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Novel Composite pH Controlled Drug Release Hydrogel Containing Dexibuprofen

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ABSTRACT

Objective: The objective of this study was to develop a gelatin-cyclodextrin hydrogel using glutaraldehyde as crosslinker containing Dexibuprofen, characterized by the mucoadhesive and controlled release of drug in the stomach and its *in vitro* characterization. All non-steroidal anti-inflammatory drugs (NSAID's) cause peptic ulcer in chronic disease condition like rheumatoid arthritis. In conventional dosage form of NSAID's the drug is released at once with reduced duration of action. On other hands in hydrogels dosage form the drug is released slowly with prolongs the duration of action & minimal side effects. This also decreases the dosing frequency.

Methods: Nine formulations were developed by varying the gelatin-cyclodextrin (Gel/CD) and glutaraldehyde. Swelling studies of hydrogels were performed at three different pH conditions (1.2, 6.5 and 7.4). The hydrogel samples were also analyzed by Fourier transformed infrared spectroscopy (FTIR), Differential scanning calorimeter (DSC), X-Ray diffraction (XRD) and Scanning Electron Microscopy (SEM).

Results: The developed hydrogel showed Maximal swelling and drug release at pH 1.2. The results also revealed the development of pH-dependent swelling and drug release pattern which advocates its feasibility to be used for site-specific, pH-dependent, and controlled drug release behavior.

Conclusion: The developed hydrogels may prove to be a good plate-form for delivering drugs at a specific rate, at the specific site making the drug release pH responsive.

Keywords: Controlled release, Dexibuprofen, hydrogel, pH sensitivity.

INTRODUCTION

Hydrogels are polymeric 3D networks, hydrophilic in nature, which have the ability to absorb water and other fluids [1-3]. Hydrogels absorb water and other biological fluids due to functional groups such as –CONH, –OH, $–SO_3H$ and $CONH_2$ [4]. Hydrogels may be natural or synthetic in origin. Natural polymer based Hydrogels may not provide sufficient mechanical strength and may contain an infectious agent which initiates inflammatory responses. There are several advantageous properties such as inherent bio-friendly, biodegradable and bioactive moieties that support cellular activities. On the other hand, synthetic hydrogels usually have distinct structures that can be adjusted to yield adaptable degradability and functionality [5].

They have become progressively significant and are indeed very versatile materials with widely spread applications in various areas such as artificial implantation [6], swelling CDDS [7], artificial skin development [8], wound dressing and humidity sensor [9]. Innovative hydrogel products have thus been developed for biomedical applications such as soft contact lenses, cosmetology, and protein and gene delivery. In above mentioned applications, the biodegradation and biocompatibility of the hydrogel system are a preferred [10]. They are also termed as "Smart gels" and/or "intelligent gels" as they exhibit the changes in their physical nature upon exposure to

certain stimuli [11, 12]. The stimuli may be pH, temperature, ionic strength and electric currents [13].

Gelatin (Gel) is a polypeptide acquired from collagen by the process of hydrolysis which is obtained from the skin of the animals, tissues (connective tissues) and bones [14]. It is formed by different arrangements of amino-acids where proline and hydroxyl-proline are important to show the effect of gelling. Due to its low price, biodegradation, compatibility and natural origin gelatin are used in hydrogels as a natural polymer in great contents [15]. At a temperature of about 40 ºC, Gel aqueous solutions are in a solution state and on cooling form physical thermos-reversible gels [16]. Cyclodextrintin (Cyclodextrin) is naturally occurring hydrophilic, a polysaccharide found in the cell wall in most of the higher plants. It is extracted from apples, plumes, gooseberries, cherries, grapes, and oranges [17, 18]. Chemical structure of Cyclodextrin contains a linear chain of poly-α-(1-4)-dextro-galacturonic acid with varying level of CH_3 esterification of –C=O (carboxyl group) [19, 20].

Dexibuprofen is pharmacologically more effective than ibuprofen [21]. It inhibits COX-I and COX-II receptors at half dose as compared to Ibuprofen. Furthermore, it has less side effect on GIT [21, 22].

The main objective of this work is to develop a formulation which should be highly mucoadhesive and release its drug maximum in the stomach for fast and controlled delivery in the general circulation which otherwise either get destroyed or have minimal effect if absorbed from the intestine.

MATERIALS AND METHODS

Materials

Dexibuprofen was received as a gift sample (Global Pharmaceuticals, Pakistan). The polymer Gelatin (Merck, Germany), and β-Cyclodextrin (Cerestar, USA) and Glutaraldehyde (Uni-Chem®) were purchased. Hydrochloric acid, potassium chloride, monobasic potassium phosphate, sodium hydroxide, acetic acid and ethanol (Merck) were also purchased. All chemicals were of analytical grade and used without any further purification.

Methods

Synthesis of Hybrid Network of Gel/Cyclodextrin Hydrogels

A pre-determined amount of glacial acetic acid (3% v/v) was taken in a beaker and heated up to 60 ± 0.5 °C and gelatin was dissolved by stirring at 150-200rpm. In another beaker, cyclodextrin was dissolved by stirring and heating in glacial acetic acid (3% v/v). Cyclodextrin solution was added slowly to the gelatin solution and mixed for 25 minutes at 200rpm. Glutaraldehyde was added slowly dropwise by maintaining the temperature at 37 ± 0.5 °C. Finally, the weight was made 100g by distilled water. In order to prevent air entrapment nitrogen was bubbled continuously throughout the mixing process. The solidified hydrogels were sliced into 5 mm discs, dried again at room temperature for 48 h and then washed with distilled water for removing any access uncrosslinked polymer residue. The formulations are given in Table **1** and presumptive structure of Gel/CD hydrogels are given in Fig. **1**.

Table 1. Feed composition ratio of Gel/CD hydrogels.

Sample No.	Gel/100 g of Solution	CD/100 g of Solution	Gel/CD (Wt %)	GA/100 g of Solution	GA Wt % of Gel
S1	20	0.8	96.15/3.84	0.8	1%
S2	24	0.8	96.77/3.22	0.96	1%
S3	28	0.8	97.22/2.77	1.12	1%
A1	20	1	95.23/4.76	0.8	1%
A2	20	1.2	94.33/5.66	0.8	1%
A3	20	1.4	93.45/6.54	0.8	1%
E1	20	0.8	96.15/3.84	0.64	0.8%
E2	20	0.8	96.15/3.84	0.96	1.2%
E3	20	0.8	96.15/3.84	1.12	1.4%

Gel: Gelatine, CD: Cyclodextrin, GA: Gluteraldehyde

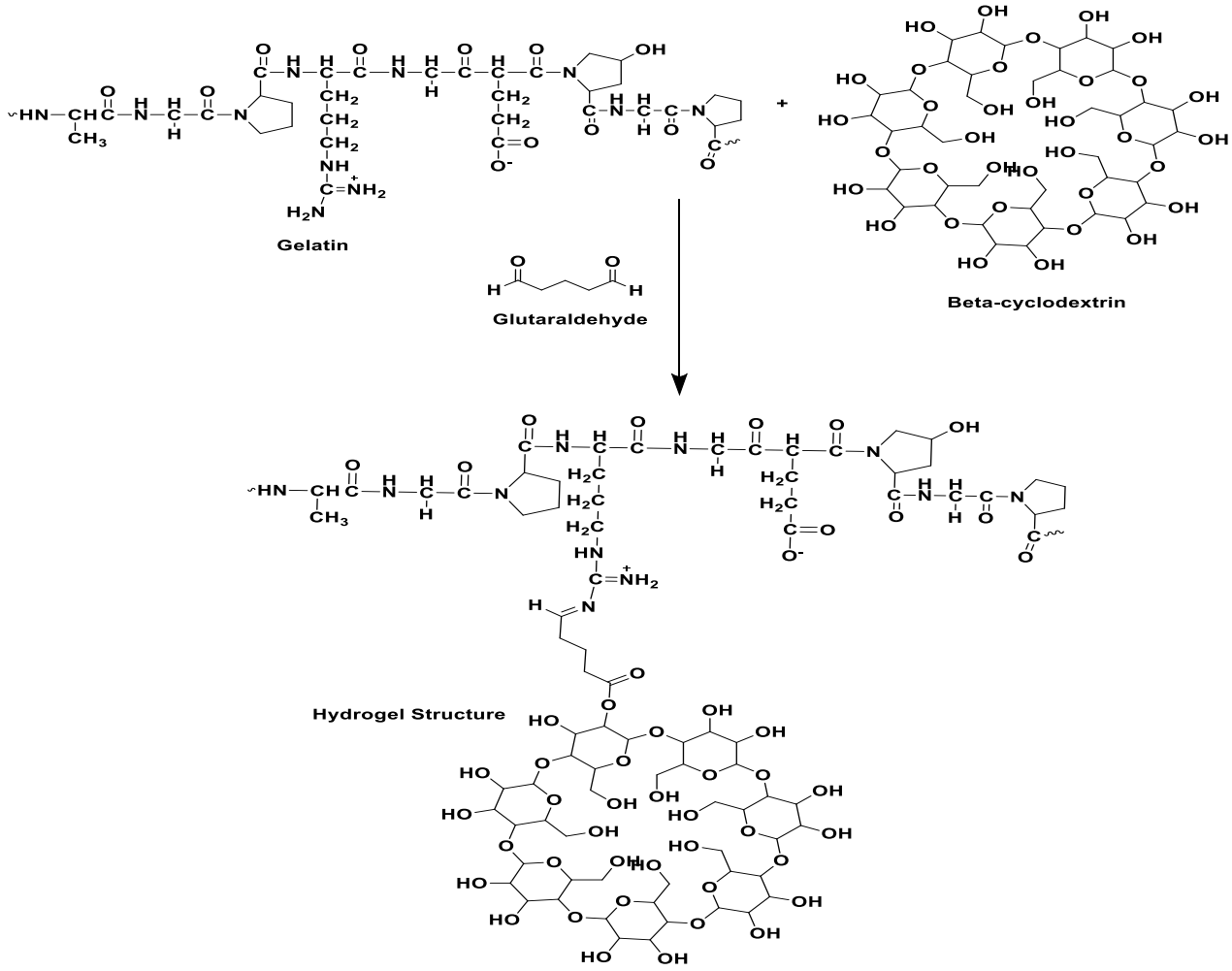

Fig. (1). Presumptive structure of Gel/CD hydrogels.

Swelling Studies

The prepared hydrogels were subjected to swelling studies and dynamic and equilibrium swelling ratios were obtained for all hydrogel samples. A medium of pH 1.2, 5.5, 6.5 and 7.5 was used for performing swelling studies in order to find out dynamic swelling ratio. The method involved the weighing of dried discs and their immersion in 150 ml of a medium solution of required pH. Samples were taken out of solution at specific time intervals and excess water present on surface removed by blot drying the sample with tissue paper.

Samples were weighed and placed back into the respective solution. The difference of weight is indicative of the amount of water hydrogel samples have taken up after definite time intervals. Following equation was used to determine dynamic swelling ratio [23]. At least triplicates were conducted and results averaged with ±SD.

$$q = \frac{W_t}{W_d}$$

Where;

q = dynamic swelling ratio

W_t = weight of swollen gel at time t and

W_d = weight of dry gel before swelling.

Equilibrium Swelling Ratio

On completion of dynamic swelling studies, the same samples were allowed to swell continuously until the weight of each sample become constant. Following equation was used to determine the swelling ratio.

$$q_{(Eq)} = \frac{W_h}{W_d}$$

Where $q_{(eq)}$ is Equilibrium swelling ratio, W_h represents the weight of swollen gel at equilibrium

and W_d represents the weight of dry gel before swelling.

Sol-gel Analysis

Amount of un-crosslinked polymer present in the gel was estimated by carrying out sol-gel analysis. The study was performed on unwashed samples of hydrogels. Hydrogels were cut into discs of 3-4 mm length and subjected to drying first at room temperature and then in an oven at 45°C until constant weight. To remove un-crosslinked material, fully dried discs were subjected to soxhlet extraction using deionized water. Extracted discs were again subjected to drying in a vacuum oven at 45°C until discs of constant weight were obtained once again. The percent gel fraction and percent sol fraction were estimated by using the following equation [24];

$$\text{Sol fraction } (\%) = \left[\frac{W_o - W_1}{W_o}\right] \times 100$$

Gel fraction (%) = 100 – Sol fraction

Where;

W_o = initial weight of dry gel and

W_1 = weight of dry gel after extraction.

Porosity Measurement

In many cases, drug release is usually dependent on the process of diffusion occurring through "pores" present in the hydrogels network. For the purpose, porosity was determined by using the solvent replacement method. Briefly, the pre-weighed dried hydrogel discs were immersed in absolute ethanol for the whole night and weighed again after removal of the leftover solvent by blotting. Porosity was determined by following equation [25].

$$Porosity = \frac{(M_2 - M_1)}{\rho V} \times 100$$

Where;

M_1 and M_2 represent the hydrogel disc weight before and after dipping in ethanol respectively.

ρ represents the density of absolute ethanol.

V is the hydrogel volume.

At least triplicates were conducted and results averaged with ± SD.

Drug Loading

The model drug was loaded into the selected hydrogel samples by swelling equilibrium method. Briefly, pre-weighed amount of drug was solubilized in distilled water to prepare 1 % w/v solution followed by immersion of dried discs and left until equilibrium is achieved. The drug-loaded discs were removed from the solution, blot dried with tissue paper and dried under ambient conditions followed by drying in vacuum oven at 45°C until the weight of each disc approached a constant value [24].

At least triplicates were conducted and results averaged with ± SD.

Measurement of Dexibuprofen Loading

To determine the percentage of loaded drug extraction and swelling method were used. In the extraction technique, the drug was extracted from hydrogel discs using 25 ml fresh phosphate buffer solution pH 7.4. This process was repeated several times until no drug remained in the solution. The samples were analyzed spectrophotometrically (Shimadzu 1800) at 264 nm. while in the swelling method the difference between the dry weight and equilibrium swelling state of hydrogel was calculated by which the amount of loaded drug was estimated [25]. Finally, triplicates were conducted and mean were calculated with their standard deviation.

In Vitro Drug Release and Kinetic Analysis

The *in vitro* drug release profile of dexibuprofen from hydrogel systems were examined at pH 1.2, 6.5 and 7.5 to simulate the pH conditions of various regions of the alimentary canal. The drug-loaded hydrogel was examined in each medium at speed of 200 rpm. Sample aliquots of 5 ml were withdrawn at specific time intervals and replaced with the fresh medium used. The samples were analyzed spectrophotometrically at 264 nm. Triplicates were conducted for each buffer and results averaged with ±SD.

FTIR Spectroscopic Analysis

For FTIR analysis, the dried crosslinked hydrogel samples were grinded into a fine powder with a pestle in an agate mortar. The crushed material was mixed with potassium bromide (Merck IR spectroscopy grade) in 1:100 ratios and compressed to a 12 mm semitransparent disc by applying a pressure of 65 kN (Pressure gauge, Shimadzu) for 2 min. The FT-IR spectrum over the wavelength range 4,500-400 cm^{-1} was recorded using FTIR spectrometer (FT-IR 8400

S, Shimadzu). Triplicates were conducted and results averaged with ± SD.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was done in the DSC unit (Netzsch DSC -200 PC Phox, Germany). A sample weighing 3 ± 0.5 mg was filled into standard aluminum pans and sealed with DSC pan sealing assembly. The pans were placed into DSC furnace and heated at a rate of 10°C/minute. Nitrogen was used as a purge gas with a flow rate of 50ml/min. Triplicates were conducted and results averaged with ± SD.

X-Ray Diffraction (XRD)

X-ray diffraction (XRD) for drug loaded and unloaded hydrogel is performed using Bruker D8 Discover (Germany) apparatus. Measurement conditions included a target (CuKα), voltage (35 KV), and current (35 mA). A system of diverging, receiving, and anti-scattering slits of 1^o, 1^o, 1^o, 0.15^o, respectively, was used. Eva software was used for the data processing (Evaluation Package Bruker, Germany). Patterns were obtained using scan speed of 4 degrees/minute with 2θ between 5^o and 80^o [25].

Scanning Electron Microscopy (SEM)

The surface and cross-sectional morphological analysis of drug loaded and unloaded hydrogel systems were done with scanning electron microscopy (S3400-N Hitachi). The samples were fixed on SEM stab and sputter coated with platinum for 5 minutes followed by viewing and the representative sections were photographed.

Statistical Analysis

All the experimental data were analyzed using student *t*-test and ANOVA (SPSS 18.0). The results with a p-value less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of pH on Swelling and Drug Release from Gel/CD Hydrogel

Dynamic swelling and effect of simulated fluid with different pH values on Gel/CD hydrogels is given in Table **2**. To evaluate the effect of pH on the swelling behavior of hydrogels, swelling studies were performed using the medium of pH 1.2, 5.5, 6.5 and 7.5. It was observed that at low pH (1.2), Gel/CD hydrogel show maximum swelling and release as compared to high pH (5.5, 6.5, and 7.5) ($p<0.05$, student *t*-test). These results predicted actually the polyelectrolyte nature of gelatin. Dexibuprofen loaded disc was introduced into different pH such as 1.2, 6.5 and 7.5. Table **3** and **4** symbolize the percent of drug release with regard to different types of Gel/CD hydrogel. At acidic pH (1.2) all of the samples display the greatest drug release. It was the similar action of the hydrogel which they have already shown in the swelling behavior (in Table **2**), in which by reducing the pH of buffer solution, swelling increases [26].

Table 2. Gel/CD hydrogels (dynamic and equilibrium swelling ratios).

Sample Codes	pH 1.2		pH 5.5		pH 6.5		pH 7.5	
	Q	Eq	Q	Eq	Q	Eq	Q	Eq
S1	6.4	12.7	3.6	6.3	5.2	8.8	5.4	8.2
S2	8.2	15.4	3.9	7.0	5.5	8.9	5.6	8.8
S3	8.9	17.2	4.4	7.1	6.9	11.4	5.7	11.1
A1	7.4	14.5	3.5	6.2	6.0	9.8	5.9	8.0
A2	7.6	16.4	3.7	6.3	6.1	9.9	6.2	9.3
A3	8.1	18.1	3.9	6.5	6.6	10.1	7.7	9.5
E1	9.2	16.1	4.1	7.8	7.2	10.8	6.9	9.1
E2	7.1	15.1	3.6	6.2	6.2	9.1	5.5	8.4
E3	6.8	13.5	3.5	6.1	5.6	8.4	5.6	8.1

Q: Dynamic swelling ratio

Eq: Equilibrium swelling ratio

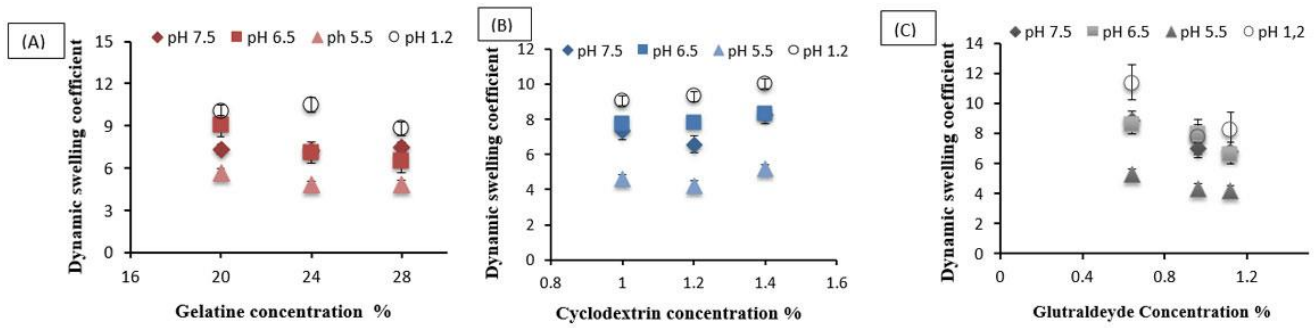

Fig. (2). Dynamic swelling coefficient of Gel/CD hydrogels. (A) Effect of Gel contents, (B) Effect of CD contents, (C) Effect of GA contents.

Effect of Gelatin on Swelling of Gel/CD Hydrogels

For the assessment of polymer impact three formulations with various gelatins concentration of 20gm, 24gm, and 28gm for each 100gm of the solution, keeping the β-cyclodextrin and GA fixation steady (1% wt of gelatin). In Table **2**, samples (S1, S2, and S3) demonstrate the impact of Gelatin on the dynamic and equilibrium swelling proportion of Gel/CD hydrogel. Fig. **2(A)** shows the Dynamic swelling ratio of Gel/CD hydrogel with different concentration of gelatin.

In acidic pH, NH_2 group of gelatin accept the proton. This prolongation (H^+) of the amino group causes the electrostatic repulsion among the polymers and separation of the hydrogen bond between the polymers, as an aftereffect of which it expands the swelling of cross-linked polymer chains [27].

Swelling and Drug Release of Gel/CD Hydrogels by Using a Different Concentration of Cyclodextrin

To analyze the effect of polymer on swelling and on the release of dexibuprofen, β-cyclodextrin has been used at various concentrations of 1gm, 1.2 gm and 1.5 gm per 100 gm of solution keeping gelatin and glutaraldehyde concentration constant (1% wt of gelatin). By increasing the concentration of β-cyclodextrin inside the formulation, swelling increased. In Table **2** the samples A1, A2 and A3 show the dynamic and equilibrium swelling ratio. Fig. **2(B)** shows the effect of CD contents on dynamic swelling coefficient. The effect of β-cyclodextrin has been investigated at pH 1.2, 6.5 & 7.5 as shown in Fig. **3(A)**. The release of the drug is higher at pH 1.2 as compared to pH 6.5 and 7.5. As the pH moves from basic to acidic, the swelling and drug release characteristics of Gel/CD hydrogel discs increased. From the result, it was predicted that the percent drug release increases by increasing the amount of β-cyclodextrin [28]. Table **3** and **4** show the release of drug from Gel/CD hydrogels.

Table 3. Amount of dexibuprofen loaded in different formulations of Gel/CD hydrogel.

Sample Codes	Amount of Dexibuprofen Loaded (g/g of dry gel)	
	By Swelling	By Extraction
A1	0.54	0.52
A2	0.75	0.73
A3	0.87	0.82
E1	0.42	0.46
E2	0.34	0.36
E3	0.22	0.23

Table 4. Amount of dexibuprofen % released in different formulations of Gel/CD hydrogel.

Sample Codes	Percent Cumulative Dexibuprofen Released		
	pH 1.2	pH 6.5	pH 7.5
A1	70.25	45.35	20.45
A2	75.49	47.53	24.64
A3	77.09	49.14	29.24
E1	80.78	47.32	16.45
E2	75.73	46.78	12.89
E3	67.58	41.98	11.23

Swelling and Drug Release of Gel/CD Hydrogels by Using a Different Concentration of Glutaraldehyde

To investigate the effect of cross-linker GA, three hydrogels samples (E1 to E3) are subjected to study the swelling pattern with different concentration of cross-linker GA (0.8, 1.2 and 1.4% wt of gelatin) at different pH values ranges from 1.2 to 7.5. Fig. **2(C)** shows the effect of GA contents on dynamic swelling coefficient. The outcomes uncover that the swelling decreases as the amount of cross-linker increased. By increasing GA the cross-section size of the hydrogel system diminishes and there is expansion in the strength of the hydrogel system. This increase in the stability toward the swelling of a hydrogel is due to the increased cross-linking strength of the polymer. Samples (E1, E2, and E3) in Table **2**, demonstrates the impact of cross-linker on the swelling by keeping the amount of gelatin and cyclodextrin contents.

The samples are also investigated for drug release performance on pH 1.2, 6.5 and 7.5. The increase in GA concentration shows decreases drug release [28]. Fig. **3(B)** represents the drug release behavior in response to variable crosslinker contents.

Sol-gel Fraction Analysis

To determine the uncrosslinked fraction of the polymer in hydrogels sol-gel analysis was to perform. The data obtained from the analysis revealed that the gel fraction increases with the increased of Gelatin as well as β-CD concentration. Sol-fraction of the samples reduced with the enhanced of the gelatin as well as β-CD concentration. The particular gel fraction increases with the increasing amount of the glutaraldehyde as shown in Table **5** [29].

Table 5. Gel fraction and porosity of different formulation of Gel/CD hydrogel.

Sample Codes	Gel/CD Ratios	Degree of Cross linking (GA %)	Gel Fraction (%)	Porosity (%)	P-Value
S1	96.15/3.84	0.8	51.82	25.3	0.05
S2	96.77/3.22	0.96	61.83	26.6	0.05
S3	97.22/2.77	1.12	89.49	34.5	0.005
A1	95.23/4.76	0.8	65.19	25.3	0.05
A2	94.33/5.66	0.8	66.29	36.2	0.05
A3	93.45/6.54	0.8	69.58	47.5	0.005
E1	96.15/3.84	0.64	71.43	76.04	0.005
E2	96.15/3.84	0.96	79.91	72.04	0.05
E3	96.15/3.84	1.12	87.22	71.5	0.05

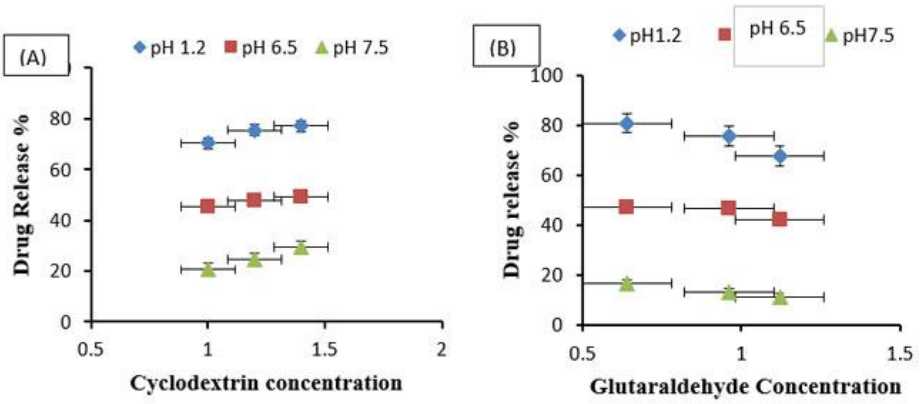

Fig. (3). Percent drug release from Gel/CD hydrogels in various phosphate buffers solutions. (A) Effect of CD contents on drug release, (B) Effect of GA contents on drug release.

Porosity Measurement

Porosity measurement is done to check the porous nature of the hydrogels. The porosity values are given in Table **5** and it is evaluated from the results that the porosity of the hydrogels increased by increasing the concentrations of both gelatin and cyclodextrin. It is due to an increase in the viscosity of a solution of the polymers because as the concentration of the polymers increases it prevents the escape of bubbles from the solution. These bubbles present in the structure of the hydrogel contribute to the porosity of the hydrogels. On the other side by increasing the concentration of the crosslinker glutaraldehyde (GA) the porosity of the hydrogel decreases gradually because of increase in the physical entanglement between the polymers [30].

Drug Release Mechanism

Regression coefficient (r) is employed for the evaluation of release kinetics of the delivery device. The most suitable model is selected on the basis of regression coefficient values (r) and defines the drug release behavior of the delivery device. Zero order, first order, Higuchi and Pappas model were used to evaluate the value of regression coefficient (r) and release constant (k). Table **6** and **7** shows values of "r" from both kinetics. Drug-loaded discs of hydrogels were subject to dissolution studies to obtain the values of "r" for zero order and first order. The experimental data reveal that most of the samples follow zero order release kinetics model. By applying the Higuchi model on the drug release data reveals that the samples follow diffusion controlled drug release behavior. The linear values of the plot of drug release versus the square root of time reveal diffusion-controlled system. Table **8** and **9** shows the effect of cyclodextrin and glutaraldehyde on release exponent (n). The slope and intercept of the plot ln Mt/Mα versus ln t were used to calculate the value of "n" for the release of dexibuprofen at different pH (1.2, 5.5 and 7.5) and results show that the value of "n" is between 0.45 and 1.0. It reveals that the anomalous or non-fickian diffusion mechanism, swelling and relaxation depends upon polymer.

Table 6. Effect of different concentration of β-cyclodextrin on drug release kinetics of Gel/CD hydrogels in solutions of different pH using glutaraldehyde as crosslinking agent (1% of gelatin).

Sample Codes	β-CD Contents	pH	Zero Order Kinetics		First Order Kinetics		Higuchi Model	
			K_0 (h^{-1})	r	K_0 (h^{-1})	r	K_0 (h^{-1})	R
A1	1	1.2	7.762	0.994	-0.134	0.970	0.258	0.967
		6.5	5.551	0.968	-0.071	0.939	0.178	0.879
		7.5	2.550	0.967	-0.022	0.929	0.082	0.878
A2	1.2	1.2	8.668	0.992	-0.154	0.929	0.284	0.94
		6.5	6.006	0.989	-0.078	0.965	0.195	0.923
		7.5	3.137	0.996	-0.035	0.991	0.103	0.949
A3	1.4	1.2	8.917	0.989	-0.159	0.911	0.291	0.929
		6.5	6.056	0.998	-0.081	0.983	0.2	0.956
		7.5	3.797	0.999	-0.044	0.997	0.126	0.968

Table 7. Effect of degree of cross-linking on drug release kinetics of Gel/CD hydrogels in solutions of different pH.

Sample Codes	GA Contents	pH	Zero Order Kinetics		First Order Kinetics		Higuchi Model	
			K_0 (h^{-1})	r	K_0 (h^{-1})	r	K_0 (h^{-1})	R
E1	0.8%	1.2	9.375	0.995	-0.182	0.942	0.312	0.970
		6.5	5.854	0.981	-0.076	0.953	0.189	0.903
		7.5	1.933	0.942	-0.020	0.929	0.061	0.846
E2	1.2%	1.2	8.667	0.995	-0.157	0.950	0.287	0.961
		6.5	5.789	0.991	-0.076	0.971	0.189	0.929
		7.5	1.480	0.957	-0.015	0.948	0.047	0.880
E3	1.4%	1.2	8.140	0.997	-0.132	0.977	0.271	0.970
		6.5	4.897	0.988	-0.062	0.971	0.160	0.933
		7.5	1.272	0.900	-0.013	0.890	0.040	0.787

Table 8. Effect of different concentration of β-cyclodextrin on drug release mechanism of Gel/CD hydrogels in solutions of different pH values using glutaraldehyde as cross-linking agent (1% of gelatin).

Sample Codes	B-CD Contents	pH	Release Exponent (n)	R	Order of Release
A1	1	1.2	0.645	0.986	Non-fickian
		6.5	0.991	0.979	Non-fickian
		7.5	0.620	0.069	Non-fickian
A2	1.2	1.2	0.779	0.988	Non-fickian
		6.5	0.935	0.929	Non-fickian
		7.5	0.575	0.142	Non-fickian
A3	1.4	1.2	0.828	0.988	Non-fickian
		6.5	0.695	0.994	Non-fickian
		7.5	0.579	0.106	Non-fickian

Table 9. Effect of degree of cross-linking on drug release mechanism of Gel/CD hydrogels in solutions of different pH.

Sample No.	GA Contents	pH	Release Exponent (n)	R	Order of Release
E1	0.8%	1.2	0.849	0.979	Non-fickian
		6.5	0.939	0.995	Non-fickian
		7.5	0.543	0.189	Non-fickian
E2	1.2%	1.2	0.751	0.992	Non-fickian
		6.5	0.892	0.985	Non-fickian
		7.5	0.621	0.244	Non-fickian
E3	1.4%	1.2	0.924	0.980	Non-fickian
		6.5	0.969	0.954	Non-fickian
		7.5	0.926	0.480	Non-fickian

FTIR Spectroscopy

FTIR spectroscopy was used to find the interaction between the drug and the polymers. Fig. **4** demonstrates spectra of pure Gelatin, pure cyclodextrin, pure drug, Gel/CD hydrogel, and drug-loaded Gel/CD hydrogel. The FTIR absorption range and band task of gelatin is specific groups as represented by absorption bands at 3260.73 cm-1 shows (N-H stretching), 1631.42 cm-1 peak shows (amide I, C-N stretching and C=O), 1536.50 cm-1 shows (amide -II) while 1241 cm-1 shows (amide -III) can be assigned to the distinctive bands of gelatin [31]. Finally, the skeletal stretching arises at 665.60 cm-1, which shows the N-H bond. In the β-cyclodextrin spectrum, there is a wide band in the 1200-1000 cm-1 area, which recognized to the glucopyranosic ring. Different peaks are observed in drug (dexibuprofen) at 2962.01, 1701.81, 1417.28, 1228.73, 940.76 corresponding to O-H (hydroxyl group), C=O (carbonyl group), C-C (carbon-carbon), C-O (carbon-oxygen) stretching and OH- bending, which confirms the presence of the pure drug. Bands observed at 3260.73 cm-1 shows (N-H stretching), while 1626.57cm-1 (amide-I C-N stretching, C=O), 1534.98 cm-1 shows (amide -II), 1450.31shows (C-H and C-C aromatic), 695.58 (C-H aromatics 675-900). Bands observed at 3279.33 cm-1 shows (N-H stretching), 1631cm-1 shows (amide I, C-N stretching and C=O), 1536.14 cm-1 shows (amide-II), 1451cm-1 shows (C-H bend). The peaks showed in Gel/CD cross-linked hydrogels are actually the peaks of polymers which show that there is no interaction between polymers and drug.

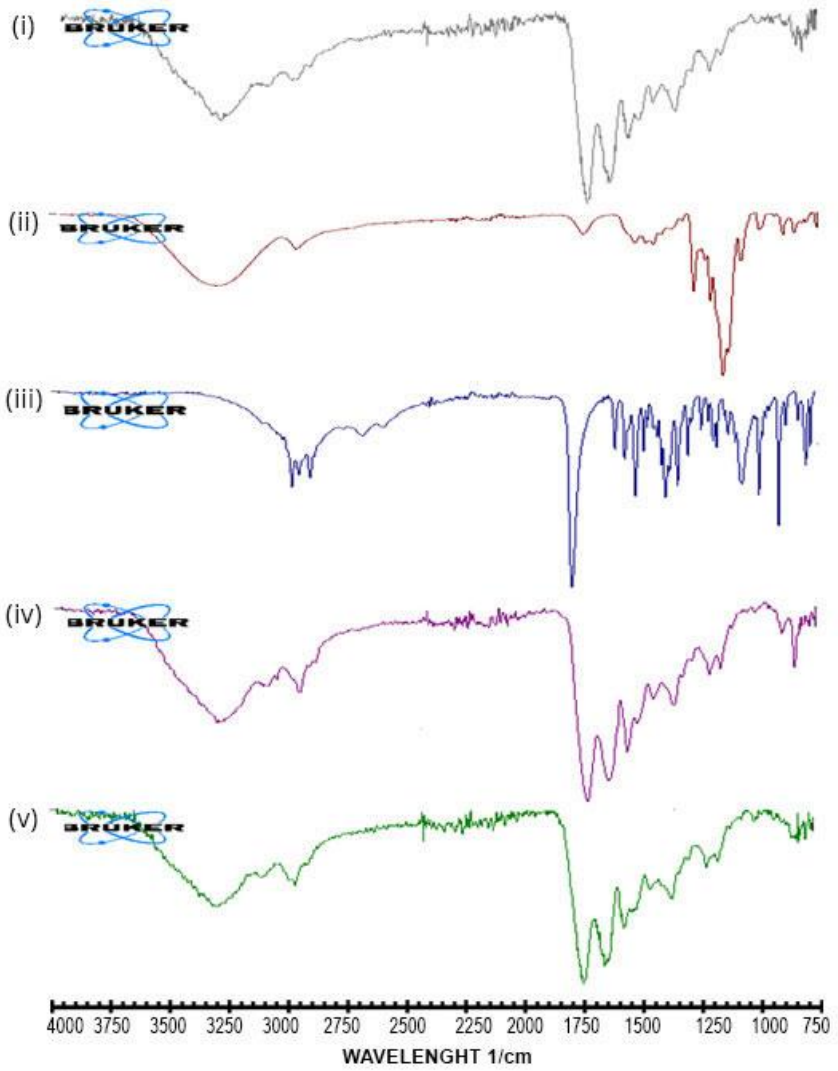

Fig. (4). FTIR spectroscopic analysis of (i) Gelatin, (ii) Cyclodextrin, (iii) Dexibuprofen, (iv) Unloaded hydrogel, (v) Drug loaded hydrogels.

Differential Scanning Calorimetry

During manufacturing, chemical and physical changes occur in the crystalline nature of drugs, due to change in enthalpy or heat capacity of the drug, in the polymer matrix. To measure this change in heat capacity, a well-developed technique called Differential scanning calorimetry is used [32]. The thermal behavior of the dexibuprofen, dexibuprofen loaded Gel/CD hydrogel was characterized using DSC unit (Netzsch DSC-200 PC Phox, Germany) as shown in Fig. **5**.

The dexibuprofen thermogram illustrated a sharp endotherm at 55.5°C followed by 400°C which is corresponding to its MP. The thermogram of loaded Gel/CD hydrogel showed a sharp peak at 50.5°C and improves the thermal stability of the Gel/CD hydrogel. This indicates that there were no changes in the thermal behavior of the drug in the manufacturing process.

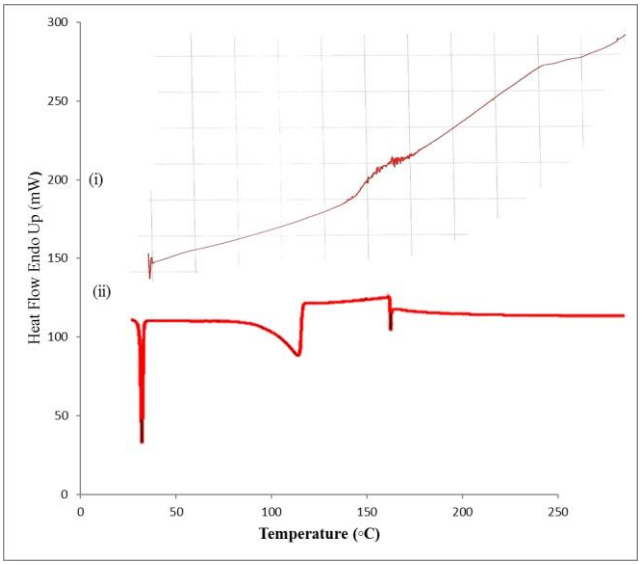

Fig. (5). DSC analysis of (i) drug loaded hydrogel, and (ii) standard thermogram of dexibuprofen.

X-Ray Diffraction

Bruker D8 Discover (Germany) apparatus was used to perform the x-ray diffraction. The pattern of unloaded and loaded Gel/CD was studied in the 2θ range between 5° to 80° in scan speed of 4 degrees/minute shown in Fig. **6**. The sharp peak of pure dexibuprofen observed at 10.47, 12.18, 19.68 25.25 and 39.65 (2θ). The XRD pattern of Gel/CD hydrogels predicted that peak of gelatin was at around 20- 2θ, having intensities of 1200. The XRD pattern of the hydrogels showed a visible peak at around 20-2θ, having an intensity of 1600. From this, it is confirmed that the crystallinity of the hydrogels is mainly due to gelatin. From the XRD patterns, the percentage of crystallinity of Gel/CD hydrogel discs was found to be 4.75, 4.56, 3.93, and 3.56, respectively. Thus, it causes lessening in crystallinity of the hydrogels. This result predicted that drug amorphization in a polymer matrix, decrease the crystallinity of the drug. So, in this case, no appreciable changes occur in the crystallinity of the drug.

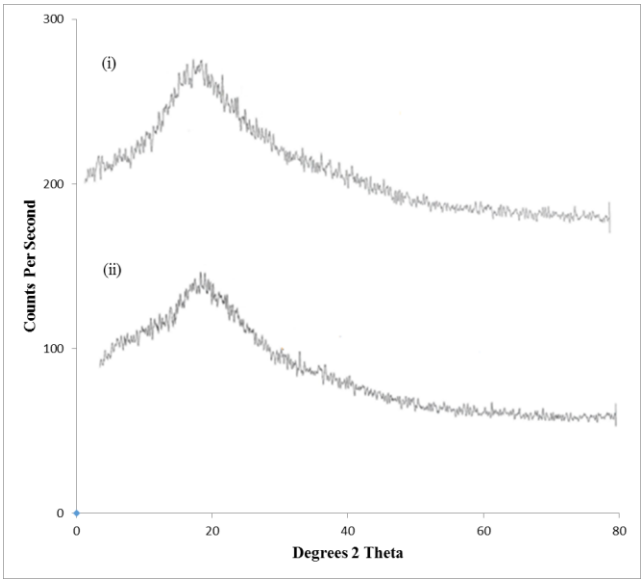

Fig. (6). XRD analysis of (i) unloaded hydrogel, (ii) drug loaded hydrogel samples.

Scanning Electron Microscopy

The surface and cross-sectional morphology of the representative hydrogel formulations were evaluated via scanning electron microscopy (Fig. **7**). Dry and swell discs of Gel/CD hydrogel are shown in Fig. **8**. The porous structure is observed with variations in the degree of porosity and heterogeneity among the gelatin and cyclodextrin polymers that were used in formulations. An increase in polymers concentration, while keeping other factors constant, increases the porosity of samples. In contrast, by increasing the amount of cross-linker (GA) in the hydrogels, which may lead to a greater crosslink density and which is responsible for a possible reduction in the pore size resulting in an increase in roughness of the surfaces of the hydrogel.

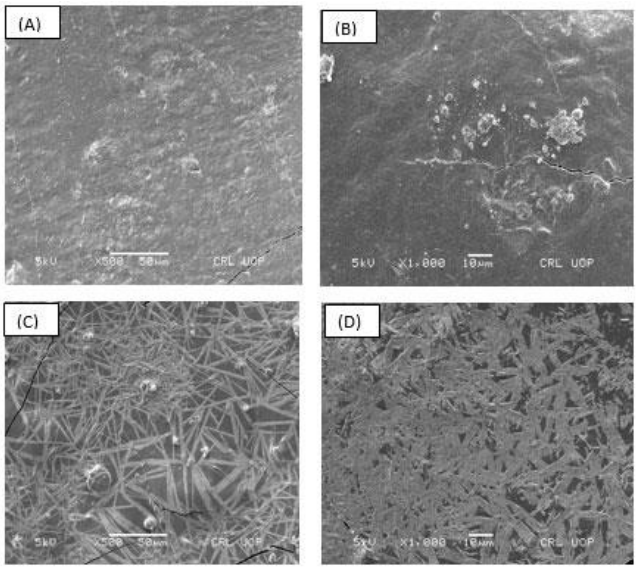

Fig. (7). SEM analysis of Gel/CD hydrogels. (A) Surface morphology without drug, (B) Surface morphology with loaded drug, (C) Cross sectional morphology without drug, (D) Cross sectional morphology with drug.

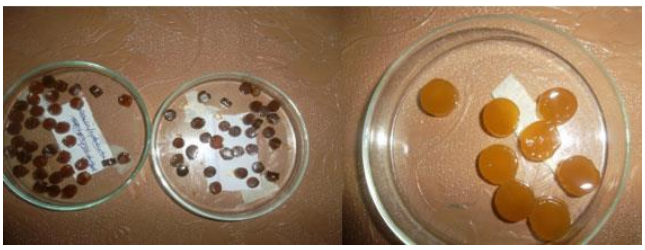

Fig. (8). Dried discs and swelling discs of Gel/CD hydrogel.

CONCLUSION

The pH-sensitive cross-linked gelatin/cyclodextrin (Gel/CD) hydrogels were developed via free radical polymerization using glutaraldehyde as a cross-linking agent to be used as a carrier for water-soluble drugs. At different pH conditions, the swelling studies result demonstrated changes in hydrogel physical structure dependent on the compositional parameters of the formulation where the swelling ratio increased with an increase in the concentration of polymers and while decreases with an increasing the concentration of cross-linker. Likewise, the *in vitro* drug release behavior was also pH-dependent where a burst and faster drug release was observed with highly acidic pH compared to alkaline pH solutions with the non-fickian mechanism of drug diffusion. Moreover, hydrogels are mucoadhesive in nature due to presence of gelatin which not only increase the duration of stay but also improves the therapeutic efficacy of drug in the stomach medium. The developed hydrogels may prove to be a good plate-form for delivering drugs at a specific rate, at the specific site making the drug release pH responsive.

DISCLOSURE

This paper is extracted from author's own research M.Phil Thesis entitled "Study and Synthesis Glutaraldehyde Cross Linked Gelatin/Cyclodextrin Hydrogel and Drug Release Kinetics", 2016. Department of Pharmaceutics, Faculty of Pharmacy, Gomal University, Dera Ismail Khan.

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