Fabrication of natural polysaccharide based hydrogel with utility to entrap pollutants

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Abstract. In the recent years of innovations hydrogels plays important role in various industrial applications. Hydrogels with combination of natural polymers are widely used for its biocompatibility and ecofriendly nature such as pectin and carboxymethyl cellulose (CMC). Plasticizers are utilized to enrich the physicochemical characteristics of hydrogels based on natural polymers. Polyethylene glycol 400 (PEG 400) is known to render thermostability. Whereas copper ions has wound healing properties and acts as biocide. These hydrogels due to its high porosity can have various utility to entrap small particles such as dust, pathogens and other pollutants and can act as very good face mask. Therefore the fabricated hydrogel is not only thermostable but also have high cytocompatibility, pH sensitivity, porosity and degree of swelling.

Keywords – Divalent cations, Hydrogel, Natural polymers,

1. Introduction

Hydrogel is a combination of polymeric networks cross-linked among each other which can entrap large amount of water inside it. This cross-linked polymeric networks of hydrogels form porous structure which has high entrapment capability and has wide application in multiple industrial applications such as wastewater treatment [1-3], medical applications [4-8], pharmaceuticals [9-11] and other industrial and non-industrial applications. The biocompatibility, abundance and eco-friendly nature of natural polysaccharides makes it ideal for hydrogel formation [12, 13]. Freeze-dried hydrogel develops a scaffold-like anatomy which has high porosity [14-21] helpful for trapping valuable molecules which plays important function in drug trapping and regulated drug release [22-25]. Other than this porosity of hydrogel also allows high cell proliferation [26-29], capturing of waste molecules and contaminants in wastewater management [30].
Pectin is a plant derived natural polysaccharide with great applications but it has low mechanical robustness which can be amended by mixing with other natural or ecofriendly polymers [31]. Pectin comprises of arrangement of rhamnogalacturonan I (RG I), homogalacturonan (HG) and rhamnogalacturonan II (RG II) which are connected to one another by covalent linkage. HG comprise of negatively charged α-1, 4 –linked galacturonic acid (GalA) monomers which are methyl-esterified to some extent at the C–O–6 carboxyl [14]. The degree of methyl esterification (DE) helps to determine the effect of pectin jellification by multivalent cations such as poor methyl esterification of pectin easily forms crosslinking among the polymer networks due to divalent cation with respect to high methoxy pectin [14]. The mechanical strength of plants are contributed by the combination of pectin and cellulose due the poroelastic effect [14]. Sodium carboxymethyl cellulose (CMC) is form of cellulose a water soluble which comprise of β-(1→4) glucopyranose residues [14]. Apart from this CMC being derivative of cellulose is non-toxic and eco-friendly along with has excellent bioadhesive and polyelectrolytic properties which provides pH sensitivity to hydrogels [32]. Since the ancient times, copper is recognized to have plentiful optimistic outcomes over tissues of human beings, chiefly on skin. It aids in production and balance of the skin proteins of the extracellular matrix and supports angiogenesis which benefits in improvement of skin elasticity and curing infections resulting in wound healing [33]. Divalent ionic salts such as copper sulfate (CuSO₄) with Cu²⁺ ion which comes to the aid of cross-linking of CMC and pectin to fabricate hydrogel. Plasticizers helps in increasing the flexibility of the polymer composite by aligning them properly [34-36]. PEG 400 has extensive utilization in the biomedical sectors like encapsulation of the protein and its stabilization [37], ointments, body caring products, capsule coats, tissue engineering etc. [38, 39, 40]. PEG earns immense importance in industrial application due to its eminent features such as the great structural flexibility, biologically compatible, amphiphilicity, absent of any steric hindrances, and high hydration capability [38, 41]. The wide application of PEG 400 in the cosmetics such as lotions, ointment base, cream, etc. makes it an industrially valuable chemical [38]. Additionally, the fluidic nature of PEG 400 at room temperature provides it easy mix-ability in solvents at room temperature. The above mentioned characteristics of PEG 400 makes it a perfect plasticizer for creation of hydrogel along with different polymers.

Since hydrogels with thermostatbility has various application in industries, therefore formation of hydrogel with pectin, CMC and PEG 400 was accomplished. CMC, Pectin, Copper ions and PEG 400 has numerous significance and applications which are already discussed above among which biocidal property is the most studied one. The hydrogel constructed is experimentally analyzed by utilization of XRD, FTIR, TGA/DTG/DTA, and DSC which indicated favorable assembly of polymer to form hydrogel with elevated thermostatbility. Other than this, experiments for example SEM analysis, swelling properties studies, and MTT assay revealed good porosity, elevated degree of swelling, high cytocompatibility of the constructed hydrogel which is discussed further.

### 2. Materials and methods

#### 2.1. Materials

Pectin and sodium carboxymethyl cellulose (CMC) were bought from Hi-media. Glycerol and polyethylene glycol 400 (PEG 400) were purchased from Merck. Copper sulfate pentahydrate (CuSO₄.5H₂O), hydrochloric acid (HCL), tris-buffer and 98% ethanol were purchased from SRL. MTT reagents and WI-38 cells were supplied by the Department of Life science and Biotechnology, Jadavpur University, India.

#### 2.2. Preparation of Hydrogel

The stock solutions of pectin (3% (w/v) and CMC 1% (w/v)) were formulated by means of double distilled water. Altogether the polymers were blended into diverse ratio for 1 hour over a magnetic stirrer. Different ratio of PEG 400 or glycerol were added to the prior blended solution and was left to
stir for another 1 hour. The obtained polymer mixture was evacuated into the cast. The polymer solution which contain glycerol was named as HGY which is considered as standard and the polymer solution with PEG 400 was named as HPEG which is our interest of study. A solution of 1 % copper sulfate (CuSO₄) was made and poured over the polymers mixture contained into the cast. Hydrogel was formed due to crosslinking done by the CuSO₄. Double distilled water was utilized to wash the hydrogel formed to eliminate leftover CuSO₄ solution and retained at -20°C for overnight for accurate hardening. The hydrogel scaffold was obtained by lyophilizing the hydrogel at room temperature. The different ratio of polymer mix are aspectin : CMC (1:0.5; 1:1; 0.5:1) with PEG 400 and glycerol variation as 1%, 3%, 5%. Lower the plasticizer brittle the hydrogel and higher the plasticizer, the polymer mix did not crosslink properly. Therefore among these polymer mixture the mix which produced proper hydrogels are pectin : CMC (1:1) with 3% PEG 400 or 3% glycerol entitled as HPEG and HGY respectively (figure 1).

![Figure 1. The image of hydrogel along with the hydrogel scaffold](image)

2.3. Characterization

The SEM (analysis scanning electron microscope) is utilised to learn regarding the structural anatomy of the casted scaffolds of hydrogels. Fourier transform infrared spectra (FTIR) of the hydrogel scaffolds were achieved at the frequency range of 400 – 4000 cm⁻¹. The study of XRD pattern (X-ray diffraction) of hydrogel was done by using a skinny segment of 1X1 cm scaffolds of hydrogel under CuKα radiation secure at 1.5418 Å wavelength with 2θ range of 0.2° s⁻¹ at room temperature. A thin segments of hydrogel scaffolds were utilized for the thermogravimetric (TGA) and differential thermal analysis (DTA) analysis to be carried out at the scanning rate of 10°C min⁻¹ followed by the atmospheric condition of having nitrogen in a temperature ranging from 20°C – 1000°C. Differential scanning calorimetry (DSC) of the hydrogel scaffolds were achieved by heating 15mg of samples from 10°C – 100°C with a speed of 10°C min⁻¹ under the nitrogen atmosphere.

2.4. Swelling properties studies

The swelling property of the scaffolds of hydrogels can be calculated as the degree of swelling (DOS). A minor portion of dehydrated scaffolds with equivalent weight (wi) was submerged in double
distilled water or buffers of diverse pH at room temperature. The final wet weight ($w_f$) of the hydrogel was obtained after seven days by softly blotting the hydrogel over the blotting paper. The DOS equation (1) was recorded corresponding to the subsequent equations [42].

\[
\text{Degree of swelling} = \frac{w_f - w_i}{w_i}
\]  

(1)

2.5. Cytocompatibility studies

Cytocompatibility of HGY and HPEG was estimated by means of MTT assay which is a spectroscopic assay that analyse the reduction of yellow color MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) through mitochondrial succinate dehydrogenase. The MTT penetrate the cells and gets reduced in mitochondria into not soluble formazan product which is dark in color. The cells are then dissolved in organic solvents and analysed spectrophotometrically [43]. The reduction of MTT only occurs in live cell due to it metabolic activity, thus it proves that the cells are living. WI-38 cells were cultured in RPMI media with 100 U/ml streptomycin, 100 U/ml penicillin and 10% fetal bovine serum (FBS) as antibiotics at 37°C in 5% CO₂ and moist atmosphere. In 96 well plate, WI-38 cells were dispersed at a density of 1 X 10⁶. An equivalent weight of HGY and HPEG were incubated in diverse well plates and allowed the cells for diverse hours (24h, 48h, and 72h). After incubation the cells were washed using 1X phosphate buffer saline (PBS) twice. Subsequently 0.5 mg/ml MTT solution was put into the well of the plate and incubated for 4 hours at 37°C until the color changes to a purple. Further the obtained product was solubilized into DMSO (Dimethyl sulfoxide) and measured spectrophotometrically at 570nm by means of a microplate reader (Biorad). The calculation of survival percentage was done bearing in mind the untreated cells having 100% viability.

![Figure 2. (a) SEM image of HGY, (b) and (c) SEM image of HPEG](image)

3. Results and discussions

3.1. Morphological analysis using SEM
The morphological analysis and porosity determination of the scaffolds of hydrogels (HGY and HPEG) was done by SEM analysis. The SEM image of HGY and HPEG shows the vastly porous structure of this scaffold with an mean pore size of 41.38 µm (figure 2 (a)) and 6 – 98 µm (figure 2 (b) and (c)) respectively. The porosity of HGY and HPEG varies greatly due to the use of glycerol and PEG 400 as plasticizer respectively. On the basis of the past studies, it has been found that the pure PEG hydrogels consist of many interconnected pores with a porosity ranging from 6 ±15 µm to 6 ± 82µm [40]. HPEG also reflect a similar type of porosity and simultaneously allows cell viability as discussed further in this paper. Therefore, the above discussion reflects that HPEG has higher and variable porosity than HGY.

![Graphical depiction of FTIR peaks of HGY and HPEG](image)

**Figure 3.** The graphical depiction of FTIR peaks of HGY and HPEG

### 3.2. Study of interaction among polymers using FTIR spectroscopy

According to Mishra et al. 2008 [44] the FTIR spectrum of pectin due to –OH stretching vibration is 3366 cm⁻¹ to 3402 cm⁻¹ and 2931 cm⁻¹ due to –CH stretching vibration. The 1734 cm⁻¹ is assigned due to C=O which is an esterified carboxyl group. 1220 cm⁻¹ represent to ether (R–O–R) and 1008 cm⁻¹ represent C–C bond which is the part of the ring structure of pectin molecules. 800 – 1000 cm⁻¹ correspond to weak symmetric carbonyl stretching vibration [44].

According to Ninan et al. 2013 [14] the FTIR spectrum of CMC represent –OH stretching vibration at 3367 cm⁻¹, 1587 cm⁻¹ and 1416 cm⁻¹ symbolize symmetric and asymmetric modes of stretching vibration or carboxylic groups. 1020 – 1080 cm⁻¹ corresponds to asymmetric stretching vibration [14, 45].
As compared to the above discussing [14, 44, 45] the FTIR spectra of HGY (figure 3) display clear absorption peak at 3424 cm\(^{-1}\) to 3491 cm\(^{-1}\) which are owing to \(-\text{OH}\) stretch vibration peaks. The peaks at 1454 cm\(^{-1}\) and 1365 cm\(^{-1}\) represents CH\(_2\) and \(-\text{OH}\) bending vibration peaks respectively. The peaks between 1706 cm\(^{-1}\) to 1770 cm\(^{-1}\)represents carboxymethyl group (\(-\text{COOCH}_3\)). The peaks 1047 cm\(^{-1}\) represents the C–O stretching vibration peak in the hydrogel [46]. The peak 1706 cm\(^{-1}\) to 1770 cm\(^{-1}\) represent C=O in the hydrogel. The alteration of peaks is due to strong crosslinking by Cu\(^{2+}\) ions in hydrogel [47]. The peaks of the carboxylic group have shifted to higher range i.e. 1625 cm\(^{-1}\) to 1706 cm\(^{-1}\) due to strong crosslinking by Cu\(^{2+}\) ions. The FTIR peaks symbolized in the graph (figure 2) verifies the satisfactory interactions among pectin and CMC which inspire to efficacious hydrogel scaffold formation [14, 44, 45, 47].

The FTIR spectra of HPEG (figure 3) display clear absorption peak at 3422 cm\(^{-1}\) to 3809 cm\(^{-1}\) which is owing to \(-\text{OH}\) stretch and 2865 cm\(^{-1}\) is owing to \(-\text{CH}\) stretching vibration peaks. The peak 2323 cm\(^{-1}\) to 2363 cm\(^{-1}\) represents \(-\text{COO}\) stretch. The peaks at 1424 cm\(^{-1}\) to 1478 cm\(^{-1}\) and 1339 cm\(^{-1}\) – 1396 cm\(^{-1}\) represents CH\(_2\) and \(-\text{OH}\) bending vibration peaks respectively. The peaks between 1705 cm\(^{-1}\) to 1770 cm\(^{-1}\) signifies the carboxymethyl group (\(-\text{COOCH}_3\)). The peaks 1011 cm\(^{-1}\) to 1063 cm\(^{-1}\) represents the C–O stretching vibration peak in the hydrogel [46]. The peaks 1626 cm\(^{-1}\) to 1693 cm\(^{-1}\) represents – COOH group and the peaks 1075 cm\(^{-1}\) to 1798 cm\(^{-1}\) represents C=O in the hydrogel. The strong crosslinking by Cu\(^{2+}\) ions in hydrogel inspire to shifting in peaks in FTIR analysis. Owing to strong cross-linking by Cu\(^{2+}\) ions, the peaks of the carboxylic group have shifted to higher range i.e. 1515 cm\(^{-1}\) to 1646 cm\(^{-1}\) [47]. The FTIR peaks symbolized in the graph (figure 2) verifies the promising interactions among the pectin and CMC which inspire to efficacious hydrogel scaffold formation [14, 44, 45, 47].

As the intensity increases the number of that particular bonds also increases. The HGY and HPEG have peaks locus at the identical wavelength range however the peak intensity has increased significantly in case of HPEG. This suggests that, at these peak locus where the intensity is high, the bond formation number is more [48, 49]. Therefore, it infers that the intensity of the peaks absorbance implies the creation of fresh bonds owing to crosslinking by Cu\(^{2+}\) ions. As PEG 400 (C\(_{2n}\)H\(_{4n+2}\)O\(_{n+1}\); n=8.2 to 9.1) has a long polymer chain than glycerol (C\(_3\)H\(_6\)O\(_3\)) thus, the \(-\text{OH}\) group of PEG 400 are more likely available for bond formation with other functional groups present in CMC and pectin during crosslinking. Therefore, hydrogel with PEG 400 (HPEG) has more intense peak absorbance than hydrogel with glycerol (HGY). Other than this, two bonds of \(-\text{CH}\) and \(-\text{COO}\) are more prominently detected in FTIR spectra of HPEG at 2865 cm\(^{-1}\) and 2323 cm\(^{-1}\) wavelength respectively which are not observed in HGY. This further confirms the ionic bond formation in HPEG during crosslinking whereas in case of HGY the bonds formation is mainly hydrogen bond formation.

### 3.3. Crystallization studies using XRD

The study of crystallization of both the hydrogels are done by XRD pattern analysis. The XRD graph (figure 4) of HGY and HPEG shows both the amorphous nature and crystalline nature of the hydrogel. The broad peak at 9.8° to 12.5° and 19° to 22.5° indicated the amorphous nature of the pectin/CMC hydrogel (figure 4). The peak at 26.5° signifies a low crystallinity of the polymer composite (figure 4) [50]. The crystalline nature of the hydrogel is affected by the plasticizer because plasticizers delivered mobility amid the polymer molecules layer [51]. Irrespective of the difference in plasticizer HGY and HPEG have similar nature of crystallization that is amorphous in nature. Thus, the XRD pattern of PCGY and PCPEG are similar. The above conversation approves the amorphous nature of the two hydrogels (HGY and HPEG) [44].
3.4. Thermogravimetric analysis (TGA) / Derivative thermogravimetric analysis (DTG) and Differential thermal analysis (DTA)

The TGA profile of hydrogel HGY and HPEG is represented by figure 5 (a) and (b) respectively. The thermal stability with respect to change of temperature is represented by TGA plot. In figure 5(a), at 160 °C the initial degradation of HGY starts and degradation end at 390 °C. The weight loss percentage of HGY is obtained as 62%. The DTA graph plot denotes the endothermic degradation of HGY. The first degradation happened at 79 °C which is owing to evaporation of water molecules intensely bound to the HGY sample [52]. An endothermic reaction occurred at 200 °C lead to major degradation of the polymer. The further degradation of polymer occurred at 300 °C due to endothermic degradation reaction. On additional heating, an endothermic dehydration reaction of the polymer follows at 750 °C. The DTG graph plot approves the breakdown of the sample and signifies the precise temperature at which the breakdown starts and ends. The deterioration because of loss of water starts at 20 °C and ends at 100 °C. At 180 °C the major breakdown of polymer starts and ends at 300 °C, whereas the final degradation of the polymer is represented by a small peak from 280 °C to 350 °C. On additional heating, a dehydration reaction occurs between 720 °C and 770 °C. In case of DTA and DTG graph the height or depth of the peaks expresses reactivity intensity [55].
Figure 5. The graphical representation of TGA/DTA analysis (a) HGY and (b) HPEG

In figure 5(b), the initial degradation of HPEG occurs from 220 °C continues till 420 °C. The first weight loss percentage of HPEG is 70% whereas the second degradation of HPEG occurs at 820 °C and continued till 920 °C with the second weight-loss percentage of HPEG as 12%. The DTA graph plot of HPEG symbolizes the endothermic degradation. The first degradation of HPEG is detected at 70 °C which an endothermic reaction where degradation occurs owing to evaporation of water molecules intensely enclosed to the sample [52]. At 220 °C a small peak of the endothermic reaction was observed representing the further degradation. At 300 °C a small broaden peak is observed representing endothermic reaction of HPEG. On additional heating, a small broaden endothermic peak was detected at 940 °C which completes the final breakdown of the polymer. The DTG graph plot approves the breakdown of the sample and denotes the precise temperature at which the breakdown starts and ends [52]. The breakdown of HPEG owing to loss of water occurs from 10 °C to 100 °C.
Correspondingly, the chief breakdown of the polymer happens at 210 °C to 280 °C and 280 °C to 450 °C. On additional heating, two broad peaks were detected at 760 °C to 820 °C and 820 °C to 950 °C which signify dehydration reaction and final degradation of HPEG [55].

So, according to the above discussion, an inference can be drawn that HPEG has more thermal stability than HGY. As HPEG roughly degrade at 300 °C and the complete degradation occurs at almost 950 °C, thus it has high thermostability which has valuable utility in industries.

![Graphical depiction of DSC analysis for HGY and HPEG](image)

**Figure 6.** The graphical depiction of DSC analysis (a) HGY and (b) HPEG

### 3.5. Thermal analysis using Differential Scanning Calorimetry (DSC)

Thermal analysis method such as DSC is used for evaluating first and second order thermal analysis like glass transition (Tg) phenomena, melting (Tm) and crystallization (Tc)h [52]. Tg temperature
represents the mobility of molecules. Tg in lower temperature represent smooth molecule movement and at higher temperature rough molecular movement [53]. HGY (figure 6(a)) has Tg value 57.5 °C whereas in case of HPEG (figure 6(b)) Tg value is 58.15 °C which are nearly similar. The Tg value of HGY and HPEG indicates that the materials are amorphous in nature as lower the temperature of Tg value higher the molecular mobility. The plasticizer molecules helps in arranging the polymer chains away from each other and upsurges the free volume which helps in easy motion of the polymer chain on top of each other at low temperature preceding in the decline of Tg of the polymer [54]. The above mentioned XRD enquiry also approves the amorphous nature of the hydrogel (HGY and HPEG) similar to DSC analysis.

3.6. Swelling properties

Table 1 describe the investigation of the degree of swelling (DOS) of HGY which is considered as standard as compared to HPEG in water and diverse pH. DOS in case of the water of HPEG is 5.54 which is slightly more than DOS in case of the water of HGY which is 5.10. With growing pH the DOS value rises to pH 5.5 and declines from pH 6 which discloses the pH-sensitive nature of hydrogel. DOS value of HPEG is highest as12.09 at pH 5 whereas HGY has maximum DOS value of 18.87 at pH 5. As glycerol has short polymer chain and PEG 400 has long polymer chain thus glycerol forms more space in the hydrogel which effects the swelling property of hydrogels [56]. Thus HGY has comparatively more DOS than HPEG. But as discussed earlier in TGA/DTG/DTA section PEG 400 provides thermal stability to the polymer thus, in summary, though HPEG has slightly low DOS value than HGY but have more enhanced property than HGY. The properties of HPEG is probably an operational drug delivery method over body parts which has a mean pH of 5 to 5.5 [57]. Other than this, the high entrapment capability, porosity and swelling properties makes it capable to entrap pollutants from the air such as dust particles, microorganism and pathogens entrap into droplets which generally spread by sneezing and more. The DOS value of HPEG is significantly high as compared to literature study and thus it has significant industrial value [14, 58].

Table 1. DOS value in water and different pH buffers of HGY and HPEG

| Sample | DOS in water | DOS in pH 4.5 | DOS in pH 5.5 | DOS in pH 6 | DOS in pH 6.5 | DOS in pH 7 | DOS in pH 7.5 |
|--------|--------------|---------------|---------------|-------------|--------------|-------------|--------------|
| PCGY   | 5.10±0.12    | 6.53±0.22     | 18.87±0.07    | 16.12±0.27  | 14±0.63      | Dissolved   | Dissolved    | Dissolved    |
| PCPEG  | 5.54±0.08    | 6.07±0.24     | 12.09±0.27    | 8.75±0.61   | 3.18±0.24    | Dissolved   | Dissolved    | Dissolved    |

Symbol “±” signifies standard deviation where n = 4

3.7. Cell compatibility studies

The cytompatibility of HGY and HPEG was measured by observing the change of survivability of WI-38 cells after cultivation with HGY and HPEG for diverse time intervals using MTT assay. The survival percentage at different time intervals is shown in figure 7. HPEG showed high survival percentage from 24 hours to 48 hours and decrease by 72 hours similarly as in HGY. Owing to the percolation of Cu²⁺ from gradually breakdown of hydrogels with respect to time because of alkaline pH of the media, the rate of cell survival decrease at 72 hours [59]. The literature study has revealed
good cell compatibility of glycerol base hydrogel with CMC and pectin thus, HGY reveals good cytocompatibility [14] but, PEG 400 with pectin and CMC are not studied. Thus, HPEG shows good cytocompatibility of PEG 400 based hydrogel.

**Figure 7.** The graphical depiction of the survival percentage of HGY and HPEG with respect to time.

4. Conclusion

As discussed earlier the efficacious manufacture of hydrogel scaffold has accompanied. HGY which is made-up by means of pectin, CMC with glycerol as the plasticizer is considered as standard hydrogel in comparison with HPEG which is made-up by means of pectin, CMC with PEG 400 as a plasticizer which is the interest of study. HGY and HPEG are both amorphous in nature as confirmed by DSC and XRD analysis which reveals that interaction of plasticizers has resulted in the loss of crystalline nature of pectin and CMC molecules. FTIR peaks confirm the satisfactory interaction among the CMC and pectin in both HGY and HPEG. PEG 400 have more reactive –OH groups than glycerol which effects the bond formation and the intensity of the peaks of HPEG. As discussed earlier the increase in the intensity of peak absorbance is directly correlated to the increase in the number of bonds formation at that particular wavelength. The higher intensity of HPEG signifies intense bond formation at that particular wavelengths. TGA/DTG/DTA confirms the high thermal stability of HPEG than HGY due to the presence of PEG 400 as the plasticizer in HPEG. As HPEG approximately degrade at 300 °C and the complete degradation occurs at approximately 950 °C, thus it conveys high thermal stability. The pore size of HGY is 41.38 µm and HPEG is 6 – 98 µm. The pore size of HGY is less than HPEG but are uniformly spread whereas HPEG is a combination heterogeneous pore size. PEG400 avoid nonspecific bindings as stated in the above context thus, this property of PEG 400 provides excellent property to HPEG. HGY and HPEG both have high DOS as 18.87 and 12.09 respectively at pH 5 which is due to glycerol and PEG 400 as plasticizer respectively.

The high and heterogeneous porosity of the HPEG provide its unique features by providing the capability to entrap molecules with small to large size. Other than this, HPEG is cyto compatible and nontoxic with a high degree of swelling which enables it to absorb moisture and the pollutants along with this moisture from the environment. The Cu^{2+} ion used for crosslinking hydrogel has biocidal property along with thermostability which is helpful for easy sterilization. The pH sensitivity of the hydrogel can be applied to discharge the entrap particles in a specific pH media and also enable easy degradation and therefore is eco-friendly in nature. The conversion of hydrogel into scaffold by lyophilize can be utilized to form thin film of hydrogel scaffold with application as filters for masks and more.
Thus, as discussed earlier HPEG is thermo-stable, pH sensitive, have high porosity, avoids nonspecific binding, high swelling property, and cytocompatibility makes it excellent hydrogel scaffold for its industrial use as compared to HGY. Hence, PEG 400 as a plasticizer enhances the properties of hydrogel and the above discussed results suggested that HPEG hydrogel has varied applications in biomedical sectors.

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