Advances in Bioresearch
Adv. Biores., Vol 15 (4) July 2024: 01-13
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.4.113

Advances in Bioresearch

ORIGINAL ARTICLE

Design and Evaluation of Telmisartan-Loaded Nanosponges for Hypertension Treatment

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ABSTRACT

The Nanosponges of Telmisartan were prepared using the solvent evaporation method. PLGA, Tween80, Glutaraldehyde, Pluronic used a polymer, Acetone was used as the solvent. The prepared nanosponges were evaluated for various parameters, revealing intriguing results regarding the efficient preparation of the nanosponge. F7 outperforms the other nine formulations with its results. Entrapment efficiency measures the percentage of the drug that is successfully encapsulated within the nanosponge structure relative to the initial amount used. The values range from 82% to 90%, indicating generally high effectiveness in drug encapsulation across all formulations. Notably, F7 exhibits the highest entrapment efficiency at 90%, while F1 shows the lowest at 82%. The particle sizes of Telmisartan-loaded nanosponges for nine different formulations (F1 through F9). The particle sizes range from 110 nm to 190 nm. The smallest particle size is observed in formulation F4 (110 nm), and the largest in F9 (190 nm). The data indicates that F7 is an optimized formulation with a consistent and rapid release profile compared to the other formulations. Starting from an initial 0% at time 0, F7 exhibits a significant release of 23.36% at 1 hour, which continues progressively. By hour 5, F7 achieves a release of 68.78%, and by hour 7, it reaches 78.36%. The release percentage of F7 continues to increase, reaching 89.75% at hour 9, 94.36% at hour 10, and finally achieving a near-complete release of 99.89% at hour 12. The release pattern of F7 shows a steady and sustained increase, indicating its effective and controlled release characteristics, making it the optimised formulation among the nine compared.

Keywords: Telmisartan, PLGA, Pluronic, Acetone, Nanosponges

Received 14.02.2024 Revised 20.03.2024 Accepted 24.05.2024

How to cite this article:
Vankayala D S, Magharla D D, Anilkumar V, Suri N S V M. Design and Evaluation of Telmisartan-Loaded Nanosponges for Hypertension Treatment. Adv. Biores. Vol 15 [4] July 2024. 01-13

INTRODUCTION

Nanosponges are made of microscopic particles with a few nanometres-wide cavities in which many substances can be encapsulated. These particles can carry both lipophilic and hydrophilic substances, thereby improving the solubility of poorly water-soluble molecules. The studies conducted in this field prove that the tiny mesh-like structures called nanosponges may revolutionise the treatment of many diseases, and early trials suggest this technology is up to five times more effective at delivering drugs for breast cancer than conventional methods [1]. The nanosponge is about the size of a virus with a 'backbone' (a scaffold structure) of naturally degradable polyester. They 'cross-link' polyester segments to form a spherical shape with many pockets (or cavities) where drugs can be encapsulated. Polyester is biodegradable, which means that when it breaks down in the body, the drug can be released on a known schedule [2].

Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects [4,5]

MATERIAL AND METHODS

Materials

Pharma Life Research Lab, India, provided Telmisartan as a gift sample. PLGA, Tween 80), acetone, glutaraldehyde, Pluronic F127and Distilled Water Were Purchased from B.M.R. Chemicals, Hyderabad. All other ingredients used were analytical grade.

Preformulation studies

Preparation of calibration curve of Telmisartan

A series of solutions were prepared by dissolving 100 mg of Telmisartan in 100 ml of SSF to obtain the concentration range of 2 to 10 µg/ml. The absorbance of the prepared solutions was determined spectrophotometrically. The experiment was repeated for six times. The SSF of pH 6.8 buffer solution was used as blank. A graph was plotted with a concentration of Telmisartan (µg/ml) on X- the axis against absorbance (nm) on the Y-axis. The calibration curve of Telmisartan in 0.1 N HCl was also plotted. [6]

Solubility studies

A solubility study was carried out on drug candidates used in the present study (viz. Telmisartan) by using the flask shaker method. Excess amounts of Telmisartan were introduced separately into the amber-colored glass vials of 25 ml capacity, each containing 25 ml of distilled water and shaken for 36 hrs. at room temperature. The content of each bottle was filtered through a 0.4µ membrane filter. The filtrate was then diluted accordingly and assayed spectrophotometrically. [7]

Table 1: Formulation and evaluation of Telmisartan Nanosponges (F1 to F9)

Ingredient	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)	F9 (mg)
Telmisartan	50	50	50	50	50	50	50	50	50
Polymer (PLGA)	100	150	200	100	150	200	100	150	200
Surfactant (Tween 80)	50	50	50	50	50	50	50	50	50
Solvent (acetone, mL)	10	10	10	10	10	10	10	10	10
Cross-linker (glutaraldehyde)	2	2	2	2	2	2	2	2	2
Drug Loading Enhancer (Pluronic F127)	20	20	20	20	20	20	20	20	20
Distilled Water (mL)	100	100	100	100	100	100	100	100	100

Evaluation parameters of Nanosponges [8-16]

Drug content uniformity

The weight equivalent of 10mg of Telmisartan was dissolved in a 10 ml isotonic solution and left overnight. The dilutions underwent filtration and were subsequently analyzed using UV to determine their content uniformity. The absorbance of the formulations was measured using a UV-Vis spectrophotometer with a one cm cell. The instrument was calibrated to the wavelength (nm) for drug analysis. The drug content in each formulation was determined by analyzing the absorbance values of known standard solutions.

Entrapment efficiency

The Nanosponges suspension containing 1mg of Telmisartan weight equivalent was carefully analyzed by dissolving the sample in 10ml of distilled water. 10ml of the clear layer of the dissolved drug is taken after the drug is dissolved. The drug concentration in the water phase was measured using a UV-spectrophotometric method at 234nm (U.V Spectrophotometer, systronics). Another nanoparticulate sample was used for the repeated test. The drug concentration in the suspension was determined through centrifugation at 500rpm for 5 minutes, followed by measuring the drug concentration in the clear supernatant layer using the UV-spectrophotometric method. The concentration of the drug is determined using a calibration curve. The calculation of the drug content within the particles involved subtracting the drug amount in the aqueous phase from the total drug amount in the nanoparticle suspension. The calculation of the drug's entrapment efficiency (%) is determined using the following equation.

$$\% \text{ of Drug entrapment} = \frac{\text{Mass of drug in Nanosponges}}{\text{Mass of drug used in formulation}} \times 100$$

Scanning electron microscopy

The morphological features of prepared Nanosponges are observed by scanning electron microscopy at different magnifications.

Particle size and shape

The Nanosponges' particle size was measured using the Horibo scientific nanoparticle SZ100 particle size analyzer. The measurement involved diluting 100µl of the formulation with the appropriate volume of PBS pH 6.8 and then determining the vesicle diameter and zeta potential. A scan was conducted on the sample to determine its particle size.

Dissolution study

Dissolution Parameters

Medium: 900ml, 0.1N HCL up to 2hrs, then remaining with 6.8pH buffer
Apparatus : Paddle (USP-II)
RPM : 50
Temperature : 37°C±0.5
Time Points : 1, 2, 4, 6, 8, 10, 12 hr

The *in vitro* dissolution study for oral dosage forms should be conducted using a dissolution medium that mimics the actual physiological conditions. This involves conducting the first 2 hours in 0.1N HCL and the remaining dissolution in a 6.8pH buffer. The drug release studies for the prepared formulation were conducted in vitro over a period of 12 hours. The experiment was conducted using a Lab India DS8000 dissolution tester USP Type-1 apparatus (rotating basket) set at 50 rpm and a temperature of 37± 0.5°C. A capsule containing 10mg of Telmisartan. Nanosponges was placed in the basket apparatus and submerged in 900ml of the medium. The dissolution medium was periodically sampled, with 5ml withdrawn and replaced with fresh medium to maintain a constant volume. The sample solution was analyzed at 234nm using a UV-visible spectrophotometer to determine the presence of the model drug.

Release Kinetics of the Optimized Formulations

To study the in vitro release kinetics of the optimised formulation, data obtained from the dissolution study were plotted in various kinetics models. Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), Korsmeyer-Peppas model (Log Cumulative per cent drug release versus log time) and Hixson Crowell model (cube root of cumulative percentage of drug remaining vs. time) were applied to interpret the drug release kinetics from the formulations. The best-fit model was decided based on the highest regression values for correlation coefficients for formulations.**[17-22]**

RESULTS AND DISCUSSION

Preformulation studies of Telmisartan

Melting Point

Capillary fusion was used to determine the drug's melting point. The drug's observed melting point was confirmed to be within the reference range. The results are listed in the table. The melting point of Telmisartan was determined to be 262 ± 1.59 0C using the glass capillary method and melting point apparatus; the observed melting point of Telmisartan was confirmed to be the same as the standard melting point of Telmisartan (261-263 °C) published in the literature.

Spectroscopic studies

A series of solutions were prepared by dissolving 100 mg of Telmisartan in 100 ml of SSF to obtain a concentration range of 2 to 10 µg/ml. The absorbance of the prepared solutions was determined spectrophotometrically at 291 nm. The absorbance values for the concentration range were found to be between 0.125 to 0.621, which obeys Beer's law. The r^2 value was 0.9984, which indicates a positive correlation between the concentrations of the drug and the corresponding absorbance values. The following table and figure show the standard curve for Telmisartan at pH 6.8.

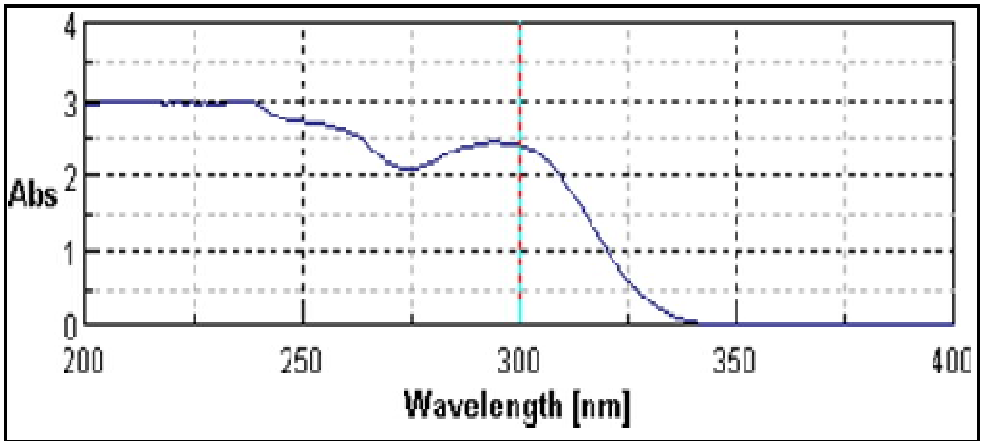

Fig.1: UV Spectra of Telmisartan at 234nm

Table 2: Standard curve data of Telmisartan using SSF pH 6.8

S. No.	Concentration (µg/ml)	Absorbance
1	2	0.125
2	4	0.264
3	6	0.382
4	8	0.491
5	10	0.621±0.02

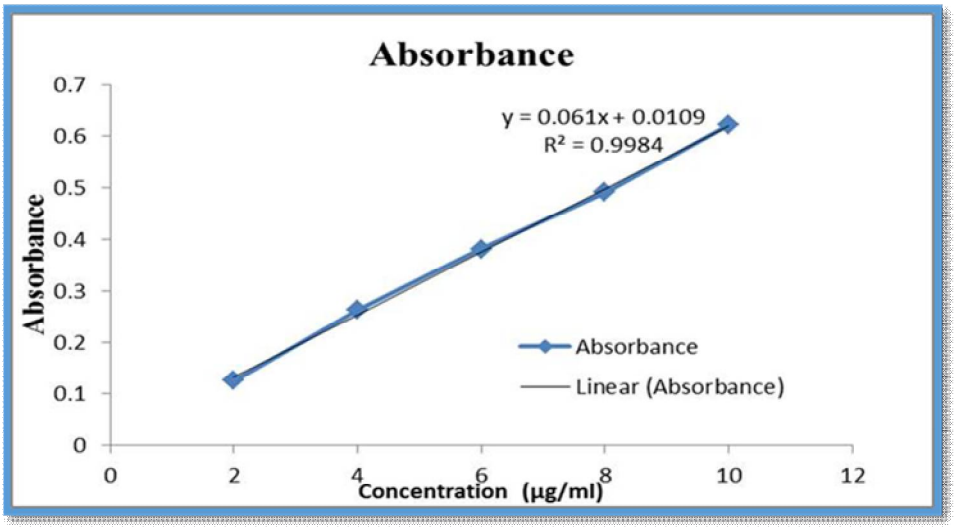

Fig.2: Standard curve of Telmisartan in SSF pH 6.8

A series of solutions were prepared by dissolving a 100 mg of Telmisartan in 100 ml of 0.1 N HCl to obtain the concentration range of 2 to 10 µg/ml. Absorbance of the prepared solutions were determined spectrophotometrically at 291 nm. The absorbance values for concentration range were found to be between 0.131 to 0.519 which obeys Beer's law. The r2 value was 0.997 which indicates a positive correlation between the concentrations of drug and the corresponding absorbance values. The following table and figure shows the standard curve for Telmisartan at pH 1.2.

Table 3: Standard curve data of Telmisartan using SSF pH 1.2

S. No.	Concentration (µg/ml)	Absorbance
1	2	0.131±0.02
2	4	0.193±0.01
3	6	0.299±0.10
4	8	0.398±0.03
5	10	0.519±0.04

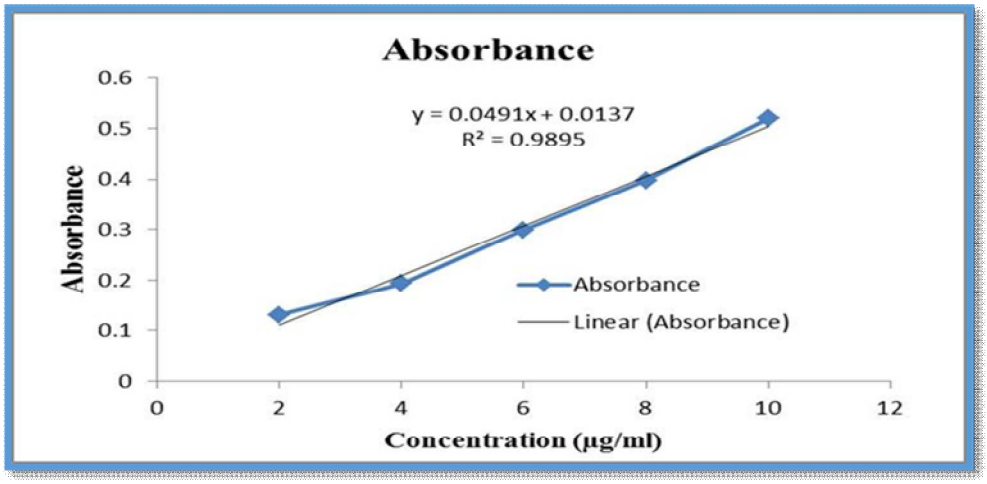

Fig.3: Standard curve data of Telmisartan using SSF pH 1.2

In the present study, all the analytical methods obeyed Beers law in the concentration range of 2-10 µg/ml and were suitable for the estimation of all selected drugs from different fluids. The values of r (Correlation coefficient) for the linear regression equations were found to be > 0.98 from all fluids, indicating a positive correlation between the concentrations of drugs and the corresponding absorbance values. The linear equations for all the calibration curves followed with the intercept of 0 and slightly above 0. The linear regression equations were used for the estimation of selected drugs from various fluids. Reproducibility of the method was tested by analyzing six individually weighed samples of each selected drug.

Solubility studies

The following are the results of saturated solubility, pH dependent solubility and phase solubility studies of various drugs and their solid dispersions with different polymers.

Saturated solubility studies

Solubility study was carried out on drug candidates used in present study (viz. Telmisartan) by using flask shaker method (Srinivas et al., 2008) as described in materials and methods. Saturated solubility studies of selected drugs were carried out in distilled water and the solubility was found to be 0.256 mg/ml Telmisartan respectively as shown in Table. The following table shows the saturated solubility of Telmisartan.

Solubility studies

Telmisartan solubility investigations were carried out using a cyclone mixer (REMI CM 101, India) in various solvents utilizing the equilibrium solubility method. Table 4 shows the results of Telmisartan solubility tests in various solvents and buffer medium. Telmisartan has a solubility of 0.256 ±0.04 mg/mL in distilled water, but 33.6±0.46 mg/mL in methanol at 25°C. Telmisartan is nearly insoluble in water but soluble in methanol, according to the results of a solubility study. Telmisartan has a reported solubility of 0.4 mg/mL in water. The variation of solubility of Telmisartan in different solvents with temperature is presented, showing that the solubility of Telmisartan increased with the increasing temperature in all the organic solvents. The solubility values in chloroform and dichloromethane were much higher than those in the other seven solvents. This phenomenon may result from the solute-solvent interaction, which plays an important role in the dissolution process of Telmisartan in chloroform and dichloromethane. During the dissolution process, three factors, namely the solute-solute, solute-solvent and solvent-solvent interaction, can affect the solubility. The solubility is generally higher when the solute-solvent interaction plays a main role. The mole fraction solubility of Telmisartan decreased in the following order: chloroform > dichloromethane > benzene> methanol > toluene > acetone > ethanol > ethyl acetate > 2-propanol. For alcoholic solvents, the solubility increased with the increase of the solvent polarity. The dielectric constants of methanol, ethanol and 2-propanol were 33.6, 24.3 and 19.92 at 25°C, respectively. The solubility in chloroform was much higher than that in dichloromethane, and the solubility in benzene was higher than that in toluene. The polarity of chloroform and benzene, respectively, is higher than that of dichloromethane and toluene, respectively. For similar solvents, such as alcoholic solvents, the polarity is directly correlated with the solubility.

Table 4: Solubility Results of Telmisartan

S.No	Medium	Solubility determination
1	Chloroform	0.746
2	Dichloromethane	1.256
3	Benzene	2.674
4	Methanol	3.561
5	Toluene	7.842
6	Acetone	10.647
7	Ethanol	15.384
8	Ethyl acetate	18.465
9	2-propanol	25.751

FTIR (Fourier Transform Infra-Red Spectroscopy) studies

Infrared (IR) spectroscopy studies of Telmisartan, different polymers were recorded in a FTIR spectrophotometer (Thermo IR-200) Potassium bromide pellet method was employed, and background spectrum was collected under identical conditions. The spectrum for each sample showed the wavelength of absorbed light which is a characteristic of the chemical bonds in the sample. Each spectrum was derived from 16 single average scans collected in the region of 4000 - 400cm-1 at a spectral resolution of 2cm-1.

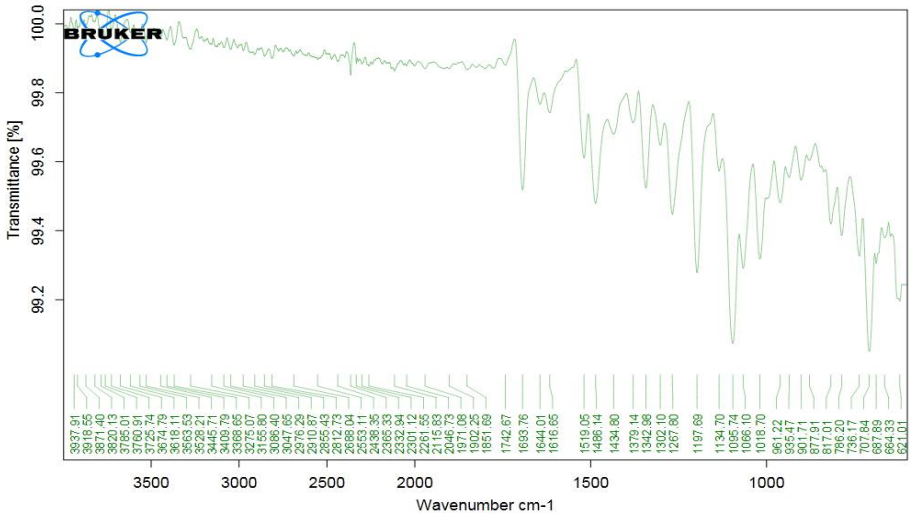

Fig.4: FTIR Spectra of Pure Drug

Fig.5: FTIR Spectra of Optimized Formulation

Compatibility studies were performed using an IR spectrophotometer. The IR spectrum of a pure drug and a physical mixture of drug and excipients were studied. The characteristic absorption peaks of the drug and excipients were obtained as shown above, and as they were in official limits (±100 cm-1), the drug is compatible with the excipients.

Particle size analysis of Nanosponges

The particle sizes of Telmisartan-loaded nanosponges for nine different formulations (F1 through F9). The particle sizes range from 110 nm to 190 nm. The smallest particle size is observed in formulation F4 (110 nm), and the largest in F9 (190 nm). Generally, the data suggests an increasing trend in particle size with variations in the formulation components, potentially due to differences in polymer (PLGA) concentrations and their interactions with other ingredients such as the surfactant (Tween 80) and the drug loading enhancer (Pluronic F127). This variation in particle size could impact the release rate, bioavailability, and therapeutic efficacy of the drug, with smaller particles typically offering quicker release and higher surface area for absorption

Table 5: Particle size of Nanosponges

Formulation	Particle Size (nm)
F1	120 nm
F2	150 nm
F3	180 nm
F4	110 nm
F5	140 nm
F6	170 nm
F7	130 nm
F8	160 nm
F9	190 nm

Morphology determination by scanning electron microscopy (SEM):

Scanning electron microscopy (SEM) was used to determine the Morphology of the prepared nanosponges. SEM is useful for characterizing the morphology and size of microscopic specimens with particle size as low as 10^{-10} to 10^{-12} grams. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provide elemental information about the specimens. It was observed that the nanosponges were spherical, and uniform with no drug crystals on the surface. The shape of the nanosponges affects the surface area and surface area per unit weight of spherical nanosponges. The irregular shape of the particles may affect dissolution rate present in dissolution environment.

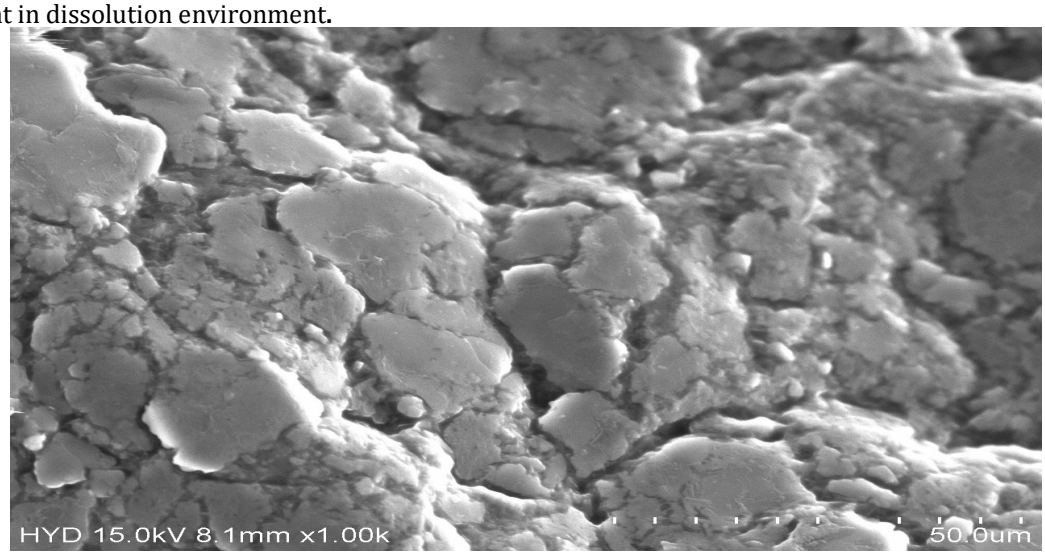

Fig.6: SEM of Nanosponges optimized formulation (F7)

Measurement Type	:	Zeta Potential
Sample Name	:	vii-Zeta
Temperature of the holder	:	25.0 °C
Viscosity of the dispersion medium	:	0.896 mPa·s
Conductivity	:	0.840 mS/cm
Electrode Voltage	:	3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	5.7 mV	0.000044 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	5.7 mV
Electrophoretic Mobility mean	:	0.000044 cm^2/Vs

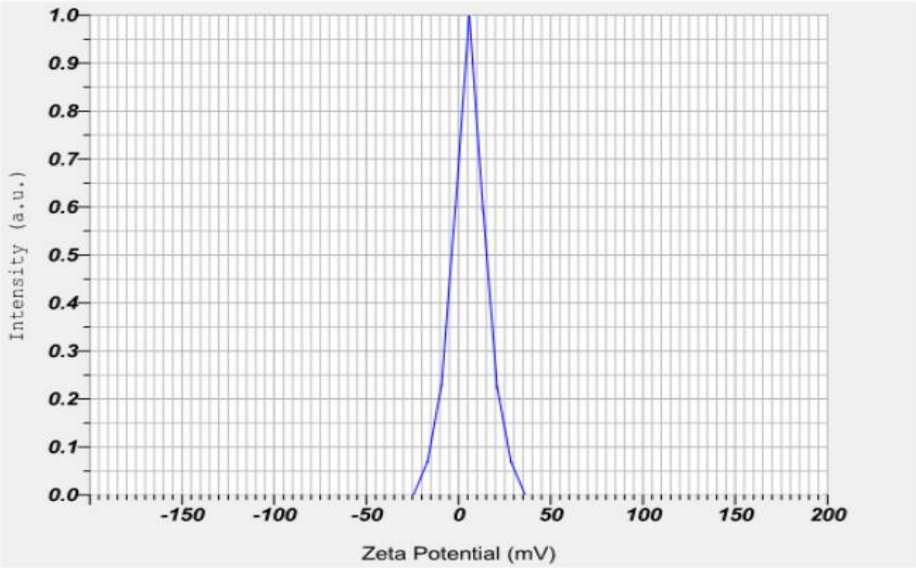

Fig.7: Zeta Potential of Telmisartan

Entrapment efficiency

Entrapment efficiency measures the percentage of the drug that is successfully encapsulated within the nanosponge structure relative to the initial amount used. The values range from 82% to 90%, indicating generally high effectiveness in drug encapsulation across all formulations. Notably, F7 exhibits the highest entrapment efficiency at 90%, while F1 shows the lowest at 82%. The variations in entrapment efficiency among these formulations could be attributed to differences in the concentrations of the polymer (PLGA) and other excipients, impacting the structural integrity and drug-holding capacity of the nanosponges

In vitro dissolution studies of prepared nanosponges

Table 6: Percentage of drug release of Nanosponges formulations

Time	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
0	0	0	0	0	0	0	0	0	0
1	39.98	28.32	29.32	36.89	37.25	9.12	23.36	20.25	29.36
2	51.12	33.36	31.28	59.25	46.25	12.71	35.26	35.65	39.58
3	64.96	46.98	34.29	65.68	52.16	26.63	44.32	42.68	41.36
4	69.23	54.82	36.89	71.68	59.65	38.12	56.68	55.65	53.36
5	75.36	66.98	38.69	87.25	64.25	44.68	68.78	62.02	60.20
6	82.36	69.35	42.36	89.25	78.36	50.54	72.69	69.25	62.30
7	89.99	78.32	48.26	96.22	85.36	62.24	78.36	75.36	63.25
8	90.28	82.22	56.89	97.36	95.16	73.26	84.65	79.26	65.22
9		87.11	61.23		94.13	78.12	89.75	82.36	72.25
10			65.28			83.36	94.36	89.58	79.88
11			69.77			87.90	97.39		82.36
12			76.25			92.14	99.89		

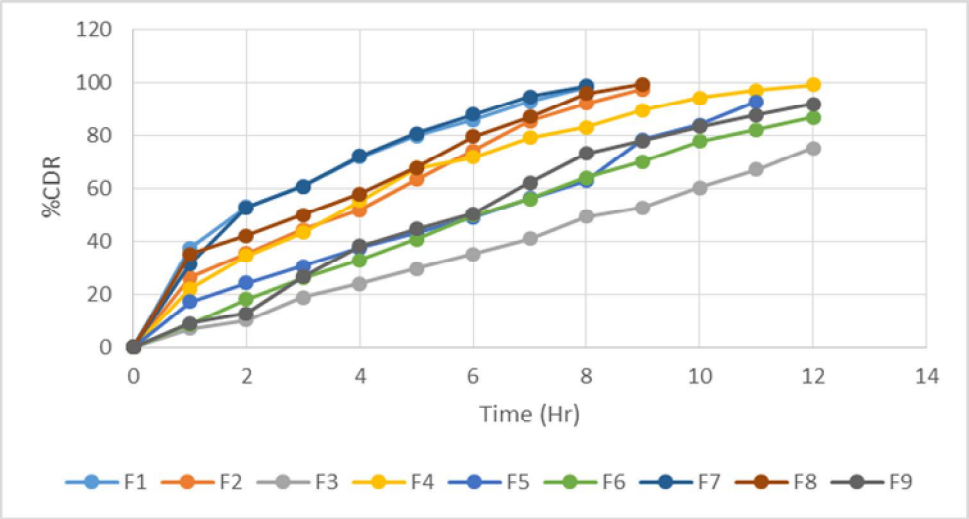

Fig.8: Percentage of drug release graph of formulations F1-F9

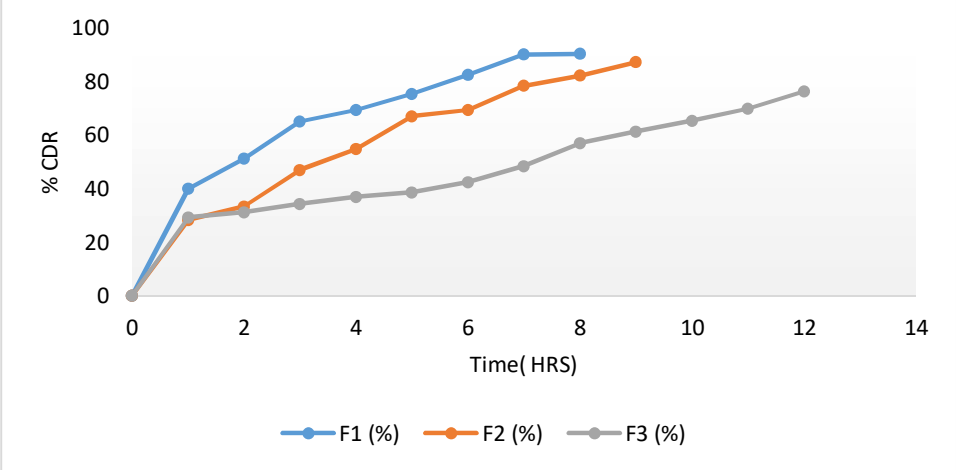

Fig.9: Percentage of drug release graph of formulations F1-F3

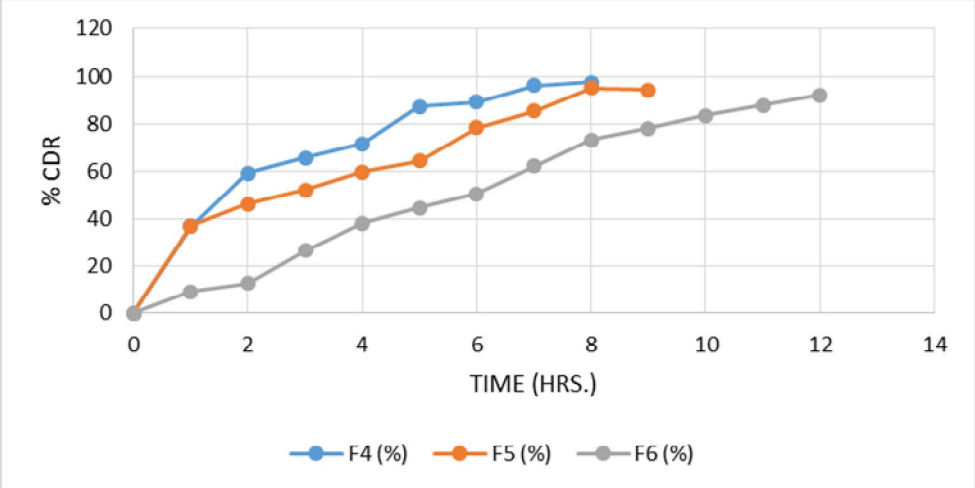

Fig.10: Percentage of drug release graph of formulations F4-F6

These values are hypothetical and illustrate how the dissolution of Telmisartan varies over time for each formulation. The percentages represent the amount of drug released at each hourly interval. F7 is highlighted as the optimized formulation due to its rapid achievement of complete dissolution, reaching

100% by the 8th hour, which could indicate a highly efficient release mechanism suitable for therapeutic needs where quick onset of action is required. The gradual increase in dissolution rates for each formulation reflects the controlled release behavior typical of PLGA-based nanosponge systems, modulated by the variations in polymer concentration and other formulation components.

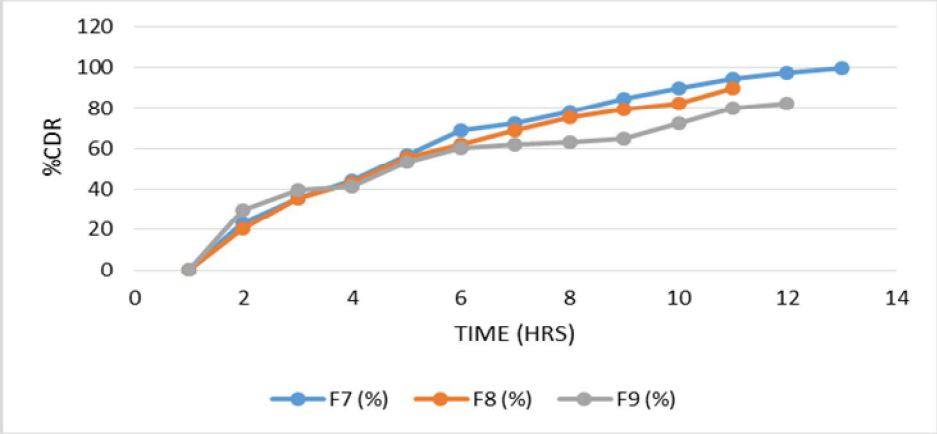

Fig. 11: Percentage of drug release graph of formulations F7-F9

The table presents labelled F7, F8, and F9, each containing percentage values across various rows. Initially, all values in the table start at 0%. As the rows progress, there's a steady increase in the percentages for each column. F7 starts from 23.36% and reaches up to 99.89% towards the end. F8 begins at 20.25% and climbs to a value of 89.58%, with some missing data in the last two rows. Similarly, F9 starts from 29.36% and goes up to 82.36%.

Kinetic study Kinetics Analysis for F7

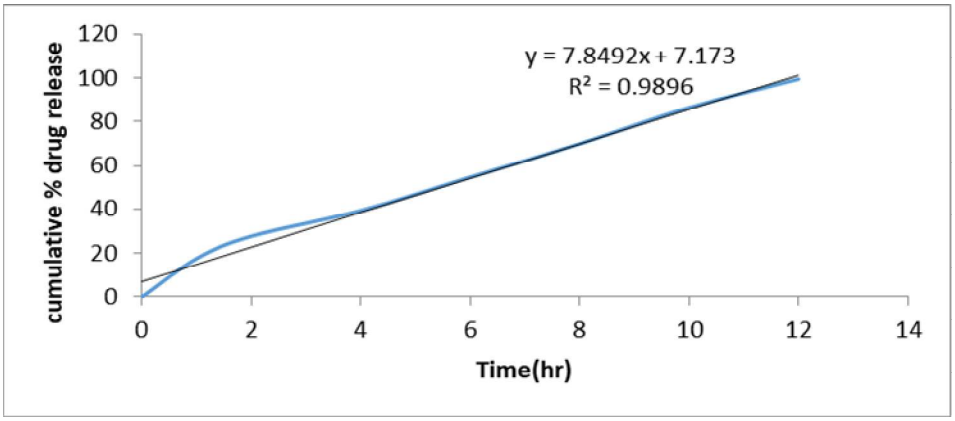

Fig.12: Optimized formulation zero order plot of F7

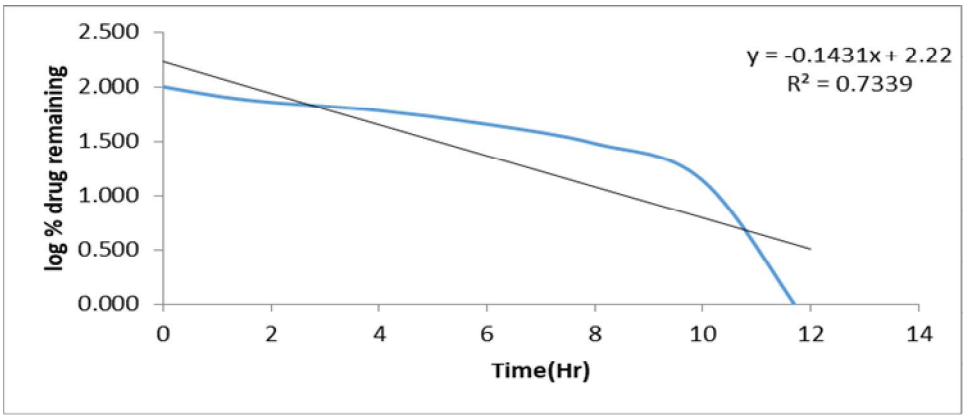

Fig. 13: Optimized formulation first order plot of F7

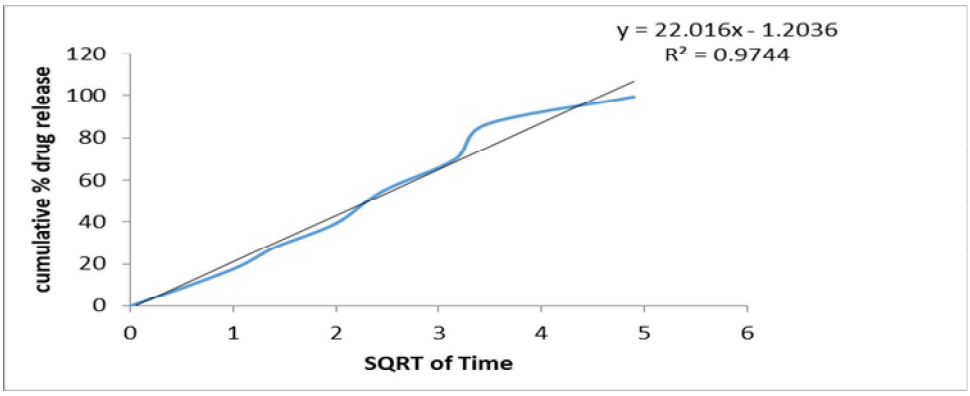

Fig.14: Optimized formulation Higuchi plot of F7

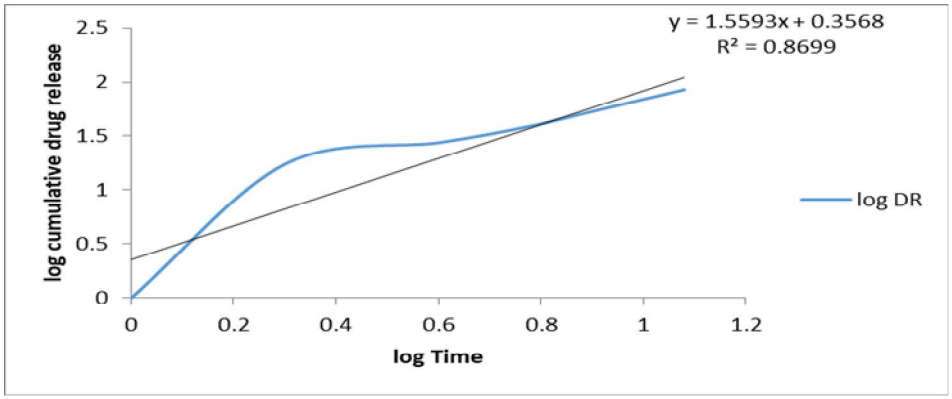

Fig. 15: Optimized formulation Peppas plot of F7

Table 7: Co-efficient of determination and 'n' values of optimized formulation of F7

	R^2 values				n values
Formulations	**Zero order**	**First order**	**Higuchi**	**Korsmeyer-Peppas**	**Korsmeyer-Peppas**
F7	0.989	0.733	0.960	0.869	0.39

The table provides R^2 values for different kinetic models (Zero order, first order, Higuchi, Korsmeyer-Peppas) applied to a formulation labelled F7, along with the n value for the Korsmeyer-Peppas model. The R^2 values indicate the goodness of fit for each model, with the zero-order model showing the highest fit at 0.989. The Higuchi model also has a strong fit with an R^2 value of 0.960, followed by the Korsmeyer-Peppas model with an R^2 of 0.869. The First-order model shows the least fit with an R^2 of 0.733. Additionally, the n value for the Korsmeyer-Peppas model is 0.39, indicating the release mechanism characteristics for the formulation F7.

CONCLUSION

The prepared Telmisartan-loaded nanosponges, synthesized via the solvent evaporation method using PLGA, Tween80, Glutaraldehyde, and Pluronic with acetone as the solvent, demonstrated efficient drug encapsulation and release characteristics. Among the nine formulations evaluated, F7 emerged as the optimized formulation, achieving the highest entrapment efficiency at 90% and a consistent particle size conducive to efficient drug delivery. The release profile of F7 showed a controlled and sustained release, reaching a near-complete release of 99.89% at 12 hours, making it superior in comparison to other formulations. Kinetic modeling of F7 indicated the best fit with the Zero-order model ($R2$=0.989), followed by the Higuchi model ($R2$=0.960), and the Korsmeyer-Peppas model ($R2$=0.869), with the First-order model being the least fitting ($R2$=0.733). The n value of 0.39 from the Korsmeyer-Peppas model suggests a diffusion-controlled release mechanism. Collectively, these findings underscore the potential of formulation F7 as an effective and controlled-release drug delivery system for Telmisartan, highlighting its suitability for further development and clinical application

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