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Formulation Optimization and Evaluation of Novel Injectable, Thermo Responsive and Cytocompatible Gel for Sustained Drug Delivery

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Abstract : The aim of the present work was formulation and evaluation of *in situ* gelling system of furosemide. Furosemide is a loop diuretic, which exhibits short half life, when given in the form of conventional injectable solutions. To overcome this, an attempt has been made to formulate temperature sensitive *in situ* gelling system of furosemide to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in temperature. The furosemide *in situ* gelling system is formulated by using polyethylene oxide and carbopol 934P which acted as drug carrier and viscosity enhancing agent respectively. All the formulations were evaluated and the results of the study showed that 0.7% to 0.9% of polyethylene oxide produces consistent, maximum and sustained drug release. The formulations were clear liquid appropriate for injection of subcutaneous route. Gelation temperature all the formulations were found in between 32^0c-42^0c and gelation time varying from 2-5 minutes. pH was found to be around 7.4. Viscosity was found out which have rheological properties. The drug content of the prepared formulation was found to be within the range of 89-99.9%. The optimized formulation F4, F5 & F6 showed sustained drug release upto 13 hours.

Keywords : Injectable *in-situ*gel, thermo sensitive, sol-gel transition, sustained release.

Introduction

Drug administration can be done through several routes such as oral, transdermal or parenteral. Of all the routes, parenteral route has more advantages over both oral & transdermal because in parenteral drug delivery system, drug reaches to systemic circulation with rapid absorption. But due to the drawback of rapid decline of drug concentration in systemic circulation, an attempt has been made to overcome this problem by developing extended release drug delivery system.[1,2]

Injectable drug delivery system has received much attention over the last few years due to several advantages. The advantages may includeease of application, prolonged drug release, decreased drug dose, frequency and better patient compliance etc. Microspheres, gels, suspensions, in situ forming implants etc. can be administered through injectable drug delivery system.[1,3]

*In-situ*gel systems are those which transform into gel from liquid phase at certain environmental conditions. The phase transformation may be thermo sensitive or pH or any other critical condition with respect to polymeric formulation. So a type of smart polymers are selected which have the ability to change its microstructure when in contact with certain enzymes, ions, or temperature etc. Moreover these systems are

designed in such a way that once injected, the formulation responds to a change in the environment to give a high viscosity or solid depot at the injection site. Thus those polymers with thermo sensitive parameters exist as a mobile viscous liquid at reduced temperatures but form a rigid semisolid gel network with an increase in temperature.[4,5,6]

Materials and Methods

Materials

Furosemide was purchased from Otto chemicals, Mumbai, India. Polyethylene oxide was bought was from Yarrow chem., Mumbai, India. Carbopol 934 was purchased from yarrow chem. Trading ltd., Mumbai, India. All other chemicals and solvents used were of analytical grade.

Methods

Drug-Excipients Compatibility Study

1. Differential Scanning Calorimetry (DSC)

DSC can be used to determine the nature and specification of crystallinityof drug and excipients through measurement of glass transition temperature and melting point temperature and their associated enthalpies. This technique has been used to study the physical and chemical interaction between drug and excipients. Firstly, required amount of furosemide was taken to obtain DSC curve and a physical mixture offurosemide and poly ethylene oxidewas also performed using DSC4000**,** Perkin Elmer.Samples were taken andsealed in aluminium pans and analyzed in an atmosphere of air at flow rate of 25 mL/min. A temperature range of 30°C to 400°C was used where rate of heating was 10°C/min.[7,8]

2. Fourier transform infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectra of furosemide was performedindividually and a physical mixture of both drug and polyethylene oxide were recorded using potassium bromide mixing method on FTIR instrument. Required amount of furosemide and polymer individually was kept on the sample holder and scanned from 400 $^{-cm}$ to 4000 $^{-cm}$ using FT-IR, Alpha, Bruker, Germany, to evaluate the physical state of the drug.[7,8]

Spectrophotometric Estimation of Furosemide

Determination of λ_{max} of Furosemide

Furosemide was dissolved in phosphate buffer having pH 7.4. The UVspectrum of the solutions was taken on Shimadzu (uv-1800) UVSpectrophotometer. The solutions exhibited UVmaxima at 277.0 nm.[8]

Preparation of Standard curve of Furosemide

10 mg of furosemide was weighed accurately and transferred to a 100ml volumetric flask which is then dissolved in 100ml phosphate buffer pH 7.4 to prepared stock solution (100µg/ml). Then 10ml of above solution was takenand diluted it with 100 ml phosphate buffer pH 7.4 in 100ml volumetric flask to prepare the solution(10µg/ml). Volumes of 2, 4, 6, 8 & 10 ml were taken in 10 ml volumetric flask from the prepared solution and diluted upto the mark with pH 7.4 phosphate buffers. Absorbance of the resulting solution was measured at 277.0 nm against a blank solution prepared without drug using Shimadzu UV Spectrophotometer. Calibration curve was prepared by plotting concentration versus absorbance as shown in Figure 1.[8]

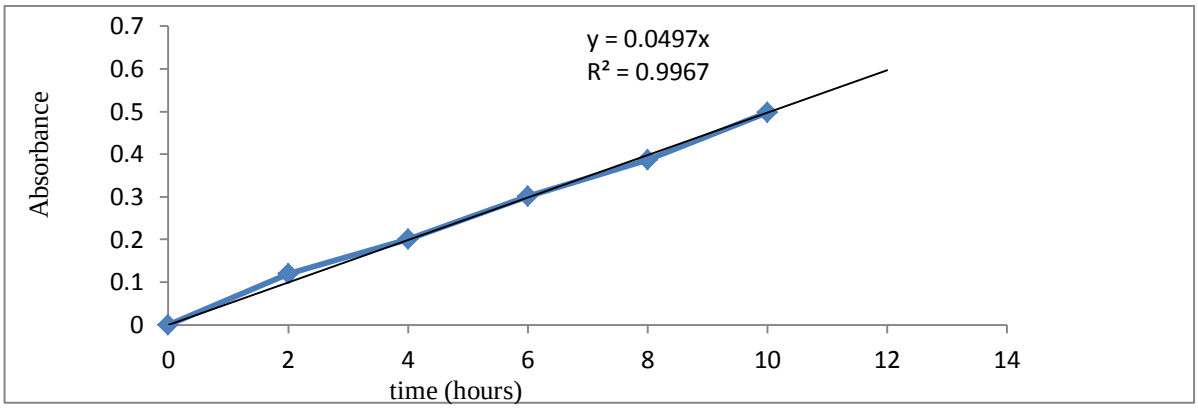

Figure 1: Standard Curve of Furosemide.

Preparation of thermo sensitive *in situ* gel formulations

For the preparation of *in situ* gel formulations, cold method was followed. Polyethylene oxide was first added to distilled water withcontinuous stirring for several hours and kept in refrigeratorand stirred periodically until clear homogenous solutions were obtained (approximately 24 hrs). By the time swelling took place and homogeneous distribution of particles were seen. Carbopol 934 was added to the polyethylene oxide solution in certain concentrationsin some particular formulations uniformly. Required quantity of furosemide was dissolved in slightly basic solution of sodium hydroxide separately and then it was added to polymer solutions under constant stirringuntil a uniform solution was obtained. Finally dilute hydrochloride acid was added to adjust the pH. Thus different concentrations ofpolymer and composition of other ingredients used for formulation of*in situ* gel are shown in Table no.1.[9,10,11]

Table no. 1: Composition of prepared in-situ gel

Formulation code	Furosemide (gm)	Polyethylene oxide (gm)	Carbopol 934 (gm)	NaOH solution (pH 10.5) (ml)	Dilute HCl (ml)	Dist. Water (ml)
F1	0.3	0.4	0.01	q s to 13	qs to 10	qs to 15
F2	0.3	0.5	0.03	q s to 13	qs to 10	qs to 15
F3	0.3	0.6	0.04	q s to 13	qs to 10	qs to 15
F4	0.3	0.7	-	q s to 13	qs to 10	qs to 15
F5	0.3	0.8	-	q s to 13	qs to 10	qs to 15
F6	0.3	0.9	-	q s to 13	qs to 10	qs to 15
F7	0.3	1.0	0.001	q s to 13	qs to 10	qs to 15
F8	0.3	1.1	0.002	q s to 13	qs to 10	qs to 15
F9	0.3	1.2	0.001	q s to 13	qs to 10	qs to 15

Characterisation of Thermo Sensitive In-Situ Gel

1. Appearance

All prepared formulations were evaluated by visual inspection. They were critically observed for clarity and transparency.[12]

2. Gelation Temperature

The estimation of gelation temperature was done by heating the solution in a thin walled tube that was placed in a low temperature digital water bathwith gentle shaking. A thermometer was placed in the sample solution and heated at the rate of 1°C/min with continuous stirring. The temperature or the point where gel formation was observed with no flow of liquid was considered as sol-gel transition temperature.[8,13]

3. Gelation Time

The gelation time was determined by test tube inverting method. Solution was taken in a thin walled tube and kept at the respective gelation temperature on a water bath. The test tube was taken out every 1 min and inverted to observe the state of the sample. The gelation time was determined by flow of the sample.[7,14]

4. Gel Melting Temperature

The sample was taken in a test tube. On heating causes gel and further heating causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature was noted and regarded as gel melting temperature.[8,15,16]

5. pH

This parameter is one of the important factors in the formulation of *in situ* gel because the solubility and stability of the formulation are directly related to it. All the formulations were prepared and then evaluated for pH byusing digital pH meter.[7,8,14]

6. SyringeAbility

All prepared formulations were transferred into an identical 5 ml syringe placed with 20 gauge needle to a constant volume (1 ml). The solutions which were easily passed from syringe was termed as pass anddifficult to pass were termed as fail.[8,15,17]

6. Viscosity

The viscosity of all the prepared formulations was measured using Digital Brookfield viscometer (LVDV-E). The measurements were carried out using spindle no.64. The readings are taken in centipoises (CP) against shear rate or rotation per minute.[16,17]

7. PercentDrug Content Determination

Accurately weighed amount of gel equivalent to 2mg of drug was taken in a 100ml volumetric flask. Phosphate buffer (pH 7.4) was added to it and kept on magnetic stirrer to dissolve the drug. The volume wasmade to 100ml with phosphate buffer (pH 7.4) and filtered using 0.45µm filter paper. 10ml aliquot of the abovesolution was taken and diluted to 100ml with phosphate buffer (pH 7.4). The absorbance of sample solutionwas determined at 277 nm against phosphate buffer (pH 7.4) by using UV-Visible Spectrophotometer-1800(Shimadzu, Japan).[7,8,17]

8. In vitro Drug Release Studies

The in vitro release of furosemide from the formulations was studied through cellophane membraneusing Franz diffusion cell. The dissolution medium was phosphate buffer (pH 7.4).Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of aspecifically designed glass cylinder (open at both ends and of 2 cm diameter). A selected volume of theformulation was accurately pipette into this assembly. The cylinder was attached to the metallic driveshaft andsuspended in 100 ml of dissolution medium maintained at 37± 0.5°C so that the membrane just touched thereceptor medium surface. The dissolution medium was stirred continuously using magnetic stirrer.Aliquots, a sample was withdrawn at regular intervals and replaced by an equal volume of the receptor medium.The aliquots were diluted with the receptor medium and were analyzed by UV-VIS spectrophotometer.[7,15,16,17]

Results and Discussions

Drug- Excipients Compatibility Study

1. Differential Scanning Calorimetry (DSC)

The DSC results provided both qualitative and quantitative information about the physicochemical state of the drug present in formulation. The thermograph of pure furosemide showed a melting endothermic peak at128^{0}C while in the thermograph of mixture peaks it was observed at 128^{0}C. The DSC thermo grams of

themixture showed distinct endothermic peaks for furosemideand the polymer. This corresponds to the peaks of individual drug and polymer without exhibiting any modification which indicates that the drug did not interactwith excipients used in the injectable gel. This confirmed that the presence of other excipients did not affect the drugstability. The thermograph is shown in Figure2.

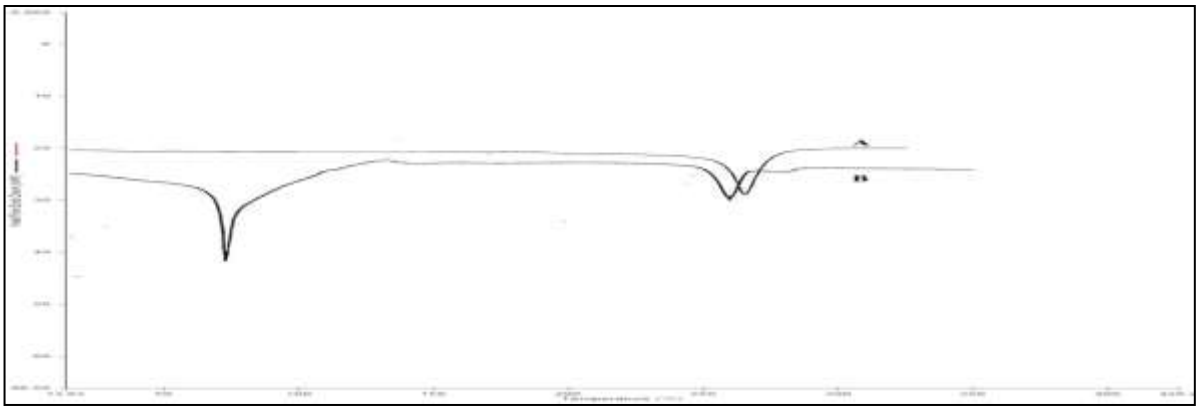

Figure 2:Thermogram(DSC) of furosemide with polymer mixture

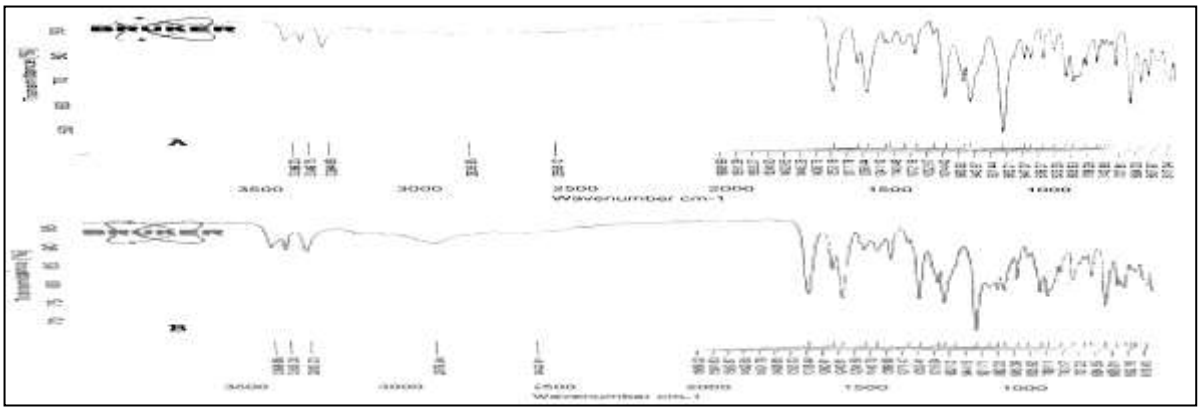

Figure 3: FTIR study of furosemide with polymer mixture

2. Fourier transform infrared (FTIR) Spectroscopy

The FTIR spectra of physical mixture of Furosemide and polyethylene oxide and optimized formulation of injectable are shown in Figure3. From this it is clear that there is no interaction between Furosemide and polymers.

Evaluation of *In Situ* Gels

1. Appearance

All the formulations were checked for clarity.The clarity of all the formulationwas evaluated by visual inspection under white background and was found as shown in Table no.2.

Table no. 2: Evaluation Parameters of in-situ gel

Formulation code	Gelation temperature (^{0}C)	Gelation time (min)	Gel melting temperature (^{0}C)	pH	% drug content	Syringe ability (pass/fail)	Appearance
F1	39.3 ± 0.5	< 5	69.4 ± 0.5	6.7 ± 0.4	97.06	Pass	clear
F2	38.6 ± 0.5	<5	67.6 ± 0.5	6.9 ± 0.5	96.77	Pass	Clear
F3	35.4 ± 0.5	<5	68.5 ± 0.5	7.4 ± 0.6	96.73	Pass	Clear
F4	36.5 ± 0.5	<5	70.5 ± 0.5	7.4 ± 0.4	98.91	Pass	Clear
F5	35.6 ± 0.5	<5	72.7 ± 0.5	7.3 ± 0.4	99.79	Pass	Clear
F6	34.7 ± 0.5	<5	74.2 ± 0.5	7.4 ± 0.6	95.16	Pass	Clear
F7	31.4 ± 0.5	<5	78.1 ± 0.5	8.1 ± 0.7	RF	Fail	clear
F8	32.3 ± 0.5	<5	77.7 ± 0.5	8.0 ± 0.4	RF	Fail	Clear
F9	33.3 ± 0.5	<5	78.8 ± 0.5	8.1 ± 0.5	RF	Fail	clear

***RF** stands for rejected formulation

2. Gelation Temperature

The prepared formulations showed a wide range of gelation temperature. It was found that as the concentration of polymer increases the gelation temperature of the formulation decreases. Gelation temperature of all the formulations is shown in Table no.2

3. Gelation Time

The time for gelation of all the formulations were observed and noted. The time for gelation of all the formulations were found to be less than 5 minutes.They are shown in Table no.2

4. Gel Melting Temperature

The temperature for melting of gel has been recorded. The values found are indicated in Table no.2

5. pH

The formulations were evaluated for pH as per described in material and methods.pH of formulation code such as F3, F4, F5, F6 were close to physiological pH. All other pH values were noted as shown in Table no.2

6. Syringe Ability

Syringe ability of all the prepared formulations were checked as per material and methods. Syringe ability of all formulations is shown Table no.2. The formulation code F1 to F6 was found to pass through the needle gauze size of 20.

7. Viscosity

Viscosities of all formulations were measured using Brookfield digital Viscometer. The gel under study was placed in the spindle S64 at different rpm at 37^{0}C. As the RPM increases, viscosity decreases from 13600 to 380 in F4, 14880 to 430 in F5 and 16240 to 480 in F6. The graph of viscosities of the optimized formulations (i.e. F4, F5, F6) are cited in Figure4.

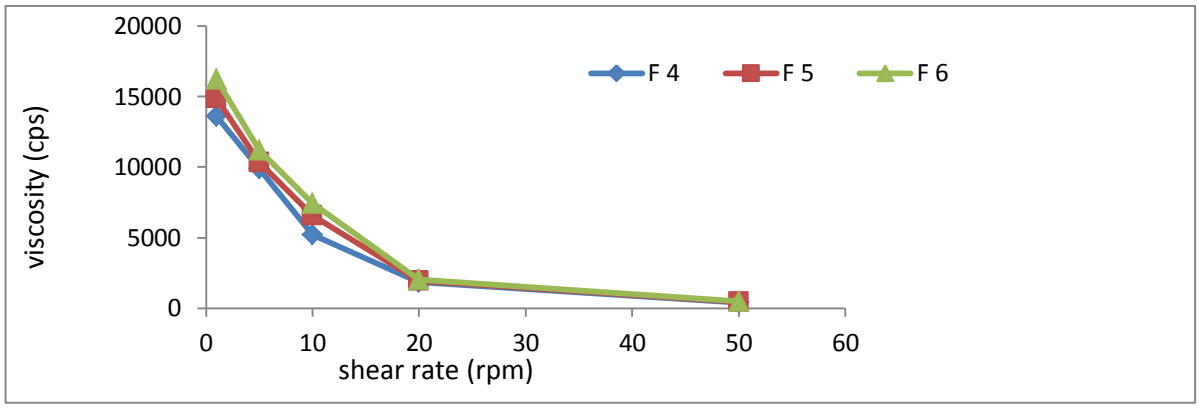

Figure 4.Viscosity of optimized formulations

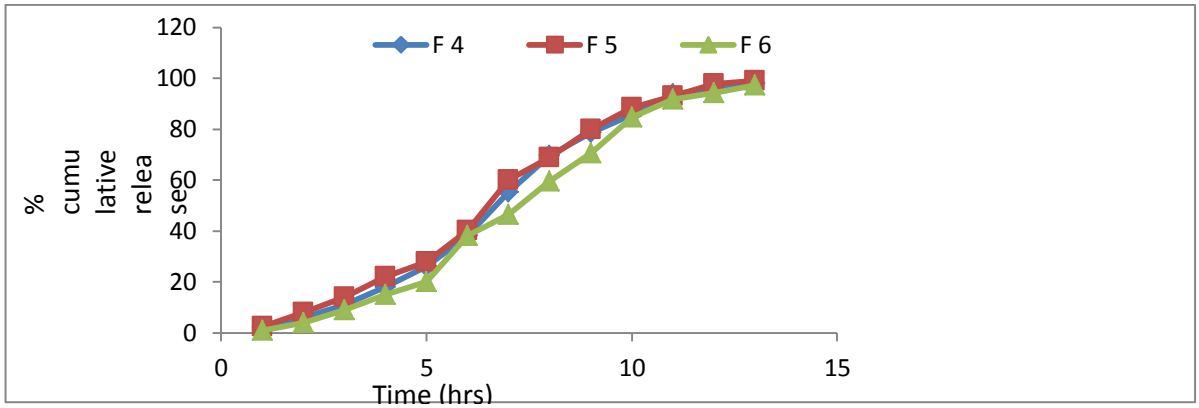

Figure 5: Graphical representation of cumulative release of drug from optimized formulations

Figure 5(a): Mathematical Models for the Diffusion Data.

Formulation code	Zero-order (r^2)	First-order (r^2)	Higuchi (r^2)	Korsemeyer-Peppas (r^2)(n)	
F4	0.9417	0.8015	0.7038	0.9628	1.248
F5	0.9546	0.8170	0.7283	0.9671	1.248
F6	0.9279	0.7830	0.6701	0.9712	1.248

8. Percent Drug Content Determination

Drug content of all batches was found to be in the range of 89.91 to 99.79. This indicates the uniformity of drug content. All the drug content percentage are stated inTable no.2

9. *In vitro* Drug Release Studies

The *in vitro* release of furosemide from the formulations was studied and evaluated as per the process indicated in materials and methods.The formulations having optimum viscosity with highest percentage of drug release are shownTable no.3. Graphical representation of cumulative drug release is shown in Figure5.

All the release data of *in vitro* drug release study was applied in various kinetic models and the release mechanism was found to be diffusion which is reported inFigure5(a). All the formulations showed a greater linearity (r^2= 0.96-0.98) indicating the korsemeyer-peppas model of diffusion. Further study on diffusion exponent (n) of korsemeyer-peppas equation (n>0.89) indicated the release mechanism as super case II transport.[19,20,21]

Table no. 3: Percentage cumulative release of furosemide

Time (hours)	%age of cumulative drug release		
	F4	**F5**	**F6**
1	2.295	2.5	1.09
2	6	8.05	4.01
3	11.05	14.08	9.04
4	18.11	22.14	15.09
5	26.18	28	20.15
6	38.26	40.28	38.2
7	55.38	60.1	46.38
8	69.55	68.89	59.46
9	78.69	79.92	70.59
10	85.78	88.47	84.7
11	93.85	92.97	91.84
12	95.93	97.71	94.26
13	97.95	99.04	97.18

Conclusions

The novel injectable thermo sensitive *in situ* gelling drug delivery was successfully formulated by using polyethylene oxide and carbopol 934P. The formulated injectable *in situ* gelling systems were characterized for appearance, clarity, pH, gelation and gel melting temperature, rheological character, *in-vitro* drug release in PBS 7.4 fluid. The formulation was slightly viscous liquid at room temperature and underwent rapid gelation upon raising the temperature. Thus from the study it can be concluded thattemperature sensitive injectable gel can be used to achieve sustained drug release over 13 hour period of time.So, this formulation is an alternate to conventional parenteral formulation of present drug to improve the bioavailability through its longer residence time and ability to sustain drug release. By reducing the frequency of administration this formulation may improve patient compliance.

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