Formulation and Evaluation of Hydrogels of Eloxatine (Anti-Neoplastic Drug)

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Abstract- **In PEGA 4000 hydrogels, FTIR analysis of PEG 4000 showed peaks at 3272 (OH), 2882 (Alkyl (-CH_2), 1095 (C-O-C), 2988 (C–H) and 1710 Wavelength (cm^{-1}); FTIR analysis of AA showed peaks at 2972 (─CH_2─), 1708 (carboxyl group), 1635 (C=O group), 1296 (C-C) and 1173 (C─O); FTIR Analysis of EGDMA confirmed peaks at wavelength (cm^{-1}) 1716 (C=O), 1637 (C=C), 1453 (CH_3), 939 and 812 (═CH_2). Subsequently, in PEGA 8000 hydrogels, FTIR of PEG 8000 showed peaks at 1094 (C-H),2880 (C–H), 1094 (C–O), 3436 (-OH), 2890 (-CH), specific peaks of EGDMA were found at 1725, 1,453, 953 and 814 and finally EXT confirmed peaks at 1643 carbonyl stretching. Analysis of DSC and TGA clearly indicated more thermal stability than single polymer contents weight loss. SEM analysis proved good attraction forces between polymer and monomer. Finally, more polymeric network = more water diffusion = higher drug Eloxatine (EXT) loading in PEGA 8000 hydrogels (high loading at pH 7.4).**

Keywords: **Eloxatine, hydrogels, polymers, colon, cancer, acrylic acid, water imbibitions, scanning electron microscopy, thermogravimetric, gel time.**

Introduction

Hydrogels

Yar *et al*., 2015, hydrogels are three dimensional cross-linked networks (polymer matrix containing a large amount of water). Hydrogels are very similar to most human tissues made of polysaccharides and proteins. (Osada *et al*., 1998)

Miyata *et al*., 2002, hydrogels application include human tissue repair, organ culture *in-vitro* and cancer treatment. The development of hydrogels depend upon factors like physical and chemical properties of the drug and polymers, nature of hydrogels (acidic or basic), pKa and pKb values, swelling, and drug release (Ahmed, 2015).

Design of Hydrogels

Bacelar *et al*., 2017, hydrogels can be made of synthetic or natural polymers (Asail *et al*., 2020)

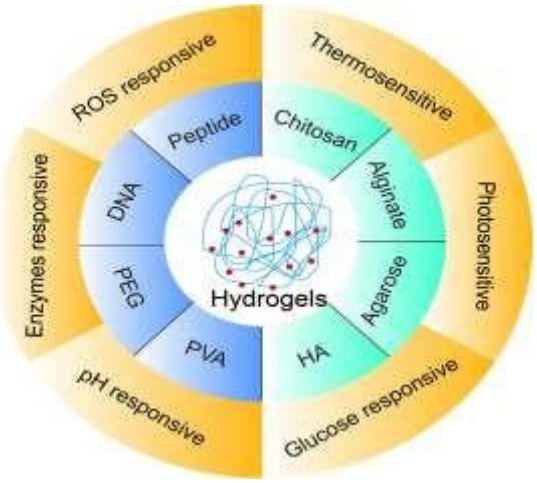

Figure 1: Rational design of smart hydrogels. (Yanyu *et al*., 2021)

Classification of Hydrogels Linking / Gelation Mechanism (Ullah *et al*., 2015)

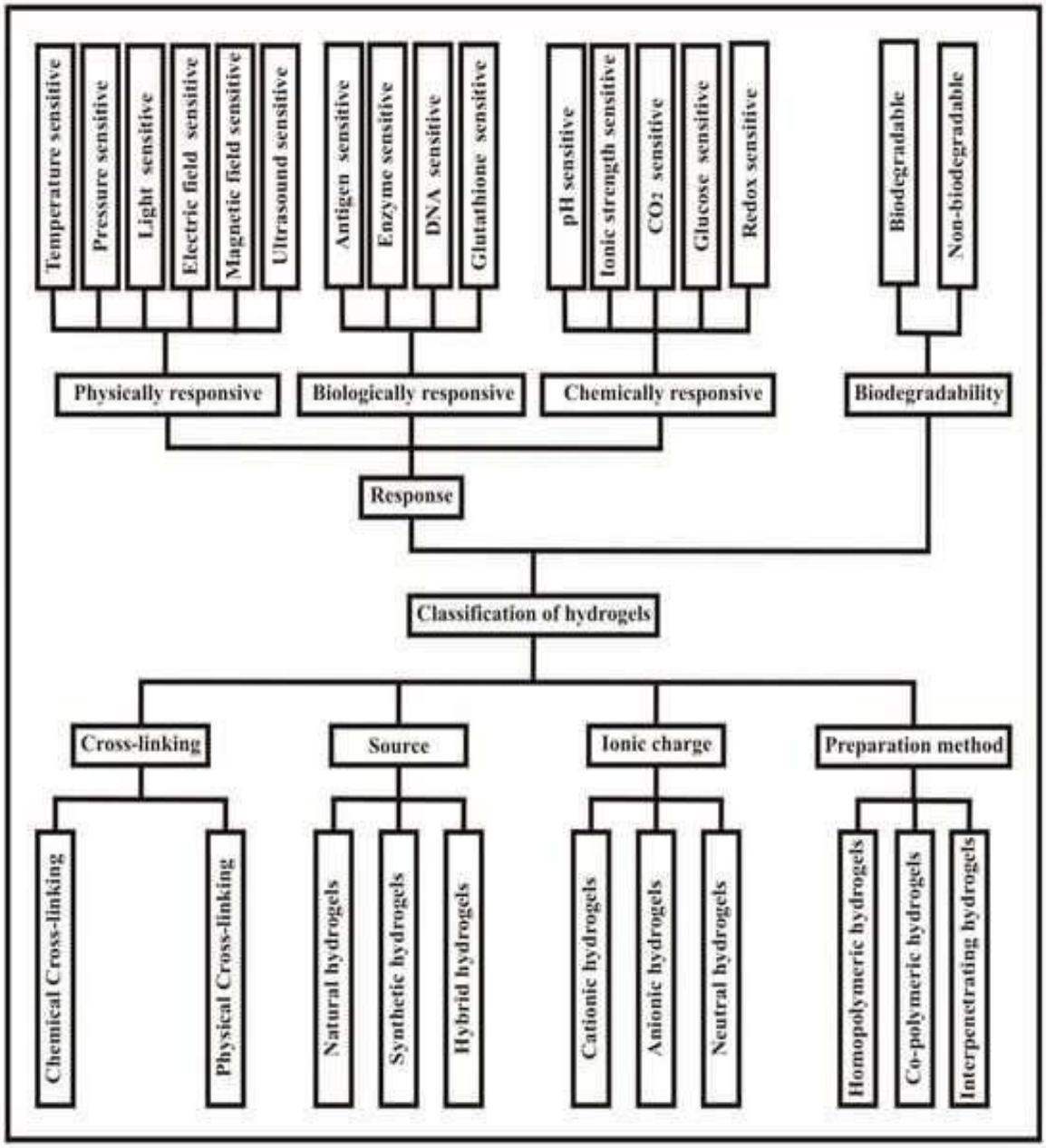

Figure 2 : Hydrogels linking.

pH Sensitive Hydrogel

Zhao *et al*., 2018, pH-sensitive hydrogels can alter their volume on the basis pH of the environment (acidic in stomach and colon (acidic hydrogels) and basic in small intestine (basic hydrogels). (Riaz *et al*., 2019)

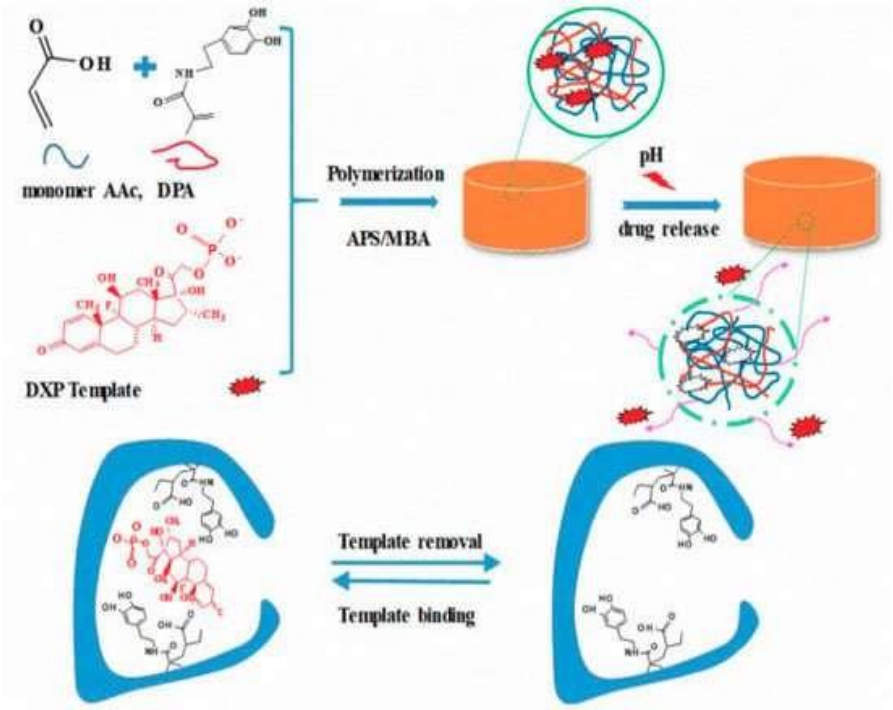

Figure 3: preparation of pH-responsive hydrogels. (Wei *et al*., 2018)

pH Sensitive Polymer for Hydrogels (Kocak *et al*., 2017)

Hu *et al*., 2014, various natural and synthetic polymers are used to formulation hydrogels depending upon cross-linking of polymers, acidic and basic nature of hydroges, chemical responsiveness and pH responsive / sensitive nature of hydrogels etc. (Hoffman, 2012)

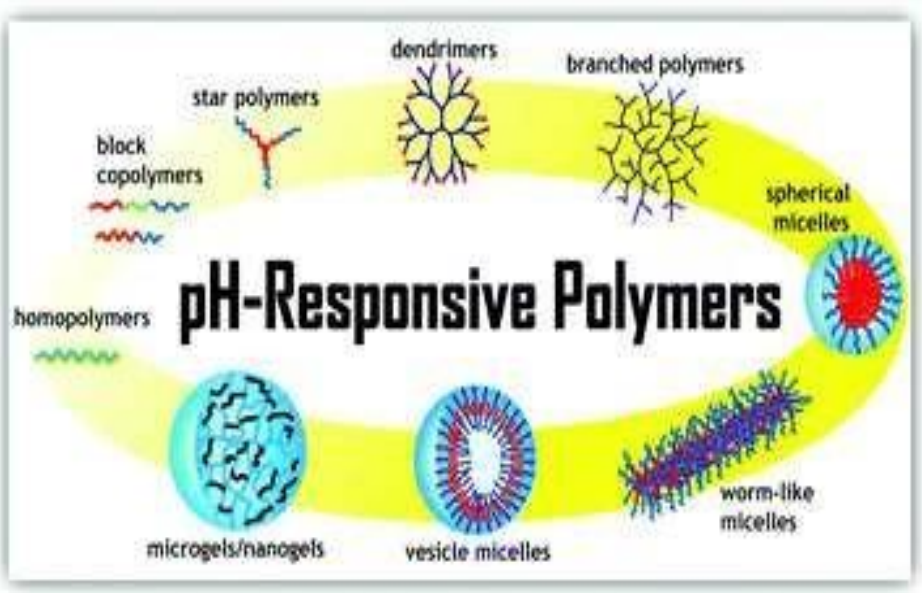

Figure 4: pH responsive / sensitive polymer for hydrogels.

Applications of Hydrogels (Nebhani *et al*., 2016)

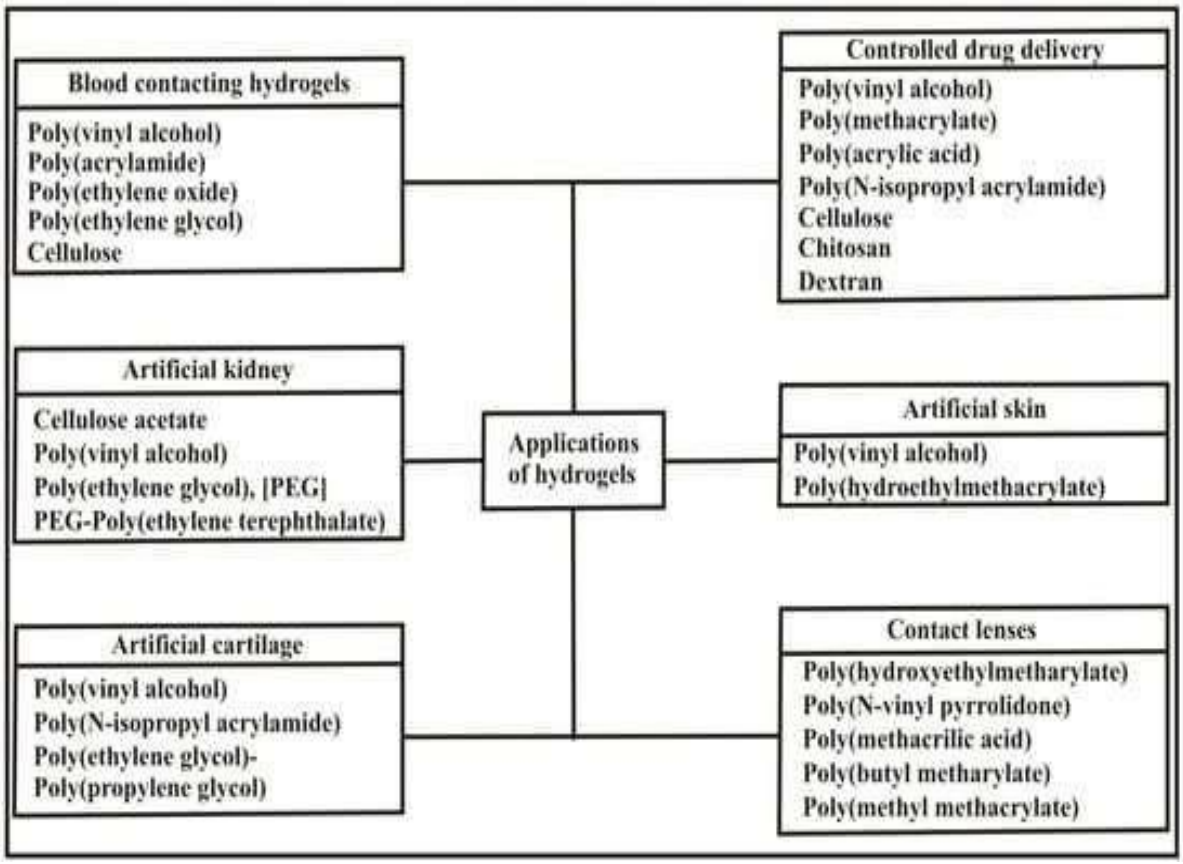

Figure 5: Applications of hydrogels. (Zhang *et al*., 2012)

Table 1: Application of polymers used in hydrogels.

Area of Application	Example of Polymer
Drug delivery, pharmaceutical	starch, poly(vinylpyrrolidone), poly(acrylic acid); polyvinyl alcohol, acrylic acid, methacrylic acid; acrylic acid, carboxymethyl cellulose; chitosan, α β-glycerophosphate; κ-carrageenan, acrylic acid, 2- acrylamido-2-methylpropanesulfonic acid; carboxy- methyl cellulose, hydroxypropyl methyl cellulose; poly(vinylpyrrolidone)
Tissue implants	Hyaluronan; collagen; poly(vinylalcohol), poly(acrylic acid)
Injectable system	β-hairpin peptide; polyesters, polyphosphazenes, polypeptides, chitosan;
WoundHealing	Polyurethane, poly(ethylene glycol), poly (propylene glycol); poly(vinylpyrrolidone), polyethylene glycol and agar; Xanthan, methyl cellulose; carboxymethyl cellulose, alginate, hyaluronan and other hydrocolloids;

Cosmetic, Pharmaceutical	Xanthan, pectin, carrageenan, gellan, welan, guar gum, locust bean gum, alginate, starch, heparin, chitin and chitosan; gum Arabic; Starch
Dental Materials	Hydrocolloids (Ghatti, Karaya, Kerensis gum)

Colon Cancer

Calva *et al*., 2009, Colorectal cancer is the second most common cause of cancer-related death Factors such as history of ulcerous colitis, Chron's disease, colon, rectal, ovarian, endometrium, breast cancer, and diabetes mellitus increase risk of colon cancer.

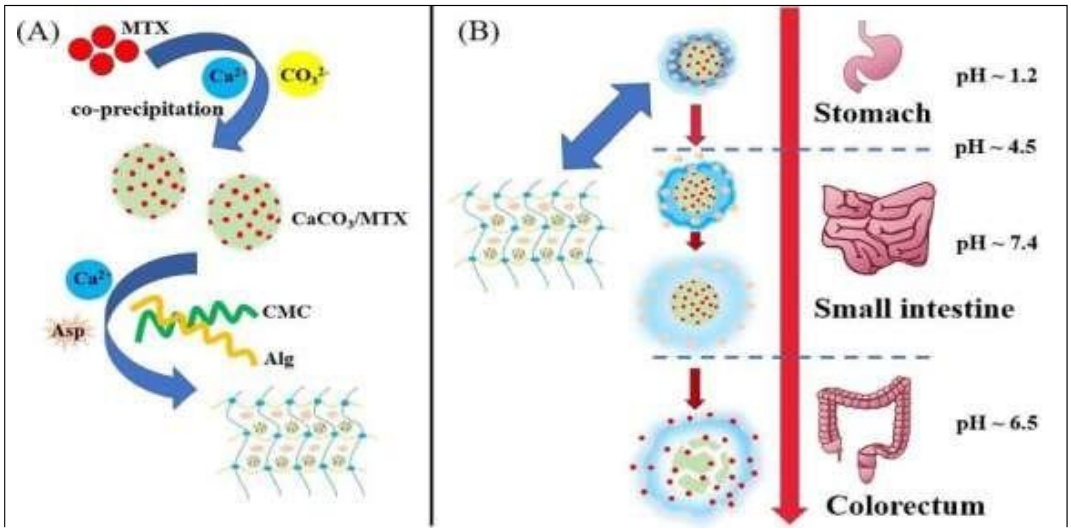

Figure 6: pH variations in digestive organs.

Causes of Colon Cancer

Recent studies suggest that the risk of colon cancer for people with İnflammatory Bowel Disease (IBD) increases by 0.5-1.0% yearly, 8-10 years afterdiagnosis. Patients diagnosed with Crohn Disease (CD) are also on increased risk of colon cancer.

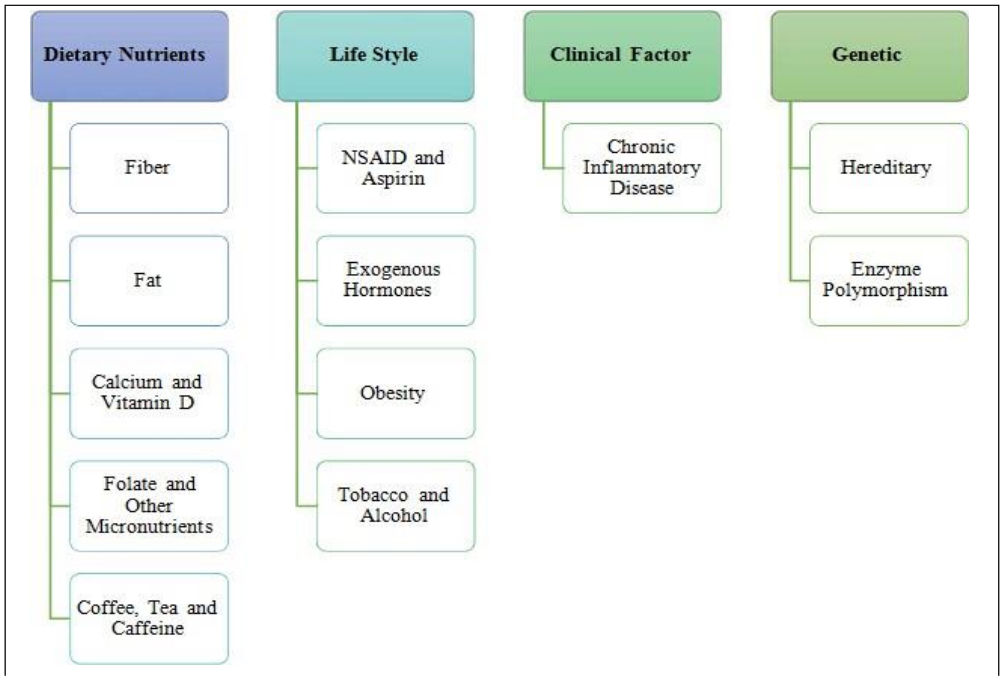

Figure 7: Causes of Colon Cancer.

The present research investigation was carried out to formulate pH-sensitive hydrogels (PEGA 4000 / PEGA 8000) for colon targeting of Eloxatine (EXT) withfollowing objectives:

- formulate Eloxatine (EXT) pH-sensitive hydrogels for colon cancer;
- optimize the concentration of polymer, monomer (Acrylic acid) and Ethylene glycol methacrylayte (EGDMA) used as crosslinking agent;
- evaluate physico-chemical characteristics like drug interaction by FTIR,SEM, DSC and swelling study etc;
- evaluate the drug release characteristics of pH sensitive hydrogelsprepared for colon targeting drug delivery system to treat colorectal cancer;
- study and evaluate water imbibitions at low (1.2) and high pH (7.4);
- evaluate the water imbibition and *in-vitro* drug release studies assessbehavior of EXT regarding its release from carrier system;
- study release kinetics models in order to determine release mechanism ofEXT.
- study the effect of pH on prepared formulations (stability studies).

Materials And Methods

The all materials used in current investigation were analytical grade or the best available AR (Analytical Reagent) as supplied by the different commercial sources / manufacturer.

Formulation of PEGA hydrogels

In this research work hydrogels were prepared PEGA 4000, AA, APS and EGDMA for colon targeting of EXT anticancer drug.

Preparation of EXT - PEG 4000 Hydrogels

- Methods used: Free radica polymerization;.
- 09 formulations were prepared (Table 4.3)
- With Magnetic stirrer PEG 4000 dissolved in H_2O.
- AA, APS and EGDMA were added to the reaction mixture;
- Prepared cylindrical hydrogelscut into 8 mm discs;
- Washed with water and ethanol (70:30) and dried at 40°C

Table 2: Chemical ingredients of prepared hydrogels.

Formulation Code	Concentration (g/100g)				
	PEG-4000	AA	APS	EGDMA	Total
F-1	1.3	33.33	0.30	0.16	34.63
F-2	2.0	33.33	0.30	0.16	35.33
F-3	2.6	33.33	0.30	0.16	35.93
F-4	2.0	26.6	0.30	0.16	28.6
F-5	2.0	33.3	0.30	0.16	35.33
F-6	2.0	40.0	0.30	0.16	42.0
F-7	2.0	33.33	0.30	0.16	35.33
F-8	2.0	33.33	0.30	0.33	35.33
F-9	2.0	33.33	0.30	0.50	35.33

Formulations Characterization Analysis

FTIR Analysis

- The KBr pellets were prepared and examined by FTIR (8400S,Shimadzu Corporation, Japan);
- The scanning was done using KBr dispersion pellets;
- Scanned between 4500-600 cm^{-1};

DSC Analysis : DSC analysis using TA instruments of Q2000 series as per SOP.
TGA Analysis : TGA analysis using Q5000series (West Sussex, UK) as per SOP.
Scanning Electron Microscopy (SEM) : SOP was used for SEM analysis using SEM, EVO 40, Zeiss Germany; AIRF at JNU, New Delhi.
Gel Percentage, Yield Percentage and Gel Time Analysis: Standard Analytical Procedure (SAP) was used for Gel%, yield% and gel timeanalysis.

$$Gel\% = \frac{m_d}{m_i} \times 100$$

$$Yield\% = \frac{m_d}{m_c} \times 100$$

Where, m_c is hydrogels total wt.
Swelling Study of Hydrogels : It was done to determine pH-sensitivity of hydrogels as per SAP withfollowing equations:

$$Q_{t=} \frac{m_{t-m_O}}{m_O}$$

$$Q_{\infty=} \frac{m_{t-m_O}}{m_O}$$

Drug (EXT) Loading

(i) **S**welling-diffusion method was used;
(ii) EXT was loaded in PEGA 4000 hydrogels;
(iii) EXT was extracted with buffer solution at pH 7.4;
(iv) EXT dilutions prepared and calibration curve plotted;
(v) UV (Systronic-2202) analysis was done used at absorption maxima.

Drug Release Analysis (DRA) : DRA analysis was undertaken at low pH (1.2) and high pH (7.4) and values wereanalyzed at λmax 205nm.

EXT Release Kinetic Analysis

(vi) Zero order release $F_t = F_0 + K_0 t$
(vii) First order release $\ln F_t = \ln F_t + K_1 t$
(viii) Higuchi's model $F_t = K_2 t^2$
(ix) Korsmeyer Peppas Model

$$\frac{Ft}{F_0} = K_3 t^n$$

Formulation of EXT, PEG-8000, AA, APS, and EGDMA Hydrogel

- Nine formulations were prepared using polymerization (Table 4.4);
- With Magnetic stirrer PEG 8000 dissolved in H_2O;
- PEG 8000 cross-linked with AA, APS and EGDMA;
- EXT loaded in hydrogels in pH 7.4 buffer solution.
- 8 mm discs cylindrical hydrogels cut;
- Washed with water and ethanol (70:30) and dried at 40°C.

➢ FTIR, TGA, and DSC analysis.

Preparation of Hydrogels (PEG-8000)

Table 3: Composition of PEG-8000 pH sensitive hydrogels.

Code	Conc. (g/100)				
	PEG 8000	AA	APS	EGDMA	Total
F-1	1.3	20.0	0.33	0.16	21.3
F-2	2.0	20.0	0.33	0.16	22.0
F-3	2.6	20.0	0.33	0.16	22.6
F-4	2.0	26.66	0.33	0.16	28.66
F-5	2.0	33.33	0.33	0.16	35.33
F-6	2.0	40.0	0.33	0.16	42.0
F-7	2.0	20.0	0.33	0.16	22.0
F-8	2.0	20.0	0.33	0.33	22.0
F-9	2.0	20.0	0.33	0.50	22.0

Characterization

FTIR: The KBr pellets were prepared; Shimadzu FTIR 8400S Instrument, Japan scanned between 4500- 600 cm^{-1}

TGA: As per SOP / SAP.

DSC: DSC analytical technique was used as per SOP / SAP.

Scanning Electron Microscopy (SEM): As per SAP / SOP.

Assessment of Gel%, Yield% and Gel time: Standard Operating Procedure (SOP) / Standard Analytical Procedure (SAP)were used.

Swelling Characteristic of PEGA 8000

It was done to determine pH-sensitivity (1.2 and 7.4) of hydrogels (PEGA8000) as per SAP with following equations:

$$Q_{t=}\frac{m_{t-m_o}}{m_o} \qquad Q_{\infty=}\frac{m_{t-m_o}}{m_o}$$

EXT Loading: Standard procedure (earlier mentioned) was used (buffer of pH 7.4).

Determination of EXT loading: Standard procedure (UV at λmax 205 nm) for determination of EXT loading (earlier mentioned) was used.

Drug Release Analysis (DRA): DRA was conducted at acidic and alkaline pH to evaluate pH-dependentrelease of EXT from hydrogels as done earlier SAP / SOP.

EXT Release Kinetic

(i) Zero order release : $F_t = F_0 + K_0 t$

(ii) First order release : $\ln F_t = \ln F_t + K_1 t$

(iii) Higuchi's model: $F_t = K_2 t^2$

(iv) Korsmeyer Peppas Model (Peppas, 1985)

$$\frac{Ft}{F_0} = K_3 t^n$$

Results and Discussions

PEGA 4000 Hydrogels
Physical Appearance : Colour : Light Yellow discNature : Rubbery; Strength : Good

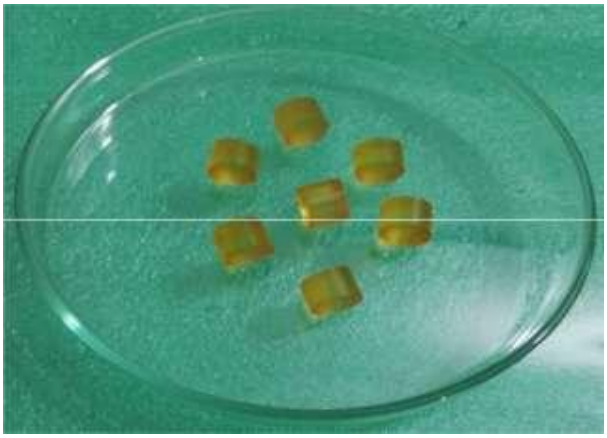

Figure 8 : Yellowish PEGA 4000 Hydrogels Disc.

FTIR Analysis :

Table 4 : FTIR spectrum Analysis of PEG 4000.

Wavelength (cm^{-1})	Bond / Stretching
3272	OH
2882	Alkyl (-CH_2)
1095	C-O-C stretching
2988	C–H stretching
1710	carboxylic acid groups

Table 5: FTIR spectrum Analysis of acrylic acid (AA).

Wavelength (cm^{-1})	Bond / Stretching
2972	─CH_2─ stretching
1708	carboxyl group
1635	C=O group
1296	C-C stretching
1173	C─O stretching

Table 6 : FTIR spectrum Analysis of EGDMA.

Wavelength (cm^{-1})	Bond / Stretching
1716	C=O stretching
1637	C=C stretching
1453	Asymmetric bending of CH_3 group
939	═CH_2 bond
812	═CH_2 bond

Table 7 : FTIR spectrum Analysis of EXT.

Wavelength (cm^{-1})	Bond / Stretching
857	N-H bending
1660	C=O
1697	C=O
3083	N-H

Differential scanning calorimetry (DSC) Analysis

- Between 20°C - 65°C (Endothermic peaks due to loss of moisture);
- Broader endothermic peak (66°C to 77°C);
- endothermic peak of prepared hydrogel (25°C to 145°C);
- Between 215°C-265°C (broader endothermic peak of prepared hydrogel);

Thermogravimetric analysis (TGA)

- 100% weight loss at 350°C for PEG 4000

Weight loss (WL) % in Prepared hydrogels	10% WL at 25°C-235°C (Phase-1);
	60% WL at 240°C-320°C (Phase-2);
	30% WL at 325°C-490°C (Phase-3);

Scanning Electron Microscopy (SEM)

- Weak bond between PEG 4000 and AA (higher swelling) **(Figure 9a);**
- Less dense structure **Figures 9b)**

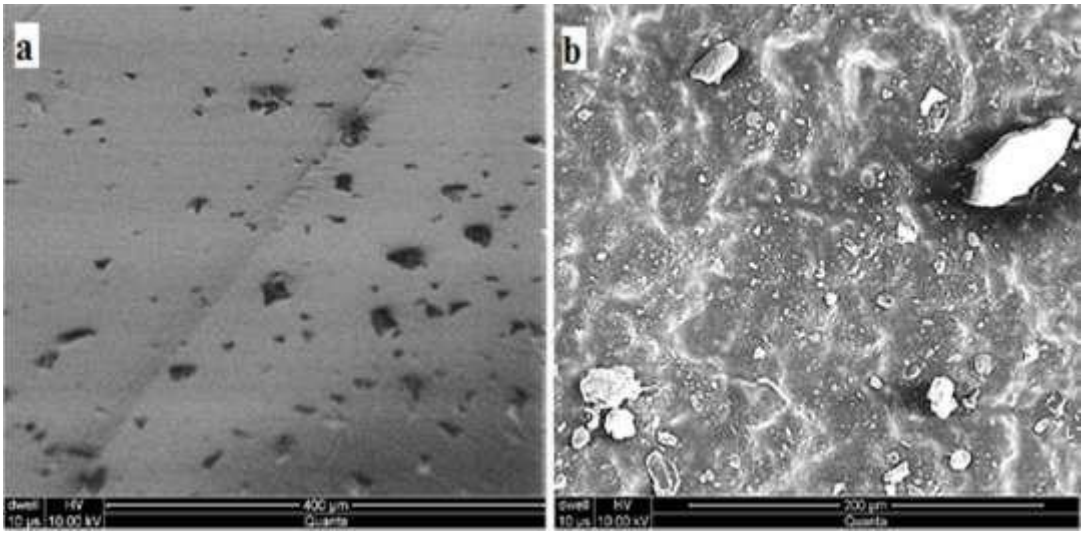

Figure 9 a-b: SEM of PEG-4000 with polymer.

Gel and yield percentage and gel time

- Gel % increased as PEG content increased (Formulation 1-3);
- Similarly, yield% increased when PEG 4000 ratio increased;
- Higher gel% and yield% in Formulation 4-6;
- Gel time increased in Formulation 7-9 ;

Impact of hydrogel components on water absorbency

- In Formulation 1-3, more equilibrium water absorbency of PEGA 4000hydrogels
- In Formulation 4-6, more water absorbency due increased conc. of AApolymeric network
- In Formulation 7-9, reduced water absorbency with increased conc. of EGDMA (high density cross-linked polymeric network)

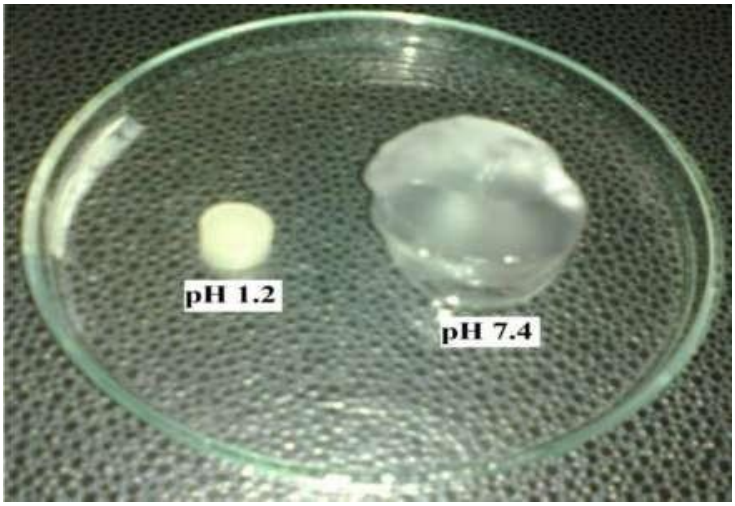

Figure 10: PEGA-4000 hydrogels water absorbency at pH 1.2and 7.4.

Drug loading was directly proportional to the water imbibition.

EXT Release Profile in PEGA 4000 hydrogels

- Cumulative % release was 19% at acidic pH 1.2 and 92% at alkaline pH 7.4;
- reduced electrostatic repulsion : decreased water imbibitions : less release ofEXT (pH 1.2).
- Enhanced electrostatic repulsion : increased water imbibitions : more water absorbency and EXT release (pH 7.4).
- EXT release profile was found increased with increased conc. of PEGA 4000and AA.
- Decreased EXT release was found with increased conc. of APS and EGDMA.

Kinetic Modeling of EXT Release

- formulations 1-9 had followed Higuchi model (R^2 values : 0.9016-0.9718);
- Korsmeyer-Peppas model (R^2 Values : 0.9028-0.9904; and "n" values :0.536-0.684)**;**

Table 8: Eloxatine (EXT) Release kinetics

Formulations (PEGA-4000)	EXT "0"	EXT 1st	EXT Higuchi	EXT Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
F1	0.7406	0.9582	0.9718	0.9796	0.562
F2	0.8676	0.9824	0.9368	0.9772	0.656
F3	0.8986	0.9956	0.9366	0.9874	0.684
F4	0.8906	0.9818	0.9426	0.9866	0.672
F5	0.8764	0.9872	0.9526	0.9904	0.654
F6	0.8842	0.9862	0.9254	0.9744	0.682
F7	0.8206	0.9874	0.9362	0.9644	0.624
F8	0.8234	0.9502	0.9166	0.9348	0.652

F9	0.6182	0.9024	0.9016	0.9028	0.536

PEGA 8000 Hydrogels
Morphology / Appearance
Colour : Light Yellow disc (Transparent)
Nature : Cylindrical & Rubbery jelly
Strength : Good

Figure 11: Morphology of PEGA 8000 hydrogel discs.

FTIR Analysis

Table 9: FTIR spectrum Analysis of PEG 8000.

Wavelength (cm^{-1})	Bond / Stretching
2880	C–H stretching
1094	C–O stretching
3436	-OH stretching
2890	- CH_2 group
1094	C-H stretching of ether

DSC and TGA Analysis

- At 71.5°C (Endothermic peak due to loss of moisture);
- At 73°C (Exothermic peak due to degradation of polymeric chain);
- Between 410°C - 415°C (Endothermic peak of PEG 8000);
- At 448°C (endothermic peak for complete degradation of -OH, ether and alkyl groups in developed hydrogel);
- TGA: At 423°C - PEG 8000 (100% weight loss);

Weight loss (WL) % in PEG 8000 prepared hydrogels	15% WL at 228°C (Phase-1);
	45% WL at 325°C (Phase-2);
	40% WL at 489°C (Phase-3);

SEM

- glossy porous and solid (Figures 12a);
- compatible with PEG-8000 and AA (Figures 12b)

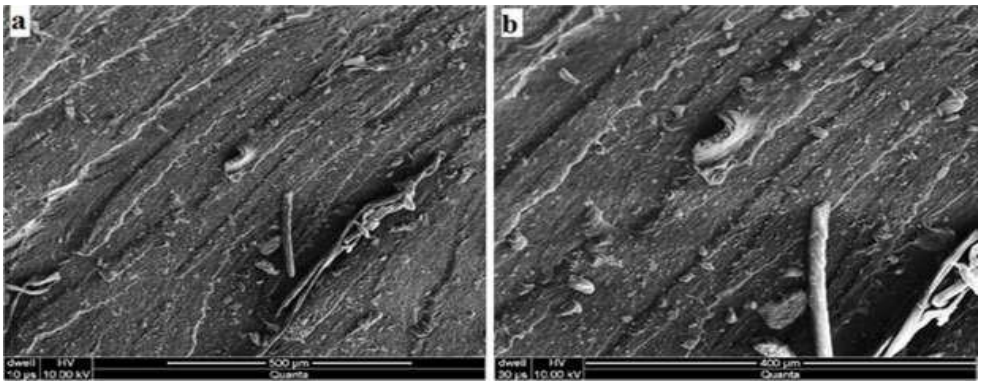

Figure 12a-b: SEM of cross-linked polymer network (PEGA 8000).

Impact of components

- increased asPEG-8000 content increased (Formulation 1-3);
- yield% (upto 92%) increased when PEG 4000 ratio increased**;**
- Higher gel% and yield% found in Formulation 4-6;
- In Formulation 7-9 with high ratio EGDMA;

Water imbibition

- In Formulation 1-3, water imbibition were increased with increased PEG 8000**;**
- In F4-F6, more water imbibition due increased conc. of AA polymeric network (increased carboxylate ions) cause electrostatic repulsion;
- In F7-F9, less equilibrium water imbibition with decreased segmental mobility of highly cross-linked polymeric chains due increased conc. of EGDMA (high density cross-linked polymeric network)**;**

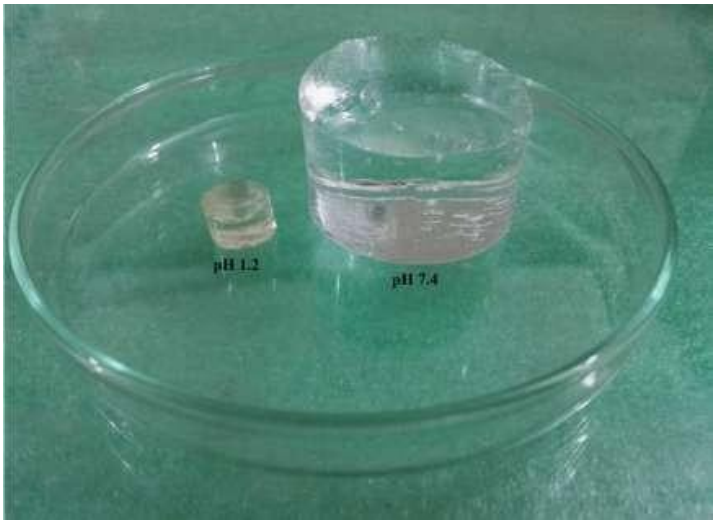

Figure 13: Water imbibition of hydrogels (PEGA 8000) at pH 1.2 &7.4.

Drug loading in PEGA 8000

- drug loading was directly proportional to pH-environment media;
- swelling and drug loading were directly proportional to increased PEG and AA content; less water uptake with increased conc. of EGDMA.

Eloxatine Release Kinetics

- high at basic pH (96%; pH7.4) than at acidic pH (11% ; pH 1.2).
- release of EXT increased with weakening of hydrogen bonding;
- drug release increased with increased of AA (26, 33, 40 wt%);
- dense structure of hydrogels = reduction in electrostatic repulsive forces =restricted drug release.

Kinetic Modeling of Drug Release

- zero order release was not favoring zero order kinetics;
- first order release (R^2 values were between 0.9078-0.9812) favored diffusion-controlled release phenomenon;
- Higuchi model : R^2 values of F1-F9 ranged between 0.9012-0.9878)**.**
- Korsmeyer-Peppas model : best porous.

Table 10: Elxatine Release.

PEGA-8000 Code	Eloxatine zero order	Eloxatine First order	Eloxatine Higuchi	Eloxatine Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
F-1	0.4178	0.9312	0.9582	0.9664	0.452
F-2	0.5698	0.9282	0.9832	0.9834	0.488
F-3	0.4882	0.9612	0.9136	0.9148	0.478
F-4	0.5958	0.9214	0.9878	0.9878	0.496
F-5	0.5978	0.9772	0.9654	0.9628	0.504
F-6	0.4312	0.9812	0.9332	0.9384	0.458
F-7	0.4586	0.9078	0.9666	0.9722	0.464
F-8	0.4534	0.9192	0.9304	0.9396	0.458
F-9	0.5386	0.9502	0.9012	0.9014	0.466

Hence, hydrogels formulations of EXT-AA-APS-EGDMA had followednon-Fickian diffusion i.e. anomalous-diffusion.

Conclusions

It was found that gel% & yield% was directly proportional to high conc. of polymers & monomers and gel time decreased as result of polymeric network formation (increased conc. of EGDMA). In PEGA 8000 hydrogels, it was further summarized that EXT release decreased as EGDMA increased (0.16, 0.33, 0.5 wt%). Finally, best porous structures for drug diffusion through water imbibitions were developed. At high pH (7.4) colon targeting of EXT can be highly effective (high EXT release; approx. 92% in 36 hrs). Finally, it was concluded that stable hydrogels (pH sensitive) of Eloxatine (EXT) in PEG 4000 / PEG 8000 can be formulated with AA, APS, EGDMA successfully and can be utilized as an ideal delivery system for EXT for colon targeting in colorectal cancer.

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