FORMULATION AND EVALUATION OF MICROSPHERES OF CANAGLIFLOZIN BY SOLVENT EVAPORATION METHOD

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Abstract

Polymeric microspheres are one of the novel drug delivery system. Canagloflozin is a first oral agent in novel class of diabetes drug as SGLT2 inhibitor. Sodium glucose co-transporter2 (SGLT2) inhibitor will reduce the risk of a first hospitalization for heart failure, possibly through glucose-independent mechanisms.

The inhibition of SGLT2 limits renal glucose reabsorption, promoting its urinary excretion and the reduction of plasma glucose levels. Thereby, SGLT2 inhibitors use a novel mechanism of action, since they do not interfere with insulin secretion.

The main objective of the work is to prepare canagliflozin loaded microspheres for controlled release and for anti-microbial property by using natural polymers Chitosan and Sodium alginate. Formulation was prepared by Solvent Evaporation method with implementation of full factorial design, varying the drug-polymer ratio and stirring speed. The 3^2 factorial design was implemented means two-factors and three-levels are fitted in a statistical model to evaluate the responses. The microspheres were evaluated for micromeritic properties, particle size, production yield, swelling index, entrapment efficiency, $t_{50,}$ drug polymer compatibility (DSC and IR Studies), *in-vitro* drug release, kinetic models of microspheres. The percentage drug release 98.85% for a period of 15 hours. The result shows that as the concentration of polymer increases it affects the particle size, percentage yield, and in vitro drug release of microspheres. As the drug-polymer ratio increases production yield will also be increased, with increasing the stirring speed the particle size will be decreased.

Keywords: Canagliflozin, natural polymers, factorial design, SGLT2 inhibitor microspheres.

Introduction

Diabetes mellitus describes a group of metabolic disorders characterized by high blood glucose levels. People with diabetes have an increased risk of developing a number of serious life-threatening health problems resulting in higher medical care costs, reduced quality of life and increased mortality. Persistently high blood glucose levels causes generalised vascular damage affecting the heart, eyes, kidneys and nerves and resulting in various complications[1].

There are two main types of diabetes type 1 and type 2. Type 2 Diabetes has leads to the introduction of new medication like sodium-glucose cotransporter 2 (SGLT2) inhibitor reduce the risk of a first hospitalization for heart failure, possibly through glucose-independent mechanisms[2]. The two members of SGLT family mediate reabsorption of glucose in the kidney. SGLT2 is a high capacity and low-affinity transporter, responsible for nearly 90% of the active renal glucose reabsorption, while SGLT1 reabsorbs the remaining 10%. SGLT2 is almost exclusively expressed in renal proximal tubules, unlike SGLT1 that is also found in the gastrointestinal tract, and its inhibition is therefore unlikely to affect other organs. Thus, inhibition of SGLT2 limits renal glucose reabsorption, promoting its urinary excretion and the reduction of plasma glucose levels[3].

Sodium-Dependent Glucose Co-Transporter 2 (SGLT2) is a large class of proteins that facilitate the transport of sugars and sodium across the plasma membrane of cells from an extensive variety of tissues. Thus, inhibition of SGLT2 limits renal glucose reabsorption, promoting its urinary excretion and the reduction of plasma glucose levels. Thereby, SGLT2 inhibitors use a novel mechanism of action, since they do not interfere with insulin secretion[4].

Microspheres are effective for administration of therapeutic active molecules in controlled manner to the target site with improved therapeutic effects. Microsphere is the good option to achieve the objective as the polymer act as a rate-controlling membrane to obtain the desired controlled release[5].

Ideal Characteristics of Microspheres[6]:

1. Ability to control the release rate for a predefined period of time.
2. Higher concentrations of the drug can be given serve as a depot.
3. Stability of the preparation after synthesis with a clinically acceptable shelf-life.
4. Biocompatibility with a controllable biodegradability.

Material and Methods

Materials:

Sodium alginate, Ethyl cellulose, Chitosan, Acetone and Liquid Paraffin were purchased from Loba Chemie Pvt. Ltd. Mumbai while Sodium alginate purchased from Chemdyes Corporation. The model drug Canagliflozin was gifted from Sun Pharma, Vadodara.

Formulation Design

For this formulation, two factors X_1 and X_2 and three levels low, medium and high are taken. So, there is a 3^2 type of formulation is possible where total 9 formulations will be prepared. X_1 is taken as a stirring speed and X_2 taken as a drug-polymer ratio.

Table: Three Levels and two factors for screening study

Factors	Levels		
	Low (-1)	**Medium (0)**	**High (+1)**
1. Stirring speed (rpm) (X_1)	800	1000	1200
2. Drug:Polymer (mg.) ratio (X_2)	1:1	1:2	1:3

Method of preparation of microspheres:

1. Solvent Evaporation Technique-

In this technique, drug and polymer (sodium alginate, chitosan, ethyl cellulose) were dissolved in a 20 ml. acetone which placed in small beaker with magnetic bead on magnetic stirrer at room temperature. The drug-polymer mixture was poured into 100 ml. liquid paraffin containing magnesium stearate maintained at a temperature of 30-40°C and subsequently stirred by mechanical stirrer at stirring speed 800, 1000 and 1200 rpm respectively for 60 minutes to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with n-hexane and air-dried for 24 hours and stored in desiccator[7-8].

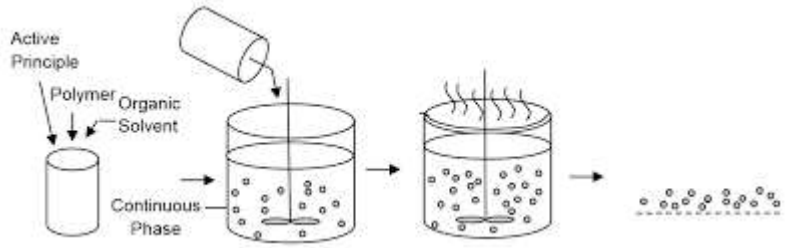

Figure: Solvent evaporation method for preparation of microsphere[9]

Table: Composition of various batches of Canagliflozin loaded Microspheres by Solvent evaporation method

Formulation	Drug:Polymer Ratio	Canagliflozin (mg.)	Polymer (CH+EC+SA) (mg.)	Stirring speed (rpm)
F1	1:1	100	100	800
F2	1:2	100	200	800
F3	1:3	100	300	800
F4	1:1	100	100	1000

F5	1:2	100	200	1000
F6	1:3	100	300	1000
F7	1:1	100	100	1200
F8	1:2	100	200	1200
F9	1:3	100	300	1200

Results and Discussion

1. Organoleptic Characteristics

The canagliflozin drug is crystalline solid, odourless and off-white in color.

2. Identification of drug by IR

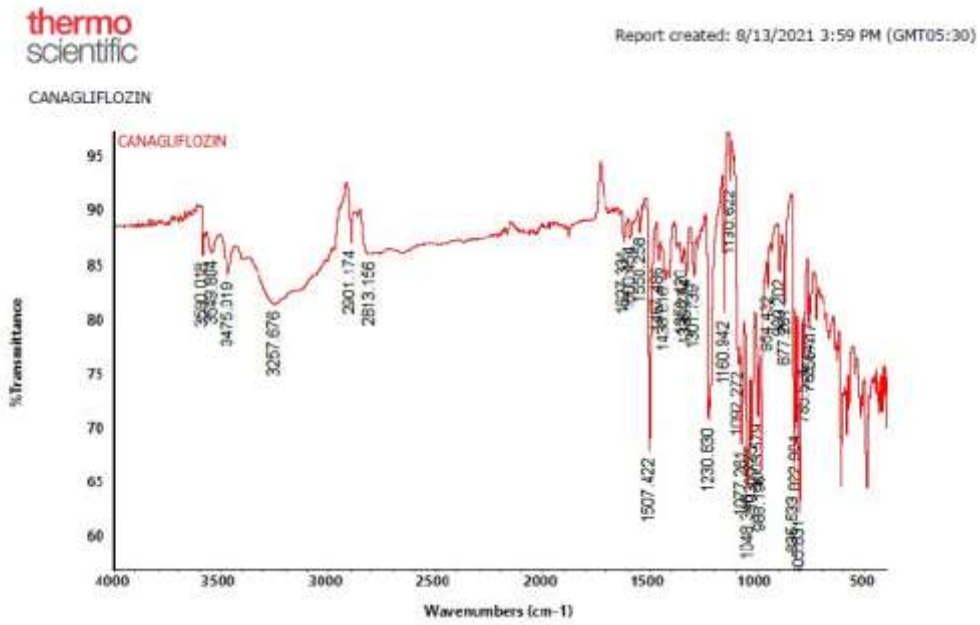

Figure: IR of pure drug

The FTIR spectrum of canagliflozin is shown in figure. The characteristics peaks were observed at 3590.01, 3549.804, 3475.01, 3257.67 cm^{-1} (-OH group), 2901.17 cm^{-1} (Ar-CH group), 1507.422 cm^{-1} (C=C group), 1230.83 cm^{-1} (C-O group). The presence of all these peaks are in conformation with the functional groups present in canagliflozin.

3. Melting point determination of pure drug

Melting point of the drug canagliflozin was observed 99-103ºC by Thiel's tube.

4. Solubility Studies

Canagliflozin is soluble in ethanol, methanol, DMSO, SGF. The drug is sparingly soluble in aqueous buffer and practically insoluble in distilled water.

5. Partition coefficient

The partition coefficient of drug was found to be 3.43 by average of triplicate readings.

6. Drug excipient compatibility data:

a. IR Spectroscopy

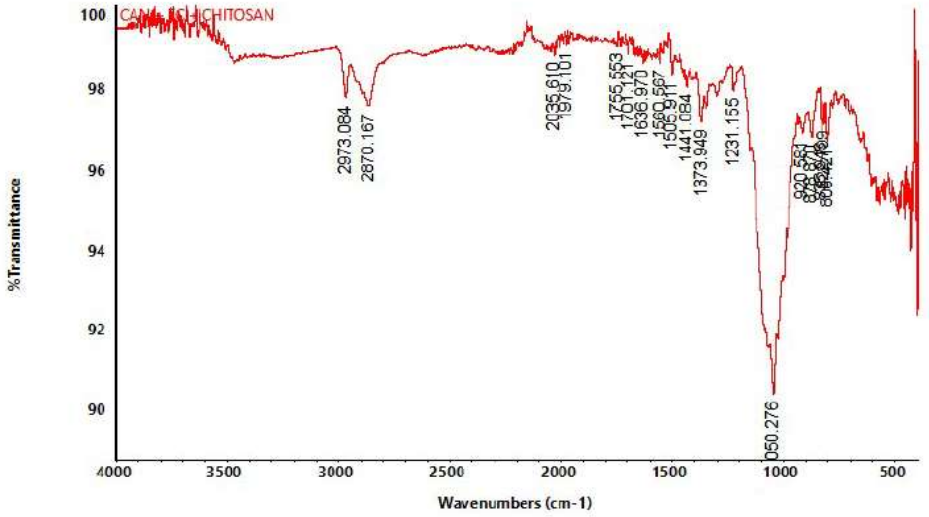

Figure: IR spectra of Drug and excipients

The characteristic peaks are reported for Canagliflozin and these peaks were not affected and appeared in the spectra with excipients. Characteristic peaks of excipients were also retained and it is indicated that there is no incompatibility was found between Canagliflozin and excipients.

b. Differential Scanning Calorimetry

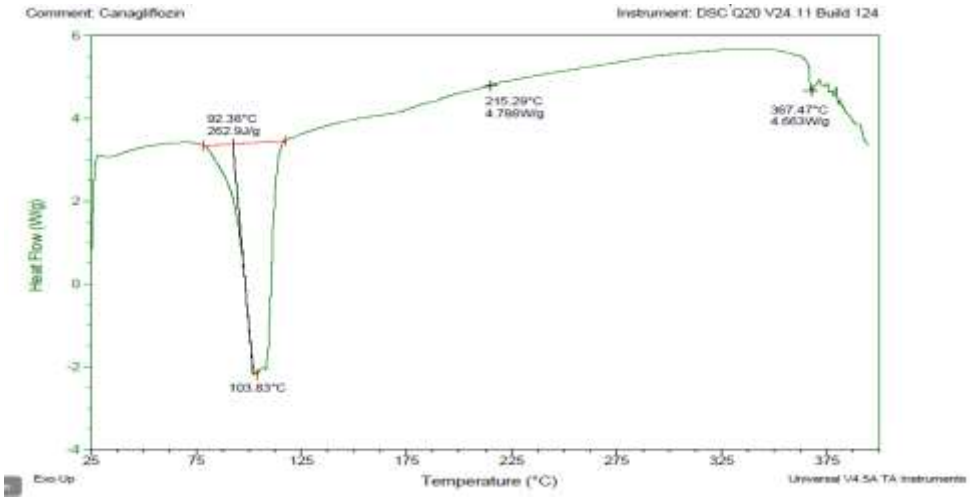

Figure: DSC thermogram of pure drug

A sharp endothermic peak was observed at 103.83ºC which is parallel to the melting point of canagliflozin, demonstrating the crystalline nature of drug.

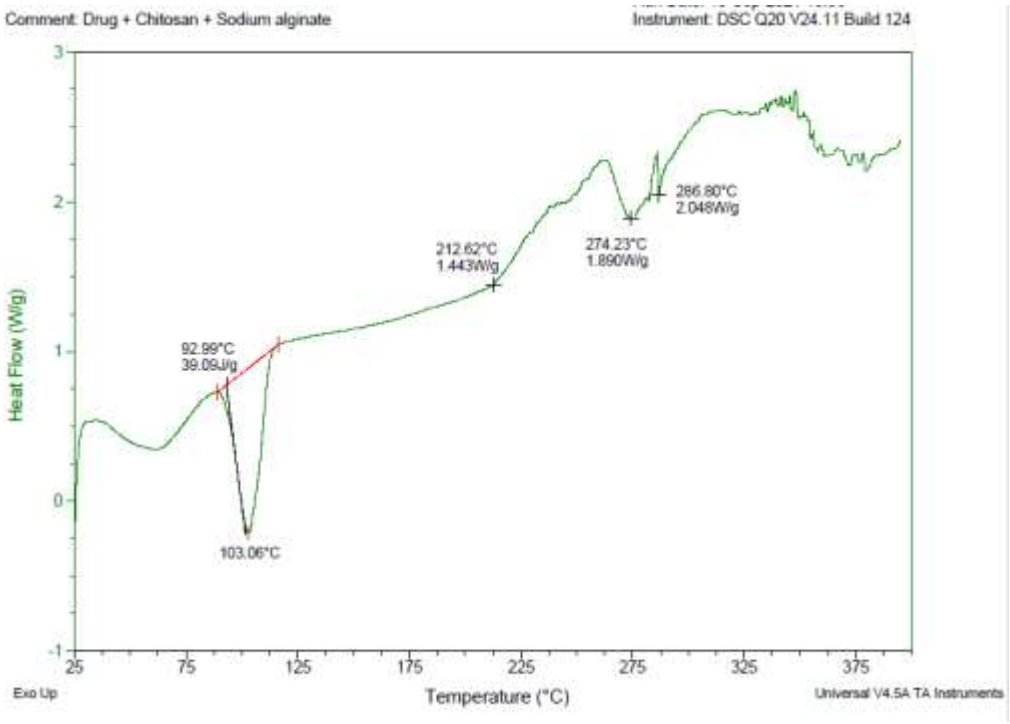

Figure: DSC thermogram of Drug and excipients

Pure Canagliflozin displays sharp peaks corresponding to its melting point suggested that there is no interaction between the Canagliflozin and excipients.

Calibration Curve

UV spectrum of Canagliflozin in Simulated Gastric Fluid showed that the drug has λ_{max} at 287 nm. The calibration curve of Canagliflozin was prepared in Simulated Gastric Fluid. The slope of the calibration curve is as follows and the solution follows Beer's law in the range of 2-20µg/ml.

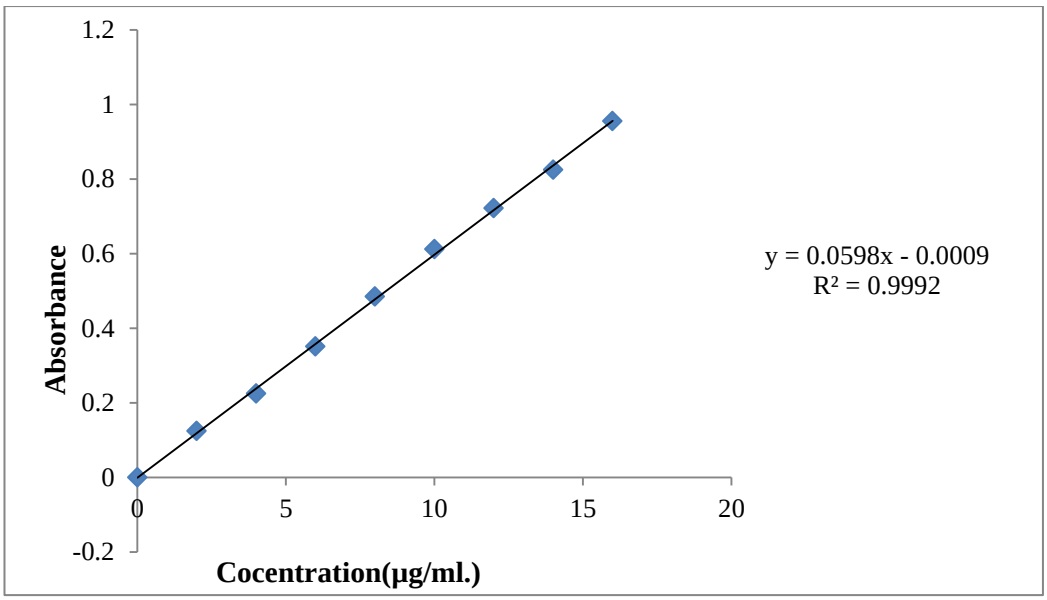

Figure: Calibration Curve of Canagliflozin in SGF

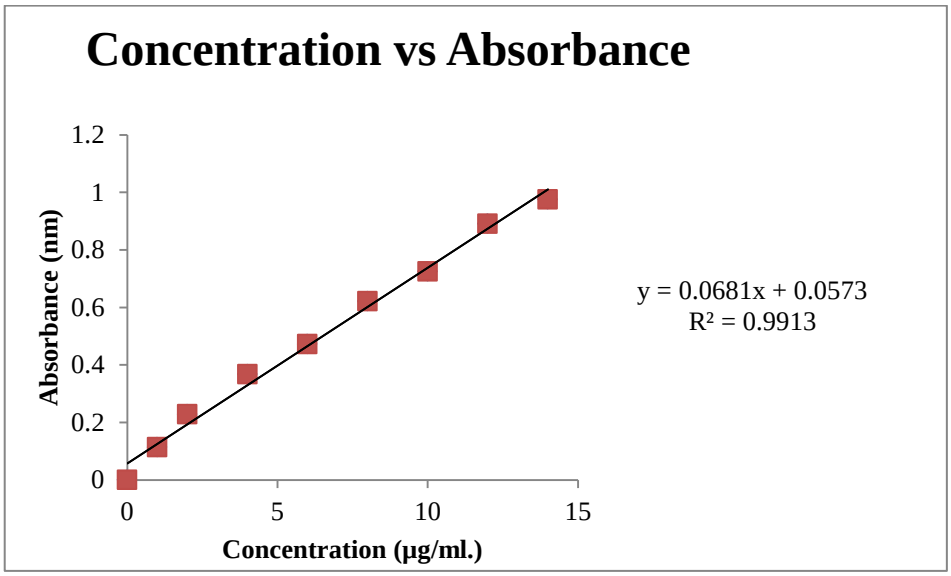

Figure: Calibration Curve of Canagliflozin in Ethanol

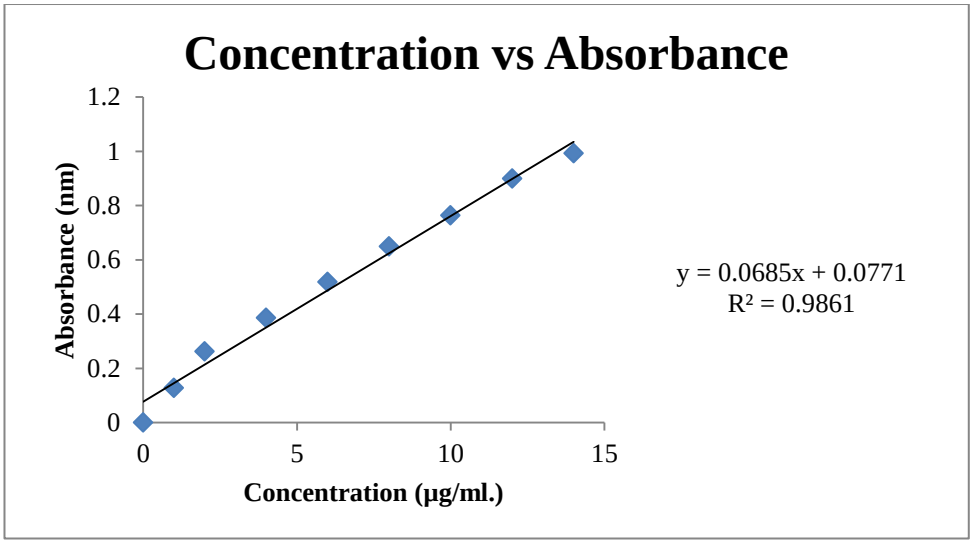

Figure: Calibration Curve of Canagliflozin in 0.1 N HCl

Indication of Flow Properties of Microspheres

F. Code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of Repose (ɵ)	Carr's Index (%)	Hausner's Ratio
F1	0.36±0.03	0.30±0.02	20.60±0.10	14.34±2.11	0.83±0.03
F2	0.38±0.05	0.32±0.05	20.58±0.21	15.92±0.24	0.84±0.01
F3	0.40±0.06	0.34±0.01	20.32±0.42	16.23±0.56	0.85±0.04
F4	0.35±0.01	0.31±0.04	24.54±0.50	14.78±0.86	0.88±0.01
F5	0.37±0.13	0.33±0.03	24.31±0.42	14.96±2.18	0.89±0.02
F6	0.38±0.02	0.34±0.12	24.22±0.63	15.78±0.32	0.89±0.05

F7	0.41±0.11	0.35±0.05	22.34±0.45	13.05±3.36	0.85±0.04
F8	0.42±0.03	0.36±0.01	22.15±0.71	14.26±2.15	0.86±0.03
F9	0.44±0.05	0.36±0.03	22.53±0.64	15.65±1.74	0.81±0.02

In these formulation of microspheres, the observation of angle of repose comes in the range of 20-30 so we can say that it has a good type of flow property. For the Carr's Index it was observed that microspheres exhibits good flow and observation of Hausner's ratio is less than 1.25 so it was concluded good type of flow.

Factorial Design

Sigma Plot 15.0 trial version software was used to design the variables. A 3^2 full factorial design was used in the present study. In this design 2 factors are evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The stirring speed (X1) and the drug-polymer ratio (X2) were selected as independent variables. The production yield, entrapment efficiency, particle size, t50 and swelling index were selected as a dependent variables. The Factorial design is a statistical model used to evaluate the responses with this polynomial equation:

$$\mathbf{Y= b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2}$$

where Y is the dependent or response variable, b_0 is the arithmetic mean response and b_1, b_2, b_{11}, b_{22} and b_{12} represents the regression coefficient. X_1 and X_2 stands for main effect, X_1X_2 are the interaction terms and shows how the response changes when two factors are simultaneously changed. X_1^2 and X_2^2 are quadratic terms of independent variables to evaluate nonlinearity. The polynomial equation can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). For drawing conclusions, grid search technique of contour plot was used.

After performing the screening studies, it was found that stirring speed and drug:polymer ratio significantly affected the selected response variables *i.e.* t_{50}, production yield, entrapment efficiency, particle size, swelling index because an effort was made to further optimize the stirring speed and drug-polymer ratio by factorial design. Further purpose, 3^2 factorial design was employed. The two factors for the study being stirring speed and drug-polymer ratio were taken and three levels as low, medium and high.

Table: Production Yield, Entrapment Efficiency, Particle Size, t_{50} and Swelling index of Microspheres of Canagliflozin by Solvent Evaporation Method

Formulation Code	Production Yield (Y1)	Entrapment Efficiency (Y2)	Particle Size (Y3)	t_{50} (Y4)	Swelling Index (Y5)
F1	72.49±2.74	55.13±1.89	311±3.76	9.96±0.36	79.55±2.88

F2	74.32±2.68	56.98±1.66	316±3.55	8.53±0.39	81.32±3.12
F3	77.68±2.93	58.26±1.78	320±3.36	8.21±0.28	87.56±3.56
F4	71.87±2.55	61.82±1.86	287±3.19	11.03±0.41	80.23±3.33
F5	74.42±3.02	66.14±1.66	299±3.75	9.88±0.37	82.45±3.76
F6	79.96±3.33	70.08±2.06	305±3.27	8.25±0.35	84.26±3.45
F7	78.98±3.21	62.21±1.56	260±3.88	10.32±0.46	82.99±3.65
F8	79.23±3.43	65.38±1.78	263±2.34	9.97±0.41	88.28±3.23
F9	80.64±3.56	68.24±1.57	268±3.55	8.82±0.35	92.51±3.76

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Table: Summary of Results of Regression Analysis

Responses	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
Y1	89.42	7.06	2.16	-0.64	0.49	-2.32
Y2	64.14	9.08	-1.26	2.04	-2.19	-1.72
Y3	310	31.67	-11.67	-6	-2	-2
Y4	7.98	-0.38	0.21	0.41	0.84	-1.15
Y5	81.45	7.89	1.41	2.85	-0.17	-1.72

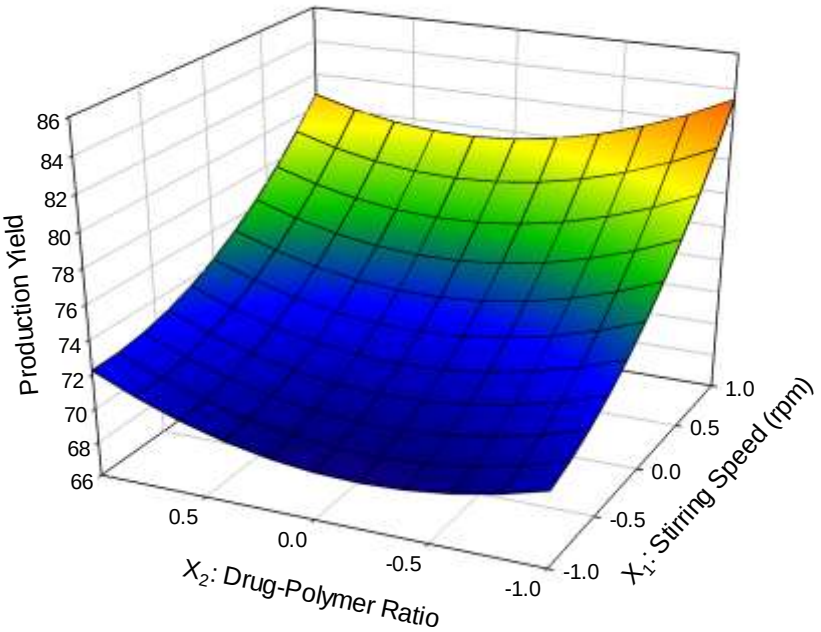

Figure: Three-Dimensional surface response plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Production Yield

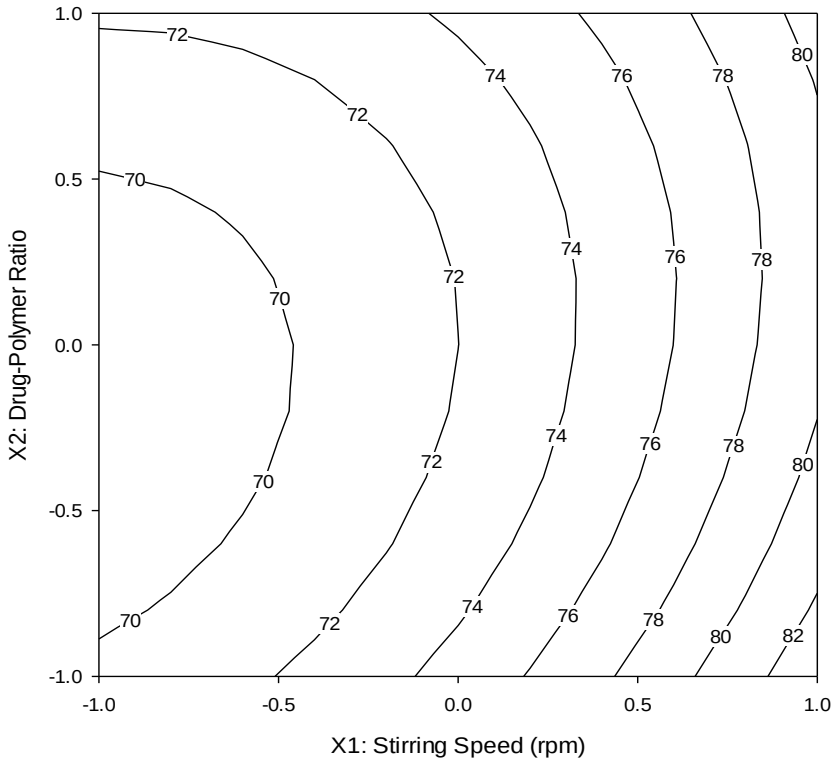

Figure: Contour Plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Production Yield

The higher the conc. of polymer then higher will be production yield. The value of production yield for different batches of microspheres was found to be in range of 71.87±1.86 (F4) to 80.64±2.84% (F9) by solvent evaporation method.

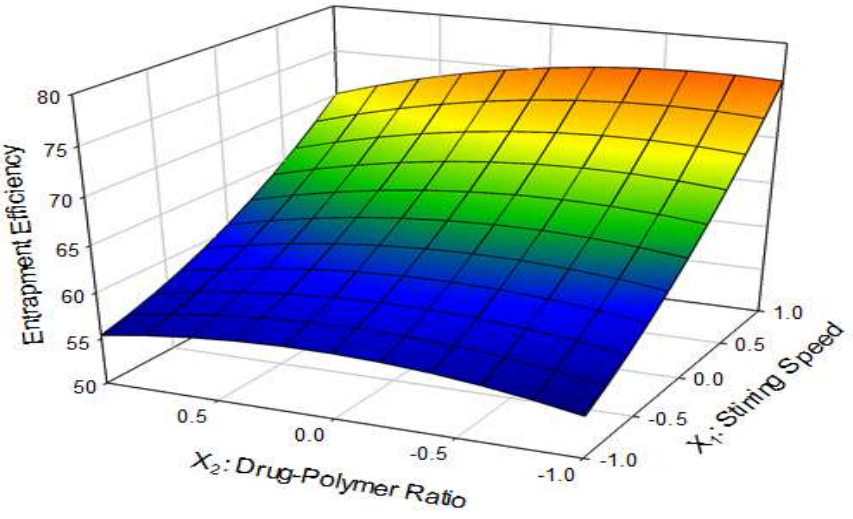

Figure: Three-Dimensional surface response plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Entrapment Efficiency

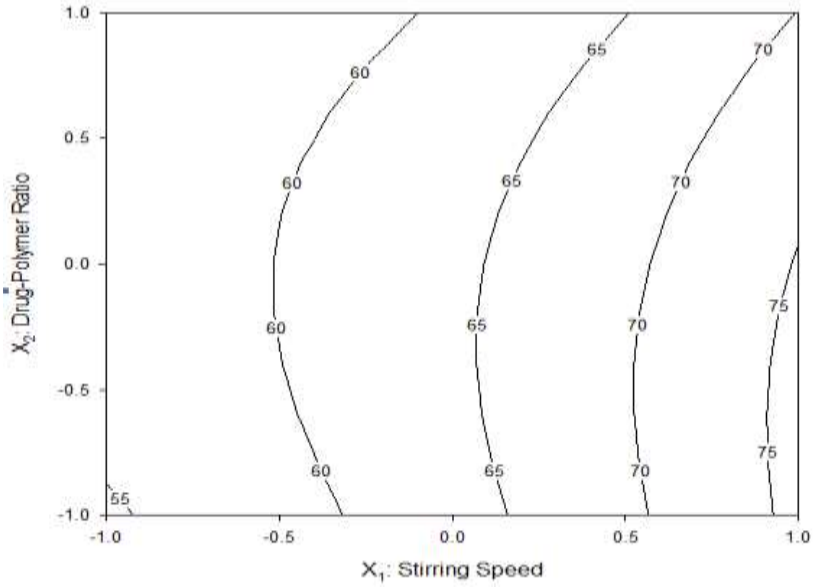

Figure: Contour Plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Entrapment Efficiency

Polymer concentration has positive effect on entrapment efficiency of microspheres which indicates that the higher amount of polymer contributes to increase in entrapment of drug. Canagliflozin loaded microspheres exhibited a highest entrapment efficiency of 70.08±2.16% (F6) by solvent evaporation method. It increases as the value of the drug-polymer ratio increased.

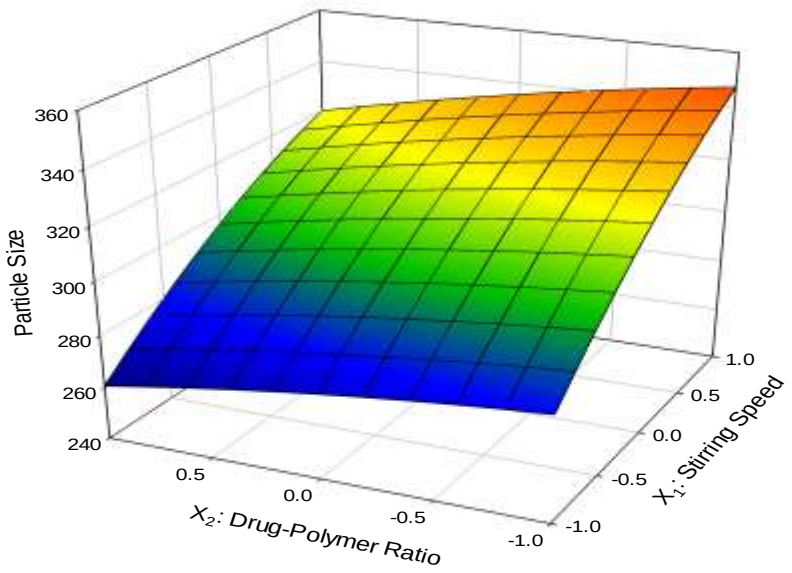

Figure: Three-Dimensional surface response plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Particle Size

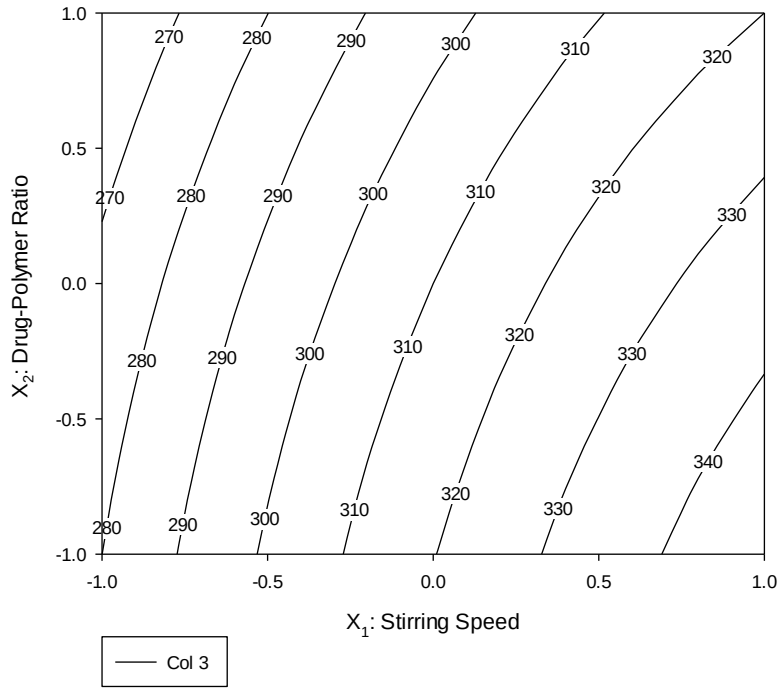

Figure: Contour Plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Particle Size

Particle size for different batches of developed canagliflozin loaded microspheres was in range of 260±2.58 (F7) to 320±3.65µm (F3) by solvent evaporation method. Drug to polymer exhibited a positive impact on particle size. A significant decreasing effect of stirring speed on microspheres was observed means particle size will decrease with increasing the stirring speed.

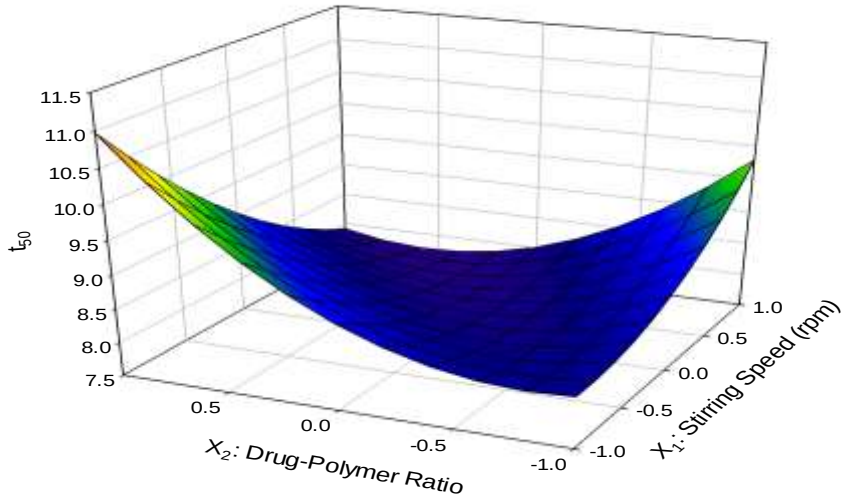

Figure: Three-Dimensional surface response plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on t_{50}

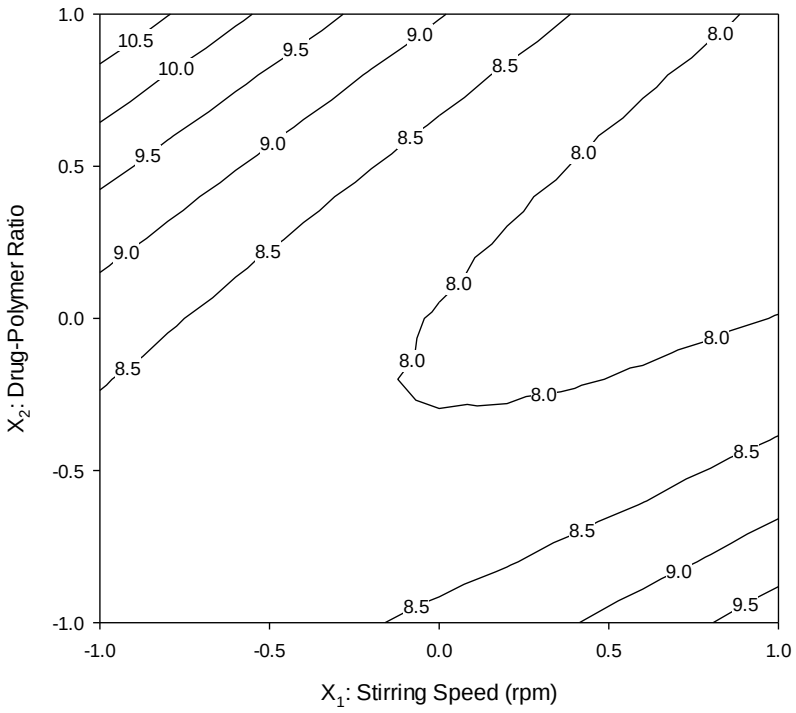

Figure: Contour Plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on t_{50}

The time required to release 50 % drug was found in the range of 8.21±0.57 (F3) to 11.03±1.23 % (F4) by solvent evaporation method. The t_{50} will decrease with increase in drug-polymer ratio.

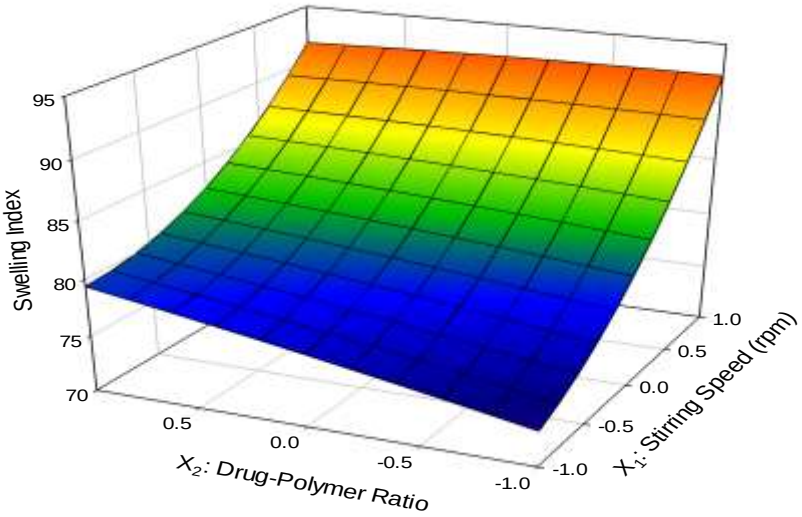

Figure : Three-Dimensional surface response plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Swelling Index

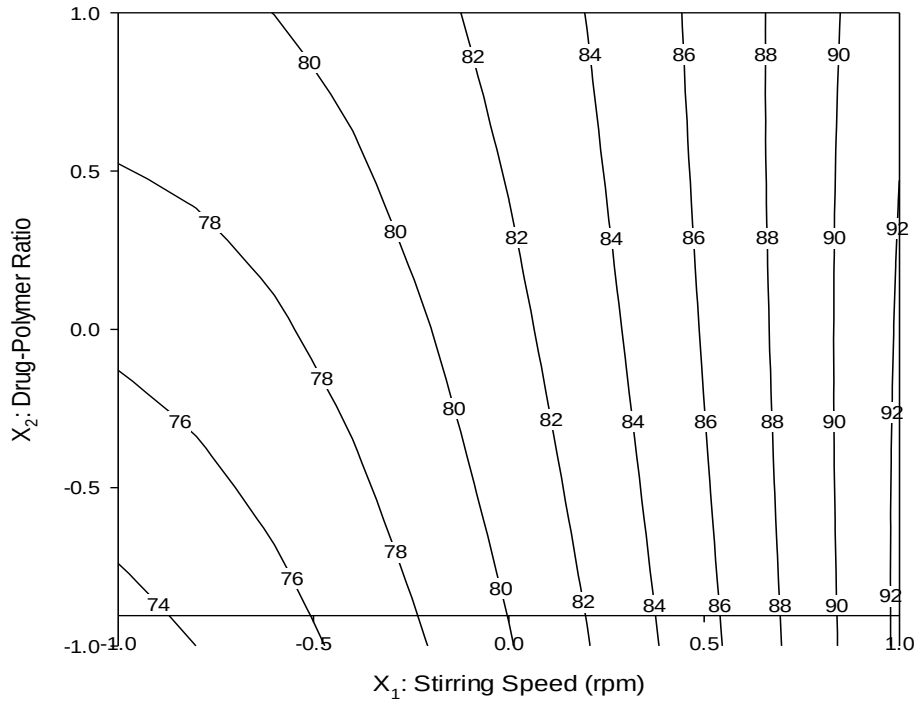

Figure: Contour Plot for the effect of stirring speed (X_1) and drug:polymer ratio (X_2) on Swelling Index

The swelling capacity of developed Canagliflozin loaded microspheres determined in water. The swelling index of microspheres was found between 79.55±2.11 (F1) to 92.51±2.83% (F9) by solvent evaporation method. The concentration of polymers has a positive effect on the swelling index as it increases with increasing the concentration of polymers especially with the amount of Chitosan because it having good swelling capability.

***In-vitro* drug release**

The in vitro drug release data were fitted to zero order, first order kinetics and Higuchi model. The results of in-vitro dissolution studies obtained in these formulations were plotted in three models of data treatment as follows:

1. Cumulative percentage of drug release v/s time (Zero-order plot).
2. Log cumulative percentage of drug remained v/s time (First-order plot).
3. Cumulative percentage of drug released v/s Square root of time (Higuchi's plot).

By Solvent Evaporation Method

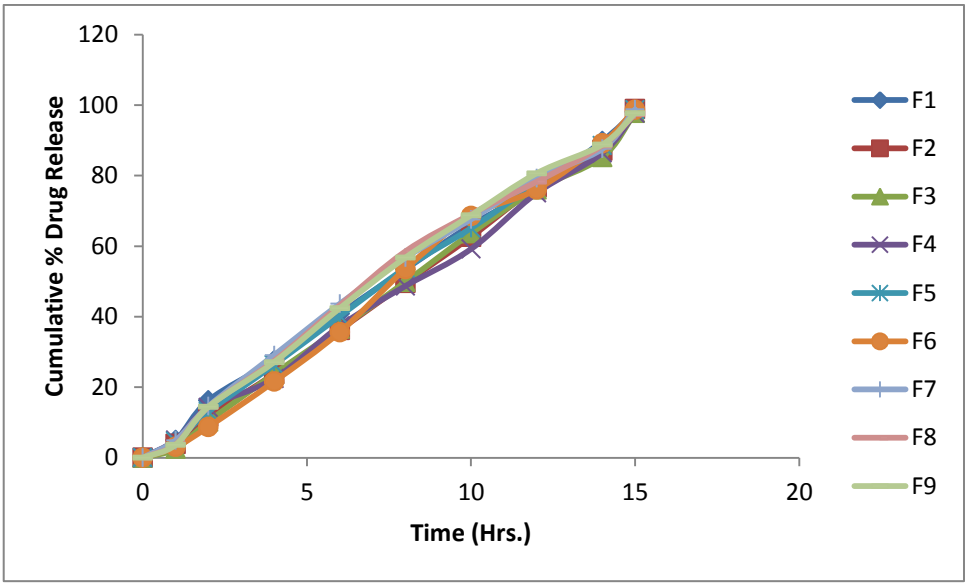

Figure: Cumulative % Drug Release *v/s* Time of Canagliflozin from Formulation F1 to F9

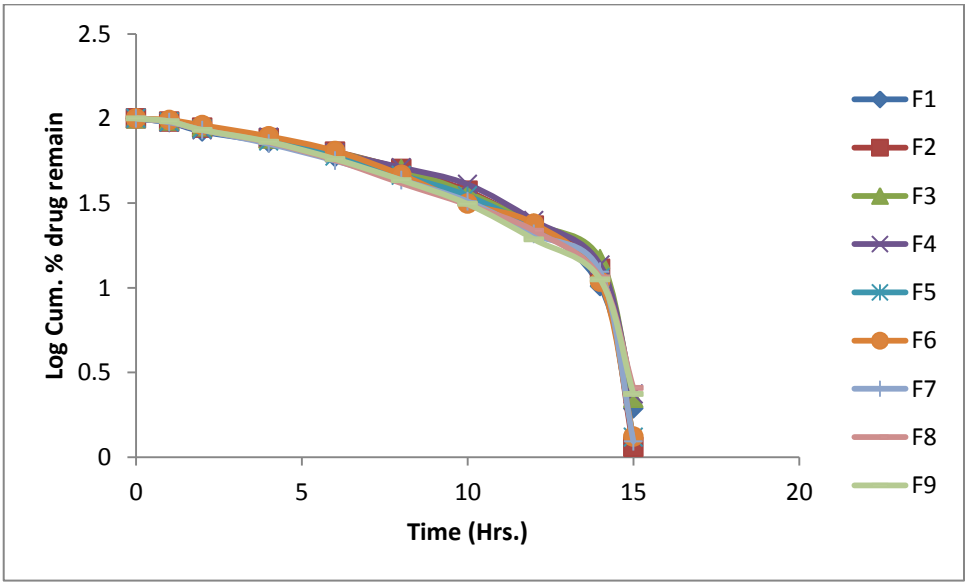

Figure: Log Cumulative % Drug Remain *v/s* Time of Canagliflozin microspheres from F1 to F9

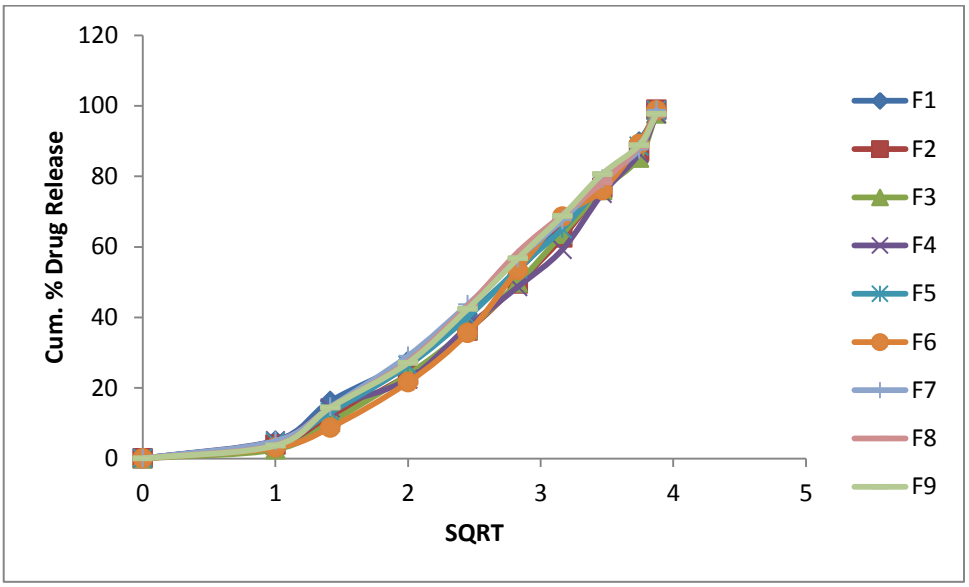

Figure: Cumulative % Drug Release *v/s* SQRT of Canagliflozin microspheres from F1 to F9

Kinetics Data Obtained from *in-vitro* release profile for Microspheres of Canagliflozin by Solvent Evaporation method

Form. Code	Zero-order kinetic data	First-order kinetic data	Higuchi matrix data
	Regression Coefficient (R^2)	Regression Coefficient (R^2)	Regression Coefficient (R^2)
F1	0.9980	0.7878	0.9688
F2	0.9982	0.7114	0.9558
F3	0.9976	0.7689	0.9686
F4	0.9965	0.7627	0.9652
F5	0.9986	0.7466	0.9531
F6	0.9954	0.7552	0.9636
F7	0.9947	0.7490	0.9665
F8	0.9931	0.8344	0.9556
F9	0.9952	0.8333	0.9625

From the correlation coefficient values obtained it was concluded that the drug release from microspheres followed zero order kinetics. A lower variation was also obtained for zero order release rate constants indicating a Zero order release pattern from the microspheres.

Higuchi model explained the matrix diffusion mechanism of drug release for all the formulations of microspheres. The coefficient of determination of R^2 values were much closer to 1 for Higuchi model that indicating that drug release followed matrix diffusion mechanism or Higuchi pattern release from prepared microspheres.

Scanning Electron Microscopy

The shape and surface morphology of the microspheres were examined by scanning electron microscopy (Supra 55, Carl Zeiss). The Supra 55 offers the highest resolution comparable to in-lens instruments. The samples were mounted directly on to the fully eucentric 5" stage multi-functional specimen chamber. Scanning electron micrographs indicated a rough surface and irregular shape of microspheres prepared with Ethyl cellulose, chitosan, sodium alginate.

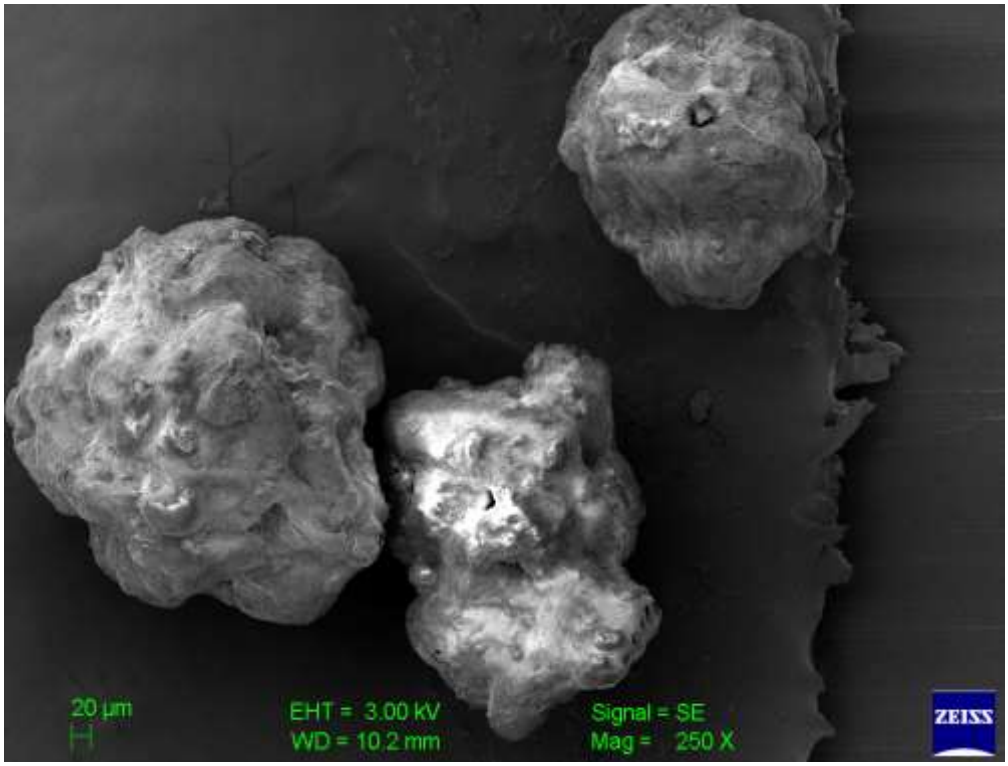

Figure: SEM of microspheres

Stability Studies

The developed optimized microspheres formulation (F5) was subjected to stability studies for 6 months at 40ºC and 75%RH and at room temperature. After the study, it was found that observed parameters like production yield, entrapment efficiency, particle size, t_{50} and swelling index were not differed significantly ($P< 0.05$) from zero-order data. The good stability of canagliflozin microspheres might be due to the combined effects of Chitosan and EC. EC is a water insoluble polymer and expectedly provides the mechanical property to the developed

microspheres. Chitosan provides a more compact coating on EC core of microspheres and ensures longer retention of the drug. During evaporation, partial precipitation of EC led to formation of a more compact chitosan layer around EC core of microspheres which is responsible for providing the stability to developed microspheres. Hence, the developed microspheres formulation was considered a stable formulation.

Conclusion

The concept of formulating microspheres containing Canagliflozin and natural polymers offers a suitable, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medication over extended period of time. The use of natural polymers in microspheres is expected that may reduce its side effects because of antimicrobial properties of natural polymers. Micropsheres were prepared successfully by solvent evaporation method using 3^2 full factorial design revealed that the stirring speed (X_1) and the drug-polymer ratio (X_2) as independent variables significantly affected the dependent variables such as production yield, entrapment efficiency, particle size, t50 and swelling index of the microspheres. The best fitted model is Higuchi model and zero order kinetics.

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References

[1]. Singh, K. et al. (2014). Canagliflozin: an anti-diabetic drug. *North American Journal of Medical Sciences*, 6(3), 107-113. DOI: 10.4103/1947-2714.128471

[2]. Demaris, K.M. and White, J.R. (2013). Depagliflozin, an SGLT2 inhibitor for the treatment of type 2 diabetes. *Drugs Today (Barc)*, 49, 289-301.

[3]. Garg, S.K. and Shah, V.N. (2014). SGLT2 inhibitors offer treatment pathway for type 2 diabetes. *Diabetes Technology and Therapeutics*, 16(S1). https://doi.org/10.1089/dia.2014.1514

[4]. Wilding, John P.H. (2014). The role of kidney in glucose homeostasis in type 2 diabetes: Clinical implications and therapeutic significance through SGLT2 inhibitors. *Metabolism Clinical and Experimental*, 63, 1228-1237. http://dx.doi.org/10.1016/j.metabol.2014.06.018

[5]. Kataria, S. et al. (2011). Microsphere: A review. *International Journal of Research In Pharmacy and Chemistry*, 1(4), 1184-1198.

[6]. Jyothi, N.V.N. et al. (2010). Microencapsulation techniques, factors influencing encapsulation efficiency. *Journal of Microencapsulation*, 27(3), 187-197.

[7]. Gholap, S.B. et al. (2010). Hollow microsphere: a review. *International Journal of Pharmaceutical Sciences Review and Research*, 1(1), 74-79.

[8]. Kar, M. (2009). Controlled release metformin hydrochloride microspheres of ethylcellulose prepared by different methods and study on the polymer affected parameters. *Journal of Microencapsulation*, 26(1), 46-53. DOI: 10.1080/02652040802130503

[9]. Scarfato, P. et al. (2008). Quercetin microspheres by solvent evaporation: Preparation, Characterization and Release Behavior. *Journal of Applied Polymer Science*, 109, 2994-3001.