Original Article

Development and Evaluation of Injectable Hydrogel as a Controlled Drug Delivery System for Metformin

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Abstract

Aim: Chitosan-dialdehyde cellulose/DAC-based injectable hydrogel for controlled release of Metformin. **Materials and Methods:** Biomaterial-based injectable hydrogel was prepared by incorporating chitosan and dialdehyde cellulose. Dialdehyde cellulose (A cross-linker) was prepared by periodate oxidation method. The antidiabetic agent metformin was easily mixed with the chitosan and dialdehyde cellulose cross-linked solution, for the controlled drug delivery applications. The prepared injectable hydrogel showed the shear thinning property. **Results:** IR spectra confirmed the presence of cross-linked network between chitosan and dialdehyde cellulose. The physical appearance, injectability, pH, sol–gel phase transition, drug content, DSC, FTIR, and SEM studies were investigated. DSC and SEM studies revealed the degradation pattern and the topographical nature of prepared injectable hydrogel, respectively. The %drug release of metformin was found to be 87.25% prolonged for 84 h. The drug release pattern revealed the effective controlled drug delivery of metformin as compared to marketed tablet formulation. **Conclusion:** The study suggested that the controlled drug delivery system can be incorporated into the injectable hydrogel system; it would be more potential as compared to conventional controlled drug delivery system and preformed hydrogel system.

Keywords: *Controlled drug delivery, FTIR, injectable hydrogel, metformin, shear-thinning property*

Introduction

Controlled drug release coatings have been around for more than 50 years and their performance has increased significantly since the beginning. The main limitations of conventional controlled drug delivery system are its decreased systemic availability, poor *in vitro, in vivo* co-relation, poor bioavailability, high-dose requirements, possibility of dose dumping, adverse side effects, low therapeutic indices, development of multiple drug resistance, and nonspecific targeting.[1,2] To overcome this limitation, researcher focused on injectable hydrogel system.[3] These is the emerging trend in the field of biomaterial drug delivery system.[4] They overcome the limitation of preformed hydrogel, as they are injected with minimum invasive procedure into target sites and used for irregularly shaped sites. They have biocompatibility with the living tissue, that is, they have flexibility similar to natural tissue.[5,6] The recent studies of injectable hydrogel for the drug delivery applications suggested that shear thinning injectable hydrogel is capable of entrapping and systematically delivering the therapeutic agents like Metformin.[7-9] The article mainly emphasizes the shear-thinning property of Injectable hydrogel. In particular, *in situ* forming injectable hydrogel, the shear-thinning property of the gel plays an important role; it is the state of art that the clear free-flowing polymer sol transforms into viscoelastic gel upon exposure to the stimuli such as pH and temperature [Figure 1]. Injectable hydrogels have received much attention and they have expected to be a promising biomaterial for controlled drug delivery system and various biomedical applications.[10,11] Herein, we report a biomaterial-based injectable hydrogel derived from two natural polymers such as chitosan and dialdehyde cellulose with the incorporation of metformin for controlled drug delivery applications in diabetic patients.

Materials and Methods

Materials

Chitosan (cell culture tested-high–molecular-weight grade) was obtained from Himedia, Mumbai; hydroxyethyl cellulose and sodium metaperiodate were obtained from Loba chemicals. Metformin tablets were purchased

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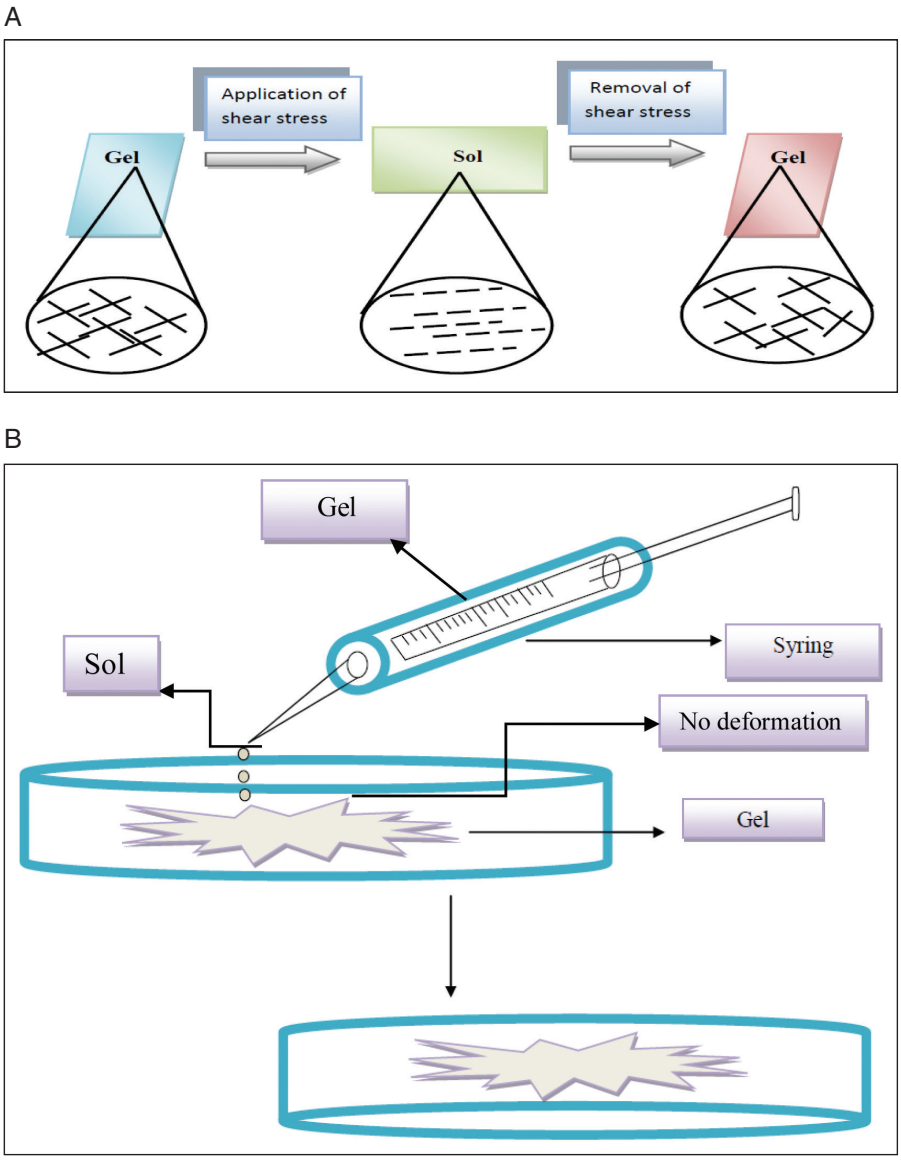

Figure 1: (A and B) Shear thinning property of injectable hydrogel

from medical store Pimpri. All other chemicals used were of analytical grade and highest purity.

Methods: preparation of chitosan-dialdehyde cellulose injectable hydrogel

Preparation of dialdehyde cellulose/DAC

Procedure: Hydroxyethyl cellulose, 10 g, and sodium metaperiodate 6.6 g were added in 500 mL of deionized water. The mixture was further catalyzed by using sodium chloride; 1.4 g aluminum foil was wrapped carefully around periodates mixture to prevent oxidation. The mixtures were mechanically stirred gently at 20°C in the dark for desired reaction times at 1200 rpm. The excess of periodate was decomposed with distilled water; the products (DAC) were recovered and washed by centrifugation, and air-dried. The prepared dialdehyde cellulose was confirmed by IR spectra. Dried DAC was kept in water at 4°C for further use.[12-15]

Preparation of injectable hydrogel

Procedure

Batch optimization

The five different batches were prepared with the different concentration range of chitosan, dialdehyde cellulose, and metformin [Table 1A]. The final batch of hydrogel was optimized by considering the cross-linking between dialdehyde cellulose and chitosan [Table 1B]. The cross-linking between the DAC and chitosan was confirmed by IR spectra. The cross-linking revealed the controlled drug release pattern.

Characterization of metformin

The characterizations of the drugs were carried out by conducting various physicochemical tests, including description, melting point determination, and spectral analysis, such as UV spectrum and IR spectrum.[16,17]

Table 1: (A) Formulae for the chitosan-dialdehyde cellulose-based hydrogel

Sr. no.	Drug and excipients	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
1	Chitosan	1 g	1 g	0.8 g	0.8 g	0.8 g
2	Dialdehyde cellulose	2 g	1.6 g	2.4 g	1.6 g	0.8 g
3	Sodium tripolyphosphate	0.26 g	0.18 g	0.18 g	0.26 g	1.8 mg
4	Metformin	50 mg	50 mg	50 mg	50 mg	50 mg
5	1% acetic acid	50 mL	50 mL	50 mL	50 mL	50 mL

Table 1: (B) Formulae for the optimized batch

Sr. no.	Drug and excipients	Batch 3
1	Chitosan	0.8 g
2	Dialdehyde cellulose	2.4 g
3	Sodium tripolyphosphate	0.18 g
4	Metformin	50 mg
5	1% acetic acid	50 mL

Characterizations of prepared injectable hydrogel formulation

Injectability of hydrogel

Tested by injecting hydrogel through gauge needle no. 26. This needle is widely used in the biomedical injection at subcutaneous injection site.[18-20]

Rheology studies: viscosity

Procedure: The spindle was mounted on the viscometer. For the viscosity measurement, turn the motor switch "ON," this energies the viscometer drive motor. Allow time for the indicated reading to stabilize. The sample was fit in sample holder and viscosity was measured by calculating % torque. The gel viscosity was measured at two points, first; gel viscosity before injecting from needle and second; viscosity after injecting from needle.

Drug content studies

Procedure: For the assay, 1 g of prepared hydrogel was mixed with the 20 mL of phosphate buffer pH 7.4 for 30 min. Mixture was further filtered through membrane filter having pore size 0.45 µm. The absorbance of sample was determined spectrophotometrically at 232 nm. The dilution was done with phosphate buffer of pH 7.4. Concentration of drug was estimated from calibration curve[21,22]

Drug entrapment efficiency

Procedure: The drug-loaded 5 g of prepared hydrogel was incubated in 50 mL of methanolic phosphate buffer (pH 7.4) at 25°C for 1 h. Centrifugation was carried for 10 min. The amount of free drug (metformin) was determined spectrophotometrically by collecting clear supernatant at 232 nm. The supernatant from empty hydrogel was taken as a blank.[23]

Sol–gel phase transition

Procedure: The inverting tube method was employed for the determination of sol–gel behavior of the hydrogel. This behavior was extremely important in case of the *in situ* gelling system. The prepared hydrogel solution at 4°C was heated at various temperatures ranging from 0.5°C to 37°C in water bath. The gelling temperature is the temperature at which no flow being observed within 1 min by inverting the glass tubes.[24,25]

Differential scanning calorimetric

Determination of differential scanning calorimetry of hydrogel formulation

The samples were heated from 400°C to 2200°C at the rate of 100°C/min. The nitrogen gas was used as a purging gas to maintain the inert atmosphere throughout the experiment at the rate of 40 mL/min. The 1–4 mg of samples were carefully transferred and heated in a crimped aluminum pan for accurate results.[26]

Scanning electron microscopy/SEM

Procedure: The surface topography and morphology of the hydrogel was examined using a scanning electron microscope (FEI ESEM quanta 200 analyzer). Samples were coated with gold film under vacuum using a sputter coater (SPI Sputter TM Coating Unit, SPI Supplier, Division of Structure Probe, Pennsylvania), then investigated at 200–1000 nm.[27,28]

Swelling behavior

Procedure: Swelling degrees/SDs of hydrogel was measured at 37°C. The lyophilized hydrogel was preweighed and added in excess of phosphate buffer pH 7.4. At various time intervals, the swollen hydrogel was removed using spatula and place on filter paper to remove the excess of water and reweighed. The procedure was repeated and continues until no weight increase was observed for at least 3 measured.[29,30]

The swelling degrees (Q) was calculated as follows:

$$Q = (Ms - Md) / Md$$

where Ms is the mass in swollen state and

Md is the mass in dried state.

In vitro drug release study

Diffusion study for metformin in hydrogel formulation

Procedure: The *in vitro* diffusion study of the prepared hydrogel was carried out in Franz-Diffusion Cell. The Phosphate buffer (pH 7.4) at 37±1ºC was used as diffusion media. The acceptor compartment of the cell was filled with the 25 mL phosphate buffer (pH 7.4). The donor compartment was filled with the hydrogel (equivalent to 50 mg of metformin). The semi-

permeable membrane (S50) was used for the permeation of the hydrogel into the acceptor compartment. At predetermined intervals, 1 mL samples were withdrawn and replaced with an equal amount of fresh buffer (pH 7.4).[31,32]

In vitro dissolution study

Metformin marketed tablet formulation (metformin tablets 500 mg bp)

Procedure: The *in vitro* dissolution was carried out using tablet dissolution test apparatus (VEEGO) USP type II. The dissolution medium consists of 900 mL of distilled water maintained at 37ºC±0.5°C. The drug release at different time interval was measured using an UV–visible spectrophotometer. The test sample was introduced inside the dissolution jar. The medium is allowed to attain the set position and medium is stirred at 100 rpm; 1 mL of samples were withdrawn at various time intervals such as 30 min, 1 h, 2 h.…up to 40 h. Simultaneously the sink condition was maintained. The sample withdrawn is diluted by 10 mL and absorbance is measured at 233 nm. The percentage release was calculated by using the calibration curve equation.[33,34]

Results

Characterization of metformin (standard)

Description

The metformin from tablet formulation was found to be white powder with characteristic odor.

Melting point

The melting point of metformin was determined by the programmable melting point apparatus and the melting point was found to be 216–220°C.

Spectroscopic characteristics

Preparation of standard curve (calibration curve) of metformin

Metformin obeys beers-lamberts law over this range of 2–10 µg/mL. The absorbance was measured at 232 nm in distilled water.

Assay of metformin

The percentage purity of the metformin in the formulation was found to be 99.96% (STD–100% ± 5% IP)

IR spectrum interpretation

Metformin [Figure 2]: The IR spectra interpretations of metformin are described in Table 2.

Chitosan [Figure 3]: The IR spectra interpretations of chitosan are described in Table 3.

Dialdehyde cellulose [Figure 4]:

The IR frequency at 1725 cm suggested the conversion of cellulose to dialdehyde cellulose.

Sodium tripolyphosphate [Figure 5]: The IR spectra interpretations of sodium tripolyphosphate are described in Table 4.

Chitosan-dialdehyde cellulose hydrogel [Figure 6]: The IR spectra interpretations of chitosan-dialdehyde cellulose hydrogel are described in Table 5.

Characterization of prepared injectable hydrogel formulation

Physical appearance

The blank hydrogel (chitosan) appeared as a slight translucent gel and dialdehyde cellulose-based hydrogel appeared as a whitish creamy colored opaque gel.

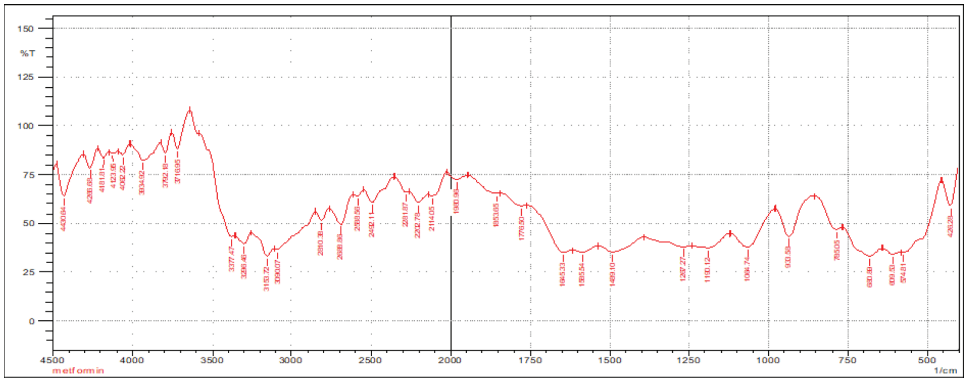

Figure 2: IR spectra of metformin

Table 2: IR absorption of functional groups of metformin

Sr. no.	Functional group	Observed frequency (cm^{-1})	Standard Frequency (cm^{-1})
1.	Amino compound N–H stretching	3239.51	3200–3300
2.	Secondary amine N–H stretching	3173.92	3000–3100
3.	Primary amine N–H bending	1630.84	1600–1650
4.	Primary amine N–H Stretching	3370.66	3200–3300
5	C–H bending	1486.18	1400–1600

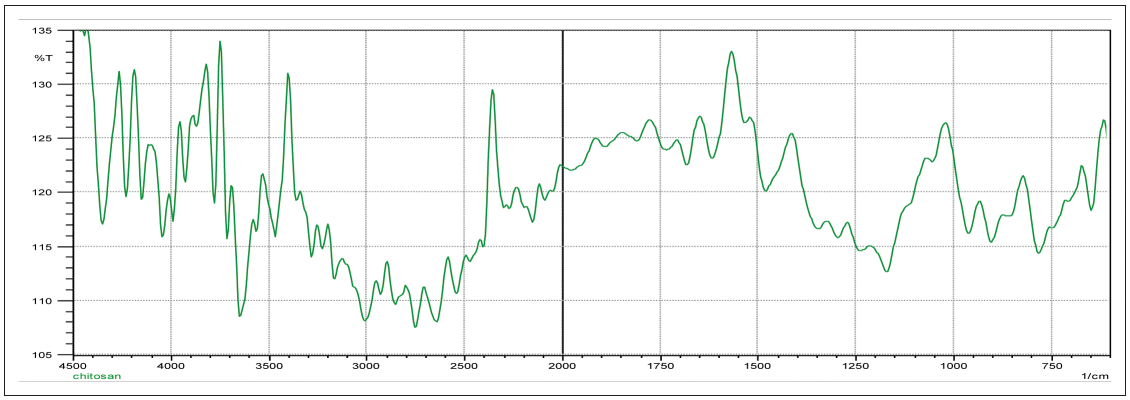

Figure 3: IR spectra of chitosan

Table 3: IR absorptions of functional groups of chitosan

Sr. no.	Functional group	Observed frequency (cm^{-1})	Standard frequency (cm^{-1})
1.	OH stretching `	3628.22	3630–3620
2.	N–H	3379	3400
3.	CH stretching of CH_3 and CH_2 groups	3000	3000–2400
4.	NH–C=O amide I	1705	1600

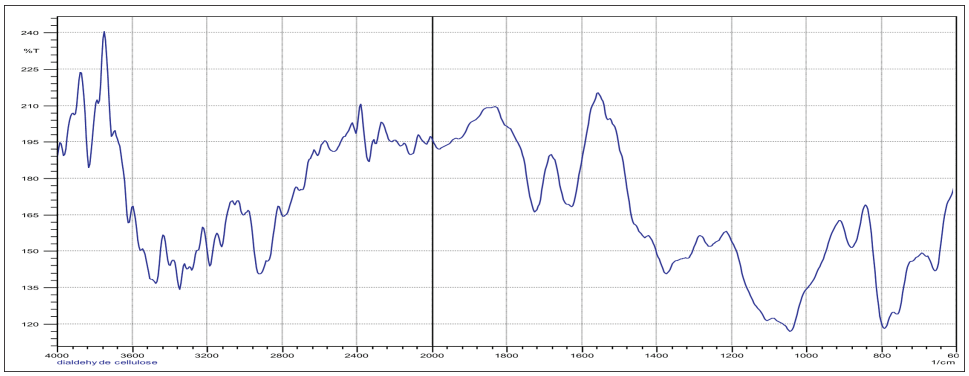

Figure 4: IR spectra of dialdehyde cellulose

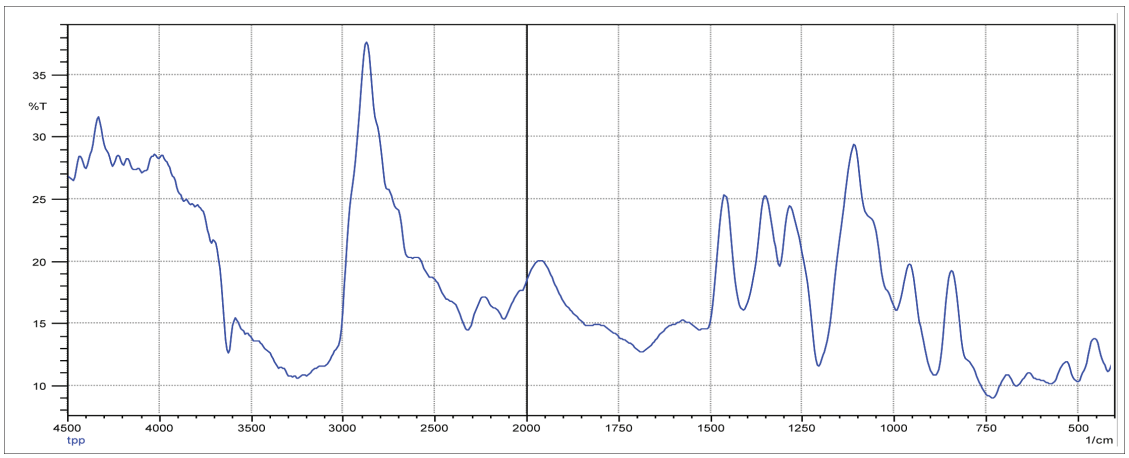

Figure 5: IR spectra of sodium tripolyphosphate

Injectability of hydrogel

The prepared hydrogel was free-flowing through 26 gauge needle.

pH determination

The pH of the hydrogel was measured and it was found to be within a satisfactory limit of 6.5 to 7.5.

Table 4: IR absorptions of functional groups of sodium tripolyphosphate

Sr. no.	Functional group	Observed frequency (cm^{-1})	Standard Frequency (cm^{-1})
1.	O-H stretching	3326.60	3300–3500
2.	C-H stretching	2935.12	3000–3300
3.	CH Stretching of CH_3 and CH_2 groups	3000	3000–2400
4.	C=O Stretching	1654.62	1685
5.	CH_2 bending	1587.12	1500–1600
6.	C–O stretching	1089.56	1300–1000

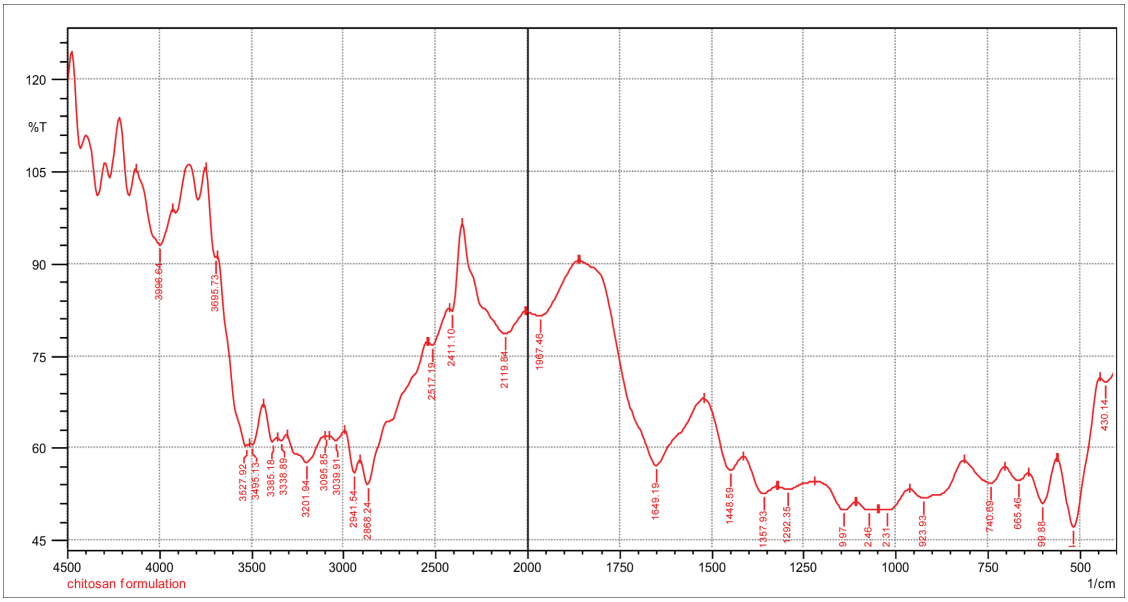

Figure 6: IR spectra of chitosan-dialdehyde cellulose hydrogel

Table 5: IR absorptions of functional groups of chitosan-dialdehyde cellulose injectable hydrogel

Functional groups	Observed frequency	Standard frequency
CH_2 stretching	3039	3300–3000
O–H stretching	3495	3500–3600
C–O Stretching	1022	1000–1300
C=O Stretching	1649	1685

Rheology studies: viscosity studies

The viscosity measurements are shown in Table 6A and B.

Drug content studies

The %drug content was found to be 98.20%.

Drug entrapment efficiency

The entrapment efficiency was found to be 99.65%.

Sol–gel phase transition study

The *in situ* gelling temperature was found to be 37°C. The study ensures that the prepared injectable hydrogel was able to transform into the gel at the body temp after injecting into the body [Figure 7].

Table 6: (A) Viscosity of gel before injection—gel form

Sr. no.	RPM	CP	Torque
1	10	3300	8.6
2	20	2400	12.5
3	30	2064	25
4	40	1684	42.5

Table 6: (B) Viscosity of gel after injection—sol form

Sr. no.	RPM	CP	Torque
1	10	796	5.1
2	20	754	8.6
3	30	624	12.5
4	40	574	18.6

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed and the result found that hydrogel formulation and TPP had melting point 102.57°C and 117.53°C, respectively, and it indicates the amorphous nature of compounds. The formulated hydrogel shows a sharp peak at 103.57◦C due to its better-organized structure. The DSC spectra of chitosan, TPP, and hydrogel formulation were summarized as follows:

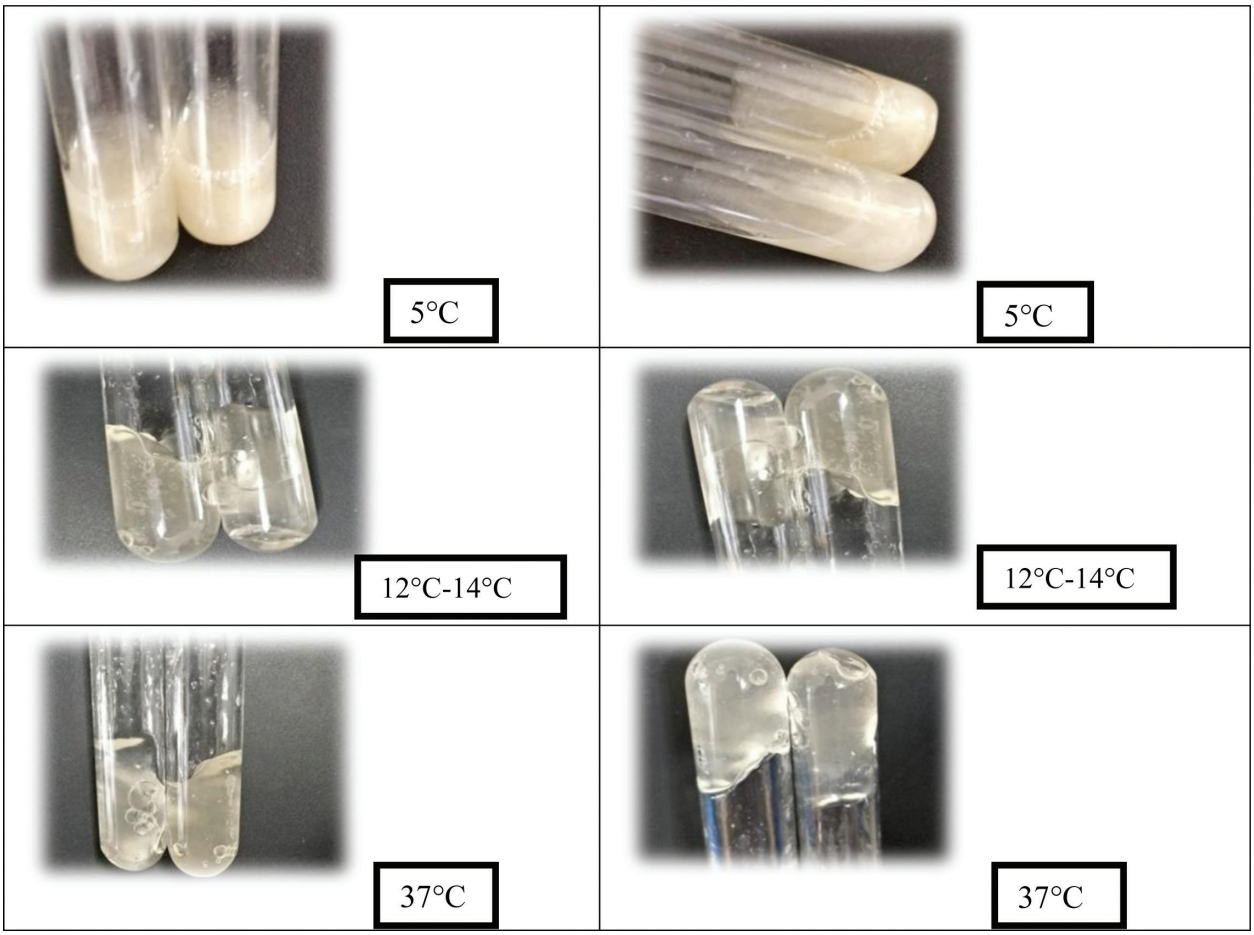

Figure 7: Sol–gel phase transition study

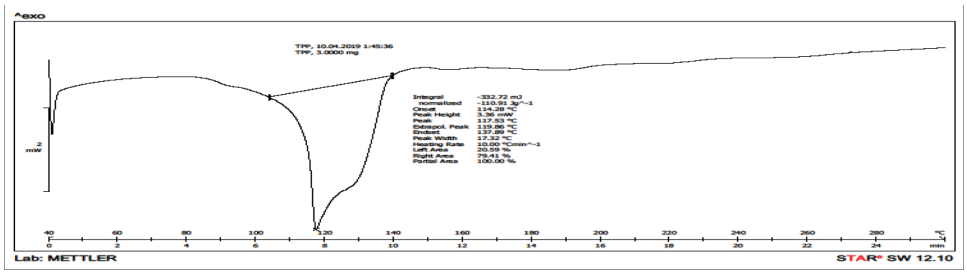

Figure 8: DSC spectra of chitosan

Table 7: DSC of chitosan

Parameters	Observation
Onset	55.03°C
Peak height	1.52 mw
Peak	91.71°C
Extrapol peak	87.58°C
End set	118.33°C
Peak width	38.21°C
Heating range	10.00°Cmin^{-1}

Differential scanning calorimetry of chitosan [Figure 8 and Table 7].

Differential scanning calorimetry of TPP [Figure 9 and Table 8].

Differential scanning calorimetry of prepared hydrogel formulation [Figure 10 and Table 9].

Scanning electron microscopy/SEM

SEM images shows morphology of the prepared hydrogel formulation. The obtained hydrogel was irregular in particle size ranging from 20 to 300 µm, when observed at 500 xs. The image shows a more porous nature of hydrogel [Figure 11].

Swelling study of hydrogel

The medium water uptake ability, that is, 594.23% was assessed by determining the swelling index of Hydrogel in phosphate buffer (pH—7.4) at 37°C. The above results show that after

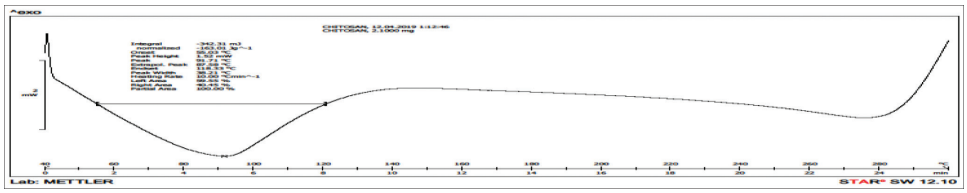

Figure 9: DSC spectra of TPP

Table 8: DSC of sodium tripolyphosphate

Parameters	Observation
Onset	114.28°C
Peak height	3.36 mw
Peak	117.53°C
Extrapol peak	119.86°C
End set	137.89°C
Peak width	17.32°C
Heating range	10.00°Cmin^{-1}

60 min no significant increase was observed in the swelling ability [Figure 12].

In vitro drug release studies

In vitro diffusion of metformin from hydrogel formulation

The *in vitro* diffusion of metformin from hydrogel formulation showed 87.28% drug release prolonged up to 84 h [Figure 13].

In vitro drug dissolution study

The studies were done on the marketed formulation of controlled release metformin tablet (500 mg). The results of the study were used for the comparative evaluation of drug release pattern of marketed formulation and prepared hydrogel [Figure 14].

The *in vitro* drug dissolution study suggested that tablet formulation containing metformin showed controlled release up to 12 h and the % drug release was found to be 99.56%.

Discussion

An injectable hydrogel was prepared from chitosan and dialdehyde cellulose with the incorporation of metformin. The percentage purity (assay) of the metformin in formulation was found to be 99.96% (STD—100% ± 5% IP). The visual inspection showed that the prepared hydrogel was opaque in nature and whitish creamy in color. The pH of formulation was found to be within a satisfactory limit of 6.5 to 7.5. The injectability study revealed that prepared hydrogel was easily passed through 26 gauge needle. Hydrogel showed maximum swelling (594.23%) in phosphate buffer of pH 7.4, the reported work also revealed the shear-thinning behavior of hydrogel. The result showed that the viscosity of hydrogel was more at the gel state but after injection in sol state it was found to be less viscous. The high drug content of hydrogel ensures the effective drug loading at the time of formulation. It also ensures the potency of drug to show the therapeutic efficacy. Entrapment efficiency was found to 99.65%. The sol–gel transition study of injectable hydrogel showed that *in situ* gelling temperature for injectable hydrogel was at 37°C. Differential scanning calorimetry was performed and the result found that hydrogel formulation and TPP had melting point 102.57°C and 117.53°C, respectively, and it indicates the amorphous nature of compounds. The formulated hydrogel showed a sharp peak at 103.57°C due to its better-organized structure. SEM images of hydrogel shows morphology of the resulted hydrogels. The obtained hydrogel was irregular in particle size ranging from 20 to 300 µm, when observed at 500 xs. The image shows more porous nature of hydrogel. The % drug release of Metformin from hydrogel formulation was found to be 87.25% sustained for 84 h. The drug release study suggested that the Metformin can be incorporated into the injectable hydrogel formulation for controlled drug release. The comparative drug release study of metformin hydrogel formulation with the marketed tablet formulation suggested that the metformin from hydrogel formulation herein gained high potential of controlled drug delivery for antidiabetic activity.

Conclusion

A novel cross-linked chitosan-dialdehyde cellulose blend with incorporation of drug for controlled release of metformin has been synthesized and IR spectra of the prepared hydrogel confirmed the presence of cross-linking network between incorporated components. The prepared hydrogel showed the controlled drug release pattern for metformin. The Metformin release was prolonged for 84 h and the %drug release was found to be 87.25%. Thus, the delivery of drugs through the injectable hydrogel system at the specific site herein gained the high potential regarding the targeted drug delivery system. Thus, an attempt has been made to incorporate metformin in injectable hydrogel formulation for the controlled drug delivery application would be more beneficial as compared to the conventional controlled drug delivery system and preformed hydrogel system.

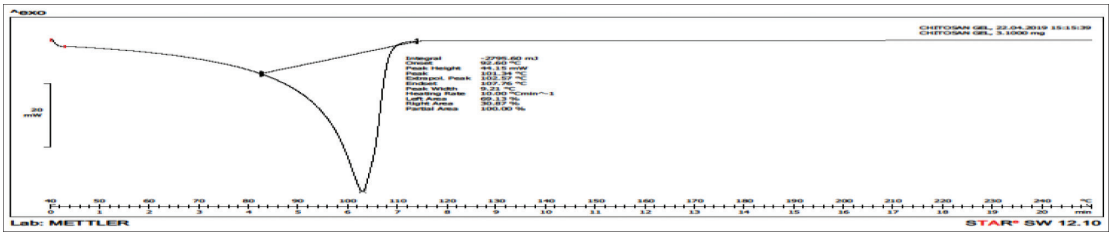

Figure 10: DSC spectra of chitosan-dialdehyde cellulose hydrogel formulation

Table 9: DSC of chitosan-dialdehyde cellulose hydrogel

Parameters	Observation
Onset	92.60°C
Peak height	44.15 Mw
Peak	101.34°C
Extrapol. peak	102.57°C
End set	107.76°C
Peak width	9.21
Heating range	100.0°C min^{-1}

Future prospective of work

Sterilization, animal studies, injectable dose optimization, and degradation study of hydrogel.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

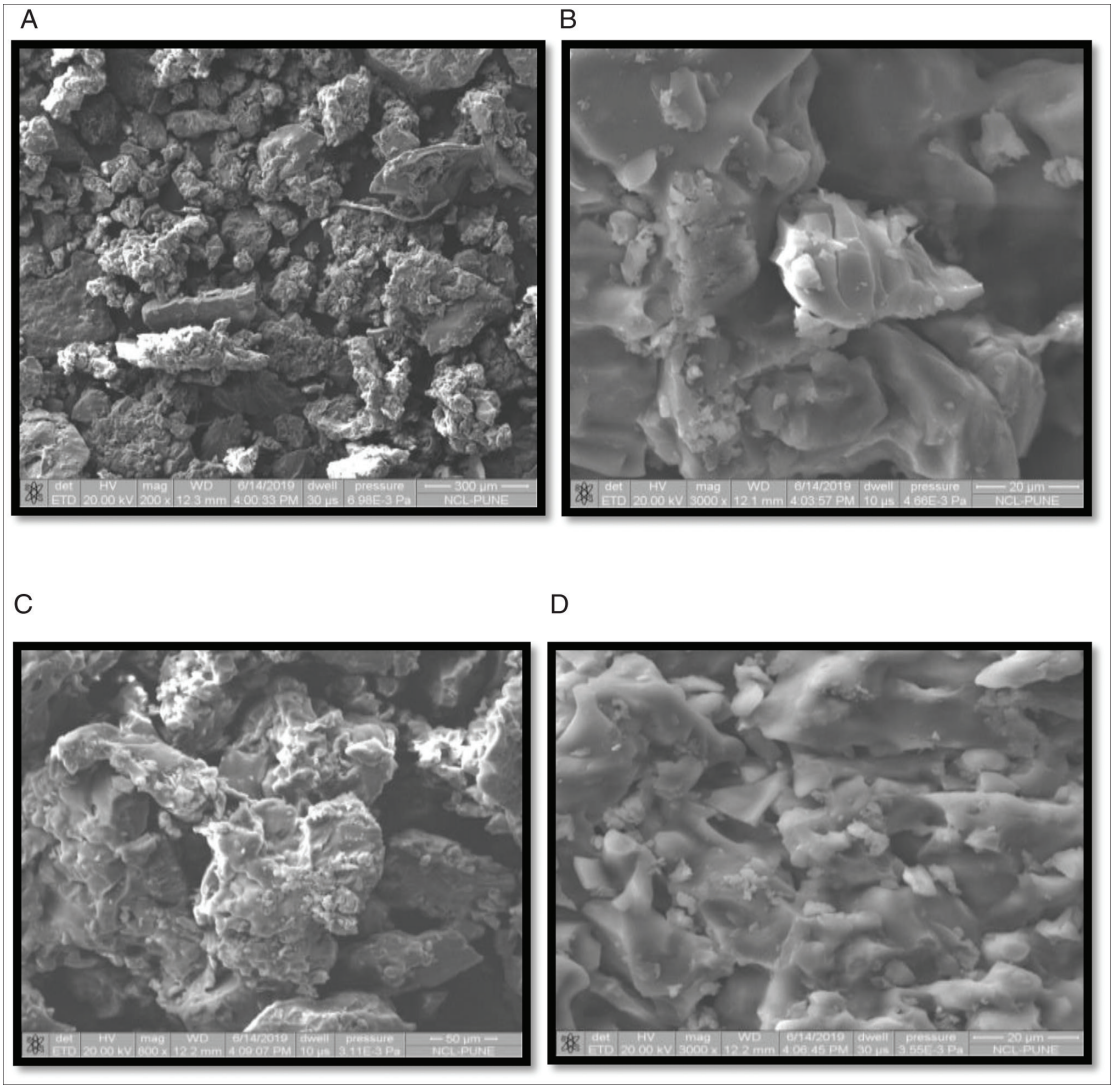

Figure 11: (A–D) SEM images for the chitosan-dialdehyde cellulose hydrogel

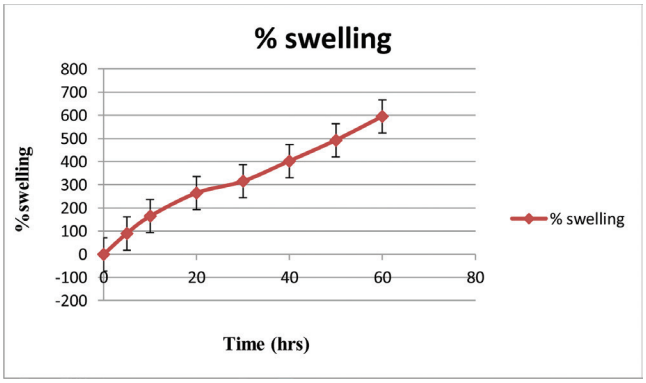

Figure 12: Swelling index of hydrogel

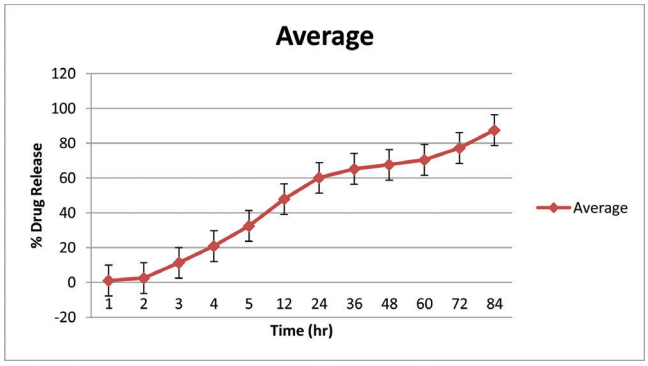

Figure 13: *In vitro* diffusion of metformin (100 mg) from hydrogel formulation

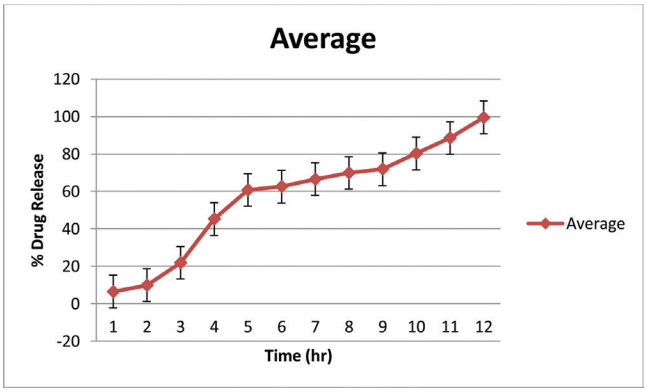

Figure 14: *In vitro* dissolution of metformin from formulation

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