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Preparation, Characterization, *In-Vitro* Drug Release and Kinetics Studies Canagliflozin Polymeric Nanopartciles

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Abstract
Nanosuspension is one of the most promising approaches for the enhancement of oral bio-availability of poorly soluble drugs. In this current research work, 13 different formulations of surfactant-stabilized nanosuspension of canagliflozin polymeric nanoparticles were developed and optimized based on average particle size distribution, entrapment efficiency, and zeta potential. In-vitro drug release and kinetics experiments were also investigated. The nanoparticle formulation CGF-12 was found with optimum results in particle size distribution and zeta potential analysis. The %EE of drug in the prepared nanosuspension was in the range of 49.5 ± 0.70 to 100 ± 2. In in-vitro drug release studies, CGF-12 formulation showed maximum drug release of 100% in distilled water within 3 hours, 93% in HCl media at the end of 8 hours and reached 100% in phosphate buffer media within 2 hours. Hence, the formulated canagliflozin polymeric nanoparticles are good choice to improve physicochemical properties of the drug and these formulations improve canagliflozin drug efficacy.
Keywords: *Canagliflozin, Nanosuspension, Physicochemical, Drug release and Drug kinetics.*

Introduction

Developing nations fall as a prey to number of disorders due to changes in lifestyle that have raised the risk of obesity and diabetes. According to World Health Organization Global reports on diabetes (2018),422 million adults were suffering from diabetes, 1.6 million population deaths are directly caused by diabetes every year because of obese[1]. Sulphonyl Urea such as Tolbutamide, Chlorpropamide, Glibenclamide, Glipizide, Gliclazide medications will activate β-cells and increase the insulin secretion and also increase sensitivity of cells for insulin upregulation in its receptor. Usage of high dose of the drugs ultimately results in adverse side effects.To resolve these disadvantages, anti-diabetic medications have been tested using nanotechnology-based drug delivery techniques to improve efficacy and safety while reducing dose and adverse effects[2,3,4].

Canagliflozin (CGF) is an SGLT2 inhibitor drug with a molecular formula of $C_{24}H_{25}FO_5S$ (Figure 1). Canagliflozin inhibits the reabsorption of glucose that has been filtered by inhibiting SGLT2[5]. CGF also helps to improve pancreatic function by lowering glucose toxicity. CGF is used as supportive combination drug therapy with Metformin, Sulfonylurea, Sitagliptin, which is also used as monotherapy for glycemic control trials in adults with type 2 diabetes mellitus[6]. One of the most intriguing ways for improving the oral bioavailability of poorly soluble medicines is nanosuspension[7].

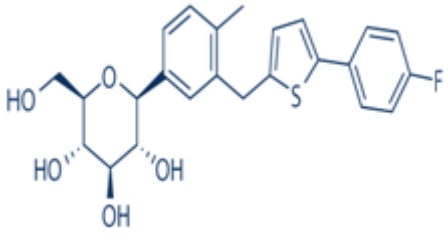


Figure 1: Molecular structure of Canagliflozin

Surfactants and polymers are used to stabilizenanosuspensions. Polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), Pluronic F127, and Eudragit E100 are among the surfactants and polymers employed (EE100). Stabilizing agents should be included in nanosuspensions to prevent crystal development and increase the solubility, dissolution profile, and hence bioavailability of the medications[8,9]. This research study mainly focused on the synthesis of polymeric nanoparticle formulations of Canagliflozin, their optimization and to evaluate the *in-vitro* drug release studies of canagliflozin polymeric formulations.

Materials and Methods

Materials

Canagliflozin drug was purchased from Gift sample from Heet HealthcarePvt. Ltd., India and Poly vinyl alcohol &Poly Vinyl Pyrrolidone were purchased from Hi Media Laboratories Pvt. Ltd., India and SD Fine-Chem. Ltd., India respectively. Potassium DihydrogenOrthophosphate&Pluronic F127 were bought from Sigma Aldrich Pvt. Ltd., India. Eudragit E100 was purchased from Evonik Nutrition & Care GmbH, Germany.

Methods

Calibration of the drug

Calibration of the drug was performed using different media such as distilled water, 0.1N hydrochloric acid (pH- 1.2) and phosphate buffer (pH- 7.4). The λ max of the drug was observed by scanning the drug solutions between 200– 800 nm using double beam UV- Visible Spectrophotometer. The standard calibration curve was plotted using concentration vs absorbance to get the linearity and regression factor.

Nanoparticles synthesis and Characterization

Nanosuspensions were prepared by bottom-up approach named as Nanoprecipitation-Solvent evaporation method with varying concentrations of surfactants (PVA, PVP, Pluronic F 127) and polymer (Eudragit E100) as shown in Table 1.

This organic phase was added drop wise using a syringe into an aqueous solution containing the specific amount of surfactant PVA or PVP or Pluronic F-127, which was kept in a magnetic stirrer of about 1000 rpm at a constant stirring rate at the temperature of 30°C .The evaporation of organic solvent was completed in 4 to5 hours, which resulted in the precipitation of the drug and polymer in the form of nanoparticles. The synthesized polymeric nanoparticles were characterized by particles size distribution and zeta potential techniques. The entrapment efficiency was also calculated[10,8].

In-vitro drug release studies

The *in-vitro* drug release studies was analyzed by USP type-II (paddle) dissolution apparatus using dialysis bag method. In this method, about 0.5 mL of nanoformulation was sealed in a dialysis membrane bag, which was then immersed in a basket containing 100 mL of respective media (distilled water, 0.1 N Hydrochloric acid pH-1.2 and Phosphate buffer pH-7.4) and maintained at 37°C ± 2°C and stirred at 100 rpm. The basket was kept in a water bath maintained at the temperature of 37°C ± 2°C. The samples were taken away at regular intervals of time continuously for 8 hours and again replaced with same volume of media to maintain the perfect bath condition. The absorbance of the taken samples was measured using UV-spectrophotometer and the cumulative amount of drug release was calculated based on estimation of drugconcentration[11].

In-vitro drug release kinetics

In-Vitro drug release kineticsis a mathematical modeling of drug release kinetics, which exhibits a basis for the study of mass particles transport mechanisms, that are required in the control of drug release. Several models have been proposed for the description of drug release systems. The

mathematical expressions or models such as zero order, first order, Higuchi, Hopfenberg, Hixon-Crowell, Baker-Lonsdale, Kosmeyer-peppas, Weibull and Gompertz models were used to find the best fit model with highest R^2 (correlation factor) and lowest SSR (sum of squared residuals) values to describe the kinetics of drug release[12].

Table 1: Formulation of Polymeric nanoparticles of Canagliflozin

FORMULATION CODE	DRUG : POLYMER (CGF: E100)	SURFACTANT (%)
CGF 1	1:0.5	PVA – 1
CGF 2	1:1	PVA – 1
CGF 3	1:1.5	PVA – 1
CGF 4	1:0.5	PLU – 1
CGF 5	1:1	PLU – 1
CGF 6	1:1.5	PLU – 1
CGF 7	1:0.5	PVP – 1
CGF 8	1:1	PVP – 1
CGF 9	1:1.5	PVP – 1
CGF 10	1:1	PLU - 1.5
CGF 11	1:1	PLU – 2
CGF 12	1:1	PVP- 1 &PLU-1
CGF 13	1:1.5	PVP- 1 & PLU-1

Table 2: Physio-Chemical Analysis of Canagliflozin Polymeric Nanoparticles

FORMULATION CODE	DRUG : POLYMER (CGF: E100)	SURFACTANT (%)	PARTICLE SIZE (nm)	PDI	ZETA POTENTIAL (mV)	ENTRAPMENT EFFICIENCY (%)
CGF 1	1:0.5	PVA - 1	195.1	0.471	+3.33	67.44 ± 14
CGF 2	1:1	PVA - 1	438.3	0.132	+0.582	69.88 ± 8.3
CGF 3	1:1.5	PVA - 1	203.1	0.147	+7.40	100 ± 3.5
CGF 4	1:0.5	PLU – 1	90.59	0.255	+8.75	100 ± 13
CGF 5	1:1	PLU – 1	117.0	0.146	+7.72	96.71 ± 5.1
CGF 6	1:1.5	PLU – 1	117.2	0.138	+11.6	100 ± 8.4
CGF 7	1:0.5	PVP – 1	2270	0.954	+2.97	49.5 ± 0.70
CGF 8	1:1	PVP – 1	706.7	0.274	+7.07	77.93 ± 3.6
CGF 9	1:1.5	PVP – 1	181.3	0.282	+29.2	66 ± 2.82
CGF 10	1:1	PLU - 1.5	107.1	0.140	+3.83	100 ± 5.3
CGF 11	1:1	PLU – 2	90.66	0.173	+5.17	100 ± 2.75
CGF 12	1:1	PVP-1 &PLU-1	171.5	0.259	+6.55	94 ± 5.0911
CGF 13	1:!:5	PVP-1 & PLU-1	207.5	0.189	+1.64	51.62 ± 2.121

Results and Discussion

Calibration curve of Canagliflozin

The λ max of Canagliflozin in distilled water, HCl buffer (pH- 1.2) and with the phosphate buffer (pH-7.4) was found to be 290 nm (Figure 2). The serial diluted samples with known concentration of drug were analyzed using UV- Spectrophotometer and the calibration curve was plotted for different media, which showed the linearity with $R^2 = 0.99$. Hence, the drug obeyed Beer Lambert's law for the concentration range of5 to 25 µg/mL, in distilled water, HCl and phosphate buffer media.

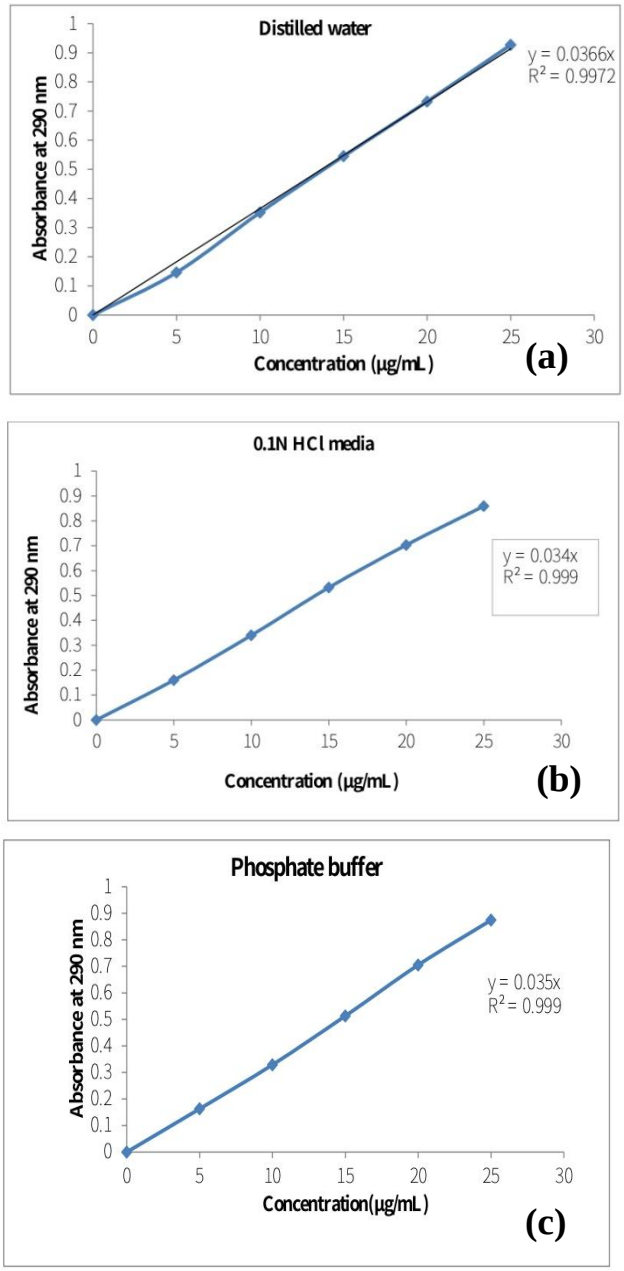


Figure 2: The λ max of Canagliflozin in distilled water (a), HCl buffer (b) and with the phosphate buffer (c).

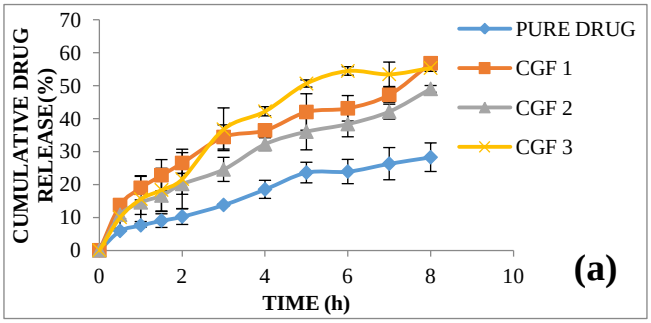
Nanoparticles synthesis and characterization

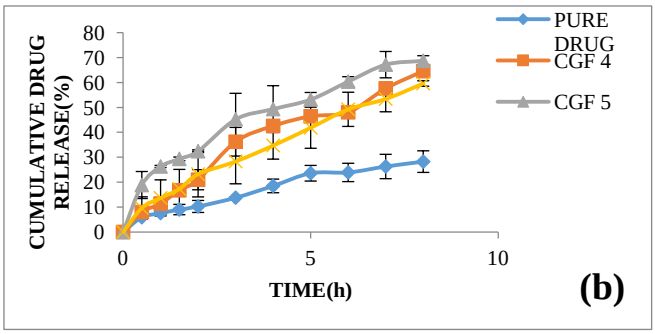
The formulated Canagliflozin polymeric nanoparticles confirmed the average size variation between 90 to 706nm for various ratio of surfactants. The polydispersity index of the CGF 12 nanoparticles was 0.259, which confirmed the monodisperse nature of the particles. The zeta potential value of +6.55mV showed the moderate colloidal stability of the nanosuspension[13].The %EE of drug in the prepared nanosuspension was in the range of 49.5 ± 0.70 to 100 ± 2. Similarly, Guncum *et.al.,* have reported that their amoxicillin polymeric nanoparticles showed average size at 300 to 500 nm, z-value at -30 to -47 mV and EE% of the amoxicillin polymeric nanopartciles at 26 to 62%[14]. Hence, the optimized formulation was selected based on the improvement in drug dissolution and the formulations were lyophilized for better stability.

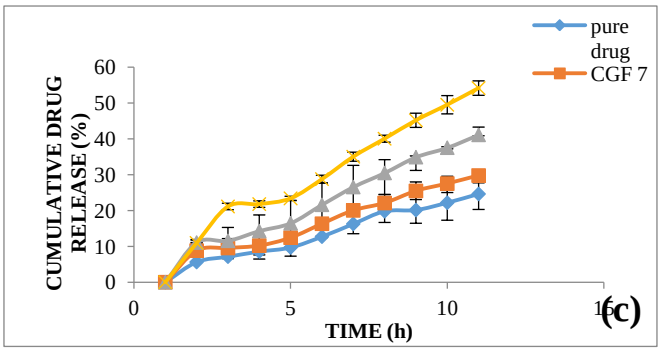
In-vitro drug release studies

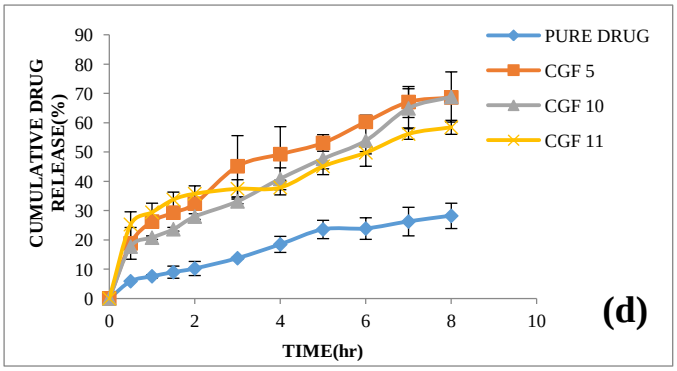
In-vitro drug release studies of Canagliflozin-Eudragit E100 polymeric nanoparticles was studied with different media like distilled water, HCl buffer (pH-1.2) and phosphate buffer (pH-7.4) media, and compared with the pure drug aqueous dispersion. In distilled water, the pure drug sample exhibited only 28% drug release at the end of 8 hours due to its poor aqueous solubility. The nanoparticles prepared with PVA (1%) as surfactant CGF 1, 2 and 3 showed about 55% drug release at the end of 8 hours. When comparing the pure drug, the nanoformulation showed an enhanced dissolution of the drug as shown in Figure 3 (a). The nanoparticles prepared with Pluronic (1%) as surfactant CGF 4, 5 and 6 showed about 64 to68% drug release at the end of 8 hours. However, the increase in the polymer ratio did not display proportionate increase or decrease in the drug release. Among the three formulations (CGF 4, 5 and 6), the formulation containing drug: polymerat 1:1 ratio was considered to exhibit linear and optimum drug release profile Figure 3 (b).Due to stability issues, the PVP surfactant based polymeric nanoparticles (CGF 7, 8, 9) displayed lesser drug release compared to other surfactant stabilized nanosuspensions (Figure 3c). For the enhancement of drug release, the percentage of pluronic surfactant was increased from 1% to 1.5% and 2%. But, these formulations exhibited the maximum of only 68% of drug release in distilled water at the end of 8 hours. Increase in concentration of surfactant does not exhibit proportionate increase in drug release (Figure 3d). Similar profile was observed for the formulations CGF 5, 10, 11 in other 0.1 N HCl and Phosphate buffer with maximum drug release of 68% (Figure 3e and f). To improve the stability and dissolution of the polymeric nanosuspensions, formulations were

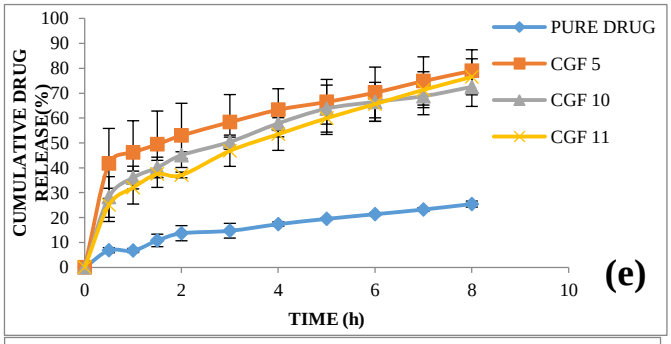
trailed with combination of both PVP & Pluronic surfactant and drug release profile obtained in different media (distilled water, 0.1N HCl media and phosphate buffer media), which displayed the maximum drug release as shown in Figure 3 (g) (h) and (i). While comparing CGF 12 and CGF 13 with respect to the particle size, zeta potential, entrapment efficiency and drug release profile, CGF 12 was considered as optimum formulation with maximum drug release of 100% in distilled water within 3 hours, 93% in HCl media at the end of 8 hours and reached 100% in phosphate buffer media within 2 hours.

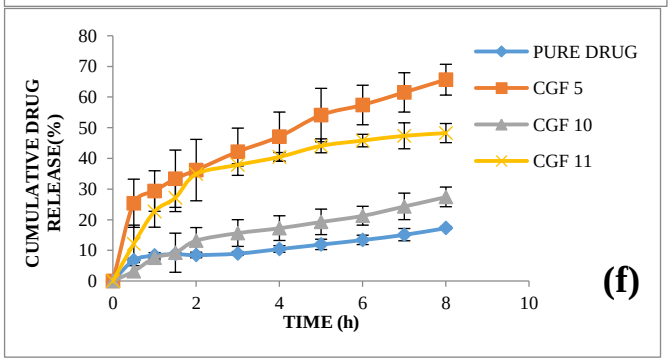


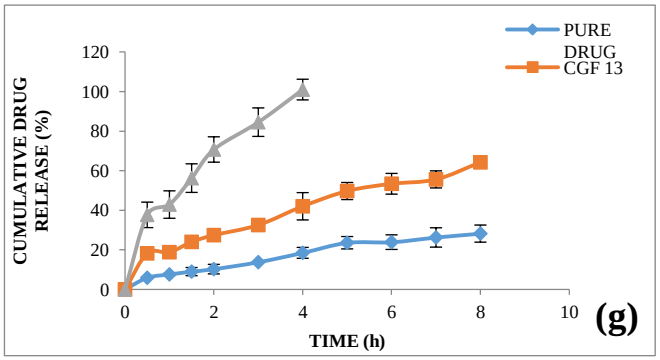


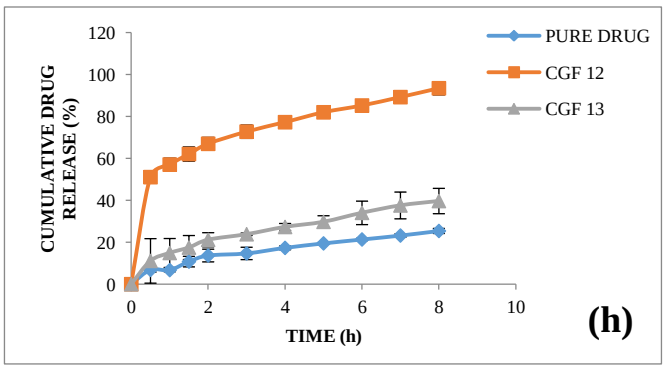












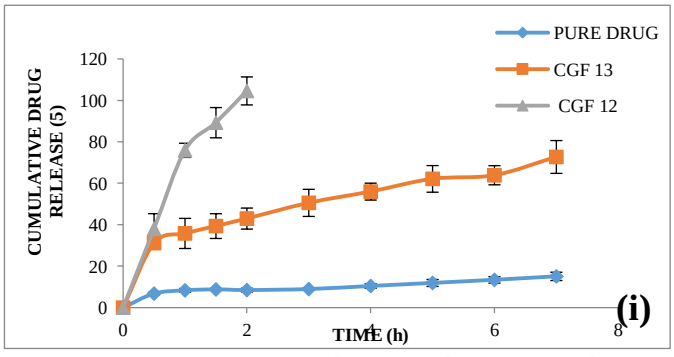


Figure 3: *In-vitro* drug release studies of Canagliflozin-Eydragit E100 with 1% PVA (a), 1% Pluronic (b) & 1% PVP (c) surfactant in distilled water. *In-vitro* drug release studies of Canagliflozin-

Eudragit E100 with Pluronic (1%, 1.5%, 2%) surfactant in distilled water (d) and 0.1 N HCl media €, *In-vitro* drug release studies of Canagliflozin-Eudragit E100 with Pluronic (1%, 1.5%, 2%) surfactant in phosphate buffer (f). *In-vitro* drug release studies of Canagliflozin-Eudragit E100 with PVP & Pluronic acid (1%) surfactant in distilled water (g), 0.1 N HCl (h) and Phosphate buffer (i).

***In-vitro* drug release kinetics**

The drug release kinetics of the formulations was established by fitting the *in-vitro* drug release data in different kinetic models to find the excellent fit. The kinetic model, which showed highest R^2 value and lowest SSR was considered. The *in-vitro* drug release of the polymeric drug nanoparticles in 0.1 N HCl (pH-1.2) and phosphate buffer (pH-7.4) followed by Weibull model, which confirmed that the nanoparticles are matrixtype. The Canagliflozin polymeric nanoparticles followed Korsemeyer Peppas model indicating that the drug release was predominantly influenced by diffusion mechanism. The n-value in Korsemeyer- Peppas was found to be less than 0.4, which confirmed that the drug release from the polymeric nanoparticles followed the Fick's law of diffusion.

Table 3: Drug release kinetics of Canagliflozin nanoparticles in distilledwater

		CGF 1	CGF 2	CGF 3	CGF 4	CGF 5	CGF 6	CGF 7	CGF 8	CGF 9	CGF 10	CGF 11	CGF 12	CGF 13
Zero	**R^2**	0.93	0.90	0.85	0.94	0.73	0.95	0.55	0.86	0.94	0.88	0.27	0.38	0.80
	K_0	6.68	6.71	8.55	8.72	10.29	8.08	3.94	6.08	5.11	9.45	8.78	10.17	24.31
	SS	188.34	262.82	602.12	271.55	1261.41	198.88	272.39	252.24	90.85	557.54	1927.85	2281.19	5548.64
First	**R^2**	0.97	0.96	0.96	0.99	0.92	0.99	0.64	0.92	0.97	0.95	0.55	0.68	0.58
	K_1	0.09	0.09	0.13	0.13	0.17	0.11	0.05	0.08	0.06	0.14	0.14	0.17	0.94
	SS	80.15	108.66	175.67	64.39	381.90	43.92	216.70	141.71	39.97	239.75	1182.08	1172.00	11285.53
Higuchi	**R^2**	0.92	0.94	0.95	0.93	0.99	0.95	0.97	0.97	0.95	0.97	0.89	0.92	0.99
	K_H	15.55	15.73	20.19	20.29	24.64	18.86	9.54	14.36	11.93	22.27	21.50	24.78	57.91
	SS	208.56	156.75	192.43	319.91	26.23	175.02	20.72	50.23	72.47	149.39	297.98	293.63	205.78
Korsemeyer Peppas	**R^2**	0.97	0.97	0.97	0.98	0.99	1.00	0.98	0.98	0.99	0.98	0.97	0.97	0.99
	K_{KP}	10.71	11.91	16.82	13.91	24.94	13.40	10.97	12.27	8.57	18.55	28.31	31.27	54.98
	SS	70.76	71.69	125.12	73.12	25.79	8.64	10.80	28.25	8.63	80.45	85.41	100.27	162.53
	n	0.73	0.67	0.62	0.74	0.49	0.71	0.41	0.60	0.71	0.62	0.32	0.35	0.53
Hixson-Crowell	**R^2**	0.96	0.94	0.93	0.98	0.87	0.98	0.61	0.91	0.96	0.93	0.47	0.60	0.89
	K_{HC}	0.03	0.03	0.04	0.04	0.05	0.03	0.02	0.02	0.02	0.04	0.04	0.05	0.24
	SS	103.57	146.46	272.44	96.18	585.21	71.31	234.24	171.69	53.46	300.18	1398.72	1473.84	2962.62
Hopfenberg	**R^2**	0.97	0.96	0.96	0.99	0.92	0.99	0.64	0.92	0.97	0.95	0.55	0.68	0.59
	K_{HB}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
	SS	80.26	108.83	175.81	64.46	382.24	43.95	216.78	141.85	40.11	239.90	1182.95	1172.96	11171.50
Baker-Lonsda	**R^2**	0.90	0.92	0.93	0.90	0.99	0.93	0.97	0.96	0.94	0.94	0.92	0.95	0.53
	K_{BL}	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.06
	SS	262.71	200.44	263.24	455.60	60.88	272.35	17.20	72.32	93.00	265.91	207.29	188.55	12626.73
Weibull	**R^2**	0.98	0.98	0.98	0.99	0.99	0.99	0.98	0.98	0.99	0.97	0.96	0.96	0.58
	α	10.01	8.41	5.32	7.13	3.62	8.39	8.66	8.29	11.79	7.50	3.00	2.66	1.31
	SS	63.01	61.27	86.30	59.54	45.57	21.21	11.73	36.58	8.96	131.71	104.96	141.58	11239.36
Gompertz	**R^2**	0.96	0.97	0.98	0.98	0.97	0.97	0.97	0.95	0.98	0.92	0.94	0.94	0.54
	α	2.55	2.36	1.99	2.33	1.42	2.21	2.21	2.15	2.62	1.72	1.24	1.13	0.32
	SS	98.02	78.95	93.58	103.54	153.53	123.20	18.42	89.74	27.93	357.73	149.06	229.97	12405.89

Table 4: Drug release kinetics of Canagliflozin polymeric nanoparticles in 0.1 N HCl

		CGF 9	CGF 10	CGF 11	CGF 12	CGF 13
Zero	**R^2**	0.65	0.47	0.62	-0.29	0.700
	K_0	7.73	11.88	11.31	15.16	5.83
	SS	853.30	2782.67	1944.87	8683.98	433.07
First	**R^2**	0.82	0.83	0.87	0.77	0.81
	K_1	0.11	0.23	0.21	0.59	0.07
	SS	452.17	866.73	641.39	1527.14	276.45
Higuchi	**R^2**	0.99	0.97	0.98	0.73	0.99
	K_H	18.62	28.89	27.27	37.80	13.98
	SS	23.14	181.72	79.84	1808.10	7.80
Korsemeyer- Peppas	**R^2**	1.00	1.00	0.99	1.00	1.00
	K_{KP}	20.10	34.78	30.18	57.37	14.57
	SS	12.17	12.88	38.57	10.48	6.08
	n	0.45	0.38	0.44	0.23	0.47
Hixson-Crowell	**R^2**	0.77	0.75	0.82	0.65	0.78
	K_{HC}	0.03	0.06	0.06	0.16	0.02
	SS	564.63	1305.04	937.09	2353.40	323.03
Hopfenberg	**R^2**	0.82	0.83	0.87	0.77	0.81
	K_{HB}	0.00	0.00	0.00	0.00	0.00
	SS	452.70	867.10	641.66	1528.78	276.62
Baker-Lonsdale	**R^2**	0.99	0.99	0.99	0.92	0.99
	K_{BL}	0.01	0.02	0.02	0.05	0.00
	SS	15.57	43.67	62.27	517.21	7.41
Weibull	**R^2**	0.99	0.99	0.98	0.99	0.99
	α	4.53	2.38	2.84	1.17	6.42
	SS	20.62	51.88	95.76	68.92	9.09
Gompertz	**R^2**	0.98	0.97	0.95	0.98	0.98
	α	1.61	1.04	1.18	0.52	1.94
	SS	58.61	167.52	249.48	164.66	25.93

Table 5: Drug release kinetics of Canagliflozin polymeric nanoparticles in Phosphate buffer media

		CGF 9	CGF 10	CGF 11	CGF 12	CGF 13
Zero	R^2	-0.26	0.79	0.38	0.44	0.02
	K_0	8.48	3.55	7.78	11.65	27.56
	SS	2769.55	134.05	1484.47	2767.75	23951.81
First	R^2	0.09	0.84	0.64	0.79	0.27
	K_1	0.13	0.04	0.11	0.22	2.88
	SS	1989.81	98.87	852.41	1039.08	17867.21
Higuchi	R^2	0.63	0.98	0.93	0.95	0.85
	K_H	21.02	8.48	19.07	28.35	68.25
	SS	818.58	12.68	175.42	251.84	3753.04
Korsemeyer-Peppas	R^2	0.63	0.98	0.93	0.95	0.00
	K_{KP}	21.02	8.48	19.07	28.35	47.38
	SS	818.58	12.68	175.42	251.84	9172.92
	n	0.17	0.67	0.45	0.34	0.40
Hixson-Crowell	R^2	-0.01	0.83	0.57	0.70	0.30
	K_{HC}	0.04	0.01	0.03	0.06	0.20
	SS	2224.61	109.84	1039.35	1454.97	6451.90
Hopfenberg	R^2	0.09	0.84	0.64	0.79	1.00
	K_{HB}	0.00	0.00	0.00	0.00	0.55
	SS	1990.53	98.95	852.65	1039.35	0.01
Baker-Lonsdale	R^2	0.69	0.98	0.95	0.98	0.35
	K_{BL}	0.01	0.00	0.01	0.02	0.06
	SS	672.59	14.08	108.61	118.37	5968.53
Weibull	R^2	0.88	1.00	0.99	0.98	1.00
	α	2.49	9.54	2.99	2.36	0.31
	SS	254.40	2.51	14.68	119.64	0.17
Gompertz	R^2	0.87	0.99	0.99	0.95	1.00
	α	1.08	2.60	1.49	1.03	0.04
	SS	288.79	3.76	26.72	252.17	1.43

Conclusion

The nanoparticles had a polydispersity index of 0.259 and an average particle size of 171nm, which confirmed the monodisperse uniform particles. *In-vitro* drug release studies revealed that the pure drug sample exhibited only 28% drug release at the end of 8 hours in distilled water media due to its poor aqueous solubility. Nanoformulations of canagliflozin showed 55 to 90% drug release at various time intervals. Among them, CGF 12 was considered as optimum formulation with maximum drug release of 100% in distilled water within 3 hours, 93% in HCl media at the end of 8 hours and reached 100% in phosphate buffer media within 2 hours. Thus, This study suggests that CGf-12 formulation have great efficiency to improve the physicochemical properties of drug and this formulation showed maximum drug release which confirmed it has great potential to improve the drug solubility of canagliflozin.

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