Synthesis and Characterization of Carboxymethyl Cellulose-g-poly(acrylamide) as Polymeric Drug Carrier

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Abstract

The grafted acrylamide copolymerization on Carboxymethyl Cellulose (CMC) in aqueous media was synthesis and studied via ceric ammonium nitrate (CAN) with thiourea as initiators. The graft polymer was illustrated through FTIR, DSC, TGA and DTG analysis. The effect of the monomer ratio to CMC was examined at the optimal grafting conditions found. Swelling properties of both CMC and the grafted polymer also the degree of swelling as a function of time have been studied and calculated in diverse media to get the time needed to achieve maximum swelling. The new prepared polymers with the maximum grafting percentage, PG have been used as polymeric drug carrier for 2-deoxy-D-glucose, DOG in two different media also the amount of DOG release from the polymers was found via spectrofluorometer.

Key words: Carboxymethyl Cellulose, Drug release, Graft polymerization.

تحضير وتشخيص Carboxymethyl Cellulose-g-poly(acrylamide) وأستخدامه كناقل دوائي

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الكلمات المفتاحية: كاربوكسي مثيل سيليلوز , التحرر الدوائي , البلمرة التطعيمية.

الخلاصة

تم تحضير وتشخيص ودراسة تطعيم مونمر الاكريلامايد على البوليمر الطبيعي كاربوكسي مثيل سيليلوز بأستخدام نترات ثنائي الامونيوم السيريوم (الرباعي) وثايويوريا كبوادئ للتفاعل في وسط مائي وشخصت البوليمرات بأستخدام عدة تقنيات: طيف الاشعة تحت الحمراء , والتحاليل الحرارية (التحليل الحراري الوزني و التحليل الحراري الوزني المشتق و المسعر الحراري التفاضلي).

تمت دراسة نسبة المونمر المضاف على نسبة التطعيم في الظروف المثلى ومن ثم تم اختبار خاصية الانتفاخ لكل من البوليمر الأساس والبوليمر المطعم في وسطين مختلفين وحساب درجة الانتفاخ بمرور الزمن لمعرفة الزمن اللازم للوصول لأعلى درجة انتفاخ. البوليمر المطعم الذي له اعلى نسبة تطعيم تمت دراسته كحامل دوائي للمركب الدوائي ٢- ديوكسي-D-كلوكوز وقياس تحرره بوسطين مختلفين بأستخدام جهاز مطيافية الفلورة.

1. Introduction

Owing to their benefits, drug release procedures have a consideration to the usual dosage types such as extended the liberate time; decline the poisoning effect also dipping the rate of drug release [1,2]. Polysaccharides are observed as the mainly polymeric substance to nano-particles synthesis for drug release methods, also accepted to be the mainly gifted substances since their excellent behaviors for instance being abundant, safe, inexpensive also their simply adjusted properties [3-5].

CMC as a cellulosic ethers is a water-soluble polysaccharide having carboxylate also hydroxyl groups which let this green plus economical poly-sugar to apply large interactions with metal elements [6,7]. Macromolecules CMC are so essential cellulose derivatives which have excellent solubility also chemical stability furthermore they are non-toxic, bio-compatible as well as ecological, additionally, polysaccharides gate from natural supplies have efficient advantages than synthetic polymers. CMC sodium salt had been establish in many uses in several sides involving; food, cosmetic, paper, chemical, textile as well as pharmaceutical industries as together stabilizing also plunging reagent [4,8-13].

In several researches, CMC has been applied for drug delivery through the aim of controlled drug liberate by diverse ways also employed for a micro-gel matrix for a novel sticking plaster, furthermore, CMC was used as bio-adhesive matter due to its adhesive properties chiefly as muco-adhesive polymer for nasal [14] buccal drug delivery systems [15]. In this research the adjustment of CMC by chemical grafted of acryl amide monomer was illustrated as well as tested the effect of monomer to CMC ratio on grafting percentage. FTIR, TGA, DTG and DSC thermal analysis was utilized for description of new polymers. Swelling properties of CMC and acrylamide grafted CMC were studied in distilled water and buffer solution at pH= 7.4. DOG drug was used in controlled release system via the highest grafting percentage of the prepared polymers (PG) in different media; the released DOG from the polymers was estimated by using spectrofluorometer.

2. Experimental

2.1. Materials

The reagents, solvents and materials were commercially obtainable at suitable purity grade also immediately used with no more purification.

2.2. Measurements

Shimadzu 8400S-FTIR in the range between 4000–800 cm^{-1} was used for the polymers spectra recorded. TGA, DTG and DSC thermal analyses of the polymers were obtained at room

temperature in inert conditions; argon flow rate, 10 ml/min, heating rate, 5 °C/min using Linseis STA PT-1000. Fluorescence spectra were achieved in the region among 257–900 nm, concentration of each sample solution in, path length= 1.0 cm at room temperature by using RF-5301PC Shimadzu spectrofluorophotometer.

2.3. Synthetic procedure

2.3.1. CMC Grafted copolymerization through Acrylamide:

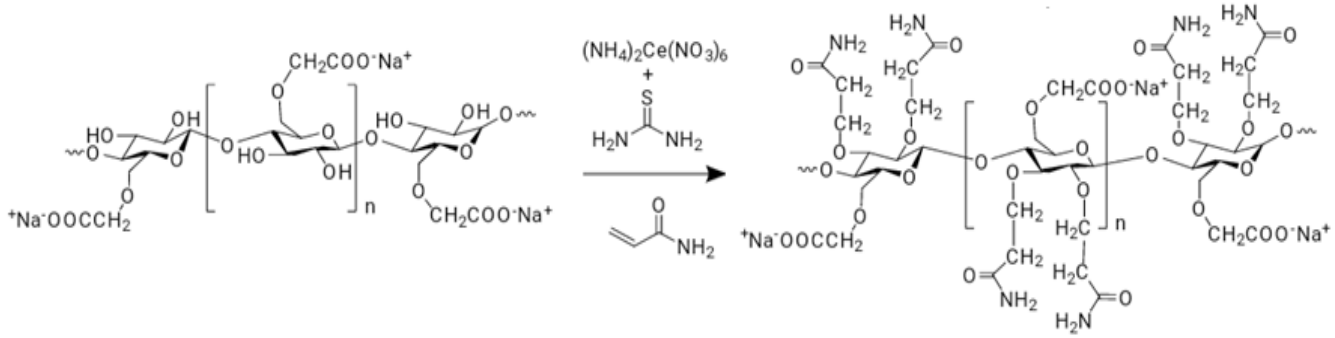
Into suitable volume, 250-500 mL polymerization flasks, grafting reactions were completed by dissolving 1g Sodium Carboxymethyl Cellulose in 60ml distilled water with overnight stirring at R.T then reflex to 70 °C followed by adding 0.165g ceric ammonium nitrate, 0.3mmol and 0.0076g thiourea, 0.1mmol. After 30min, a different amount of monomers was added to the reaction mixture for 150min, and then the mixture was treated with acetone to precipitate the polymer. The prepared polymer was filtered and washed with acetone also dried under vacuum oven to obtain stable weight. The dried polymers were extracted by 40:60 acetone–water mixture for 24 hrs to eliminate the acrylamide polymer. The synthetic diagram for the Carboxymethyl Cellulose grafted polymers preparation is illustrated in Scheme 1.

2.4. Swelling measurements

CMC and its grafted copolymer were accurately weighted in suitable closed thumb usually used for soxhlate apparatus and sited in closured erlenmeyer flask having 100ml of distilled water and remain in a thermo-stated chamber at 30 °C every 1hr for the first 6 hrs., then every 24 hrs [16]. Excess water was decanted from the thumb and the gel was held for about 30sec. then fallen in a weighing bottle which is covered and weighed. The swelling percentage was estimated through equation below:

$$\alpha = \frac{(W_1 - W_0)}{W_0} \times 100 \qquad - - - - - - - - - - \qquad (1)$$

Where W_0 is the original polymer weight, W_1 is the equilibrium swelled sample weight. This procedure was performed once more with pH= 7.4 buffer solution.



Scheme 1: General route of CMC grafted acrylamide

2.5. Spectrofluorometer determination of DOG

10mg of a pure DOG drug was dissolved in 100ml D.W. The excited wavelength of DOG was determinate. Diverse DOG concentrations were prepared by talking accurate volume of the initial solution to 10ml graduated volumetric flask within the study range, Table 4. The solution intensity at the specific λ_{ex} was measured beside the blank, also was plotted versus the concentration to get the calibration curve. This procedure was returning without D.W however buffer solution at pH=7.4.

2.6. Drug release study

The release was achieved via acrylamide grafted CMC. In a suitable closed thumb, 0.1g exactly weighs of acrylamide grafted was placed in covered vial contain 100ml D.W. The vial was still in a thermo-stated chamber at 30 °C for 4 days as the time needed to reached the maximum swelling, 7mg of aqueous drug solution was distributed in the swelled polymer with efficient shaking. The elution medium was sampled and the drug content in the samples was obtained spectrofluorometrically at 252 nm λ_{ex} after first 6hr followed by each 24 hrs, finally, the drug release concentration was illustrated through the drug calibration graph. This procedure was repeated once more without D.W but via buffer solution buffer solution at pH= 7.4.

2.7. Calibration curve of DOG

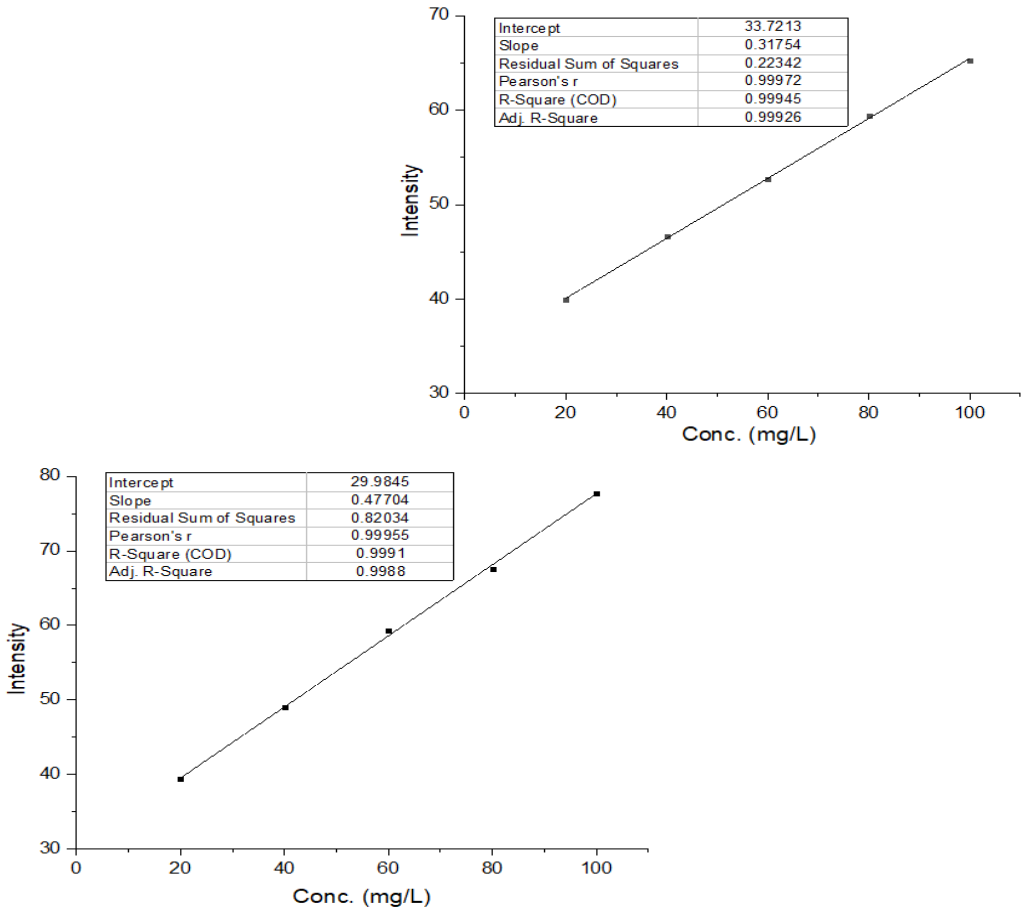
Graft copolymer was used to test the controlled release of 2-deoxy-D-glucose (DOG) in different media, a calibration curve for its quantitative analysis was constructed in the concentration range (20 mg/L to 100 mg/L) at λ_{ex} = 252nm. A working curve of (DOG) in two different media as a function of concentration is shown in Table 1 (a) and (b).

Table (1): The intensity of (DOG) at various concentration a) In Water b) In buffer pH=7.4

(a) (b)

DOG Conc., mg/L	Intensity	DOG Conc. mg/L	Intensity
20	29.373	20	29.902
40	65.988	40	66.638
60	99.302	60	102.642
80	128.599	80	129.419
100	162.771	100	165.266

These data are represented in Figure 1 (a) and (b):



(a) (b)

Figure 1: The working calibration curve for (DOG) in (a) Distilled Water (b) Buffer pH 7.4

3. Results & Discussion

3.1. Synthesis of CMC grafted acrylamide

A graft CMC copolymer is a macro-molecular chain between lone or other types of block linked to the major chain like part chains [17] was chemically changed through grafting by acrylamide in an aqueous phase with ceric ammonium nitrate and thiourea as initiators, Scheme1.

3.2. Polymerization factors effect on grafting percentage of CMC grafted copolymer

3.2.1. Monomer concentration

The monomer concentration effect of on the acrylamide grafting on CMC was studied also the grafting percentage PG was obtained through equation (2) below:

$$PG = \frac{W_P}{W_A} \times 100 \quad ----------- \quad (2)$$

Whereas W_P is the product weight; W_A is the vinyl acetate weight. Figure 2 summarized the percentage of grafting was achieved to be sure of the relative monomer amount to CMC.

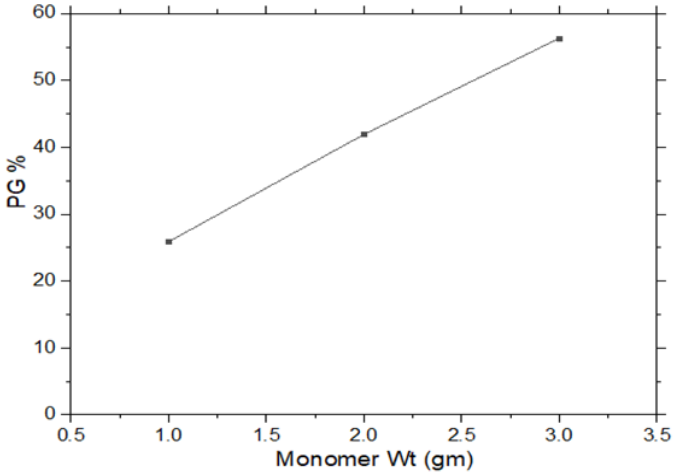
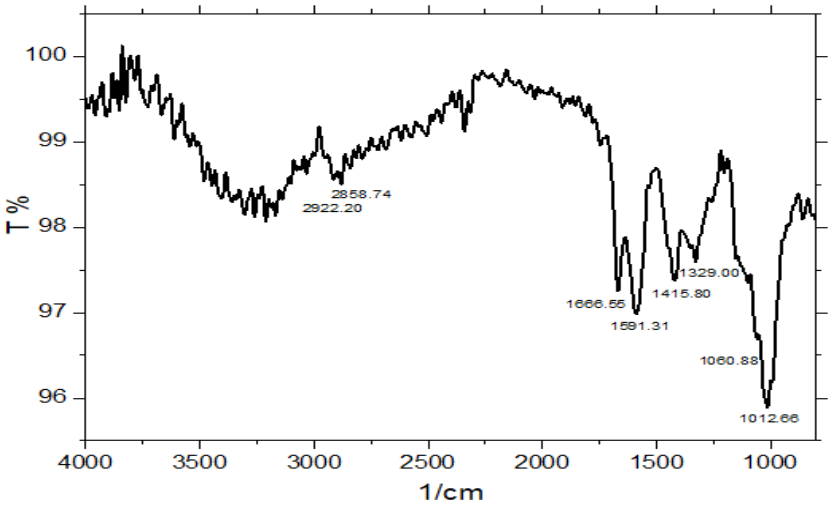


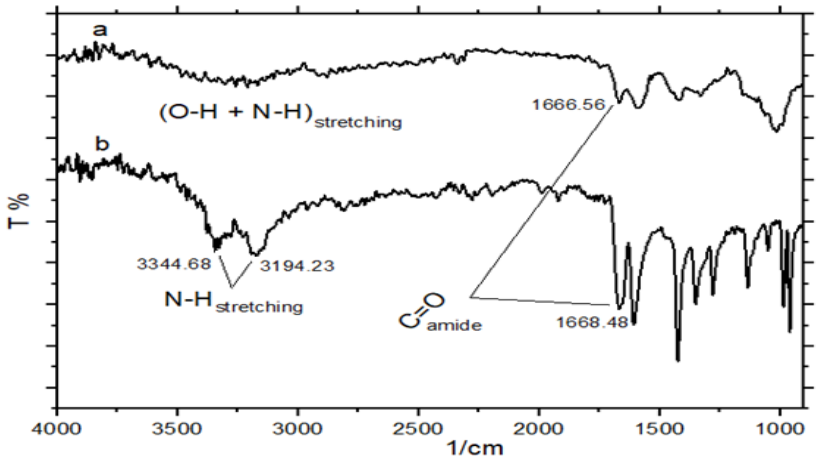
Figure 2: The monomer effect on PG of grafted polymer : CMC = 1g, CAN = 0.5mmol, thiourea = 0.1mmol, t = 150min, T = 70 °C

3.3. CMC grafted acrylamide Characterization

TGA, DTG and DSC thermal analyses also FTIR were applied for description synthetic graft copolymer. Graft copolymer FTIR, Figure 3(a) illustrate typical band at 1666cm^{-1} which signify as a stretching vibration of ester carbonyl group. This band is sited at 1668cm^{-1} in acrylamide monomer FTIR spectrum, Figure 3(b). This little lower frequency changing is possibly owing to inter-intra molecular interactions via hydrogen bond. The insolubility of graft polymer even with have a great ester group number was created as a cross-linking [18], and the band at 1591.31cm^{-1} due to COO^- stretching vibrations. Additional details of the further bands and their assignment are summarized in Table (2). DSC curve, Figure 4(a) shows single endothermic peaks at 89 °C caused by the adsorbed water evaporation also the temperature of glass transition T_g was not proofed since, it may be qualified to interference of the (T_g) transition by the endothermic moisture peak. Two exothermic peaks, one strong at 282.7 °C and weak peak at 363.3 °C which related to the decomposition of CMC [19] while CMC-g-poly(acrylamide) DSC curve, Figure 4(b) shows endothermic peak at 78.6 °C owing to the adsorbed also bound water evaporation. Furthermore endothermic peak at 202.1 °C can related to the glass transition temperature (T_g) of CMC-g-poly(acrylamide). In addition exothermic peak at 283.8°C which is due to the grafted polymer decomposition. endothermic and endothermic peaks at 425.9 and 464.4 °C respectively may be qualified to the graft poly acrylamide decomposition.



(a)



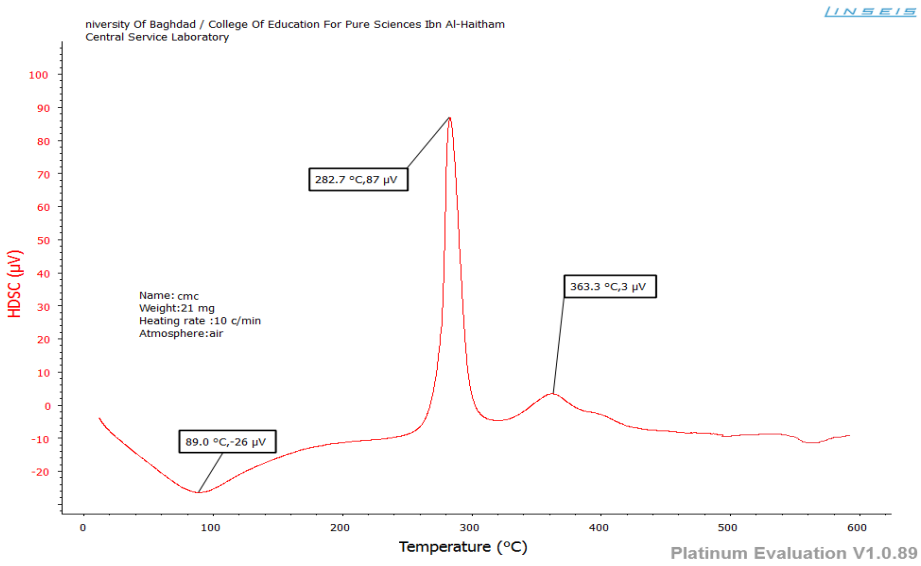
(b)

Figure 3: (a) FTIR spectrum of poly(acrylamide)-g-CMC
(b) Similarity of the FTIR spectra of a) poly(acrylamide)-g-CMC and b) acrylamide in different ranges of wave number

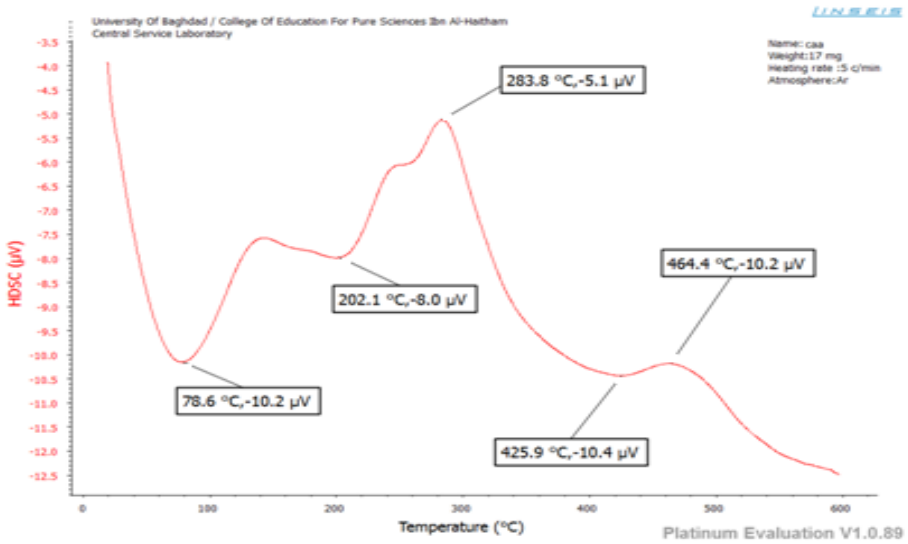
Table (2): IR spectral data of CMC grafted acrylamide

υ(N-H)+(O-H) (Hydrgen bonding) (cm^{-1})	υ(CH_2) (asymmetric) (symmetric) (cm^{-1})	υ(C=O) (cm^{-1})	δ(O-H) δ(C-H) wagging (cm^{-1})	υ(C-O-C) (Asymmetric) (Symmetric) (cm^{-1})
(3080–3670)(br.)	(2922.20)(w) (2858.74)(w)	(1666.55)(s)	(1415.80)(s) (1329.00)(m)	(1060.88)(s) (1012.66)(s)

υ=stretching, δ=bending, s=strong, m=medium, w=weak, br.=broad



(a)

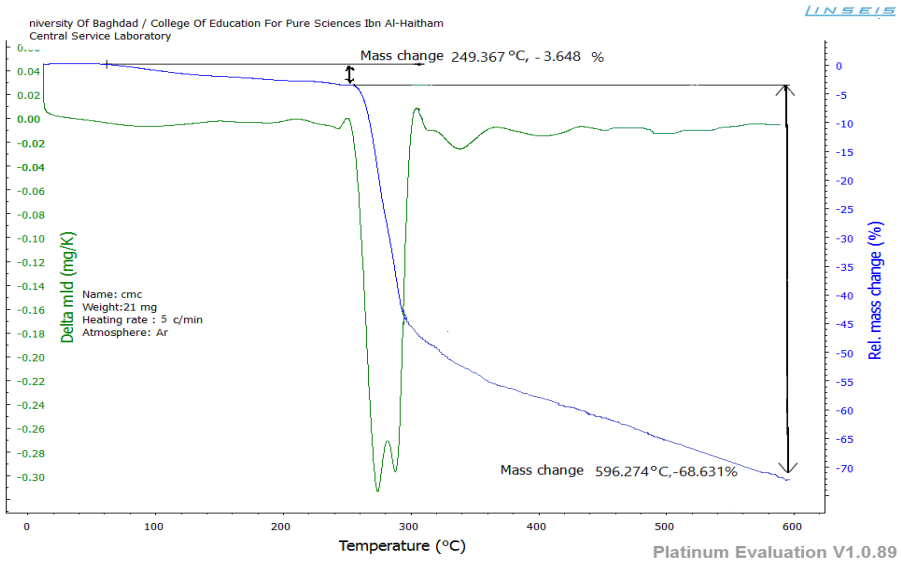


(b)

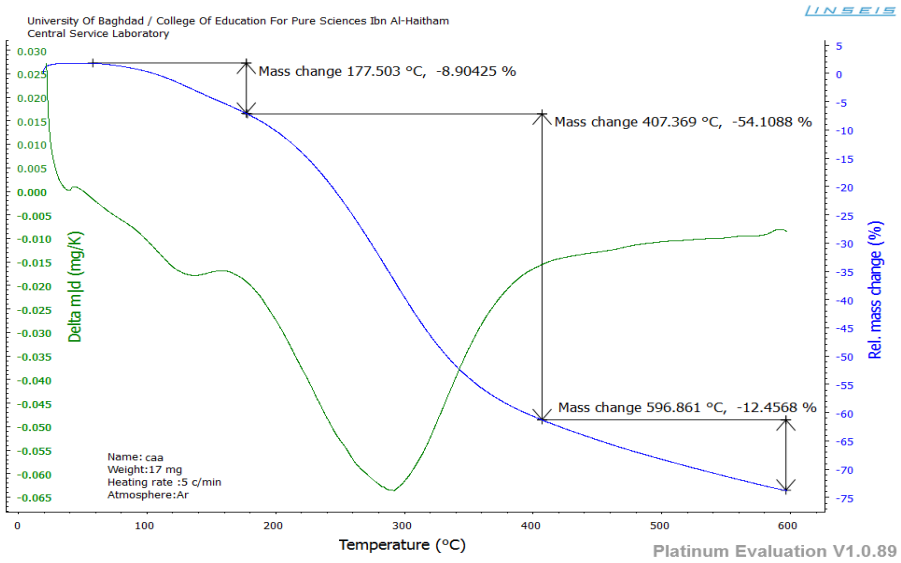
Figure 4: (a) DSC Curve of pure CMC
(b) DSC curve of CMC-g-poly(acrylamide)

The TGA and DTG curves of pure CMC, (Figure 5 (a)) exhibit two stages of diverse weight loss among 60 °C and 596.274 °C. The initial stage ranges between 60 °C to 249.376 °C with 3.648 % weight losing of the adsorbed and bound water. The next stage of weight losses begin at 249.376 °C and that keep on to 596.274 °C, T_{max} = 274 °C, which caused 68.631 % losing weight owing to the CMC degradation, while, CMC-g-poly(acrylamide) curve, (Figure 5 (b)) exhibit three separated stages of weight losing between 80 °C and 596.891 °C. The first one ranged from 80 °C

to 177.503 °C by 8.904 % losing weight, may be matched to the losses of adsorbed water. The second one initiate at 177.503 °C to 407.369 °C, T_{max} = 290 °C with 54.108 % losses weight owed to the CMC grafted copolymer degradation. A 12.456 % losing weight through the third stage begin at 407.369 °C to 596.891 °C which donated to the poly acrylamide chains grafted to CMC decomposition.



(a)

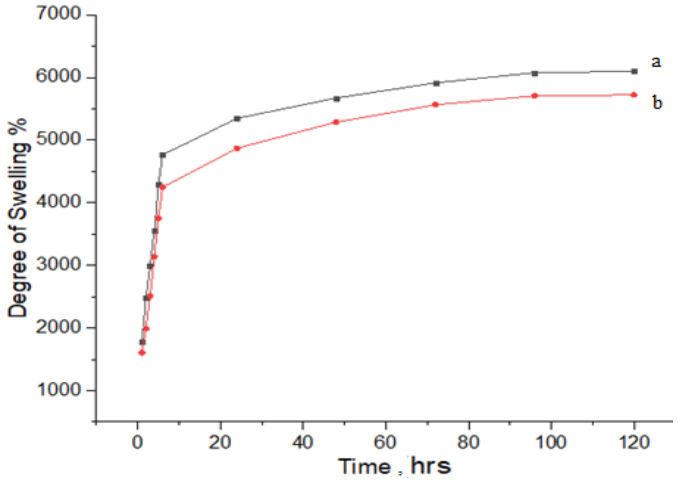


(b)

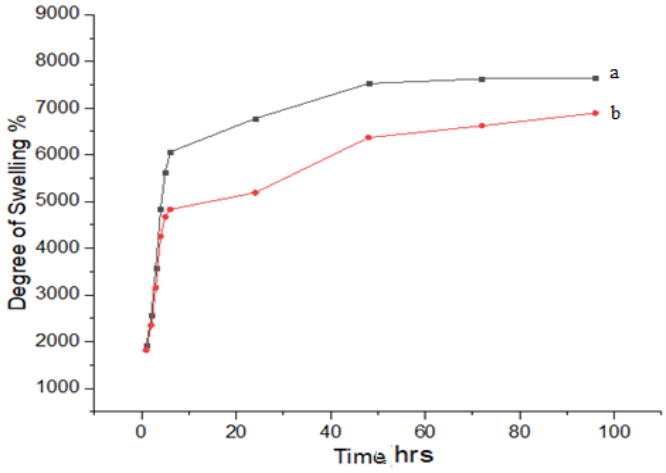
Figure 5: (a) TGA and DTG curves of pure CMC
(b) TGA and DTG curves of CMC)-g-poly(acrylamide)

3.4. Gelation of acrylamide grafted CMC

CMC is linear β-(1→4)-linked glucopyranose chains residues which have a hydrophobic polysaccharide backbone as well as several hydrophilic carboxyl groups, thus shows amphiphilic properties [20]. CMC is soluble in neutral aqueous solutions produced a sol–gel transition, CMC has been modified with monomer containing hydrophilic group to increased the swelling percentage of a hydro-gel. The effect of water also pH= 7.4 buffer solution on the swelling of CMC and its graft have been reported, the degree of swelling of CMC and its grafts in different media are shown in (Figure 6 (a) and (b)). The swelling rate in distilled water was upper than in pH= 7.4 buffer solution. The swelling process involved the interaction energy among aqueous solutions and CMC plus its graft need to be suitable to conquer the hydrogen bonding inside polymers particle. The formless particle are solvated first as well as its rapidly swelling several times its initial volume. CMC and its grafts as polysaccharides are hydrolyzed in aqueous medium which causes a highest swelling value in distilled water, therefore hydrogen bond in this solution be strong also agree to significant swelling.



(a)

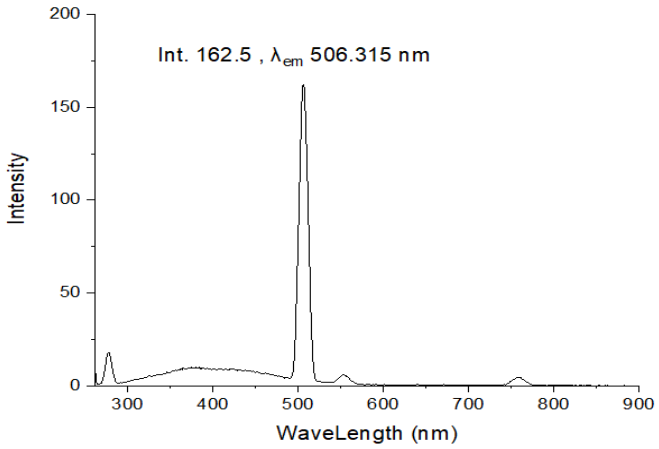


(b)

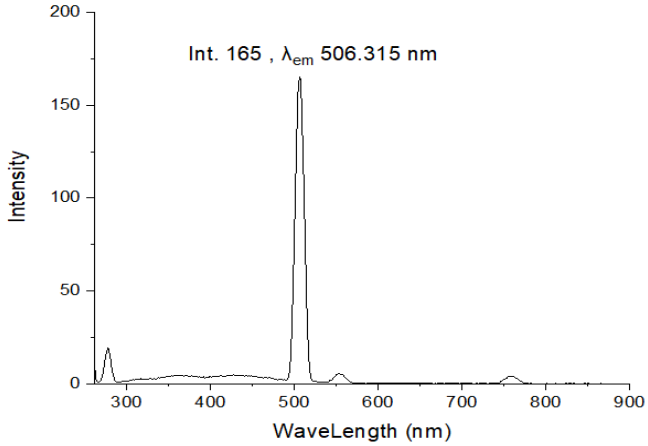
Figure 6: **(**a) Degree of swelling of CMC in a) Distilled Water b) Buffer pH 7.4 (b) Degree of swelling of poly(acrylamide)-g-CMC in a) Distilled Water b) Buffer pH 7.4

3.5. Florescence analysis for DOG

The florescence spectra of DOG drug compound showed emission wavelength at (506.315 nm) for both distilled water and buffer pH=7.4 solvent, which was the excitation wavelength is (252 nm), (Figure 7 (a) and (b)).



(a)



(b)

Figure 7: Florescence spectra of (DOG) in (a) Distilled Water (b) buffer pH=7.4

3.7. 2-deoxy-D-glucose (DOG)

DOG, 2-Deoxy-D-glucose is glucose metabolism inhibitor, Figure 8 as it reduce the primary glycolysis enzyme termed hexokinase [21]. It initiate intracellular ATP weakening [22], also autophagy induction, a cell endurance routes in response to nutrient deprivation [23]. Since the tumor depends on glycolysis, DOG has been categorize as a possible anticancer agent plus association of chimio-therapeutic agent also it has effectively used in rats [24].

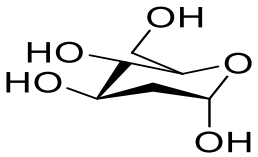


Figure 8: Chemical structure of 2-deoxy-D-glucose

DOG in D.W media showed an emission florescence at 506.315nm, also the same λ_{em} was found in pH= 7.4buffer solution. Once achieving maximum swelling at 30 °C, 7mg DOG load was distribute and test medium was checking each 24hrs after the first 6hrs, whereas excited wavelength were employed at 252nm. The percentage of DOG released with time for acrylamide grafted CMC hydrogel are graphically showed in Figure 9 also summarized in Table (3).

Table (3): Variation of drug release by time for DOG, load =7mg/0.1g grafted polymer in different media

Time/hrs	Drug concentration , mg/L	
	Distilled water	Buffer solution, pH=7.4
1	0.3549	0.40753
2	0.9672	0.57525
3	1.4217	1.07139
4	2.0653	1.2718
5	2.2013	1.35093
6	2.6569	1.6833
24	2.9627	2.92948
48	3.31	3.6781
72	3.794	3.3912
96	4.445	4.0211
120	4.9535	4.459

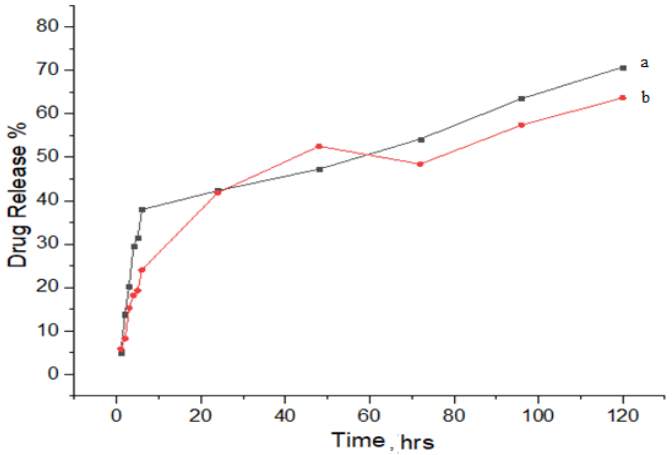


Figure 9: Percentage difference of drug release concentration through time for DOG, load = 7 mg/0.1g grafted polymer in a) Distilled Water b) Buffer pH 7.4

Conclusions

CMC-g-poly(acrylamide) was prepared by a copolymerization reaction between CMC and acrylamide monomer. Grating percentage PG found to be increase with increasing of the monomer concentration, the highest grafting percentage of the graft polymers PG polymer was used to study the releasing of DOG at different media. 70.76 % and 63.7 % releasing percentage of the loaded amount onto the graft polymer in distilled water and buffer pH 7.4 respectively in 120 hrs, this was improved that the releasing amount of the natural polymer, which confirms that upon grafting the hydrophilic monomers onto the natural polymers decreased some crystalline areas of this polymer and increased the amorphous areas of this polymer.

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