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Original Article

Preparation of O- palmitoyl pullulan micro spheres for the controlled release of hydrophillic drug: Verapamil hydrochloride

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ABSTRACT

The aim of the present investigation is to study potential of O-Palmitoyl pullulan (OPP) as a vehicle for the preparation of microspheres. In the first phase of the work the drug Verapamil hydrochloride and polymer compatibility studies is carried. Then O- Palmitoyl pullulan (OPP) is synthesised from pullulan and characterised by NMR spectrum. The microspheres of Verapamil HCl are prepared by employing the combined solution mixture of sodium alginate and O- Palmitoyl pullulan by Orifice- ionic gelation technique. The microspheres are evaluated for percentage yield, swelling index, entrapment efficiency etc. Extensive experimentation, in-vitro methods have been carried out. It was observed that more than 90% of drug is released by 10-11 hr itself. Another formulation with further increased concentration of Sodium alginate is prepared. In this formulation, drug release is more sustained up to 12 hr. Formulations followed first order release kinetics and they showed Fickian type of diffusion. The stability studies revealed that there is no change in the drug release profile after the products are stored at $37+2^0$C and 75 + 5% RH and there was no significant drug loss after storage.

Key words: O-palmitoyl pullulan, Verapamil HCl, Swelling index, Percent yield, Entrapement efficiency, Fickian diffusion.

INTRODUCTION

Among various drug delivery systems microspheric drug delivery system has acquired tremendous attention due to its wide range of applications. It targets the drug to specific site for imaging and helping the diagnostic features. In this work, attempts have been made to prepare the OPP-sodium alginate microspheres bearing verapamil HCl by ionotropic gelation method for sustained drug delivery.

Biopolymers are generally water soluble gums which have novel and unique physical characteristics. These can be produced by a wide variety of microorganisms. These polysaccharides have enormous applications in the food, pharmaceutical and other industries because of their wide diversity in structure and physical properties. Pullulan is one of the biopolymer produced by Aureobasidium pullulans. Commercially available pullulan is purified from the fermentation medium of the *Aureobasidiumpullulans* [1]. Pullulan was first described by Bernier in 1958 and the structure was discussed by Bender et al in 1959 [2]. This polymer constitutes maltotriose units connected by an α (1→4) glycosidic bond, whereas consecutive maltotriose units are connected to each other by an α (1→6) glycosidic bond **(Fig-1)**. Pullulan is non-toxic, non-mutagenic, non-carcinogenic, odourless, tasteless, and edible polymer [3,4]. Its solubility is more in water and dilute alkali. It is insoluble in alcohol and other organic solvents except dimethyl sulphoxide and formamide. Due to its high solubility in water, it can be used as a carrier for drug and it helps in sustained release of drug in plasma. Pullulan is biodegradable, impermeable to oxygen. Pullulan can be used as a carrier for oral drug delivery due to its non-attackable nature towards the digestive enzymes of the human gut. The viscosity of pullulans solutions are relatively low and resemble gum arabic. So it can be used as low-viscosity filler in beverages and sauces. Stimuli-sensitive polymer systems have been used as materials for the delivery of drug [5,6]

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Chemically modified pullulan can produce derivatives with low solubility or a modified polymer that is completely insoluble in water [7]. Derivatives of pullulan are prepared and their applications towards the above mentioned aspects are also studied by various groups [8]. The use of pullulan in biomedical field is increasing contemporarily due to its non-toxic, non-immunogenic, biocompatible and inert nature. Shingel and his co-workers developed an anionically modified pullulan via gamma irradiation which was used as a base for blood – plasma substitute [9]. Due to its high water solubility Pullulan is also explored as a potential blood-plasma substitute like that of dextrans and is used as a plasma expander [10-12]. Pullulan hydrogels as drug delivery systems in the form of microgels and nanogels have been studied [13]**.** As Pullulan has no carcinogenic, mutagenic and toxicological activities it has number of uses, in manufacturing of adhesives, cosmetics, binders and thickeners [14-17].

Sodium alginate is a linear unbranched, amorphous copolymer composed of β-d-mannuronic acid (M) and α-l-guluronic acid (G) linked by 1 → 4 glycosidic bonds **(Fig- 2)**. Commercially available Sodium alginate is usually extracted from various sea weeds. The chemical composition and sequence distribution of sodium alginate depends on the species and parts of the seaweed employed for extraction [18]. Sodium alginate is one of the biodegradable polymers, which has been extensively exploited for the preparation of nanoparticles (NPs) for controlled delivery of several therapeutic agents. Sodium alginate is principally used in food production as a thickener and emulsifier although it also has a variety of uses in areas such as printing, packaging, industrial, and dental applications. Sodium alginate is a natural, non-poisonous, and also the most efficient thickener for fabric dyes. Sodium alginate can be used in the textile craft industry to thicken liquid fabric dyes such that they can be brushed onto fabric like fabric paints. Sodium alginate acts as a good chelator for pulling radioactive substances from the body, such as iodine-131 and strontium-90 [19]. It is also used in immobilizing enzymes by inclusion.

Verapamil hydrochloride is a plenylalkylonine calcium channel blocker introduced as anti anginal agent [20]. Chemical name of the drug is 2-(3,4-dimethoxyphynyl)ethy](methyl)amino}-2(propan-2-yl)pentanenitrile. Its molecular formula is C27H38N2O4•HCl**.** Its structure is represented in **Fig – 3.** It is chiefly used in the treatment of cardio vascular diseases. It is also used as a regularly prescribed high dose for prophylactic treatment of both episodic and chronic cluster headaches [21]. It has also been shown to be an effective therapeutic agent for the treatment of hypertrophic cardiomyopathy in children [22]. It is administered orally (tablets, capsules, sustained release tablets/capsules) and parent rally (intravenous). The usual dose of verapamil is 80-280 mg/day. Conventional tablet of Verapamil is administered 3 or 4 times a day due to its short biological half-life of about 2-5 hr. The problems of frequent administration and variable low bioavailability (40-60%) after oral administration of conventional tablet or capsules have been attenuated by designing verapamil in the form of sustained release tablet. Sustained release forms are administered two times a day due to its controlled release of drug for adequate time in the gastrointestinal tract.

In the present work a trail is made to prepare Sodium alginate and OPP microspheres of Verapamil, which would prolong the residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability. An attempt is also made to develop microspheres with high entrapment efficiency. The method is based on Ionic gelation technique involving alginate polymers alone and in combination with other polymers.

MATERIALS AND METHODS

Materials: Verapamil HCl is received as a gift sample from CRS, Vizag. Sodium alginate, calcium chloride, pyridine are purchased from Loba chemicals, Mumbai. Pulluan is purchased from Sigma chem, Mumbai. Palmitoyl chloride is obtained from Himedia, Mumbai. Dimethial Form amide, Ethanol, Di ethyl ether and Hydrochloric acid are purchased from Merk Ltd,Mumbai.
Deionised water is processed through a milli-Q water purification system (MilliPore,USA). All the chemicals and reagents are of highest grade available.

Instrumentation: Double Beam UV visible Spectro photometer(UV-3000+Lab-India Pvt Ltd. Thane, India)synchronized to a computer work station using UV-Win software, DS8000 model:8-basket dissolution test apparatus (Lab-India Pvt Ltd. Thane, India), magnetic stirrers with hot plate (REMI instruments India), ultra sonicator (Front line electronics Pvt Ltd), CX-220 series (d =0.1mg) model semi micro balance(Citizen Scales India Pvt Ltd) and melting point apparatus (Shanta Engineering ,Thane, India) are used in the present study.

EXPERIMENTAL DESIGN

Pre-formulation study [23]: In order to investigate physicochemical properties of a drug substance alone and when combined with the excipients, it is necessary to generate data useful to the formulation in developing safe, stable, potent, bioavailable and efficous dosage form which can be mass produced.
Hence the following parameters are selected for the pre-formulation studies for the pure drug.

Solubility: The solubility of Verapamil HCl is tested in various solvents such as distilled water, methanol, ethanol (95%), DMF, isopropyl alcohol and pH 7.4 phosphate buffers.

Determination of melting point: Melting point of Verapamil HCl is determined by "Thiele's tube apparatus". The presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range.

Identification of drug: The IR spectra of Verapamil HCl **(Fig – 7)** are recorded using Fourier transform Infrared spectrophotometer with diffuse reflectance principle. Sample preparation involved mixing the samples with potassium bromide (KBr), triturating in glass mortar and finally placing in the sample holder. The spectrum is scanned over a frequency range 4000-500 cm^{-1}.

Compatibility studies: The compatibility study of drug and polymer under experimental condition is an important prerequisite before formulation. FTIR spectroscopy is carried out to study the compatibility of pure drug verapamil HCl with OPP as co-polymer and sodium alginate as polymer used in the formulation of spheres.

Determination of λmax at pH 7.4 phosphate buffer: 10mg of Verapamil HCl is accurately weighed on an electronic balance(sensitive with 100µg) and dissolved in 100ml of phosphate buffer (100µg/ml) having pH7.4. 10ml of this solution is taken in 100ml volumetric flask and the volume is made up to the mark with pH 7.4 phosphate buffer (10µg/ml), and obtained solution is scanned on UV scanner between 200-400 nm. The maximum absorbance obtained in the graph is considered as λmax for the pure drug.

Preparation of the standard calibration curve of Verapamil HCl: Verapamil HCl (10mg) is dissolved in 2ml of pH 7.4 phosphate buffer and volume is made upto 10ml in volumetric flask using buffer solution (concentration of this solution is 1000µg/ml). From this primary stock solution 1ml is withdrawn and diluted to 10ml in volumetric flask which gives the concentration of 100µg/ml. From this secondary stock solution 1ml is withdrawn and diluted to 10ml in volumetric flask which gives the concentration of 10µg/ml. From this solution aliquots of 1,2,3,4 and 5ml are withdrawn and diluted upto 5ml with pH7.4 phosphate buffer to give concentrations of 2,4,6,8 and 10 µg/ml. Absorbance of each solution is measured at 278nm using UV visible double beam spectrophotometer and phosphate buffer pH 7.4 as a reference. The Beer's range is found to 2-10µg/ml.

DETAILED PROCEDURE

STEP 1:

Synthesis of O-palmitoyl pullulan: O-palmitoyl pullulan (OPP) is prepared by a method described earlier [24] by Sunamoto et al. (1992). Briefly, 1g pullulan is dissolved in 11 ml of dry dimethyl formamide at 60°C. To the resulting solution, 1 ml dry pyridine and 0.1 g palmitoyl chloride, dissolved in 0.24 ml dry dimethyl formamide are added. The mixture is stirred at 60°C for 2 hours followed by 1hatroom temperature. This mixture is then slowly poured into 70ml absolute ethanol under stirring. The precipitate so formed is collected and ished with 80 ml absolute ethanol and 60 ml dry diethyl ether. The white solid material obtained is dried in vacuum at 50°C for 2 h. The equation for the formation of O- palmitoyl pullulan from Pullulan is shown in **Fig- 4**. The synthesized polymer is characterized by IR**(Fig- 6)** and H^1 NMR **(Fig- 5)**. The IR spectrum of OPP (1%) is taken by incorporating it into a compressed KBr pellet. Similarly, a H^1 NMR spectrum is obtained in deuterated dimethyl sulfoxide solution (DMSO-d) using tetra methyl silane (TMS) as internal standard. Additionally, a H^1 NMR spectrum of pullulan is also obtained.

STEP 2:

Characterization of OPP: The pullulan used in the present study is chemically modified by esterification with palmitoylchloride. The resultant product is characterized by IR and H^1 NMR spectrum**.**

A characteristic stretching vibration is observed at about 1735cm^{-1} indicating the presence of carbonyl bond. However, here in the synthesized OPP the stretching vibration is observed at 1685 cm^{-1}. This shift in frequency may be due to the intermolecular hydrogen bonding between the carbonyl and hydroxyl group, which leads to increase in the bond order of carbonyl bond. The presence of hydrogen bonds is confirmed from the OH stretching vibration at 3397 cm^{-1}. This value indicates the existence of a polymer. However, there is a shift in band from 3600$cm^{-1}$to 3397cm^{-1}which may be

attributed to the intramolecular single bridge hydrogen bonds and the intermolecular bridge between hydrogen bonds. A characteristic C–H asymmetric stretching vibration is observed at 2924 cm^{-1} and a C–O stretching at 1152 $cm.^{-1}$

From these observations it is concluded that there exists an ester bond between pullulan and palmitoyl residues, suggesting palmitoylation of pullulan.
The OPP formation is further confirmed by H^1 NMR resonance spectroscopy. The protons corresponding to the terminal methyl group of the palmitoyl chain are observed at 0.850 ppm, that of the 12-methylene groups are observed at 1.20 ppm while those at 1.234 and 2.38 ppm are indicative of the presence of β and α methylene groups, respectively. These observations are in accordance with those found by others [25]. Two peaks at the down field at 4.996 and 4.647 ppm are observed which indicates the C position of 1,4 and 1,6 glycosidic bonds, respectively. It is possible to identify a range of protons from 2.546–3.568 ppm corresponding to the glucose residue units at positions C2–C61. This observation is in accordance with the one previously founded [26] where they reported a range of 2.60–4.20 ppm. From this observed data the formation of OPP is further confirmed.

STEP 3:

Preparation of Verapamil HCl microspheres: Verapamil HCl microspheres using sodium alginate and copolymer OPP are prepared by Orifice ionic gelation method. 0.5%,1%,1.5%,2%,3% and 4% sodium alginate solution and 1% OPP solution are prepared initially. Then drug, verapamil HCl is added to 1% OPP solution mixture and homogenized thoroughly with a magnetic stirrer to form a homogeneous dispersion. The drug-copolymer solution and sodium alginate are mixed in 2:1,1:1,1:1.5,1:2,1:3 and 1:4 ratios **(Table – 3)**. The resulting bubble free dispersion is added drop wise manually with a 10 ml syringe fitted with an 18 gauge needle, into 100 ml of (5%w/v) calcium chloride ($CaCl_2$) solution kept under stirring in a 250 ml beaker. The gelation time of 15 min is allowed to complete the curing reaction and to produce spherical rigid microspheres. The spheres so prepared are collected by decantation, washed with n-hexane and dried at < 40°C for 12h.

Evaluation of formulated microspheres:

Percentage yield: Percentage practical yield is calculated as the weight of spheres recovered from each batch in relation to the sum of starting material.
The percentage yield of prepared spheres is calculated by using the formula

$$percentage\ yield = \frac{practical\ yield}{theoritical\ yield} \times 100$$

Particle size determination: The size of 50 microspheres is measured by optical microscopy. The mean diameter is determined by measuring the number of divisions covered by microspheres using ocular micrometre previously calibrated using stage micrometre.

Determination of Bulk density, Tapped density and Carr's index [27] : The Bulk density , Tapped density, Carr's index are determined by the following formulae.

Bulk density = mass of the powder / bulk volume

Tapped density = mass of the powder / tapped volume

Carr's index = (tapped density- bulk density)/ tapped density X 100

Swelling index: Pre-weighed Verapamil HCl microspheres (W_0) formulated with polymers by employing different ratios are placed in phosphate buffer having pH 7.4 maintained at 37°C. After 8h, the microspheres are collected and blotted to remove excess water and weighed (W_t). The swelling index is calculated with the following formula.

$$swelling\ index = \frac{Wt - Wo}{Wo} \times 100$$

Where W_t = weight of microspheres observed at the end of 3h and W_0 = the initial weight of microspheres.

Drug entrapment efficiency: 5mg spheres of each batch are placed in 10ml phosphate buffer showing pH7.4 and mechanically agitated on stirrer at 200 rpm for 12hrs. The resultant solution is filtered and analysed at 278nm using UV visible spectrophotometer.
The percentage drug entrapment efficiency (%EE) of each sphere is calculated

Surface morphology: The surface and crossectional morphologies of formulations are examined with a scanning electron microscope. Microspheres are mounted on metal grids using double sided type gold coated under vacuum. The surface and crossectional details of formulation 6 are shown in **Fig – 13**.

***In-Vitro* drug release:** *In-vitro* drug release studies of verapamil HCl microspheres is carried out using USP type II dissolution rate test apparatus (LABINDIA DS 8000) with a paddle stirrer at 50

rpm in 900 ml of 0.1N HCl for first 2 hrs and in phosphate buffer of pH6.8 for next 10 hrs and temperature maintained at 37 ± 0.5°C. Microspheres equivalent to 50mg of verapamil HCl are taken in the paddle. 5ml samples of the dissolution fluid is withdrawn at regular intervals and replaced with fresh dissolution medium. The samples are filtered, diluted and analyzed using UV-Visible Spectrophotometer (LABINDIA UV3000+) at a wavelength of 278nm. The dissolution is carried out for every batch in triplicate. %Drug release, order and mechanism of the release are determined by the absorbance values obtained.

RESULTS

Solubility: The solubility of verapamil HCl is tested in various solvents. It is found to be soluble in water, phosphate buffer of PH 7.4, ethanol, acetone and isopropanol; freely soluble in DMF and methanol; sparingly soluble in chloroform and insoluble in ether.

Melting point determination: The melting point of verapamil HCl sample is found to be 141ºC, which is within the reported range 140- 144ºC. It complies with the purity of the drug sample

IR spectroscopy: The FTIR spectrum of pure verapamil HCl sample recorded by FTIR spectrometer is shown in **Fig-7**. The IR ranges for different functional groups in Verapamil HCl are reported in **Table – 1** which are compared with standard functional group frequencies of verapamil HCl. The functional group frequencies of verapamil HCl are in the reported range which indicates that the obtained sample of verapamil HCl is pure.

The FTIR spectra of pullulan and sodium alginate polymer samples are recorded by FTIR spectrometer **(Fig – 8 & Fig- 9)**. The vibrational frequencies are compared with standard functional group frequencies of the respective polymers. The functional group frequencies are in the reported range which indicates that the obtained polymer samples are pure.

Compatibility study: FTIR spectroscopy is carried out to study the compatibility of pure drug verapamil HCl with OPP as co-polymer and sodium alginate as polymer used in the formulation of spheres. The pure verapamil HCl has characteristic IR peaks at 2990.26 cm^{-1}(methyl and methylene), 2967.23 cm^{-1} (methoxy), and 2541.23 cm^{-1} (protonated amine).

From the FTIR spectra of the pure drug and the combination spectra of drug with the polymers (**Fig -10)**, it is observed that all the characteristic peaks of verapamil HCl are present in the combination spectra, this indicates the compatibility of the drug with polymers used.

Scanning of verapamil HCl : 10mg of verapamil HCl is accurately weighed on electronic balance (sensitive with 100µg) and dissolved in 100ml of pH7.4 phosphate buffer (100µg/ml).10ml of this solution is taken in 100ml volumetric flask and volume is made up to the mark with pH7.4 phosphate buffer (10µg/ml), and obtained solutions is scanned on UV scanner between 200-400nm. The maximum absorbance noted at 278nm **(Fig – 11)**.

Standard calibration linearity curve of verapamil HCl in pH 7.4 phosphate buffer: Table - 2 shows the absorbance of standard solutions of Verapamil HCl ranging from 2-10 µg/ml in phosphate buffer of pH 7.4. **Figure- 12** shows standard calibration curve for Verapamil HCl. The curve is found to be linear in the range of 2-10µg/ml at λmax 278nm. Slope, intercept and regression values of the curve are 0.006, 0.000 and 0.999 respectively.

Percentage yield: Percentage yield of all formulations are calculated and the percentage yield is found to be 48.31 to 75.43 respectively. The results are shown in the **Table - 4.**

Particle size determination: The microspheres obtained are evaluated for particle size diameter using optical microscope. The results are shown in **Table - 5**. The drug loaded microspheres are spherical in shape.

The formulated microspheres are in size range of 0.45 mm to 0.49 mm. The particle size increased with the increased sodium alginate ratio.

Drug entrapment efficiency: Percentage drug entrapment in the spheres includes drug entrapped within the polymer matrices. Values are in the range of 51 to 85 for dried microspheres shown in **Table - 6**.

Entrapment efficiency increased with increased ratios of polymer. Initially due to the high hygroscopic nature of OPP, entrapment is low and with increase in sodium alginate proportion the binding nature of OPP enhanced and its hygroscopic nature decreased.

Swelling index: Swelling index values of formulations F1 to F6 are recorded in **Table – 7.** Degree of swelling is found to be increased with increase in the polymer ratio.

Determination of bulk density, tapped density and Carr's index: Bulk density, tapped Density &

Carr's Index of formulations F1 to F6 are noted in **Table - 8**. The carr's index values of the formulations are from (13-14) indicating good flow characteristics of microspheres.

Surface morphology [28] : In SEM photograph of formulation **(Fig- 13)**,a single sphere at 40x magnification is shown. The figure produced microspheres with spherical conformation and heterogeneous size distribution.

Drug release profiles of microsphere formulations: The various formulations of microspheres prepared are evaluated for drug release study. The drug release profile is shown in **Table - 9**.Drug release kinetic parameters of F1 to F6 formulations of microspheres (Zero order & First order) are shown in **Table - 10**.

DISCUSSION

Pullulan is a naturally occurring polysaccharide produced by yeast. But due to its easy aqueous solubility it is easily washed out from the matrix preparations. So it is chemically modified by esterification with palmitoyl chloride to form O-palmitoyl pullulan (OPP). This formed OPP is used in different liposomal formulations [29], which showed good drug carrier capacity. Here OPP is used as a copolymer and due to its hygroscopic nature its proportion is fixed as 1% in all the formulations. But OPP alone could not be used as release rate retarding polymer because of its hygroscopic nature being continued inspite of its esterification. Hence sodium alginate is also used as copolymer to retard the release rate and extend the release for 12hr [30].

The release of verapamil HCl from various formulations of O-palmitoylpullulan and sodium alginate microspheres is studied in 0.1N HCl for the first 2 hr and then in phosphate buffer of pH 6.8 for the remaining period of 10 hr stimulating the gastric and intestinal pH conditions.

The verapamil HCl release is found to be retarded from the matrix of microspheres. However the extent of retarding the release is dependent upon the nature of copolymer (OPP) and gradually increasing proportion of the other hydrophilic polymer sodium alginate.

Comparison of the drug release profiles of F1 to F6, the release rate is found to be gradually decreased. This is because of constant OPP with gradual increase in the sodium alginate proportion. Literature reports indicates that the formulated microspheres exhibited higher degree of swelling [31]. The drug release from the sodium alginate microspheres normally involves initial swelling and then diffusion followed by erosion from the matrix. Since the microsphere formulations F3 to F6 contained higher proportion of sodium alginate, it probably resulted in the formation of more viscous gel barrier which slows down the release compared to F1 to F3. Here we can also notice that due to constant OPP proportion in all formulations from certain ratios onwards the combined effect of both OPP and sodium alginate are elevated.

In all the formulations F1 to F5 the release of the drug through it is retarded, is found to be completed either by the end of 10th to 12th hour or not uniformly spread throughout the expected 12 hr release. So in this regard another formulation F6 is developed in which the proportion of sodium alginate is further increased. And at this ratio of OPP and sodium alginate, it is found that the release is slow and spread over 12 hr. Thus it can be observed that the release of verapamil HCl can be altered suitably with modifications in the polymer concentration ratios to extend the drug release adequately for 12 hr.

The various kinetic parameters of drug release are shown in **Table- 10**. The data indicated a good linearity with significantly high correlation coefficient values for the first order release rate constants than for the zero order release constants. As the kinetics of release is affected by physico-chemical changes, dissolution medium and processing variables it is extremely difficult to obtain zero order kinetics for controlled release system to deliver the drug [32]. For further evaluation of drug release mechanism, a plot is drawn for square root of time vs. percent drug release as proposed by Higuchi. The plots are found to be linear in all cases and the correlation coefficient values are 0.9863, 0.9681, 0.9907, 0.9897, 0.988 and 0.9927 for F1 to F6 respectively. A plot is also drawn for time vs. cube root of fraction remained and here the correlation coefficient values are 0.9713, 0.9614, 0.9782, 0.9887, 0.9811 and 0.9901 for F1 to F6 respectively. On comparing both, it is found that Higuchi plots are more linear in all cases indicating diffusion controlled drug release mechanism from matrix formulations.

Korsemeyer Peppas plot [33] is also drawn taking log time vs. log percent drug release. The 'n' values (0.259-0.509) obtained in the Peppas plot are shown in **Table - 11**. It indicates that the drug release is by Fickian diffusion mechanism as it follows the Fick's law of diffusion. Finally it is concluded that 1% OPP with 2% sodium alginate extended the release of the drug for 12 hr with 97% total drug release.

CONCLUSIONS

In this work, attempts have been made to prepare the OPP-sodium alginate microspheres bearing verapamil HCl by ionotropic gelation method for sustained drug delivery.

These prepared microspheres though gave a slow release, it is observed that more than 90% of drug is released by 10-11 hr itself or not uniformly spread throughout the expected 12 hr. Then another formulation with further increased concentration of sodium alginate is prepared. In this formulation, drug release is more sustained and the drug release is sustained up to 12 hr. Formulations followed first order release kinetics and they showed Fickian type of diffusion. The stability studies conducted revealed that there is no change in the drug release profile after the products are stored at 37 ± 2^0Cand 75 $\pm$ 5% RH and there is no significant drug loss after storage.

Experimental results revealed that the drug release can be controlled, by the nature of the copolymer (OPP) and by gradual raising proportion of the other hydrophilic polymer sodium alginate. It is also noticed that the proportion of OPP has no effect on the drug release. Higher proportion of the sodium alginate leads to the formation of more viscous gel barrier, which controls the drug release. Thus the release of Verapamil HCl can be changed with alterations in the polymer concentration ratios in order to extend drug release adequately for 12 hours. Finally it is concluded that 1% OPP with 2% sodium alginate extended the release of the drug for 12 hours with 97% total drug release.

Thus in the present investigation, controlled release products of verapamil HCl could be designed by ionic gelation method with Opp-sodium alginate ratio at 1:4 that is F6. Due to its biodegradable and nontoxic nature OPP can be further used in different pharmaceutical formulations.

Table -1:IR Ranges for different functional groups in verapamil HCl

S.NO	Energy(Wave numbers cm^{-1})		Assignments
1	**Reported**	**Sample**	
	3030-2860	**2990.26**	**C-H stretching of methyl and methylene group**
2	**2840**	**2967.23**	**C-H stretching of methoxy group**
3	**2800-2300**	**2541.23**	**N-H stretching of protonated amine**
4	**2236**	**2236.46**	**C=N stretching of Alkyl nitrile**
5	**1607,1591,1518**	**1608.03,1591.69,1518.50**	**Stretching of benzene ring**
6	**1262**	**1259.85**	**C-O stretching**

Table - 2: Standard calibration data of verapamil HCl in PH 7.4 phosphate buffer

Concentration(µg/ml)	Absorbance
0	**0**
2	**0.015**
4	**0.029**
6	**0.042**
8	**0.055**
10	**0.069**

Table - 3:Details of microsphere formulations prepared from F1 to F6

S.NO	INGREDIENTS	F1	F2	F3	F4	F5	F6
1	Verapamil HCl (mg)	100	100	100	100	100	100
2	Ratio of opp:sodium alginate	2:1	1:1	1:1.5	1:2	1:3	1:4
3	1% OPP (gm)	0.5	0.5	0.5	0.5	0.5	0.5
4	Sodium alginate(gm)	.25	0.5	0.75	1	1.25	2

Table - 4: Percentage yield of formulations F1 to F6.

Formulation code	OPP and Sodium alginate ratio	% yield
F1	2:1	48.31
F2	1:1	56.72
F3	1:1.5	63.68
F4	1:2	67.72
F5	1:3	71.35
F6	1:4	75.43

Table - 5: Particle size of formulations F1 to F6

Formulation code	Particle size (mm)
F1	0.452±0.058
F2	0.475±0.069
F3	0.464±0.068
F4	0.478±0.078
F5	0.492±0.054
F6	0.494±0.064

Table -6:Entrapment efficiency of F1 to F6 formulations.

Formulation code	%EE
F1	51
F2	58
F3	66
F4	73
F5	80
F6	85

Table- 7:Swellingi index values Of formulations f1 to F6.

Formulation Code	Swelling Index(%)
F1	4.2
F2	5.8
F3	7.2
F4	7.8
F5	9.2
F6	10.4

Table - 8: BulkDensity,Tapped Density & Carr's Index Of Formulations F1 to F6.

Formulation code	Bulk density	Tapped density	Carr's index
F1	0.845	0.975	13.33
F2	0.85	0.98	13.26
F3	0.846	0.986	14.19
F4	0.853	1.003	14.95
F5	0.857	0.987	13.171
F6	0.832	0.962	13.513

Table -9: Percent drug release of F1 to F6 formulations of microspheres

Time (hr)	Percent Drug Released					
	F1	F2	F3	F4	F5	F6
0.25	33.635	34.789	27.900	24.632	19.417	12.684
0.5	44.929	36.165	36.600	31.265	21.2625	14.569
0.75	45.776	46.675	40.172	36.123	24.727	18.402
1	46.305	52.541	43.527	39.994	28.710	21.197
1.5	47.470	58.344	47.945	46.331	31.995	27.275
2	55.129	61.231	55.390	50.424	37.890	30.091
3	57.95	67.717	59.563	56.391	46.530	45.698
4	65.717	69.579	66.927	61.619	52.762	54.614
5	73.482	72.03	72.081	70.150	60.165	60.734
6	78.117	77.02	76.036	81.098	68.227	69.840
7	83.035	83.286	81.790	85.131	77.940	75.938
8	87.929	85.334	83.345	89.350	85.660	79.771
9	91.247	89.717	88.854	92.619	89.040	83.520
10	95.729	94.224	92.809	95.961	92.392	86.865
11			96.581	97.673	95.497	92.698
12						97.272

Table -10: Drug release kinetic parameters of F1 to F6 formulations of microspheres (Zero order & First order)

S.NO	Formula	Zero order		First order	
		K_0	R^2	K_1	R^2
1	**F1**	**5.464**	**0.9639**	**-0.082**	**0.9825**
2	**F2**	**4.845**	**0.8903**	**-0.0702**	**0.9767**
3	**F3**	**5.507**	**0.9327**	**-0.0341**	**0.9571**
4	**F4**	**6.245**	**0.9394**	**-0.0957**	**0.9909**
5	**F5**	**7.143**	**0.9785**	**-0.0931**	**0.9788**
6	**F6**	**7.265**	**0.9571**	**-0.0954**	**0.9735**

Table- 11: Drug release kinetic parameters of F1 to F6 formulations of microspheres (Higuchi,Hixson-crowell & Koresmeyer-Peppas)

S.NO	Formula	Higuchi		Hixson crowell		Koresmeyer Peppas	
		K_H	R^2	K_S	R^2	n	R^2
1	**F1**	**20.66**	**0.9863**	**-0.0407**	**0.9713**	**0.259**	**0.9637**
2	**F2**	**18.89**	**0.9681**	**-0.0354**	**0.9614**	**0.248**	**0.9869**
3	**F3**	**22.13**	**0.9907**	**-0.0412**	**0.9782**	**0.310**	**0.9966**
4	**F4**	**24.99**	**0.9897**	**-0.0466**	**0.9887**	**0.360**	**0.9945**
5	**F5**	**27.97**	**0.988**	**-0.0479**	**0.9811**	**0.457**	**0.9776**
6	**F6**	**29.98**	**0.9927**	**-0.0484**	**0.9901**	**0.509**	**0.987**

Fig – 1 : Structure of Pullulan

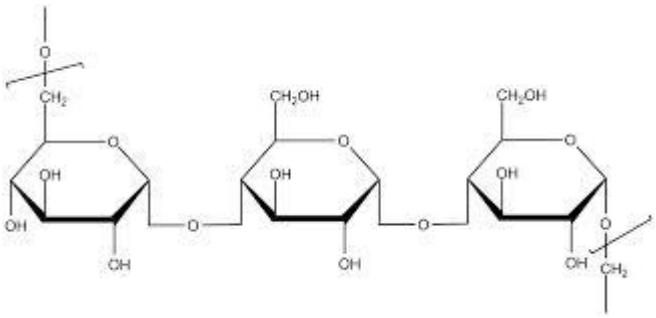
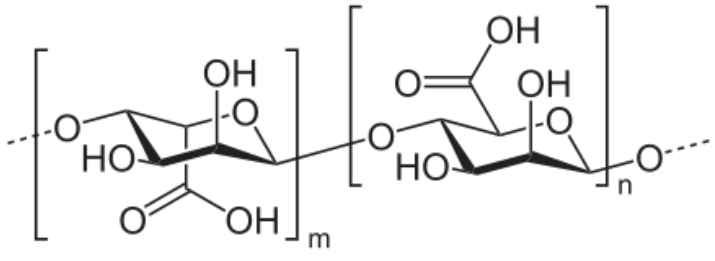


Fig – 2 : Structure of Sodium alginate



. Na

Fig – 3: Structure of Verapamil HCl

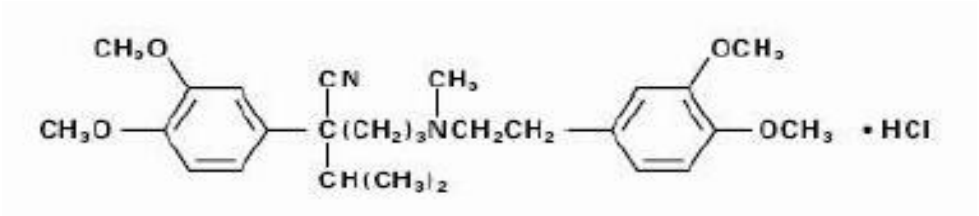


Fig – 4 : Equation for the preparation of O-palmitoyl pullulan

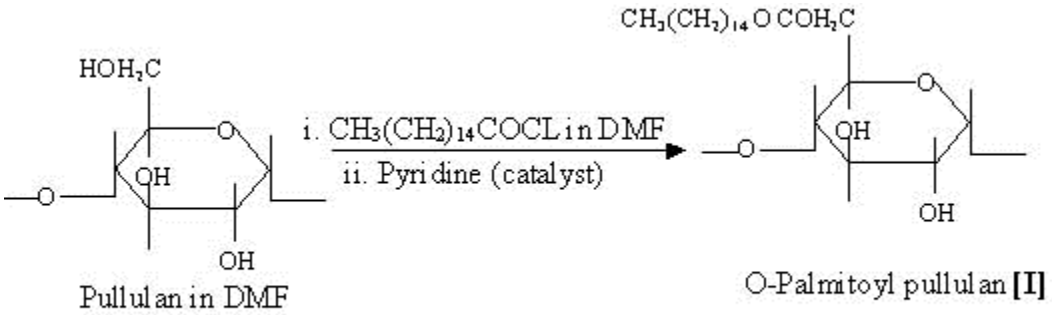


Fig – 5 : NMR spectrum of OPP

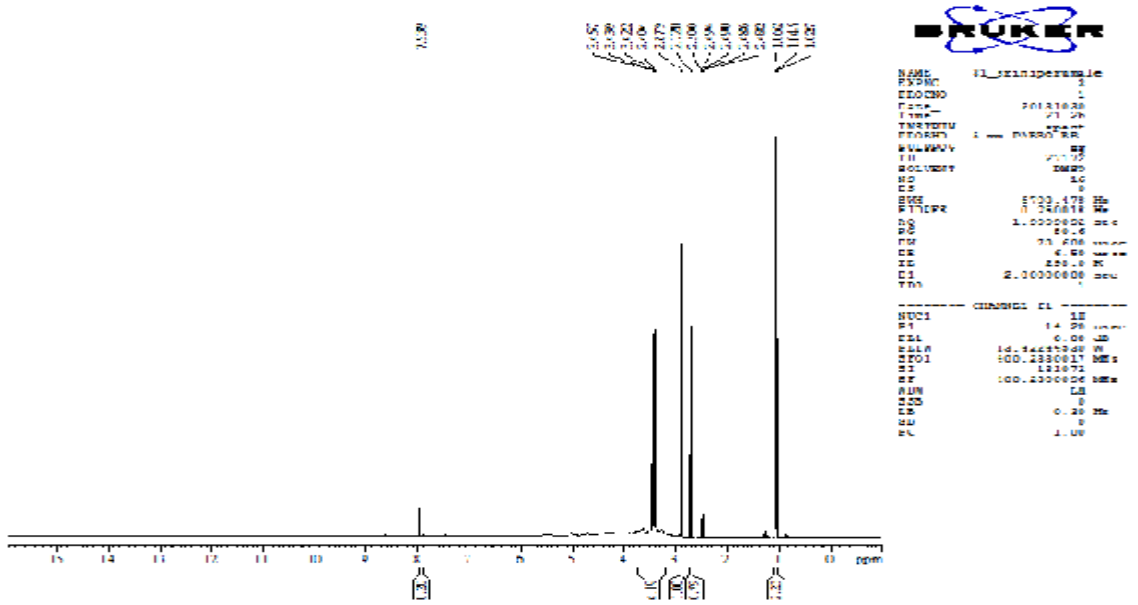


Fig- 6 : FTIR spectrum of OPP.

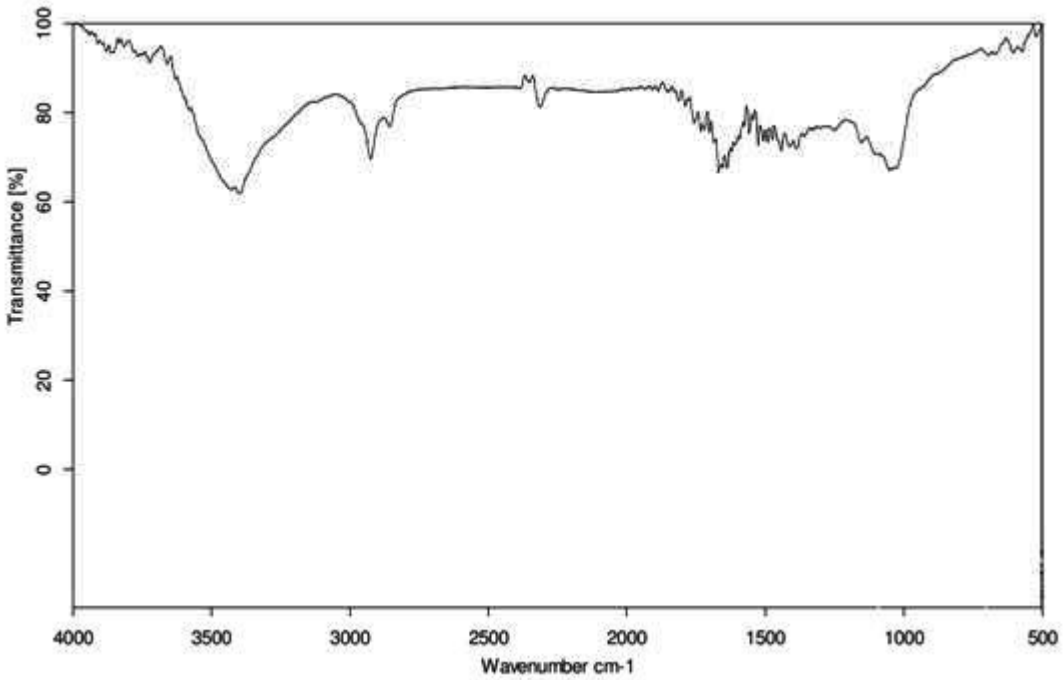


Fig – 7 : FTIR Spectrum of Verapamil HCl

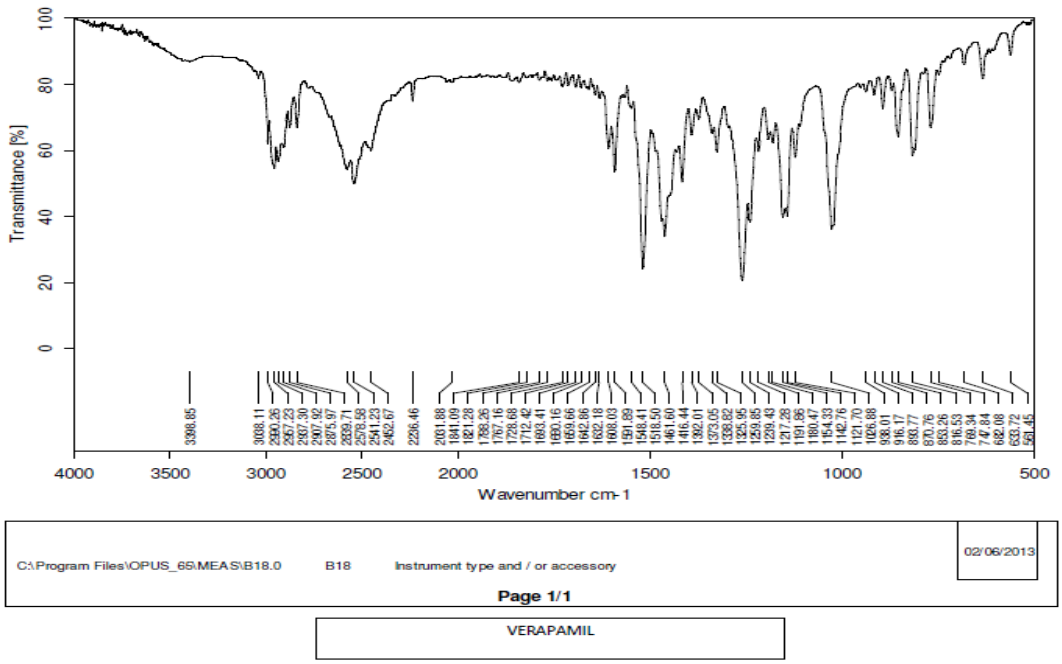


Fig – 8 : FTIR Spectrum of Pullulan

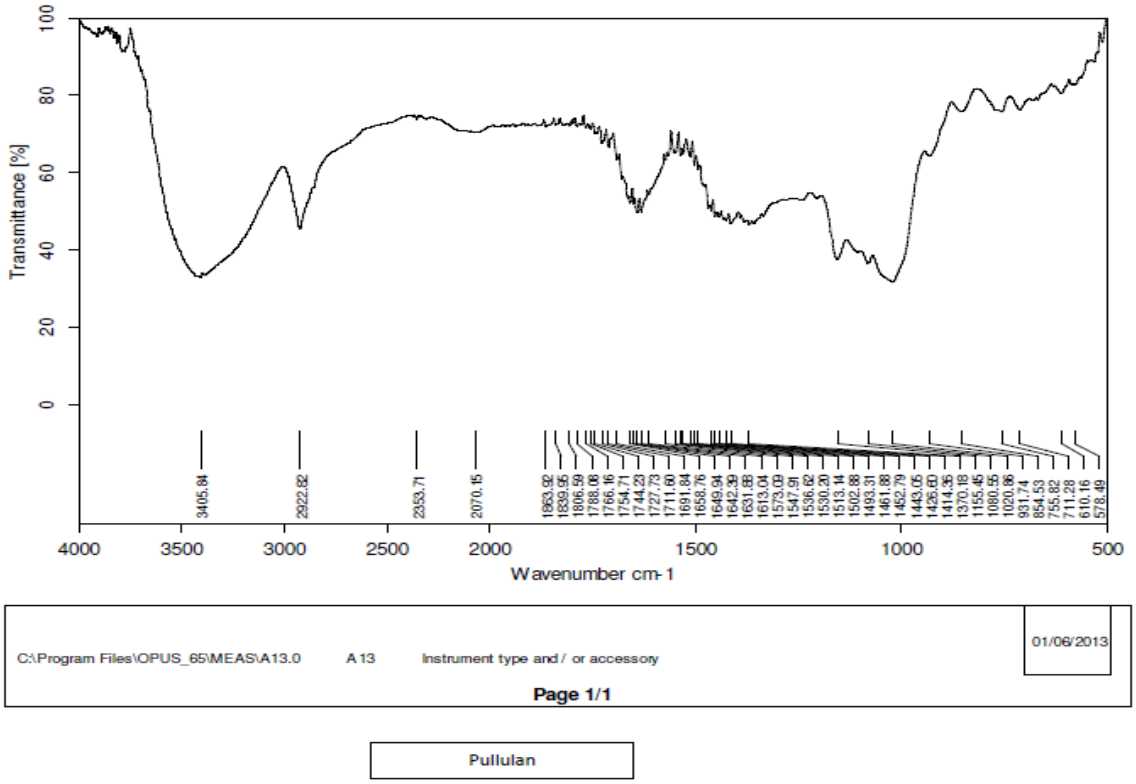


Fig – 9 :FTIR spectrum of Sodium alginate.

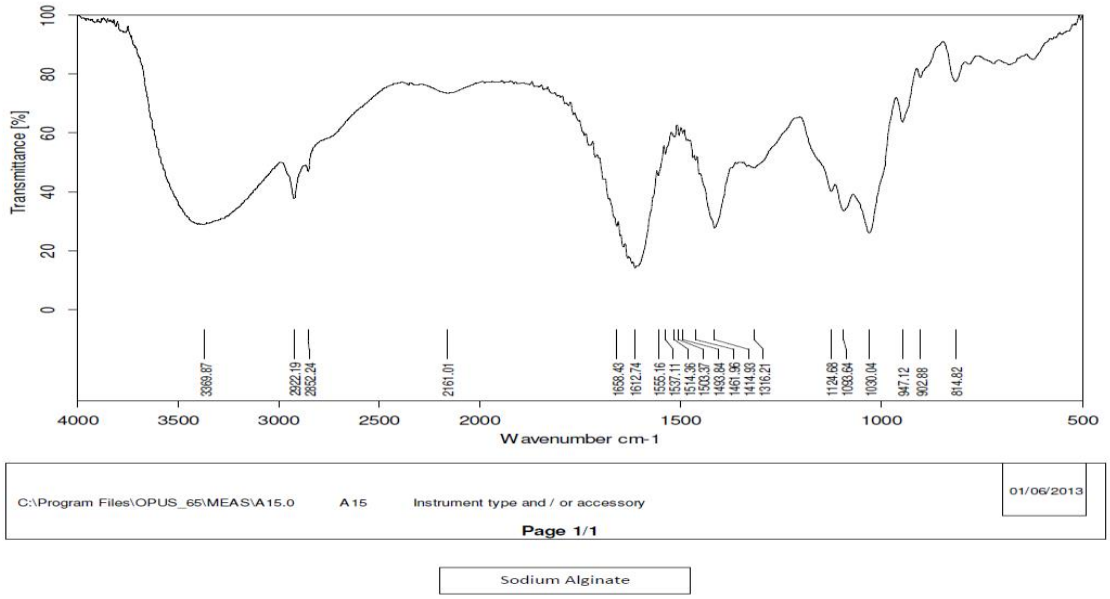


Fig- 10 :FTIR spectrum of F6 formulation.

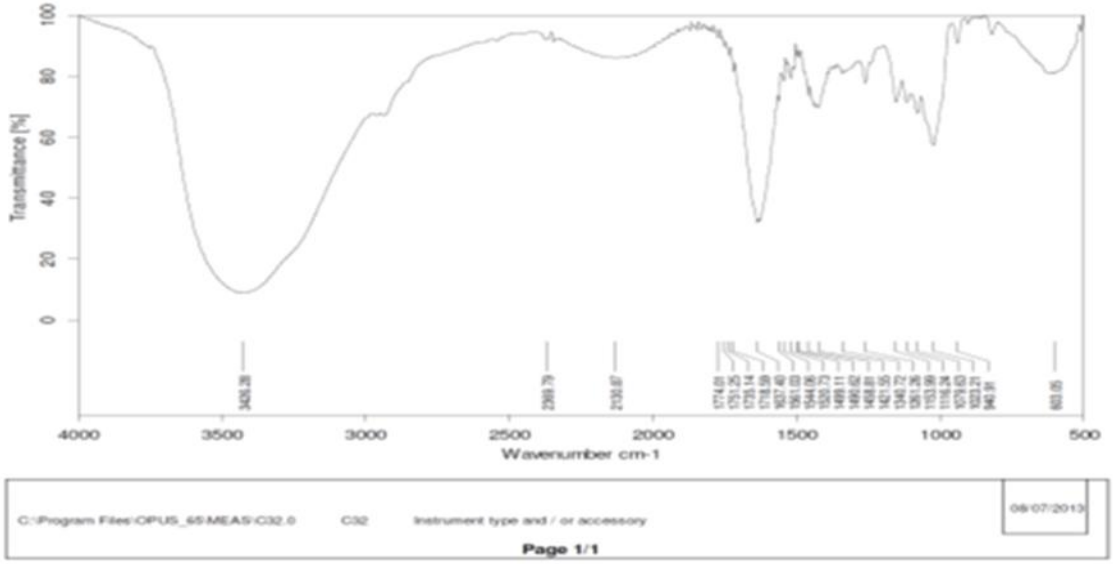


Fig - 11: Scan Spectrum curve of verapamil HCl

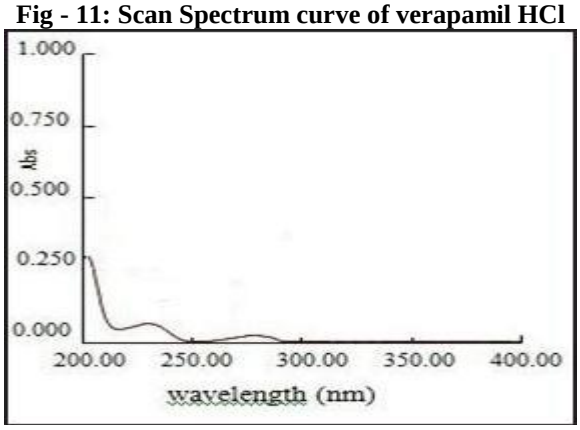


Fig - 12:Calibration Curve Of verapamil HCl

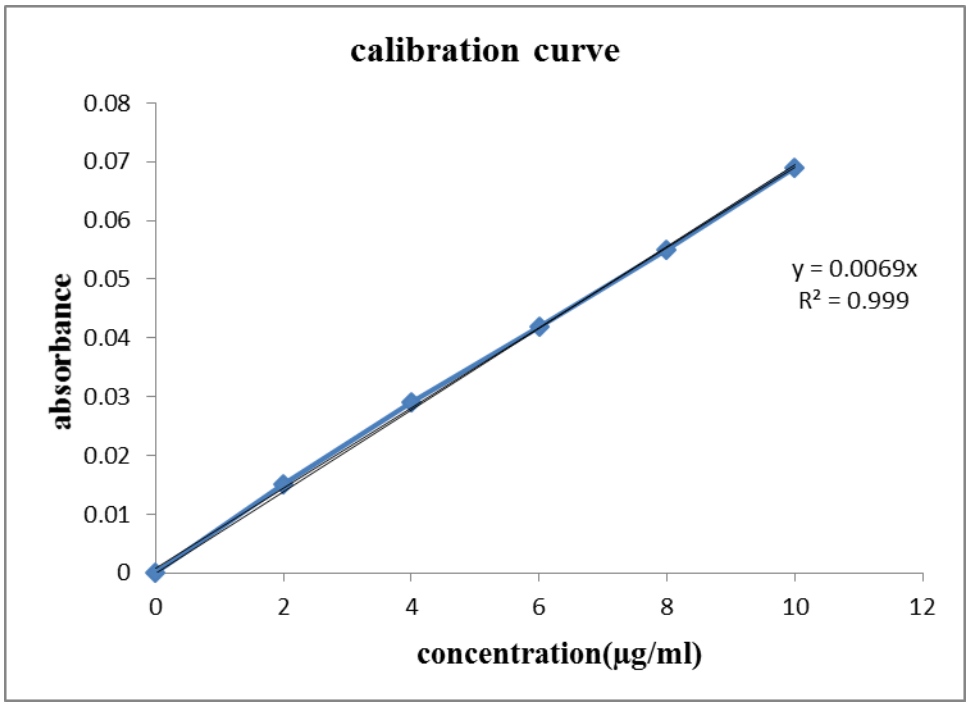


Fig- 13 :SEM photograph of formulation F6

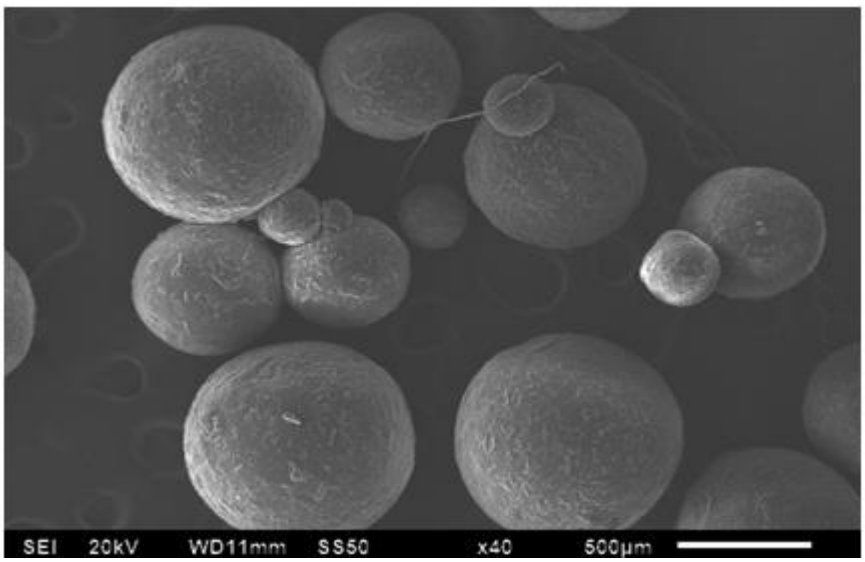


Fig - 14: Percent drug released vs. time profiles of F1 to F6 formulations of microspheres

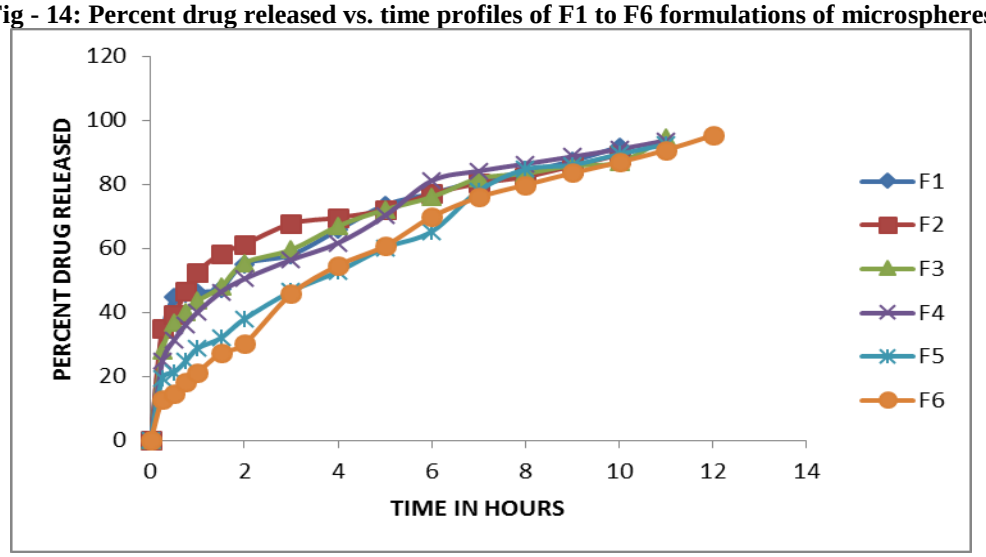


Fig- 15: First order plot of drug release from F1 to F6 formulations of microspheres

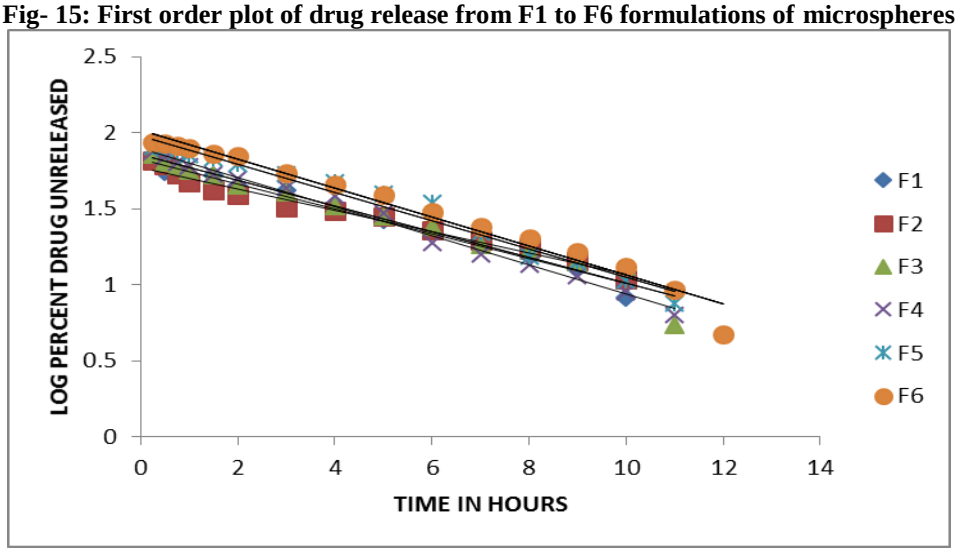


Fig - 16: Higuchi plot of drug release from F1 to F6 formulations of microspheres

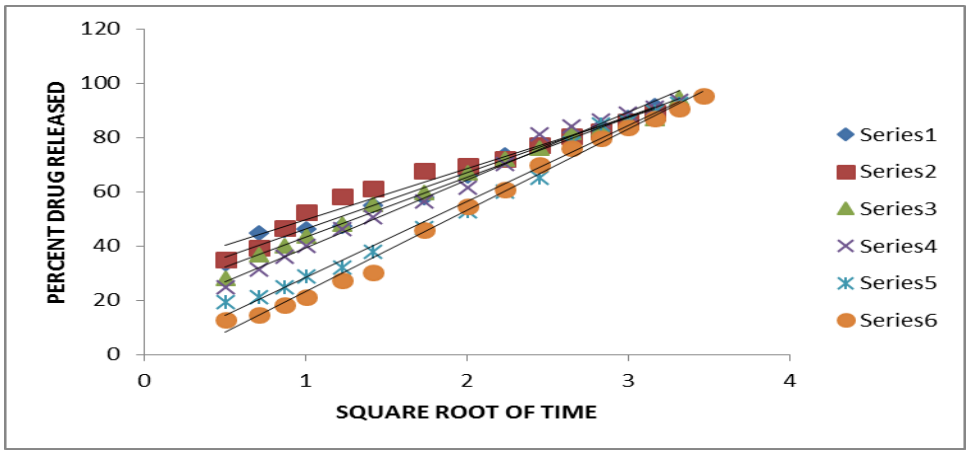


Fig - 17: Hixson Crowell plot of drug release from F1 to F6 formulations of microspheres

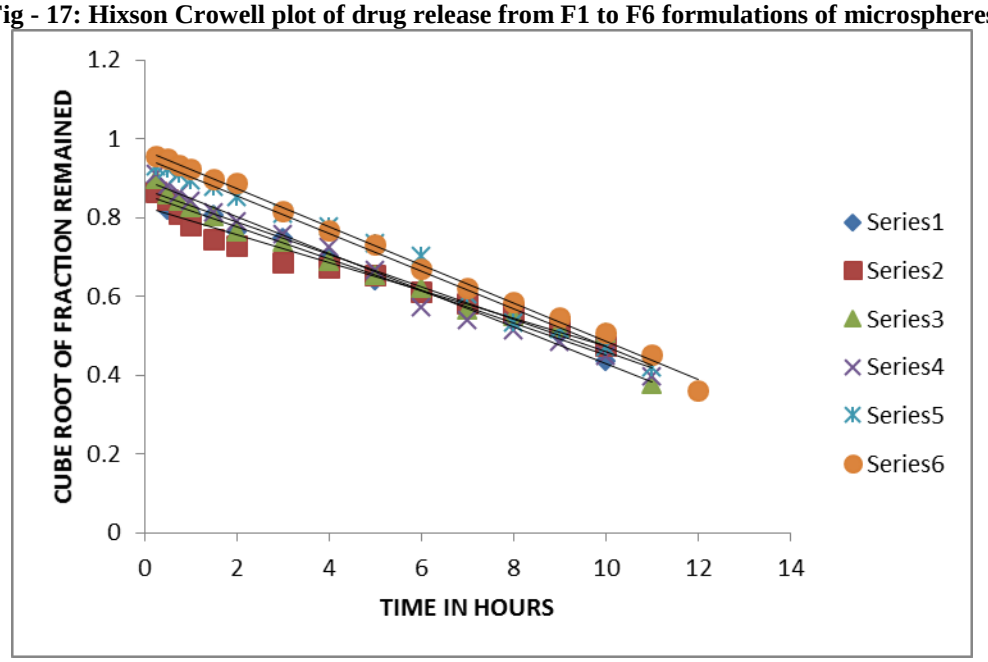
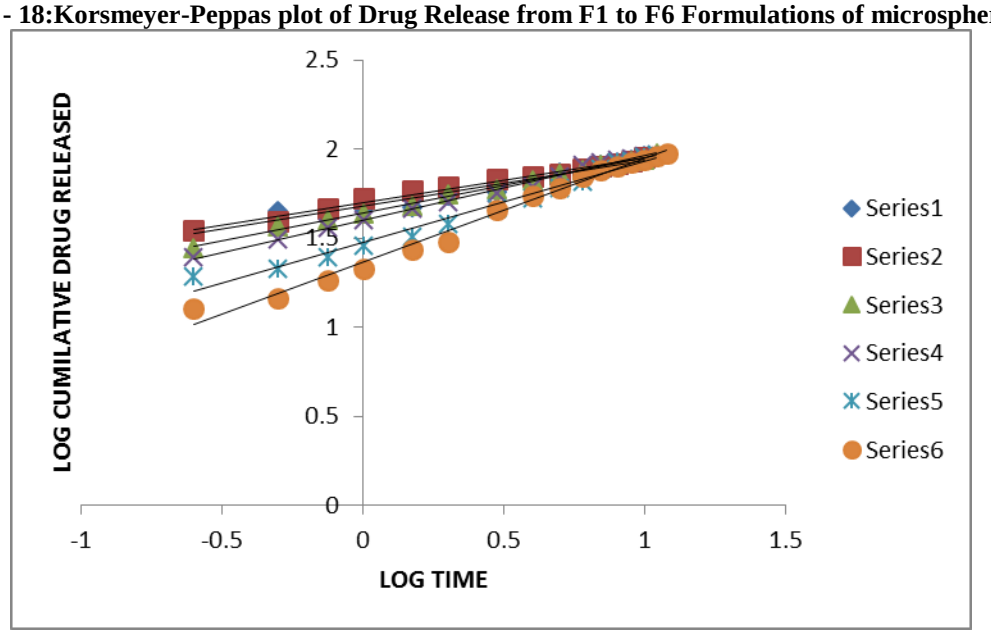


Fig - 18:Korsmeyer-Peppas plot of Drug Release from F1 to F6 Formulations of microspheres



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