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STUDY OF SOLID LIPID NANOPARTICLES AS A CARRIER FOR BACOSIDE

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ABSTRACT

Solid lipid nanoparticles (SLN) loaded with Bacoside were prepared by microemulsion probe sonicator method. Solid lipid nanoparticles (SLNs) have been proposed as suitable colloidal carriers for delivery of drugs with limited solubility. Bacoside as a model drug was incorporated into SLNs prepared from stearic acid using Tween 80 emulsifiers. SLNs in the rage of 33.3-257nm.with mean particle size of 56 nm was obtained. The characteristics of the SLNs with various lipid and surfactant composition were investigated. The mean particle size of drug loaded SLNs decreased upon mixing with Tween 80 as well as upon increasing total surfactant concentration. The zeta potential of these SLNs varied in the range of –25 to –26 (mV), suggesting the presence of similar interface properties. High drug entrapment efficiency of 74.1% revealed the ability of SLNs to incorporate a poorly water-soluble drug such as bacoside. In vitro drug release study showed upto 84.68% drug release from Solid lipid nanoparticles. The drug release from Solid lipid nanoparticles follows zero order kinetics of drug release.

KEY WORDS

Solid lipid nanoparticles , Bacoside ,Tween 80, Stearic acid. Etc.

INTRODUCTION

Present work deals with understanding of rational drug delivery i.e. to deliver the drug in proper way so that maximum amount of drug will be available for Absorption. Bacoside obtained from Bramhi (*Bacopa Monniera*) mainly used in Brain disorder and memory related disorder which is dose dependant Inhibitor of Acetylcholine esterase (AChe)[(14)].

Acetylcholineesterase is a enzyme which metabolises a neurotransmitter Acetylcholine. Bacoside also aid in repair of Damaged neurons by enhancing kinase activity,neuronal synthesis and restoration of synaptic activity and ultimately nerve impulse transmission[(44)]. *Bacopa monniera* (Bramhi) is amongst those plants which commonly grows in marshly areas in India ,It has been reported that the plant is used in traditional ayurvedic treatment for epilepsy, improvement of memory and inteluatl activity[(34)]. The pharmacological effects of *Bacopa monniera* are attributed to the presence of a number of biologically active compounds, including alkaloids, saponins and sterols. The compounds responsible for the memory enhancing effects of *Bacopa monniera* are triterpenoid saponins called "Bacosides",

Bacoside mainly given by oral route, But due Blood brain barrier the drug is unable to reach its site of action. Hence drug delivery system which is able to cross blood brain Barrier is required for administration of bacoside and for its maximum bioavailability at site of action. Recently Solid lipid nanoparticles (SLNs) have been exploited as probable possibilities as carriers for drug delivery to CNS due to their smaller size and lipid solubility [(30)]. They are considered to be a better alternative than liposomes, polymeric nanoparticles, and microemulsion[(11)]. Solid lipid nanoparticles (SLN) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery and research [(40)]. Due to their unique size dependent properties, lipid nanoparticles offer possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in

drug delivery that could use for drug targeting. Hence solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence attracted wide attention of researchers. This review presents a study of solid lipid nanoparticles of Bacosides discussing their aims, production procedures, advantages. Appropriate analytical techniques for the characterization of SLN like photon correlation spectroscopy, scanning electron microscopy, UV spectroscopy were used.

MATERIAL AND METHOD

PART-I.EXTRACTION AND CHARACTERIZATION OF PLANT MATERIAL (Kahol et al., 2003)

The fresh leaves of *Bacopa monniera* were collected from Town Hall Garden, Kolhapur, Maharashtra in the month of June 2012. The plant was identified at the department of Biotechnology Kits College of Engineering, Kolhapur, Maharashtra, India. The freshly harvested herb *Bacopa monniera* was thoroughly washed with tap water followed by distilled water, shade dried, crushed in a disintegrator to obtain ground powder and stored in air tight bottles. 44 gram of shade dried powder of plant was filled in the thimble and extracted with hexane solvent in a modified soxhlet apparatus to remove lipid from the herb. The Hexane extract will be removed from the soxhlet apparatus when the solution in the side tube of apparatus become almost clear and powder left in the apparatus will be collected and dried. The dried herb powder was extracted with acetone for a time period 4 to 8 hours to obtain an acetone extract containing unwanted colour and non-bacoside constituents and dried the herb. The dried herb was extracted with methanol to obtain a methanol extract containing bacosides. The methanol extract was concentrated under vacuum evaporator. This concentrated methanol extract was added gradually to acetone with stirring to effect preferential precipitation of bacosides. The bacosides were filtered in a Wattman filter paper to obtain a bacoside residue. This bacoside residue was dissolved in distilled water to obtain an aqueous solution. % yield of bacoside was calculated by formula,

Percentage Yield=Weight of crude extract/Weight of dried plant x 100

1. Thin layer Chromatography of Bacopa Extract

Thin layer chromatography is a technique mostly used for analytical purpose in this technique stationary phase is supported with glass plate or aluminium foil. The plate is coated with fine particle of an absorbent such as alumina or silica gel as a thin layer of about 0.2 mm thickness. The solute components get separated due to their different migration rate the separation mechanism is adsorption The Rf value is calculated by using ratio of linear distance of solute from the starting line and linear distance of mobile phase.

METHOD:

The extract or the isolated bacoside dissolved in methanol and spotted over silica gel G plates.

The plates were eluted in n-butanol: acetic acid: water (36:6:8) Then the TLC plates were sprayed with sulphuric acid (20%) in methanol. Distance travelled by sample and solvent was measured.

The Rf value calculated by formula-

Rf value= Distance travelled by sample/Distance traveled by solvent

Standard Rf value for bacoside A=0.67

2. IR SPECTROSCOPY OF BACOSIDE

IR spectroscopy of Bacoside was performed by using FTIR spectrophotometer (JASCO FTIR 410). Spectra were scanned over the wavelength region of 4000 to 400cm^{-1}. The procedure consisted of dispersing sample in KBr and compressing in to discs by applying a pressure of 5 tons for 5 min in a hydraulic press (KBr pellet method). The pellet was placed in the light path and the spectrum was obtained.

3. High Performance Liquid Chromatography of Bacoside Extract

The bacoside rich extract was subjected to HPLC to estimate Bacoside A .100mg of extract was dissolved in 25 ml of methanol by sonication and made up to 50 ml with methanol and filtered through 0.22 µ syringe filter. Bacoside from Natural remedies Bangalore was used as standard. Mobile phase was prepared by using two solvent systems; solvent A was prepared by

dissolving 0.136gm of unhydrus potassium dihydrogen orthophosphate and 0.5ml of orthophosphoric acid in 900ml of HPLC grade water and by making volume up to 1000ml with water. Solvent B was acetonitrile. The HPLC of bacoside was done by SHIMAZDU HPLC system having photo diode array detector. System was equipped with pinnacle DB C18 column (250mmX 4.6mm from restek part no.9414575). Scanning wavelength and flow rate were adjusted at 205nm and 1.5 ml /min respectively. Total run time was 45 min.

Calculation For Bacoside A

$$\frac{\text{Bacoside A area in the sample x Weight of Bacoside A in mg x Purity Std}}{\text{Bacoside A standard area x Sample weight in mg}}$$

PART-II: PREPARATION AND CHARECTERISATION OF BACOSIDE SOLID LIPID NANOPARTICLES

METHODS

1. Preparation of Solid lipid Nanoparticles.

SLN were prepared by using pre-emulsion sonication method. For preparing pre-emulsion the amount of drug was kept constant while the quantities of lipid, surfactant were varied. The stearic acid was heated to at least 10˚ above its melting point. Bacoside was dissolved in the melt of lipid by stirring until the melt appeared clear. An aqueous phase was prepared by dissolving polysorbate 80 in distilled water (sufficient to produce 100 ml of preparation) and Hot aqueous phase was added to the oil phase, and constant stirring is carried out (at 2,100 rpm and temperature 70°) using lab stirrer. Coarse hot oil in water emulsion so obtained was ultrasonicated using for 12 min. Bacoside lipid nanoparticles were obtained by allowing hot nanoemulsion to cool to room temperature.

Table No.1

EXPERIMENTAL DESIGN

Batch no	Drug in gm	Lipid in gm	Surfactant (%)	Water
1	1	2	0.5	Qs
2	1	1	1	Qs
3	1	1	1.5	Qs
4	1	1.5	0.75	Qs

2. Characterisation of bacoside solid lipid nanoparticles

Nanoparticles are characterized by using different methods mainly from materials science.

i)Surface Morphology:

Surface morphology was analyzed by using Jeol Scanning Electron microscopy INSTRUMENT JSM-6360 at X10, 000 to X30, 000 magnification.

ii) Particle size Analysis.-

Particle size analysis was carried out using Particle size analyzer (Malvern Instrument). The instrument measures the fluctuations in light intensity due to the Brownian motion of the particles in order to deduce the particle size. The measurable size ranges from 2-3 [nm] to 500[nm] – 1 micrometer.

iii) Zeta Potential Measurement.

The zeta potential was measured by using ZetaSizer Nano ZS (Malvern Instruments) having zeta cells, polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. The Henry equation is then used to calculate the zeta potential, ***z***,

$$U_e = \frac{2\varepsilon z f(\kappa a)}{3\eta}$$

Where *Ue* is the electrophoretic mobility, ε is the dielectric constant, η is the absolute zero-shear viscosity of the medium, $f(\kappa a)$ is the Henry function and k*a* is a measure of the ratio of the particle radius to the Debye length.

iv) **Encapsulation efficiency and Drug content.**

a) Encapsulation Efficiency

For encapsulation efficiency freeze dried sample of SLNs was dissolved in methanol and ultra centrifuged for 30 min at 15000 rpm.and the amount of Bacoside entrapped within the Solid lipid nanoparticles was determined by UV-visible spectrophotometer.

With known initial known concentration of drug amount of free drug will be determine

Bacoside (Encapsulation) = Bacoside (total)- Bacoside(free)

$$E\,(\%) = \frac{\text{Bacoside Encapsulated}}{\text{Bacoside Total}}$$

Where, E (%) = Percentage entrapment efficiency.

b) **Drug Content:**

The total drug content given by following formula and this indicates the amount of Bacoside encapsulated in the Solid lipid nanoparticles.

$$\text{Drug content} = \frac{\text{Amount of Bacoside encasulated}}{\text{Amount of Nanoparticles prepared}}$$

3. In Vitro drug release study from solid lipid Nanoparticles.

% yield of bacoside extract = 4.2/44 ×100 = 9 .54%

In vitro release study was performed on suspension of nanoparticles within 24 hr of preparation. 1 ml of dispersion was transferred to a dialysis tube and the sealed tube was introduced into a vial containing 10 ml of a phosphate buffer solution (pH 7.4). Samples were shaken horizontally in a shaker at 37±1 ◦C and 50 strokes per minute. At predetermined time intervals, 2ml sample of the medium was taken and replaced with the same amount of fresh medium. The amount of Bacoside released from the SLN was quantified by UV spectrophotometer.

RESULT

a) CHARACTERISATION OF BACOSIDE:

1) Bacoside extract yield-

% yield of bacoside -

% yield = Weight of crude extract / Weight of dried plant x 100

Where weight of crude extract- 5.4 gm

Weight of dried plant material-60 gm

2) TLC of Bcoside:

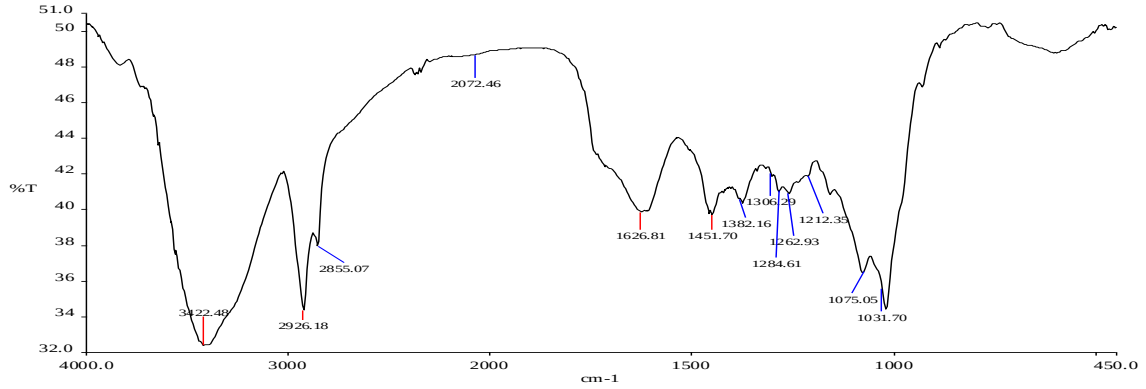
Rf value= distance traveled by sample/distance traveled by solvent

Distance travelled by sample= 5.6 cm

Distance travelled by solvent= 8.2 cm

Thus, Calculated **Rf value = 5.6 / 8.2 = 0.68** which is approximately equals to standard Rf value of bacoside A (0.67), Therefore, We confirmed the presence of bacoside A in extract.

3. FTIR SPECTROSCOPY



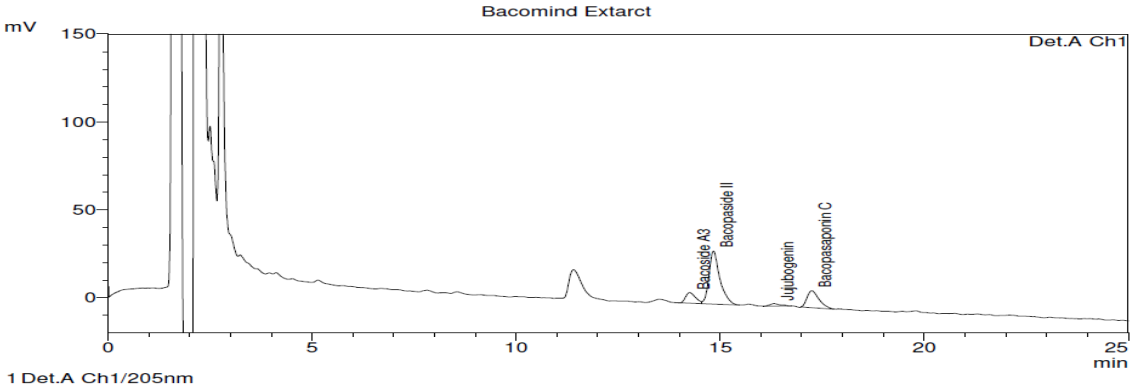
Graph No.1 FTIR SPECTRUM OF TEST BACOSIDE A SAMPLE

The IR spectrum of test Bacoside sample shows peaks at 3422.48 ,2855.07,2072.46 ,1626.81,1451.70 ,1382.16,1306.29,1284.41,1262.93 ,1212.33,1075.07 ,1031.70 respectively .which are identical with standard Bacoside A spectrum.

Hence IR spectrum of test Bacoside A is identical with standard Bacoside A

4) HPLC OF BACOSIDES

Acquired by	: Admin
Sample Name	: Bacopa Extarct- NS (1202067E)
Sample ID	:EX1202036-Tr1
Vail #	: 2
Injection Volume	: 20 uL
Data File Name	: dATA 011.lcd
Method File Name	: Bacopagradient.met.lcm
Batch File Name	: 22022012.lcb
Report File Name	: Default.lcr
Data Acquired	: 2/23/2012 4:10:17 AM
Data Processed	: 2/24/2012 1:54:51 PM



Detector A Ch1 205nm

Peak #	Ret. Time	Area	Area %	Tailing Factor	Resolution	Name
1	14.242	101620	11.480	0.000	0.000	Bacoside A3
2	14.824	559849	63.248	1.384	1.242	Bacopaside II
3	16.312	27279	3.082	1.372	3.140	Jujubogenin
4	17.232	196411	22.189	1.551	1.789	Bacopasaponin C
Total		885160	100.000			

$$\textbf{Assay}\left(\%\frac{\mathbf{w}}{\mathbf{w}}\right)\textbf{ of Bacoside A} = \frac{885160}{1547020} \times \frac{100}{100} \times 99 = 56.64\ \mathbf{w/w}$$

Assay (% w/w) of Bacoside A =56.64%

Hence Percentage purity of Bacoside A was found to be- 56.64%

b) CHARECTERISATION OF BACOSIDE SOLID LIPID NANOPARTICLES.

i. Scanning Electron Microscopy:

SEM of the nanoparticles showed a spherical shape of the dispersed particles. **Figures (1a & 1b)** show the Bacoside solid lipid nanoparticles.

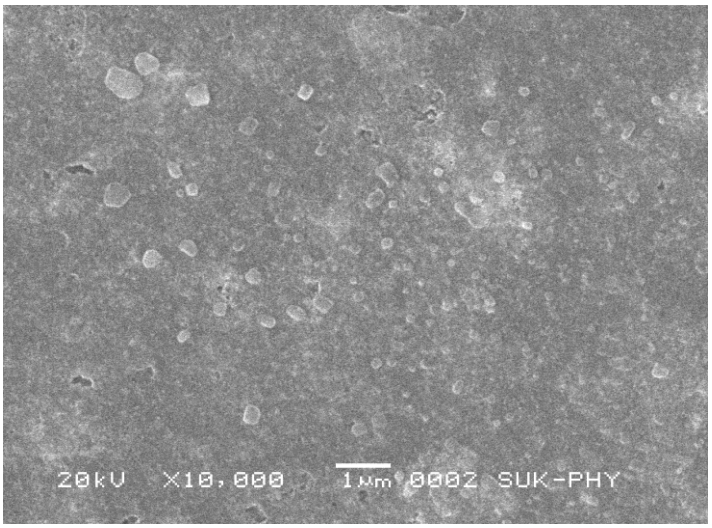


Fig:A

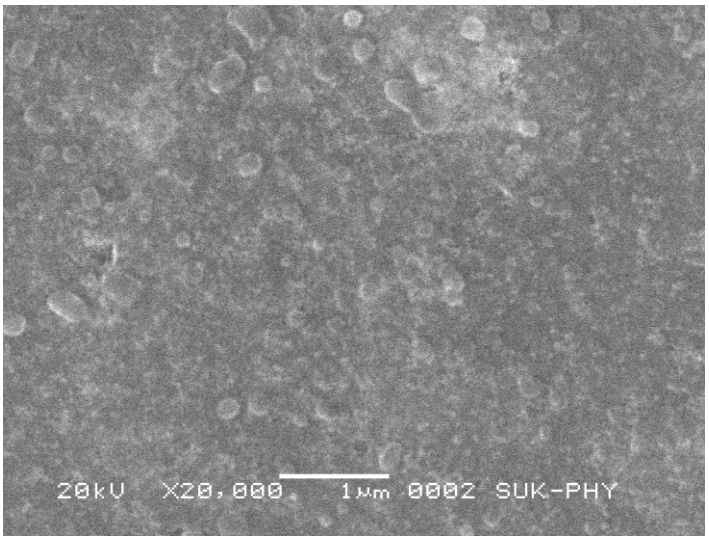


Fig:B

ii.Particle size measurement:

The table no 1 represents mean particle size for all the prepared batches of SLNs the particle size is in range from 53.4 nm to 257.1 nm. Increasing the lipid content in the prepared SLNs resulted in increased mean particle size. This increase in particle size with increasing lipid concentration may be due to the reduction of homogenization efficiency with increasing dispersed lipid phase as shown in **Table 1**

Table No.2: Mean particle diameter

BatchNo	Drug in gm	Stearic acid in gram	Tween 80	Mean Particle diameter
1	1	2	0.5	273.1
2	1	1.5	1	106.
3	1	1	1.5	53.4
4	1	1.5	0.75	203.6

iii. Zeta Potential Measurements.

Zeta potential is an important and useful tool to indicate particle surface charge, which could be used to predict and control the stability of colloidal suspensions. Since charged particles repel one another and thus overcome the natural tendency to aggregate, therefore the greater the zeta potential, the more likely the suspension to be stable. Usually particle aggregation is less likely to occur for charged particles with high zeta potential due to electric repulsion. Lipid nanoparticles are generally negatively charged on the surface.

Table No. 3: Average zeta potential

Batch No	Drug in gm	Stearic acid in gram	Tween 80	Average Zeta Potential
1	1	2	0.5	- 26.29
2	1	1.5	1	-25.75
3	1	1	1.5	-25.38
4	1	1.5	0.75	-26.12

The zeta potential of Prepared Solid lipid Nanoparticle was found to be in the range of -26.29 mv to -25.38. The –ve sign indicates that Prepared SLN has negative charge.Because of negative charge on their surface they repel each other and hence prevent agglomeration,aggregation and which leads to increased stabity of SLN.

iv) **Entrapment efficiency and Drug content**

Table No.4: Entrapment efficiency and Drug content

Batch No	Drug in gm	Stearic acid in gram	Tween 80	Entrapment efficiency (%)	Drug content mg/mgof Nanoparticles
1	1	2	0.5	61.17	0.111
2	1	1	1	55.58	0.108
3	1	1	1.5	74.1	0.139
4	1	1.5	0.75	51.59	0.094

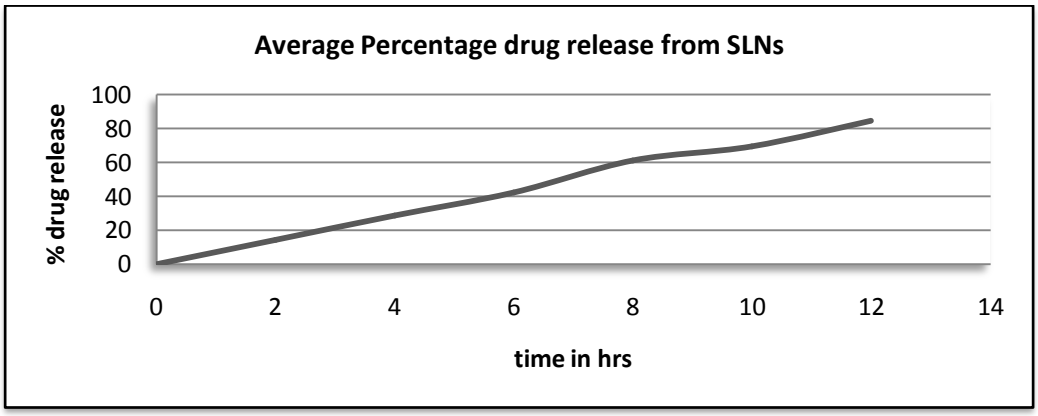
As shown in **Table No. 4.** It was found that surfactant concentration has direct effect on Entrapment efficiency and drug content with higher concentration it increases.

i. **In vitro drug release study from solid lipid nanoparticles.**

Batch No. 3: with high drug loading capacity was used for in vitro drug release study.

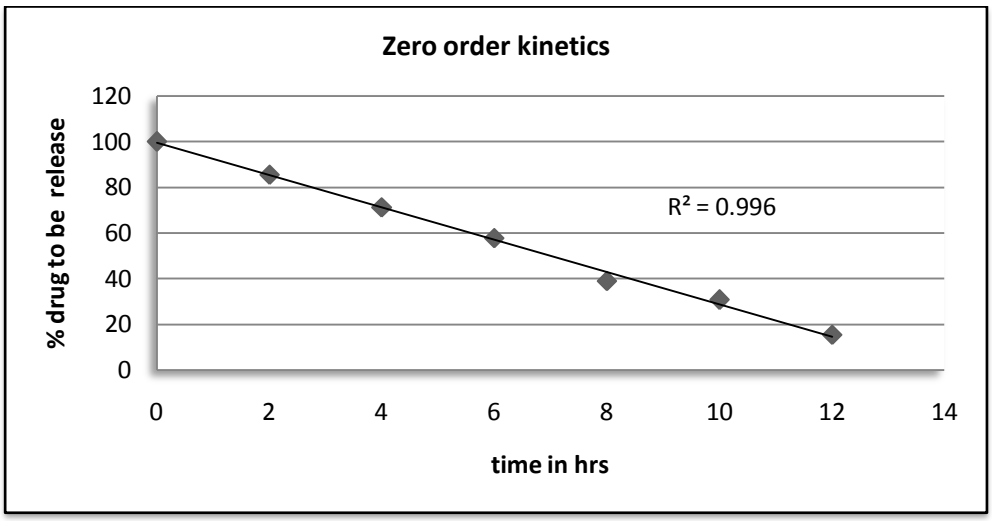
Note: Total amount of Bacoside nanoparticles containing 500 mg of Bacoside was dissolved in 5ml of distilled water and 1 ml of dispersion was used for analysis.

Graph 3 : Percentage drug release from Solid liid nanoparticles.

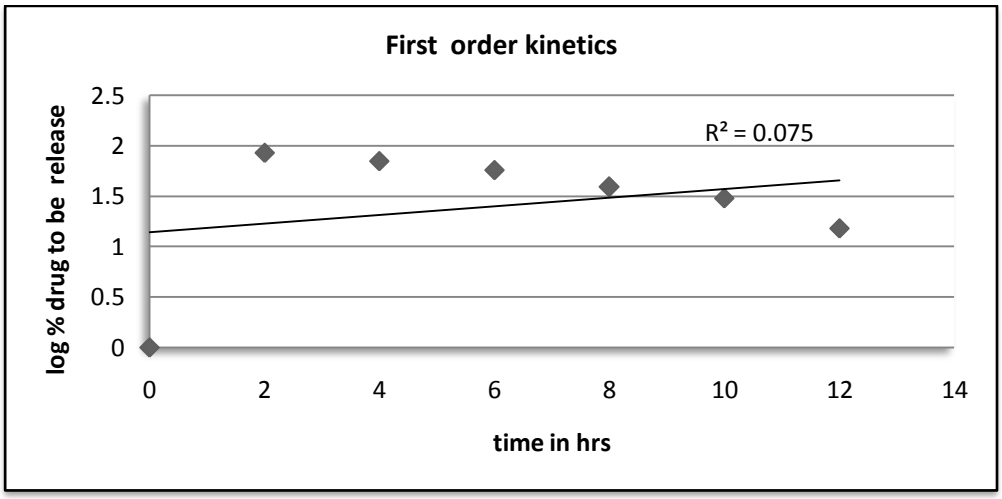


Application of kinetic models to find out order of reaction:

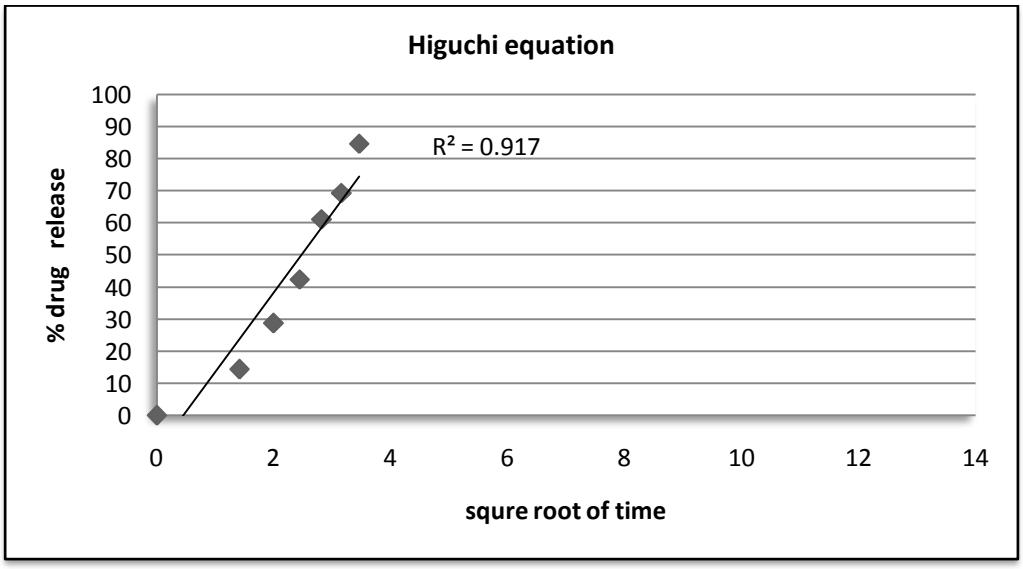
Graph 4: Zero order kinetics



Graph 5: First order kinetics



Graph 6: Higuchi Equation



Graph 7: Korsmeyer -peppas eqution

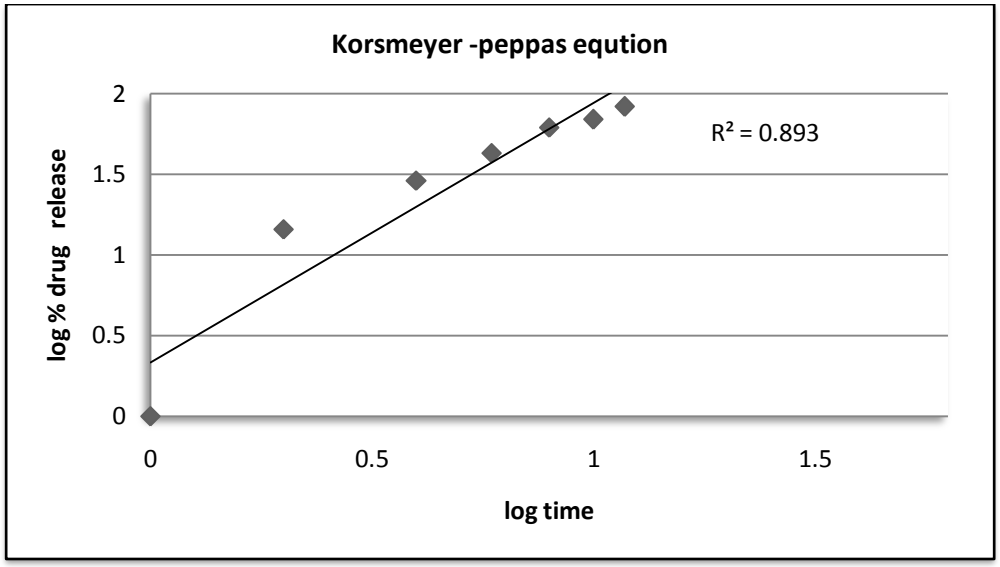


Table 5: R^2 value for drug release

Order of release kinetics	R^2
Zero order	0.996
First order	0.075
Higuchi equation	0.917
Korsmeyer –pappas equation	0.895

Hence, affter applying and analyzing data of kinetic models on drug release it was found that the given formulation follows zero order kinetics of drug release

2. DISCUSSION

The dried areal parts of plant were used for extraction and extracted first with hexane to remove unwanted lipids, plant waxes. It was done as it removes large amount of impurity present in the plant material and which can cause error if not removed initially. Second

it was extracted with acetone for decolourisation which removes chlorophyll which should be removed essentially. Finally extracted with methanol which is known as solvent for Bacoside. The methanol soluble part contains Bacoside which was recovered by using vaccum evaporation.

Then the bacoside was analysed with thin layer chromatography and Infrared spectroscopy for identification and authentication purpose which confirmed authentic Bacoside. By using High performance liquid chromatography percentage purity of Bacoside was calculated and purified bacoside was used for preparation of SLNs.

The solid lipid nanoparticles of Bacoside were prepared by using pre-emulsion sonication method for the purpose of Sonication probe sonicator was used. Sonication is the process of converting electrical signal into a physical vibration that can be directed toward a substance. Sonication is usually performed to break apart compound.During the process of SLN preparation oil in water emulsion was prepared and that emulsion was ultrasonicated, Because of sonication the emulsion is converted into nanoemulsion containing nanoparticles after cooling that nanoemulsion nanoparticles were obtained. Four batches of SLNs were prepared with different lipid and surfactant concentration keeping drug concentration and evaluated for particle size, distribution, drug content, encapsulation efficiency and zeta potential to find out optimum combination of lipid, drug and surfactant concentration for preparation of solid lipid nanoparticles of bacoside .

To check in vitro release from prepared solid lipid nanoparticles dialysis bag method was used. In invitro drug release study upto 85% of drug was released by SLNs. After application of different kinetics model it was found that the drug release from SLNs follows zero order kinetics.

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