolution study of different MT pulsin cap systems

3.6. Drug Release kinetics data

Table (6) showed kinetic parameters for the *in-vitro* release of MT from prepared pulsincap systems according to Zero order, First or der, Higuchi-diffusion model and Korsmeyer peppa's model. The release exponent values (n) of all the prepared pulsincap were from 1.787 to 3.487. It can be concluded that all the prepared pulsincap systems showed Supercase II transport [24]. All the pre pared pulsincap systems except P_2 showed higher (r) values for zero order plots indicating that drug release followed zero order kinetics. While P_2 showed higher (r) values for first order plots indi cating that drug release followed first order kinetics.

Table (6): The calculated correlation coefficient (r) and (n) value for MT pulsincap systems based on *in-vitro* release study.

Code	(n) value	r	Zero-order kinetic		First-order kinetic		Higuchi model		Possible order & mechanism of drug release
			r	k	r	K	R	k	
P_1	3.487	0.9977	0.9825	0.2371	0.9049	0.0059	0.9725	10.7703	Zero order, Super Case II transport
P_2	2.668	0.9185	0.9703	0.2032	0.9905	0.0052	0.9801	9.4234	First order, Super Case II transport
P_3	2.311	0.9793	0.9722	0.2073	0.7219	0.0133	0.9654	9.4467	Zero order, Super Case II transport
P_4	1.787	0.9776	0.9832	0.1858	0.7388	0.0132	0.9813	8.5129	Zero order , Super Case II transport
P_5	2.497	0.9398	0.9743	0.2173	0.9020	0.0076	0.9719	9.9476	Zero order , Super Case II transport
P_6	2.815	0.9968	0.9896	0.2335	0.8583	0.0079	0.9819	10.6336	Zero order , Super Case II transport

4. Conclusion

Our aim in this study was to target MT as per the circadian rhythm for controlling sleeping problem especially for travelers or night working people. So we aimed to design a novel pulsincap system that should release MT after a lag time of minimum 5 hours and maximum portion of the drug should be released between 6 to 8 hours. This was achieved by P_4 pulsincap system. It is prepared from microspnge formulae (M_2) and plugged by NaCMC in 40 % concentration showed 100 % release of MT after 12 hr with lag time of about 5 hr. Microsonge formulation M_2 showed optimum loading efficiency and smallest particle size.

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