Engineering of Gelatin for Drug Delivery Applications

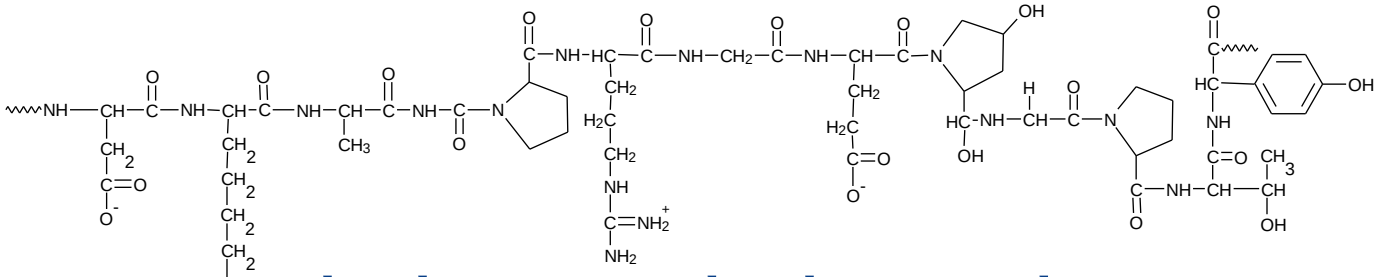
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Gelatin-important features

* Gelatin (A/ B)-a translucent, colorless, brittle, flavorless denatured protein-obtained by acid (A) or base (B)hydrolysis of collagens from animal skin & bone .
* Gelatin A (pI=7–9) and B(pI=4-5) – have different isoelectric point , mol.wt, amino acid composition, and viscosities.
* It is a heterogeneous mixture of single or multi-stranded random coiled poly- peptides, each with extended left-handed proline helix having 300 to 4000 amino acids. versatile biomaterial commonly used as a gelling agent in food and pharmaceuticals

Gelatin-features

Intrinsic features- great potential to modify at the level of amino acids, low level of immunogenicity and cytotoxicity, FDA approval as a clotting agent and exudate absorbing construct, hydrogel formation and biodegradability.



-Asp-Lys -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-Tyr-

Gelatin-composition of amino acids

* Gly 21%, Pro 12%, HyP 12%, Glu 10%, Ala 9%, Arg 8%, Asp 6%, Lys 4%, Ser 4%, Leu 3%, Val 2%, Phe 2%, Thr 2%, Ile 1%, HLys 1%, Met and His <1% and Tyr <0.5%
* Gelatin-a polyampholyte , ~12% -vely charged due to Glu & Asp, ~13% +vely charged due to Arg and Lys residues , and ~11% of its chain hydrophobic due to Ile, Met, Leu and Val residues, in an approximate ratio of 1:1:1 respectively. Pro, Gly & HPro occurring as a sequence of repeating triplet constitute the rest of gelatin

Features of gelatin

* Use of gelatin,a safe excipient approved by FDA of US, in various pharmaceutical dosage forms dates back to the early nineteenth century or before.
* Today, million pounds of gelatin is used annually as hard and soft elastic capsules, tableting, tablet coating, granulation, encapsulation, and micro-encapsulation.
* The good adhesive properties and easy shattering of its agglomerates are its additional advantages.

Features of gelatin

* pI values of gelatin can be modified to get either a -vely charged acidic gelatin, or a +vely charged basic gelatin at physiological pH to facilitate electrostatic interactions with charged biomolecule (protein, plasmid DNA etc)
* Its heterogeneity in mol.wt, high water solubility, poor structural & thermal stability- make it a poor carrier to achieve controlled and prolonged drug release

Features of Gelatin

* But its primary structural features offers many chemical modification that enable the design of different drug carrier systems, such as microparticles and nanoparticles, fibers and hydrogels for controlled release.
* Gelatin microparticles can serve as vehicles for cell amplification and for delivery of large bioactive molecules & its nanoparticles are better suited for intravenous delivery or for drug delivery to the brain.
* Gelatin fibers contain a high surface area-to-volume ratio
* Gelatin hydrogels can trap drugs between the polymer's crosslink gaps, allowing them to diffuse into the blood stream. Gelatin forms complexes with different drugs

Gelatin modifications

* Nanoparticle and microparticle formation
* Chemical Crosslinking –PEG-dialdehyde, diisocyanate, genipin, glutaraldehyde etc
* Enzymatic crosslinking- using transglutaminase
* PEGylation
* Thiolation via ε-amino groups
* Blending with other polymers
* IPN formation with appropriate polymer
* Grafting
* Conjugation with synthetic polymers and antibodies
* Anionization/cationization
* Entrapping green synthesized Ag nanoparticles
* Converting carboxylic group to amido group
* Introducing hydrophobic alkyl group via pendant amino group

Effect of gelatin modification

* Alters biofunctional properties such as solubility, swellability, biodistribution, biocompatibility, targetability, biodegradability, hydrophilicity/ hydrophobicity, morphology, and stability of the formulations
* Improve its flexibility to overcome challenges in finding ideal carrier systems that enable specific, targeted and controlled release in response to body demands.

Diisocyanate crosslinker

* (i) isophorone diisocyanate (IPDI) &
* (ii) isocyanate terminated oligomeric poly(ethylene glycol) (PEG-600) (ICTPE)-prepared by reacting IPDI and PEG-600 in DMSO

These were used as crosslinkers to modify gelatin properties because of the less toxicity of IPDI compared to other diisocyanate crosslinkers and biocompatibility of PEG moiety in ICTPE

Diisocyanate crosslinked gelatins

IPDI crosslinked gelatins using 1, 2, 5, 10, 20, and 30 wt% of IPDI with reference to the weight of gelatin taken were designated as GEIP-1, GEIP-2, GEIP-5, GEIP-10, GEIP-20, and GEIP-30,

ICTPE Crosslinked gelatin	Feed Composition for prepolymer (ICTPE) (mole)		Gelatin (g)
	PEG-600 ($\times 10^3$)	IPDI ($\times 10^3$)	
GEPI-5	0.4483	0.8966	2
GEPI-10	0.896	1.793	2
GEPI-20	1.799	3.599	2
GEPI-30	5.398	5.398	2

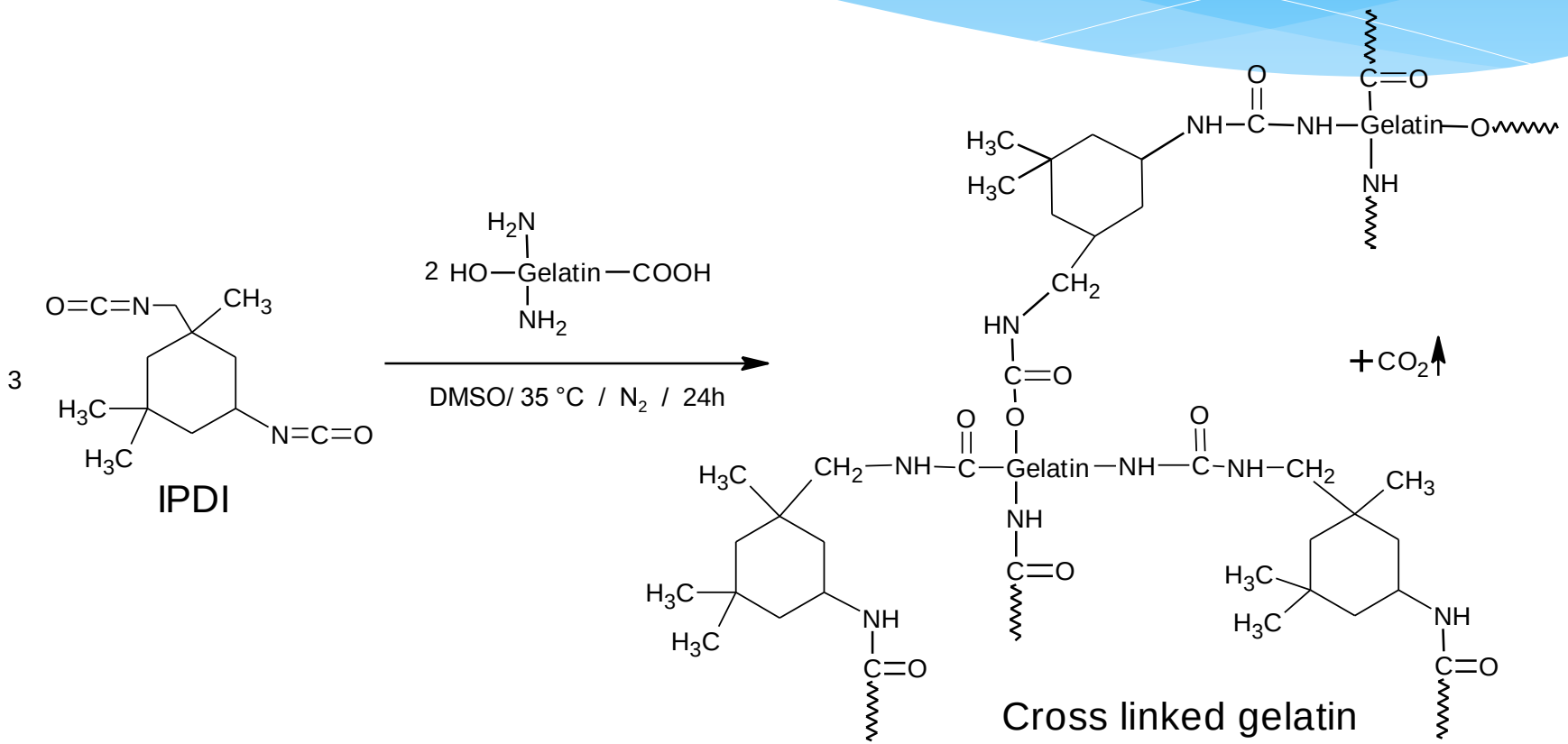
Isocyanate reactions with gelatin

* Free pendant hydroxyl, amino, carboxyl, etc. of gelatin react with isocyanate in the rate order: $–NH_2$ > –OH > –COOH to form urea (–NH–C(O)–NH–), urethane (–NH–C(O)–O–), and carbamate (–NH–C(O)–O–C(O)) links, respectively.
* $-NH_2$ + -NCO ----→ -NH-C(O)-NH- (Urea)
* -OH + -NCO ----→ -O-C(O)-NH- (Urethane)
* -COOH + -NCO ---→ -NH-C(O)-O-C(O)--- (Carbamate, Unstable)

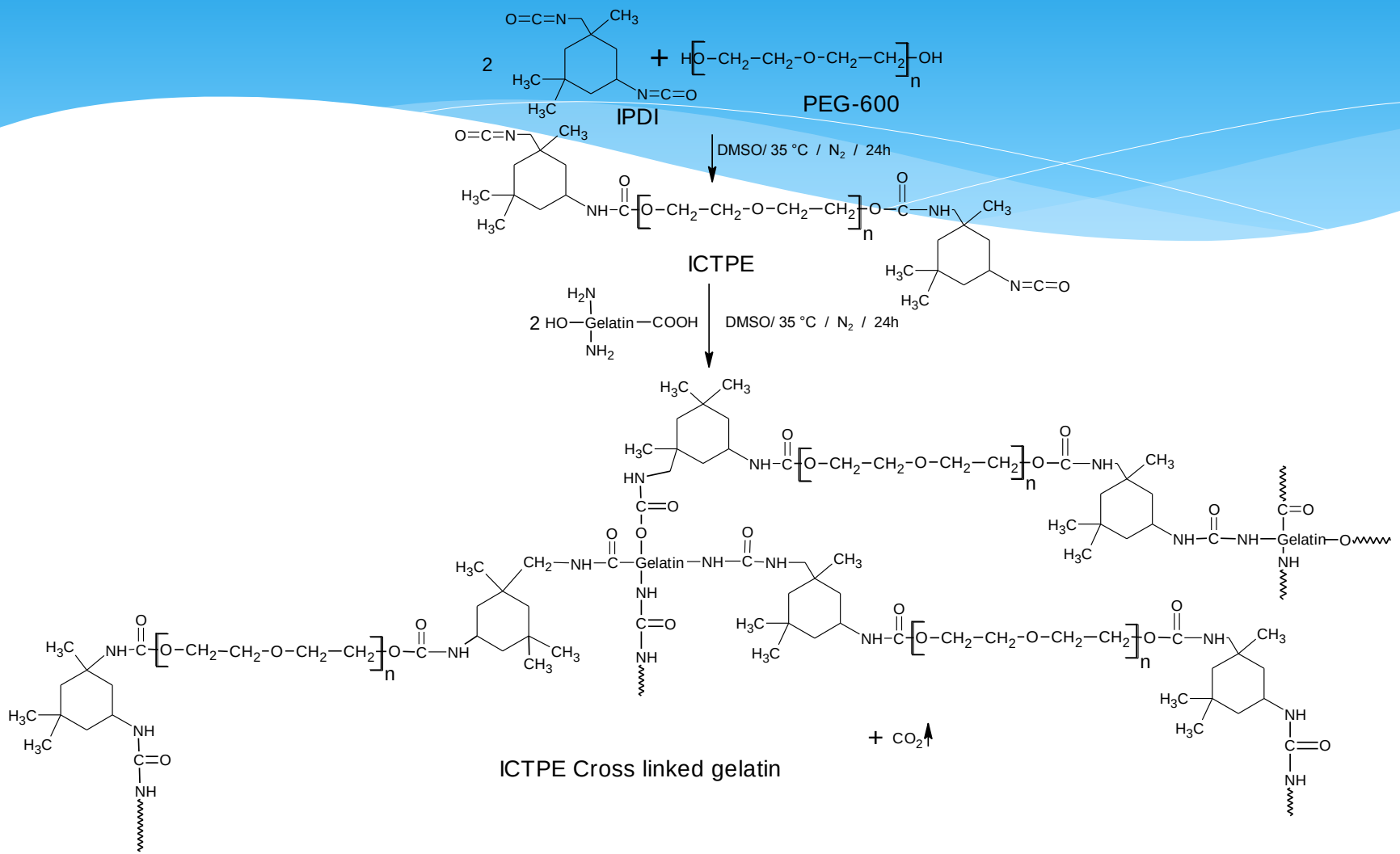
Since the carbamate is unstable, it is converted into amide bond with release of carbon dioxide

* (–NH–C(O)–O–C(O)-- -→ –NH–C(O)–NH– + CO2

IPDI crosslinked gelatin



ICTPE crosslinked gelatin



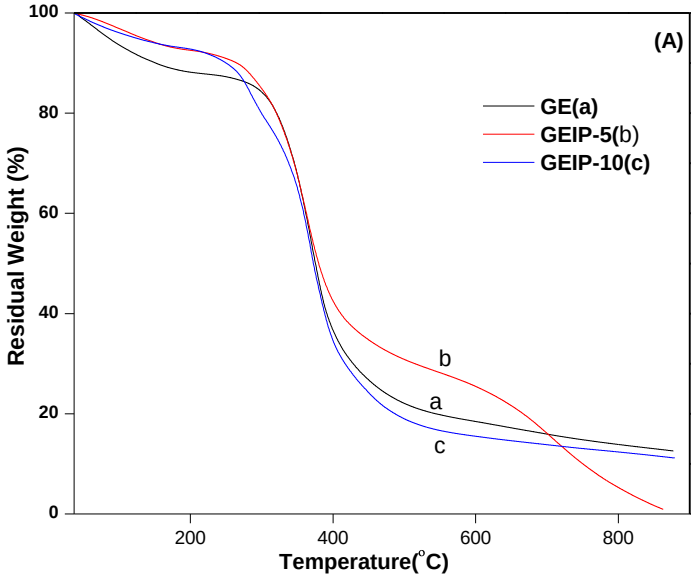
DIISOCYANATE CROSSLINKED GELATIN

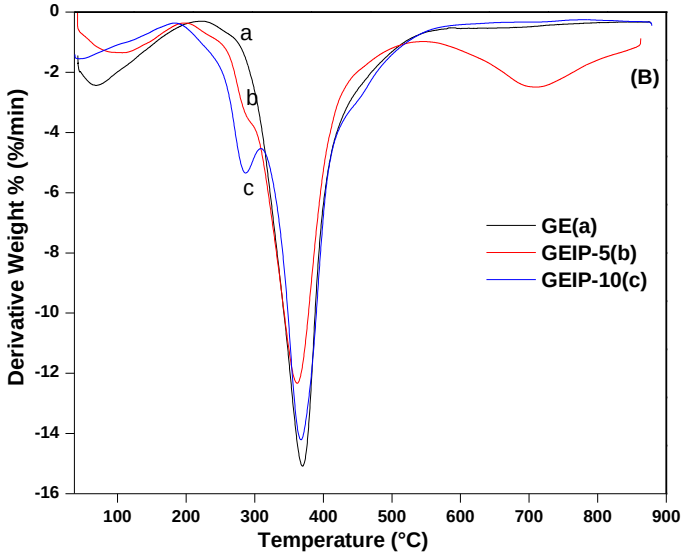
* Reaction of IPDI and ICTPE generates urethane, amide, and urea linkages- it is anticipated that they will display different mucoadhesive and swelling characteristics, which may influence the drug release characteristics.
* Moreover, the PEG spacer may minimize the loss in the flexibility and swellability of the gelatin due to crosslinking.
* The crosslinked gelatins carriers were evaluated by *in vitro* release studies THP and 5-FU tablets in SIF & SGF.
* Structural effect of drugs on their release features were also tested for a typical crosslinked gelatin carrier taking 5-FU, AMP Na & THPas specific examples.

IPDI and ICTPE Crosslinked gelatin Synthesis and characterization - NMR, FT-IR, XRD etc

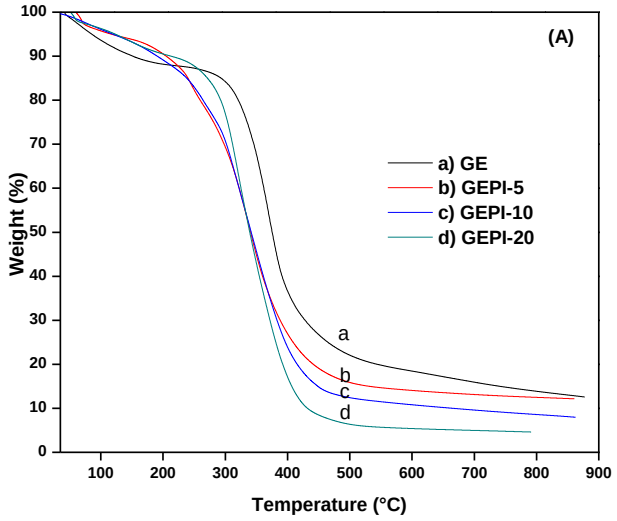
* Subramanian K, Vijayakumar V. Polymer Bulletin, 2013, 70(3): 733–753
* V.Vijayakumar, K. Subramanian, Saudi Pharmaceutical Journal,2014, 22: 43-51.

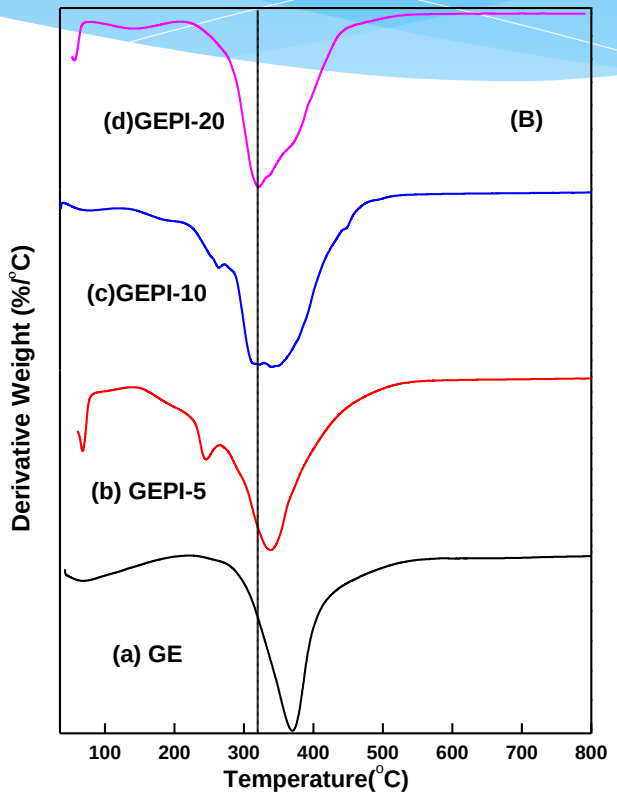
(A) TG and (B) DTG traces of gelatin (a) and crosslinked gelatins [GEIP-5 (b), GEIP-10 (c)]



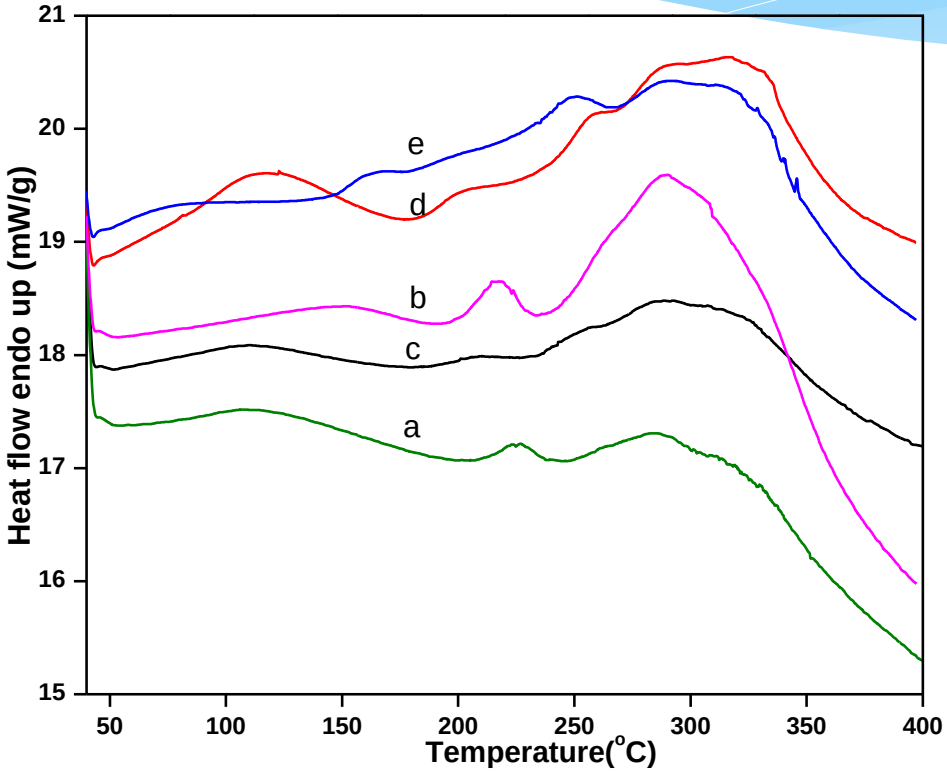


TGA (A) and DTG (B) traces of, (a) GE, (b) GEPI-5, (c) GEPI-10 and (d) GEPI-20

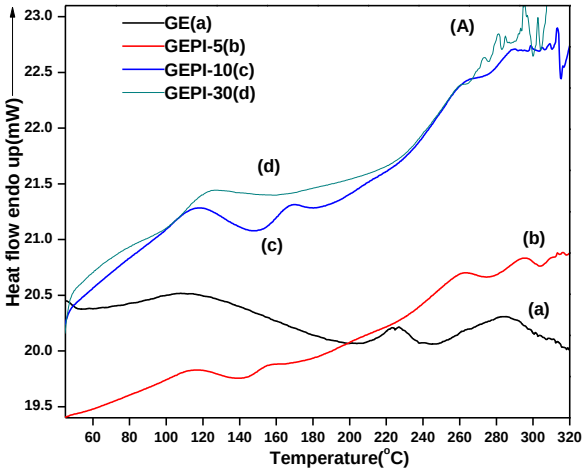


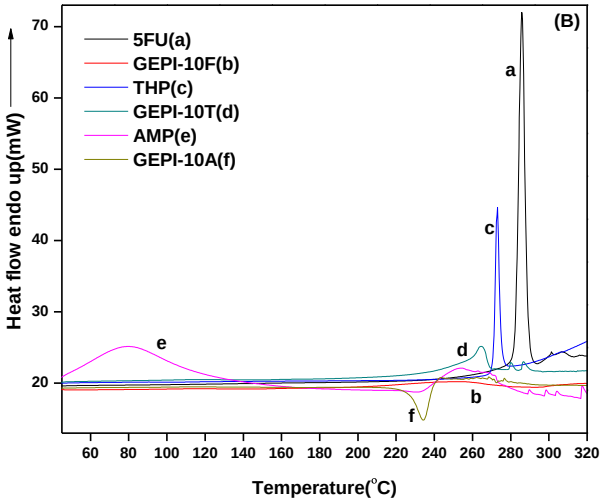


DSC thermograms of gelatin (a), regenerated gelatin (b) and crosslinked gelatins GEIP-1(c); GEIP-5(d); & GEIP-10 (e)

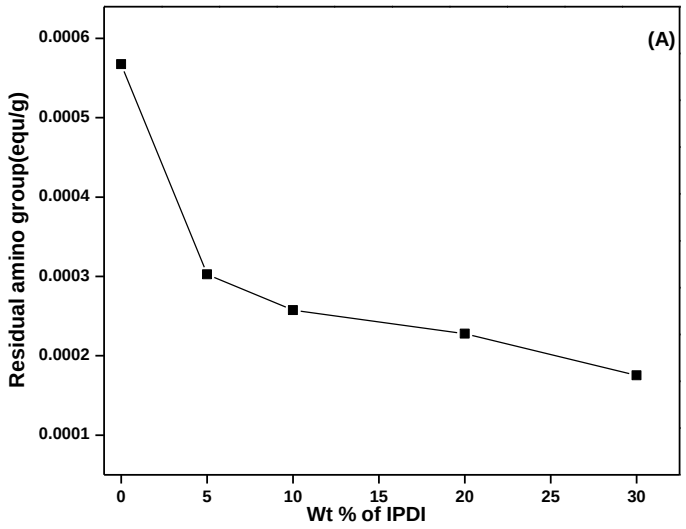


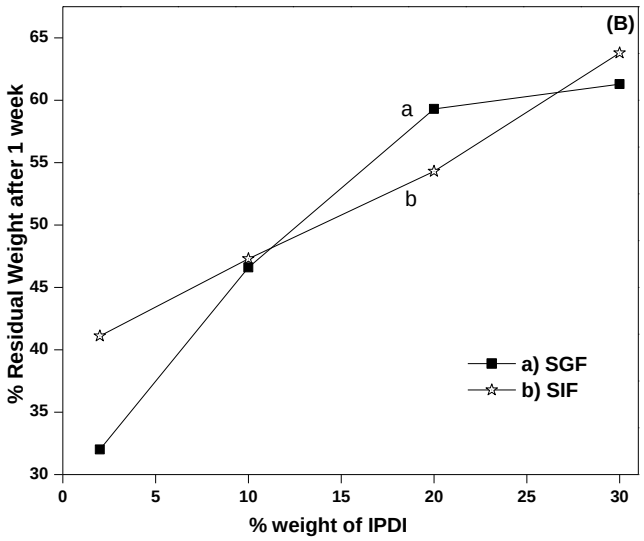
DSC traces of , (A) gelatin and crosslinked gelatins and (B) drugs and tablets



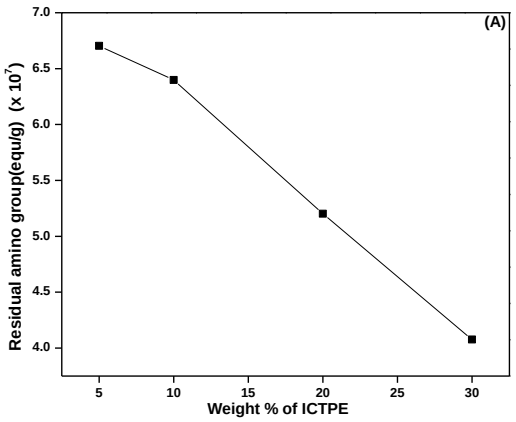


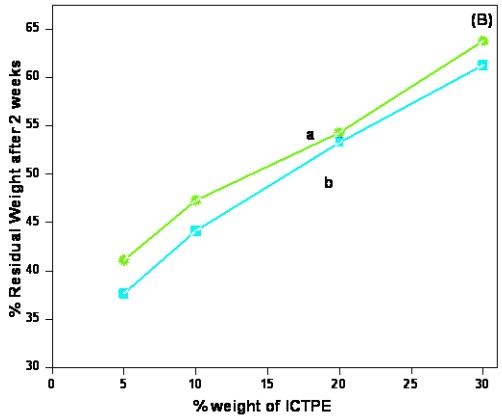
Effect of wt % of IPDI on B) % acetone insoluble fraction (A) and residual amino group (b) in aged IPDI cross linked gelatin

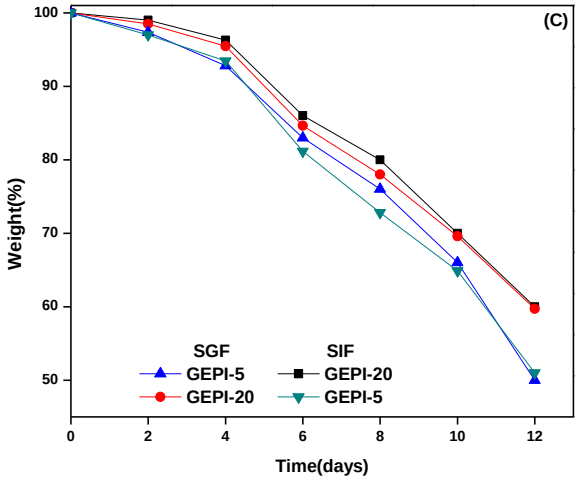




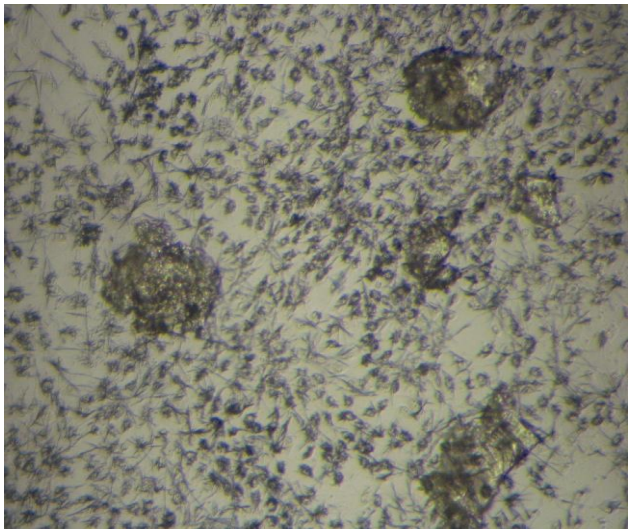
Effect of ICTPE wt% on residual amino group (A) and% acetone insoluble fraction(B) of aged cross linked gelatin in SGF (a) and SIF (b), and biodegradation of GEPI-5 and GEPI-20 with time (C) in SGF and SIF.

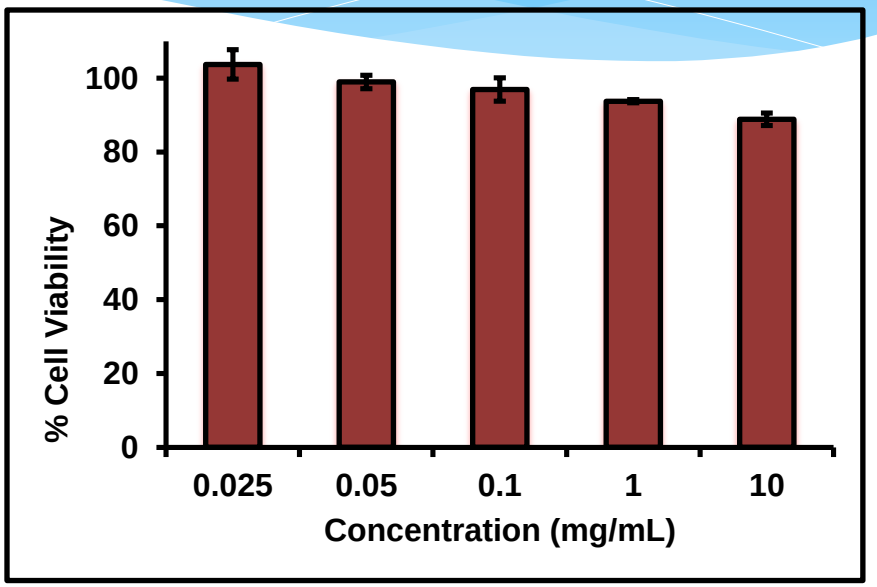




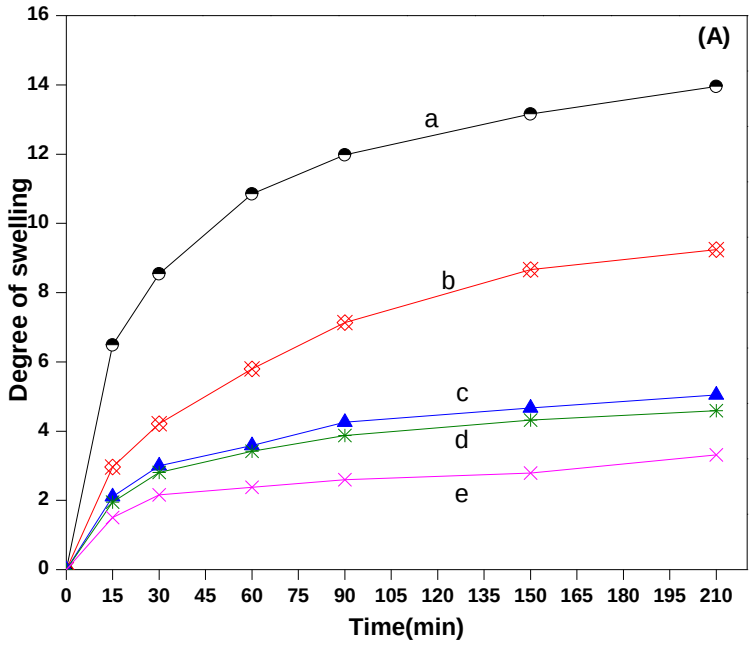


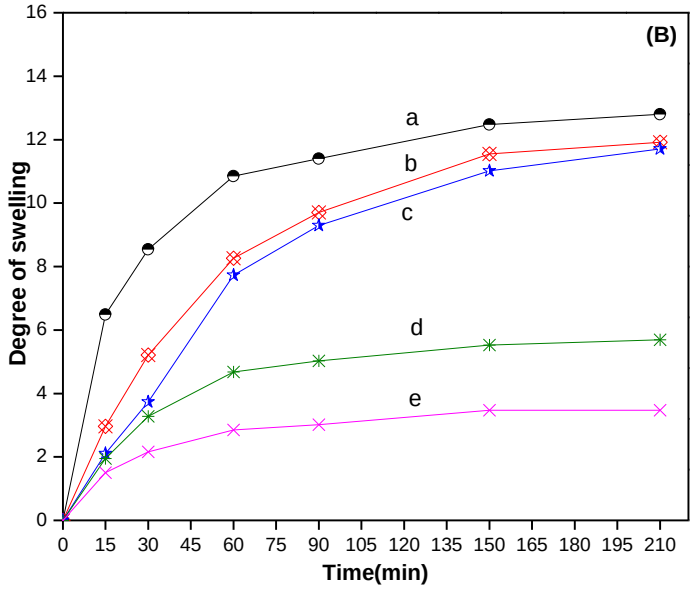
A)Optical microscopic photographs and B)Histogram - % cell viability of NIH 3T3 cell growth on GEIP-5



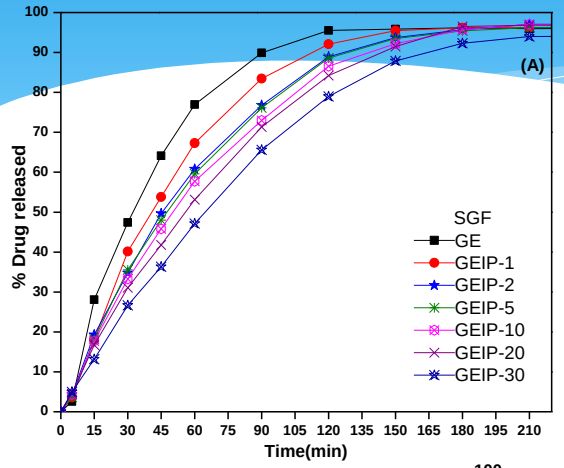


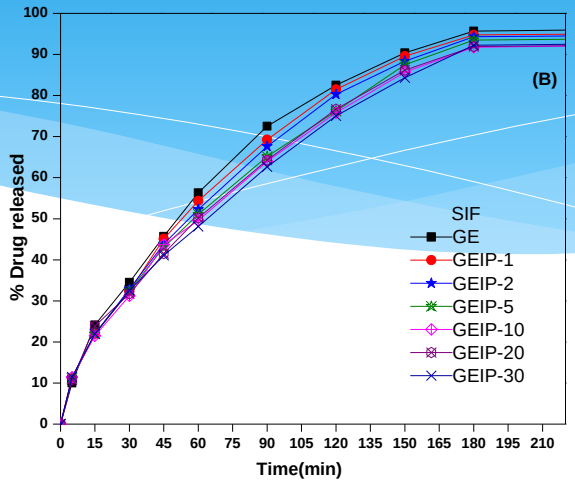
Degree of swelling of IPDI cross-linked gelatins (GEIP-2(a), GEIP-5(b),GEIP-10(c), GEIP-20 (d) and GEIP-30(e)) in SGF (A) and SIF (B) at 37°C

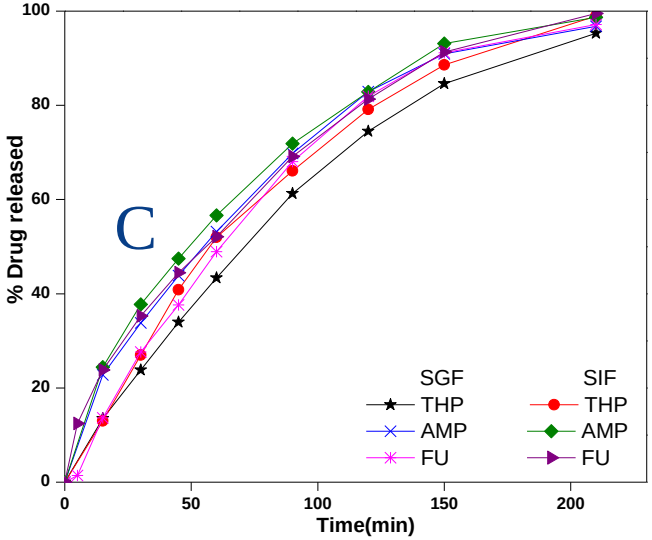




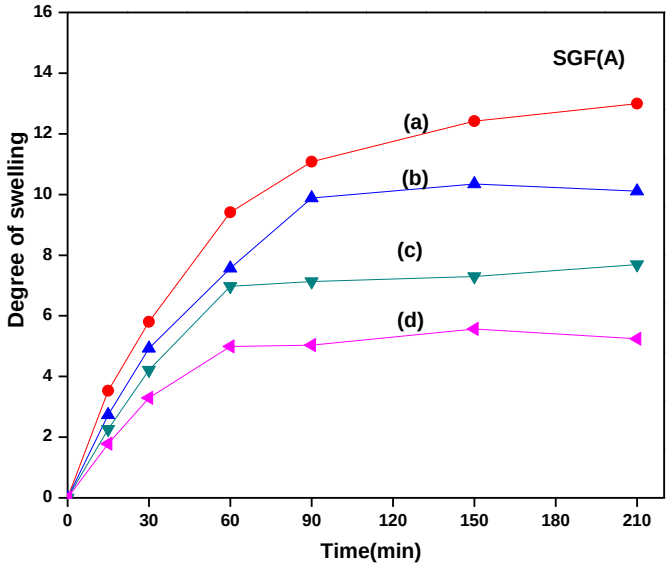
Release profiles -tablets , 5-FU with IPDI cross linked gelatin in SGF (A) and SIF (B) & THP, FU & AMP with GEIP-30 (C) at 37°C

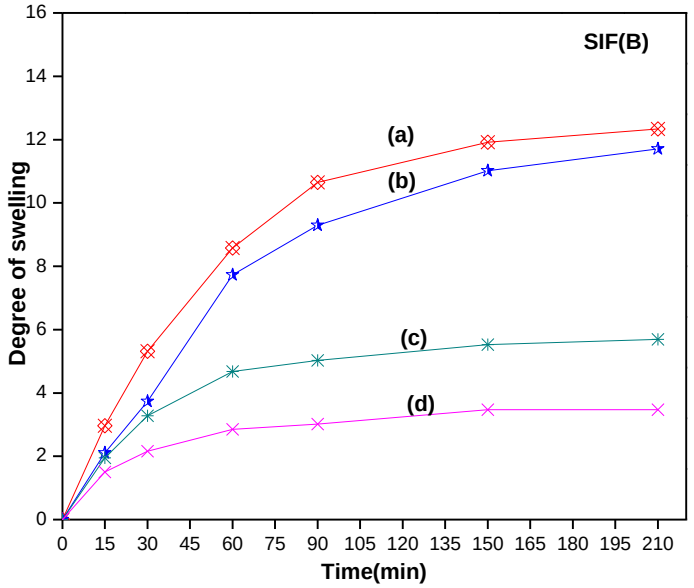




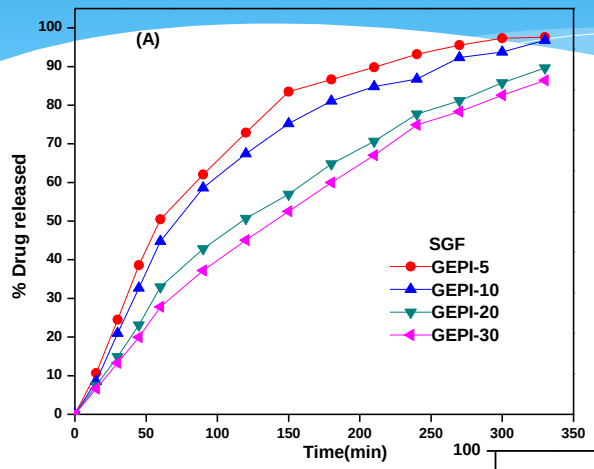


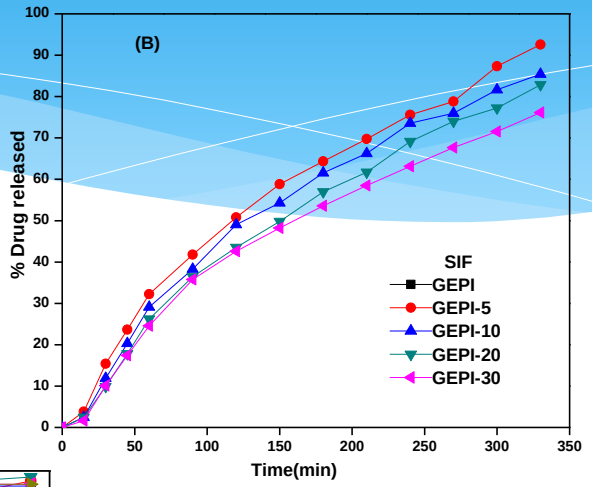
Swelling profiles of ICTPE crosslinked gelatins GEPI-5(a), GEPI-10(b), GEPI-20 (c) and GEPI-30(d) in SGF (A) and SIF (B) at 37°C

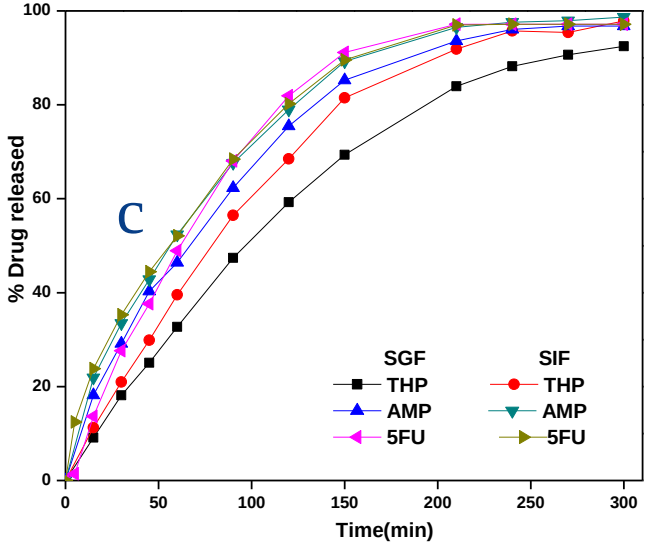




Release profiles of, THP with (a) GEPI-5, (b) GEPI-10, (c) GEPI-20 and (d) GEPI-30 in SGF (A) and SIF (B) & THP, AMP and 5-FU in SGF and SIF with GEPI-30 (C) at 37 °C







Drug release mechanism

* Drug release was modeled using the Korsemeyer-Pappas empirical equation

$$M_t/M_\infty = kt^n$$

where M_t/M_∞ - fractional release at time t, k- rate constant & n - diffusional exponent.

For IPDI and ICTPE crosslinked gelatin the n values were in the range 0.6–0.9-non-Fickian

Conclusions

* Crosslinking of gelatin with IPDI & ICTPE-decreased its water solubility, biodegradability, and swellability and enhanced amorphous nature
* *In vitro* release profiles of 5-FU -decreased drug release rate with increased crosslinking. The 5-FU release rate is more in SGF than in SIF
* Comparison of typical release profiles for AMP sodium, THP, and 5-FU for a typical crosslinked carrier indicated only marginal differences in release rates due to variation in hydrophobicity / hydrophilicity of drugs
* Non-Fickian (anamalous transport,n=0.6 to 0.9)release mech.

Conclusions

* The cross linked gelatin - biodegradable in SIF and SGF & biodegradability decreased with increased degree of cross linking. Drug release by mainly by swelling-diffusion. Minor gelatin degradation in SGF and SIF may also contribute for the drug release rate.
* The results suggested that the cross linked gelatin might be a promising vehicle for sustained release preparations in oral therapy.
* This material can also be used in tissue engineering as scaffold in medical field because of its biocompatibility, structural integrity and biodegradability.

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