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Research Article

CHEMICALLY MODIFIED PEO-PPO-PEO TRI-BLOCK COPOLYMERS AND ITS HYDROGEL WITH ALGINATE FOR SOLUBILIZATION OF LAMOTRIGINE DRUG

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Abstract

The tri-block copolymer $(PEO)_{13}$-$(PPO)_{30}$-$(PEO)_{13}$(code name L64, with PEO and PPO representing polyethylene oxide and polypropylene oxide, respectively), a member of Pluronics® polymers (*BASF*, NJ, USA) was modified by introducing benzaldehyde moiety at the terminal end of the polymer chain through condensation polymerization. The synthesized Benzaldehyde-capped Pluronic®L64 (code name, BCPL64) was characterized using FTIR, ^{1}NMR and TGA analysis. Polymers, L64 and BCPL64, are formed hydrogels' with alginate through physical blending under physiological conditions. Results show that both the polymers are mixed homogeneously with alginate and giving effective cross-linking. The BCPL64 was shown better properties than L64 and pure alginate gel. Swelling studies also elaborated the properties of the respective gels. Solubilization of an antiepileptic agent, Lamotrigine(LTG) in L64 and all the prepared hydrogels were investigated by UV-Visible spectroscopy. All the data proves that the solubilization of LTG is found better in L64 and BCPL64 based alginate gel compared to pure alginate gel

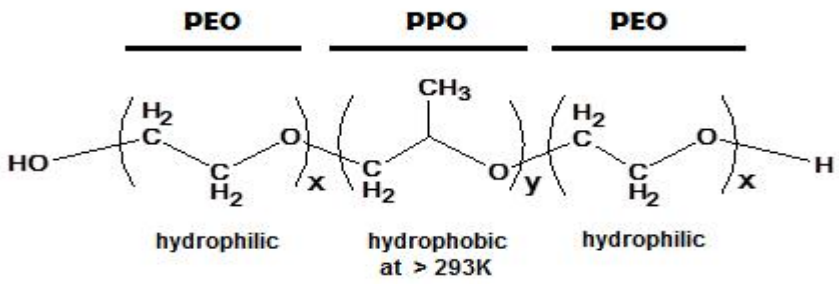
Keywords: Pluronic® polymer, Micellization, Alginate gel, Solubilization, Lamotrigine,

Introduction

Hydrophobic interactions always play a dominant role in the formation of large biological systems. These interactions can be generated in synthetic polymer systems by incorporation of hydrophobic sequences/character within the hydrophilic chains of polymer. Aqueous and nonaqueous solutions of hydrophobically modified polymers constitute a class of soft materials with remarkable rheological properties [1,2]. Above a certain polymer concentration, the hydrophobic groups in such associative polymers are involved in intermolecular associations that act as reversible breakable crosslinks creating a transient 3D polymer network.

Synthetic triblock copolymers of poly(ethylene oxide)(PEO) and poly(propylene oxide) (PPO) have gained increasing interest during the last decades because of their large variety of applications including biomedical and pharmaceutical applications[3-6]. These triblock copolymers are also known as Pluronics®, Poloxamers, Synperonics and Proxanols [7,8]. The molecular structure of Pluronic® polymers is shown in Scheme.1. The hydrophobic PPO block(at > 20^0C) is sandwiched between two hydrophilic PEO blocks. They are typical amphiphilic polymers have the ability to form micelles similar to conventional low molecular weight surfactants. These copolymers undergo thermoreversible micellization and gelation in aqueouss solutions via associations of hydrophobic PPO blocks[3-8]. The PPO center blocks form the core of Pluronic micelles while the relatively hydrophilic PEO blocks forming the micelle coronas interact with those of neighboring micelles[6]. As the temperature is increased, the number of Pluronic micelles also increases leading to the formation of thermoreversible hydrogels via intermicellar

Scheme.1 : Chemical structure of Pluronic Block copolymers



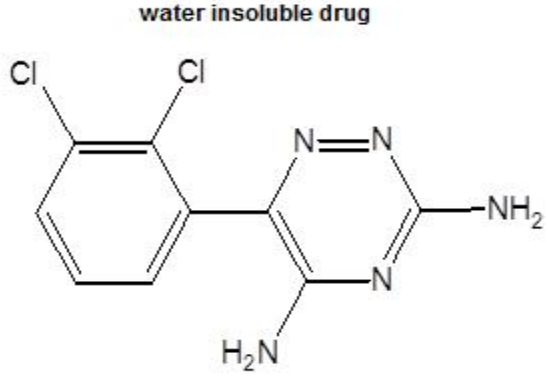
entanglements between PEO segments[9]. Hydrogels fabricated from pure unmodified Pluronics can form a physical gel at body temperature. Unfortunately, the 'gels' formed may disintegrate in a relatively short time when exposed in an aqueous environment with continuous removal of the polymer in its micellar state. Thus, such a physical hydrogel needs to be modified in order to meet the implant needs for lumpectomy patients [10]. Dependent on the degree of cross-linking, partial chemical cross-linking of Pluronics can turn the physical 'gel' into a more stable, partially covalently bonded chemical gel. This chemically cross-linked gel will no longer be completely thermoreversible.

The applications of non-modified Pluronic® polymers have several disadvantages[11]. Low viscosity at 37^0C and limited in vivo residence times renders the material unsuitable for certain clinical applications[12]. In order to overcome these drawbacks, cross-linkable derivatives have been developed. Pluronics have been functionalized with methacrylate[12], acrylate[13,14], benzaldehyde[15], azide[16] moieties etc. for better cross-linking in the preparation of hydrogel for pharma applications. Since to the best of our knowledge, mainly F127 has been applied so far for the production of covalently cross-linked hydrogels, we wanted to evaluate the feasibility of modifying different types of Pluronic® polymer.

In our present work, we have synthesized chemically modified benzaldehyde capped Polyethylene oxide-Polypropylene oxide-Polyethylene oxide tri-block copolymer (BCPL64) using the hydrophobic Pluronic®L64. The synthesized end capped Pluronic®polymer was utilized in the preparation of alginate gel for potent solubilizers of hydrophobic drug in pharma applications.

Lamotrigine(LTG) is an antiepileptic agent used as a monotherapy and as an adjunct to treatment with other antiepileptic agents for partial seizures and primary and secondary generalized tonic-clonic seizures. The molecular structure of this drug is shown in Scheme.2. It is also used for seizures associated with the Lennox-Gastantsyndrome[17,18]. It is extensively metabolized in the liver following oral administration. Therefore, the delivery of LTG for systemic use via buccal mucosa is always the concern of research. Also it has very poor aqueous solubility (0.17 mg/mL at 298K)[19] and hence, many researchers have tried to improve the solubility and dissolution rate of LTG for better therapeutic efficacy[20,21]. The solubilization of LTG in the prepared alginate gels based on Pluronic®polymers was investigated using UV-VIS spectroscopy measurements.

Scheme.2 : Molecular structure of Lamotrigine- an antiepileptic agent



Experimental Section

Materials

PEO-PPO-PEO tri-block copolymer (Pluronic®L64) is purchased from Sigma-Aldrich and used as received. Specification of the Pluronic®L64 is shown in Table-1. p-formyl benzoic acid was highly pure obtained from Sigma-Aldrich. Dicyclocarbodiimide(DCC) and dichloromethane (DCM) were purchased from *Merck*, India. The 4-dimethyl amino pyridine (DMAP) and sodium alginate were of Loba Chemie of AnalaR grade. Highly pure lamotrigine (LTG) drug is of pharmaceutical grade gifted by PAB organics, Vadodara, Gujarat, India. The molecular characteristic of the drug is shown in Table-2. Other required reagents like methanol, ethanol, isopropanol are of AnalaR grade Qulaigens reagents. Triple distilled water from an all- Pyrex™ glass apparatus was always used.

Table.1 : Specification of Pluronic®L64

Formula	Avg. mol. wt.(Da)	%EO	EO/PO ratio
$EO_{13}PO_{30}EO_{13}$	2900	40	0.638
CP, °C (1 %w/v)	**HLB**	**Mw of PPO**	**Pour point**
60 °C	12 – 18	1750	25 °C

Table.2 : Molecular characteristic of LTG drug

Category	Mol. Form.	Mw, g mol-1	MP, ^{0}C	Physical appearance
BCS class II	$C_9H_7N_5Cl_2$	256.091	214^0	White powder

Chemical modification of Pluronic®L64:

Benzaldehyde-capped Pluronic®L64(BCPL64)

In a 250 ml of three neck round bottom flask, Pluronic®L64(3.0 gm), p-formyl benzoic acid(2.4gm) and DMAP (0.244gm) dissolved in 50 mL DCM were taken, which was equipped with a reflux condenser, dropping funnel, gas inlet/outlet, and magnetic stirrer. To this was added 3.3gm of DCC in 20 mL of DCM and the mixture was stirred for 24 hours at room temperature under the nitrogen atmosphere before it was filtered. The condensed filtrate was dissolved in isopropanol and cooled at 0^0C for morethan 2 hours. The resultant product was collected and dialyzed against 3 L of methanol/water solution. This was further washed with water. The dialysate was dried in vacuum oven to grain off white colored product[15]. This product was kept in clean glass bottle sealed with paraffin.

Preparations of Alginate based Gel

Alginate Gel (Alg)

Briefly, alginate gel in the form of beads can be prepared by the dropwise addition of 10ml of 10% w/v sodium alginate in 50 ml of 0.45M $CaCl_2$ solution through syringe. The reformed gel were kept to stand in gelling medium for sometimes and separated from it through proper decantation(Fig.1a). The gel obtained was dried by keeping it in vacuum oven.

Pluronic based Alginate Gel (PL64-Alg)

First, stock solution of Pluronic®L64 was prepared with cold method. In brief, Pluronic®L64 was slowly added to distilled water contained in a beaker kept at 8^0C. Subsequently, the solution was stirred until complete polymer dissolution and then kept at 4^0C for 12 h before experimental analysis. PL64-Alg gel can be prepared by the dropwise addition of mixture of 10ml of 10% w/v sodium alginate and 10 ml of 1% w/v Pluronic®L64 through syringe in 50ml of 0.45M $CaCl_2$ solution. The reformed gel was kept to stand in gelling medium for sometimes and separated from it through proper decantation(Fig.1b). The gel obtained was dried by keeping it in vacuum oven.

Chemically Modified Pluronic based Alginate Gel (BCPL64-Alg)

Similar like PL64-Alg, stock solution of BCPL64 was also prepared with cold method. BCPL64-Alg gel can be prepared by the dropwise addition of mixture of 10 ml of 10% w/v sodium alginate and 10 ml of 1% w/v BCPL64 through syringe in 50 ml of 0.45M $CaCl_2$ solution. The reformed gels were kept to stand in gelling medium for sometimes and separated from it through proper decantation(Fig.1c). The gel obtained was dried by keeping it in vacuum oven.

Figure.1 : Image of beads of (a) Alg gel, (b) PL64-Alg gel, and (c) BCPL64-Alg gel

Methods for characterization of synthesized BCPL64 and Alginate based gels

The FTIR spectra of synthesized BCPL64 was recorded using a spectrophotometer (FTIR- 8400S, Shimadzu Co., Kyoto, Japan) using potassium bromide (KBr) pellet method. The spectrum was obtained over the frequency range 4000-400 cm^{-1} at a resolution of 4 cm^{-1}. ^{1}H- NMR spectra made on Bruker Avance II 400 NMR spectrometer operated at 400 MHz using TMS as an internal standard, and the $CDCl_3$ solvent provided the deuterium lock frequency at 25δC. The thermal gravimetry analysis was conducted for the degradation pattern of the modified Pluronic®polymer was done on Shimadzu (TGA-50) at a heating rate of 10ºC/min. The morphology of all the prepared Alginate gel and Pluronic based alginate gels were observed by scanning electron microscopy (FESEM), Model JSM-5610LV, JEOL Inc. Japan.

Solubilization of LTG drug

The solubilization of LTG in alginate based gels was determined by taking 0.1 gm quantity of Alg/ PL64-Alg / BCPL64-Alg gel in 10 ml of screw-capped glass vials filled with water. The excess amount of drug was added into the glass vials and then shaken at 303K for 24hr in a thermostatically controlled water bath. The samples were filtered through syringe membrane filter(0.2 µm). The filtrate was suitably diluted and analyzed spectrophotometrically at 306 nm wavelength on Shimadzu (UV-2450) UV–visible double beam spectrophotometer with matched pair of stoppered fused silica cells of 1 cm optical path length. The calibration curve of the LTG drug was prepared using methanol-water mixture(1:100) at 303K.

Results and Discussion

As outlined in the introduction, Pluronics® have found widespread application in the biomedical field. Within the large number of commercially available Pluronics®, only a limited number of Pluronics® have been applied for biomedical applications including F127[22-24], F68[25,26], L64[27,28] and P85[29,30]. The former Pluronics® have attracted the largest attention as evidenced by the vast amount of available scientific literature and patents. With the aim to investigate whether other Pluronics® may serve biomedical purposes, we selected benzaldehyde end-capping as our preferred strategy for the Pluronic®L64.

Synthesis and Characterization of BCPL64

In a first part of the work, we optimized the end-capping reaction for the Pluronics®L64. The general reaction scheme depicting the functionalization of the –OH terminated PEO–PPO– PEO triblock copolymer(Pluronics®L64) using p-formyl benzoic acid is shown in Scheme.3[15]. The IR spectra of BCPL64 (see in Fig.2 and Table.3) indicate that the copolymer L64 end- group conversion into benzaldehyde moieties was successful. Due to the esterification occurring, the signal related to the hydroxyl function at 3480–3300 cm^{-1} disappeared, while peaks at 3069 cm^{-1} for the substituted benzene and 2920 cm^{-1} for methylene group appeared.

Scheme.3 : Preparation of BCPL64

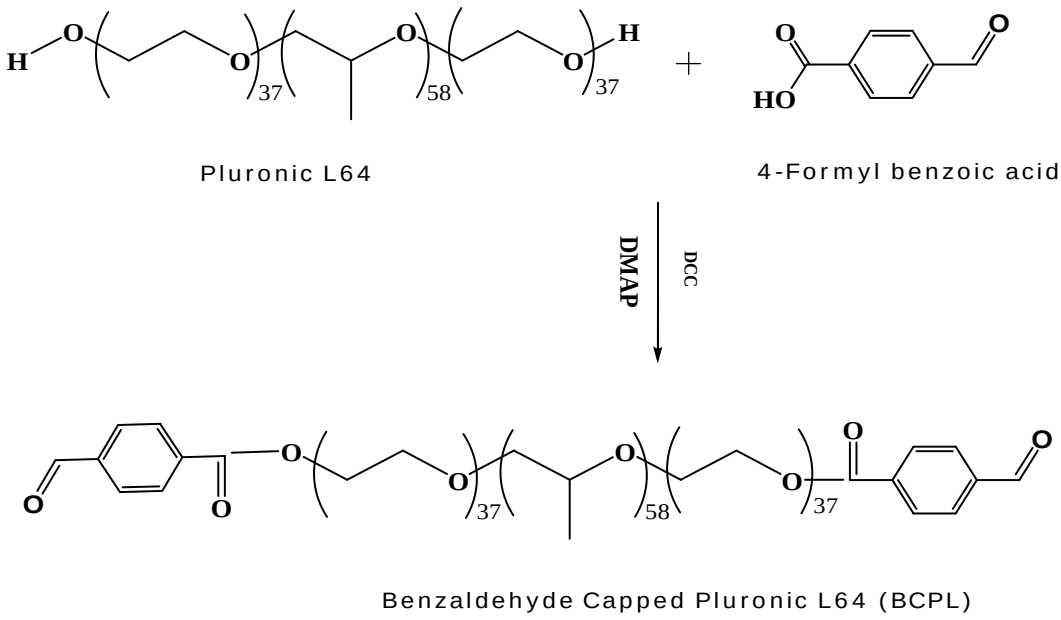


Figure.2 : FTIR spectra of BCPL64

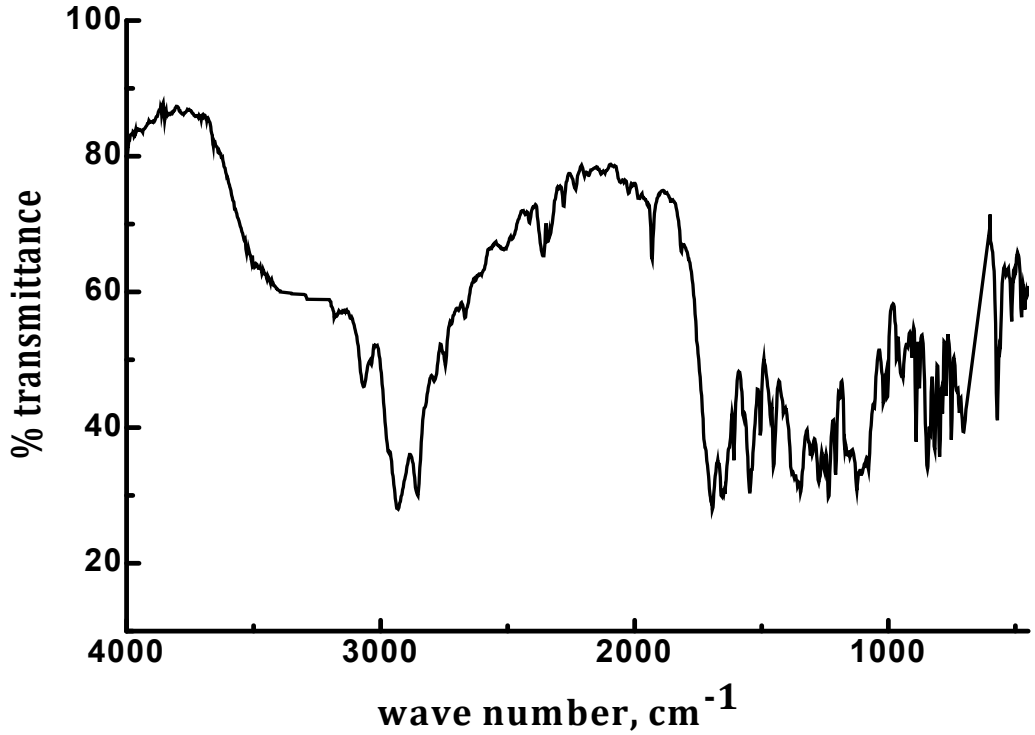


Table.3 : Spectral data of synthesized BCPL64

Signals of BCPL64	
IR Bands (cm^{-1})	3069 cm^{-1} C-H substituted benzene, 2930cm^{-1} methylene grp, 2840cm^{-1} C-H of aldehyde, 1705 cm^{-1} C=O stretching, 1535 cm^{-1} aromatic C=C, 1225 cm^{-1} C-O of ester, 836cm^{-1} *p*-substituted aromatic.
PMR Bands (ppm)	10.07δ ppm for aldehyde group, 7.69and 7.93 δ ppm for protons in phenyl ring, 0.9 δ ppm for $–CH_3$(methyl) group, 3.3-4.1 δ ppm for the aliphatic protons attached with oxygen, 1.2-2.2 δ ppm for the aliphatic protons R-CH_2 , RCH, present in the polymer

Peak at 1700 cm^{-1} in the above spectra shows C=O of aldehyde and ester fingerprints, band at 1232 cm^{-1} indicates C-O stretching of ester and ether, while peaks at around 1535 cm^{-1} indicates aromatic C=C stretching. In addition to this qualitative proof, 1H NMR spectra (see Fig.3 and Table.3) enabled the quantification of the benzaldehyde moieties introduced. There was no any peak above 10.5 ppm suggested the absence of acid group in the product.

The sharp Peaks at 7 to 8.5 ppm appeared for phenyl ring protons. Similar like IR spectra, the ^{1}H- NMR spectra also confirmed the preparation of BCPL64. Fig.4 shows the TGA spectra of BCPL64. The thermal degradation of BCPL64 indicates multistage decomposition without any stable intermediates with weight loss from 151° to 565°C. Result indicates the step by step benzaldehyde moiety and polymer degradation in BCPL64.

Figure.3 : ^{1}H-NMR spectra of BCPL64

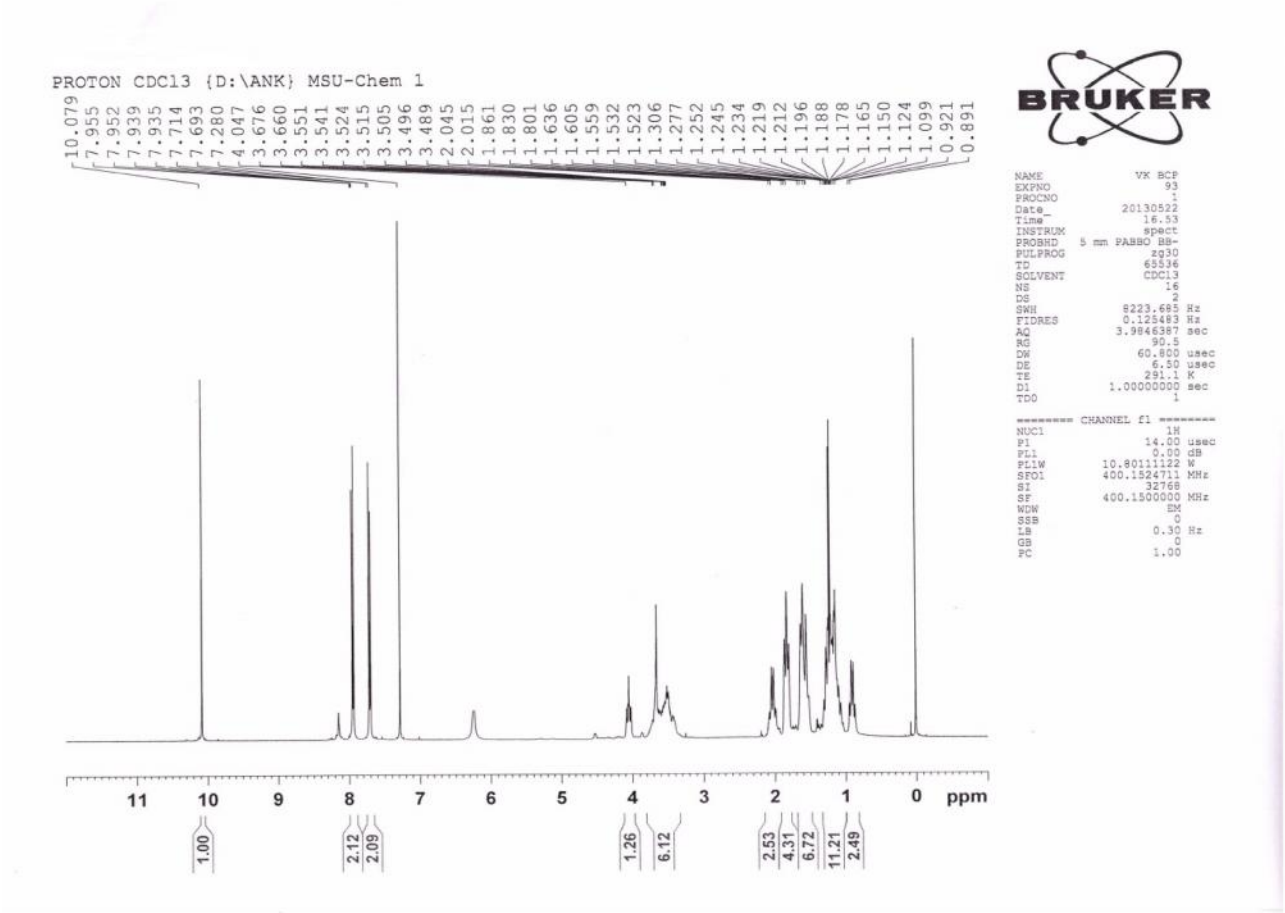
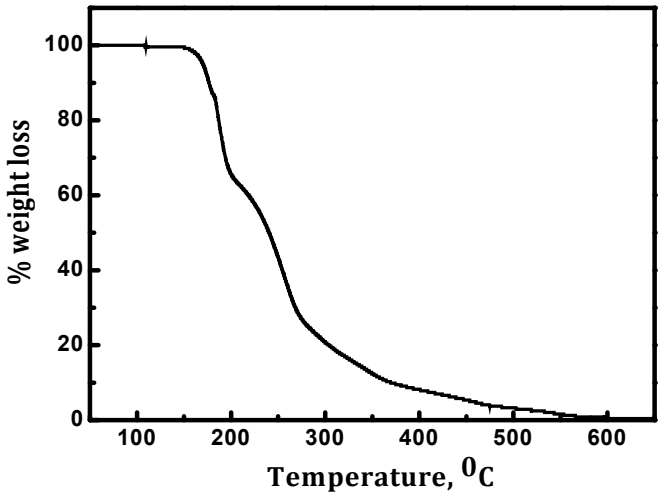


Figure.4 : TGA spectra of BCPL64



Characterization of Alginate based gels

The simple aqueous based gel formation of sodium alginate in the presence of divalent cations such as Ca^{+2} ($CaCl_2$) as nontoxic and biodegradable has been used for drug delivery[31, 32]. The combination of Pluronic and calcium alginate within one drug carrier system could be a tool to control the release kinetics of hydrophilic drugs, such as peptides and proteins. Therefore, alginate–Pluronic based microparticles were prepared by a w/o-emulsion method[33] and used in drug formulations. Fig.5 shows the SEM images of alginate gel, PL64-Alg gel and with modified polymer the BCPL64-Alg gel. In Alg gel, Alg demonstrates rough surfaces due to the singleness of molecular structure unit. While in case of PL64-Alg, the whole structure of gel are towards the smooth surface like semicrystalline nature was displayed. This is due to the more soluble (hydrophilic) micellar environment created by Pluronic®L64. The synthesized BCPL64-Alg gel demonstrates synergic cooperation of alginate and BCPL64 in its physical blending, where both polymers undergo structural changes simultaneously. It shows that polymers have filled the pores of alginate gel. This was happened due to the increase in hydrophobic environment created by BCPL64. The representation of gels(shown in scheme.4) also confirmed that the presence of aromatic moiety in BCPL64 produced larger hydrophobic core made more cross-linked gel.

Scheme.4 : Representation of a) Alg gel, b) PL64-Alg gel, and c) BCPL64-Alg gel

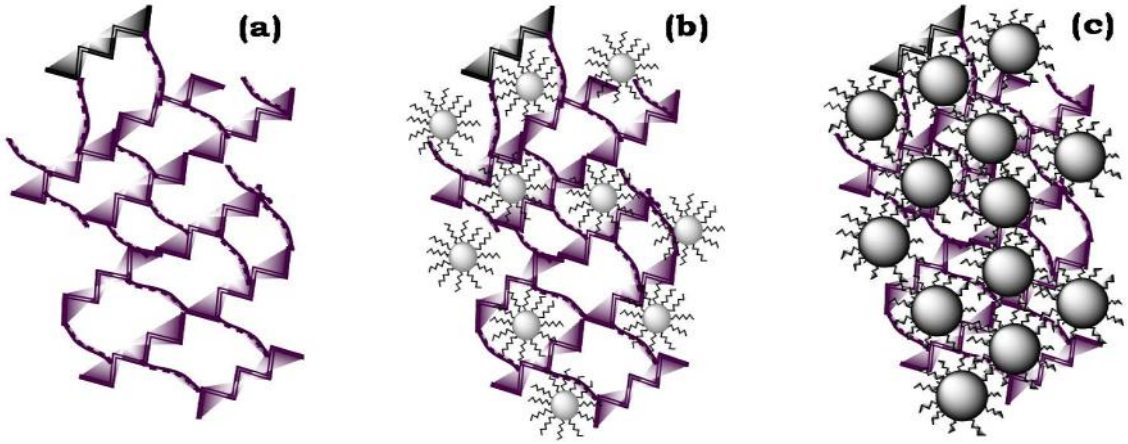
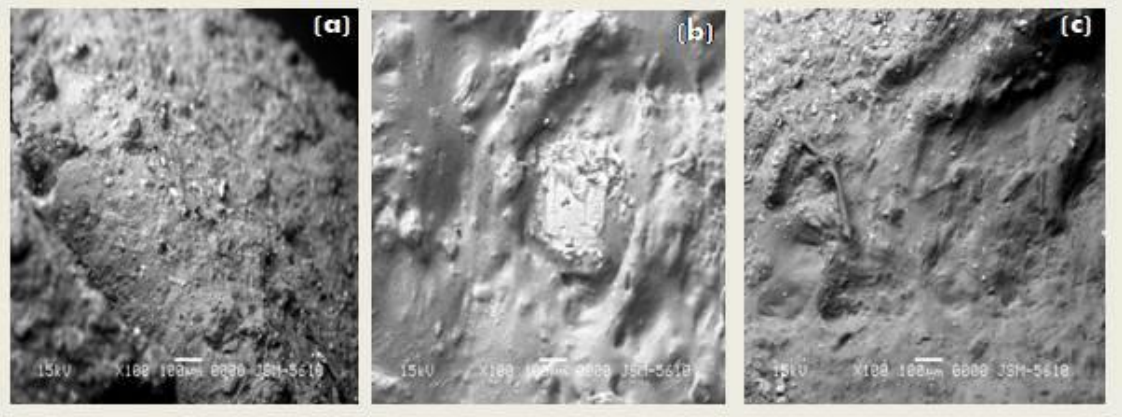


Figure.5 : SEM images of a) Alg gel, b) PL64-Alg gel, and c) BCPL64-Alg gel



Swelling studies

The swelling behavior of the prepared Alg based gel beads were examined in aqueous media, i.e. deionized water. An accurately weighed amount of beads was immersed in aqueous media and removed from the medium in a predetermined time interval. Immediately, the beads were wiped gently and weighed. The swelling ratio was computed by using the following equation;

$$\text{Swelling ratio} = (W_S - W_d) / W_d$$

where Ws is the swollen hydrogel and W_d is the weight of dry hydrogel[34].

Results of the swelling studies were listed in Table.4. The swelling ratio of PL64Alg gel is high as compared to that of BCPL64Alg gel which shows that the presence of benzaldehyde moieties(nonpolar aromatic structure) at the end parts increases the hydrophobicity of the polymer L64 which does not allow water to enter in the alginate network. It also reflected that the blending of alginate with BCPL64 polymers was hindered as compare to L64 due to aromatic ring of end moiety (Scheme.4).

Table.4 : Swelling ratio of various alginate gels

Gel	Swelling Ratio
Alg	2.357
PL64Alg	8.603
BCPL64Alg	3.142

Solubilization of LTG drug in synthesized Gels

Despite extensive research and development, poor solubility of LTG in aqueous solution remains a major barrier in its bioavailability and clinical efficacy. Being hydrophobic in nature, it is insoluble in water but soluble in methanol, chloroform, etc. To increase its solubility and bioavailability, attempts have been made through encapsulation in variety of materials.

The UV-Vis spectra of LTG drug in presence of fixed concentration of 1%w/v of the Alg gel, PL64-Alg and BCPL64-Alg gel at 303K is shown in Fig.6. The spectrum of pure LTG drug in water was inserted in Fig.6. The spectra clearly show that the LTG drug has no peak at 307 nm in water as its hydrophobic nature. While clear intense peaks at 307 nm have found in case of UV-Vis spectra of all gels. This can be ascribed to the solubility of drug in the respective gels. The solubility of LTG drug in 1 %w/v aqueous solutions of gels is shown in Fig.7. The order of solubility of LTG drug in alginate gels is : BCPL64-Alg > PL64-Alg > Alg gel. Here, BCPL64 polymer based alginate gel has highest solubility of drug in comparison to Pluronic® L64 and pure alginate gel. It indicates that the BCPL64-Alg is morehydrophobic structure due to end capping through benzaldehyde moieties which favours the solubilization of the drug (also see Scheme.4). The solubility of drug was also good in PL64- Alg gel in comparison to Alg gel due to the micelles of L64.

Figure.6. UV-Vis spectra of LTG drug in 1 wt% aqueous solutions of Alg gels at 303K.(*Inserts drug in water*)

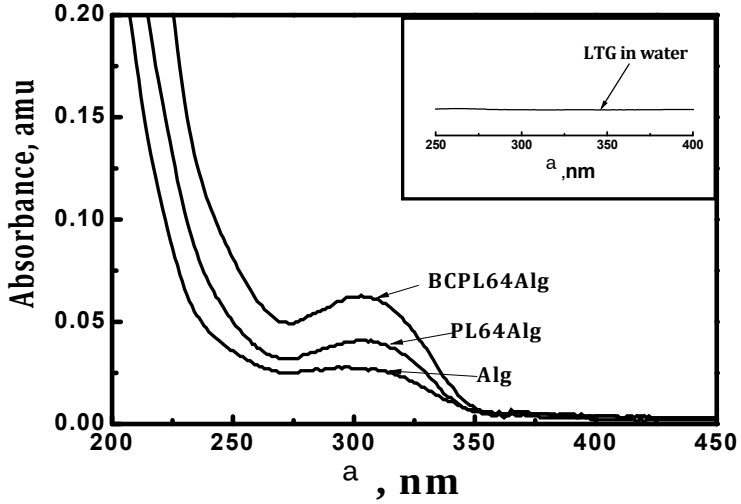
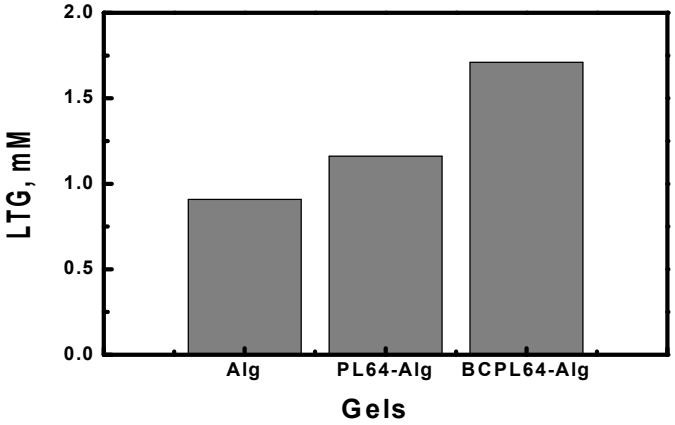


Figure.7. Solubility of LTG drug in 1 wt% aqueous solutions of Alg gels at 303K.



Conclusion

Pluronics, PEO–PPO–PEO triblock copolymers are non-ionic macromolecular surfactants widely used in industry, pharmacy, bioprocessing and separation. Various techniques were employed to modify such polymers for better applications in medical sciences.

In this context, Benzaldehyde-capped Pluronic® L64 (BCPL64) was successfully synthesized using simple condensation polymerization. The synthesized BCPL64 was well characterized by FTIR, NMR and TGA techniques. Hydrogels of Pluronic® L64 and BCPL64 with alginate in Ca^{2+} ion solutions under physiological conditions were prepared for drug solubilization. Results show that all the Pluronic® polymers are homogeneously mixed with alginate and giving effective crosslinking structure. The BCPL64 was found better amongst all. Solubilization of an antiepileptic agent, LTG drug in the prepared alginate gels were also studied by UV-Visible spectroscopy. Results indicate the solubilization of LTG in Pluronic- based alginate gel increases well compared to pure alginate gel.

This work will be correlated in future for making Pluronic® polymers/alginate based formulations can become a potential nanocarrier for poorly soluble an antiepileptic drug, LTG.

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