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# Casein Grafted Maleic Anhydride Copolymer and Substituted With Procaine

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**Abstract**
In this work**,** Casein as a natural polymer was modified with Maleic anhydride a grafted copolymer ($A_1$) ,then the substitution reaction was undergo between grafted maleic anhydride and procaine as amino drug, producing Casein grafted N- procienyl meleamic acid copolymer ($A_2$) as biodegradable polymers a suitable design of drug carrier for controlled delivery of therapeutic agent to extend period of time and increase the residence time of drug in the system . The prepare new drug copolymer was characterized by FTIR spectroscopy and controlled release was studied in different pH values 1.1and 7.4 at $37C^0$ .Thermo gravemetric analysis was carried out, which indicated the thermal stability of ($A_1$) and ($A_2$).
**Keywords:**Casein ,Natural polymer ,Grafted copolymer .

## Introduction

Natural polymers have potential pharmaceutical applications because of their low toxicity ,biocompatibility, and biodegradability Unstructured proteins are special types of polyelectrolyte with amino acids as their repeating units. [1-3] Casein in milk products is such an unstructured protein and charged regulation its basic properties can be described by the general theory for casein, [4] a predominant phosphoprotein accounting for nearly 80 percent of proteins which is a naturally occurring and low-cytoxic. [5–6].Biopolymers are an interesting alternative to synthetic polymers because of their potential loading for both hydrophilic and hydrophobic using synthetic chemical reagents and organic solvents is obviously desirable for biomedical applications drugs[7-8]. Dairy proteins, such as sodium Caseinate (SC), a widely used in the food industry as functional ingredients because of their simple production, excellent nutritional value,[9-10] ,and versatile techno-functional properties[11–12]. . Caseins and SC have been conjugated under controlled conditions with different mono-, di-, oligo-, and, polysaccharides including glucose, ribose, fructose , saccharides, maltodextrins, pectins, and dextran; these glycol conjugates exhibit enhanced emulsifying properties over the native protein .[13-14].

Casein, as a drug carrier mainly for the sustained delivery of cytotoxic drugs [15-16-17]. Glutaraldehyde cross-linked casein microspheres were found to be resistant in proteolysis' tract for more than 24 hours and suggested that it could be used as a matrix for the controlled delivery of oral drugs[18].

Casein appears to be a promising carrier for the sustained release of many orally as well as parenteral administered drugs ecosphere carrier systems made from the naturally occurring proteins have attracted considerable attention for several years as a matrix for controlled and sustained release delivery of many drugs [19-20]. Procaine was first synthesize [21-24] shortly after amylocaine, and is the oldest man-made local anesthetic still in clinical use Procaine is a local anesthetic drug of the amino ester group. It is used primarily to reduce the pain of intramuscular injection of penicillin, and it was also used in dentistry, in some regions procaine is referred to generically as novocaine. It acts mainly by being a sodium channel blocker [22].

## Experimental

**Materials and Instruments**:- Casein type Alpha was purchased from Aldrich ,Procaine was purchased from Fluka ,Malic anhydride and Ammonium persulphate (APS)were purchased from Merck .FTIR Spectra were receded by(4000-400)$cm^{-1}$on a Shimadzu Spectrophotometer .Melting points were determined on Callencamp MF B-600 melting point apparatus. Electronic Spectra measurements using CINTRAS-UV.visible Spectrophotometers.Thermogravimetric Analysis (TGA) and Deferential Scanning Calorimetry(DSC) were carried out Shimadzu model 50 WS thermal analysis instruments respectively .An accurately weighted of sample was placed in an aluminum cup ad sealed .The experiment consisted of heating the sample from $500C^0$ under the continues flow of dry nitrogen gas (50ml-$min^{-1}$) at a heating rate 10 $C^0$ $min^{-1}$.

### Graft Copolymerization of Casein with Maleic anhydride (A1) [23]

In a screw capped polymerization bottle (2g),(0.02mol) of maleic anhydride was dissolved in 5ml of acetone ,and 5g of Casein was added to the mixture .0.05% of monomer weight of (APS)was dissolved in 1ml of distilled water, then it was added to the mixture. The bottle was flashed with nitrogen gas for few minutes

inside a glove and firmly stopped .The mixture was heated at 60-70C$^0$for 20min, the graft copolymer was collected and ,washed two times with ether and dried in a vacuum oven at 40C$^0$ the yield %was 90% as white product m.p.177-185C$^{0.}$

**Substitution of procaine with Casein maleic anhydride Copolymer (A2):-**

1g of prepared copolymer (A1) was dissolved in 1ml of Dioxane and (o.5g),(0.02mol) of procaine ,the mixture was heated at 60C$^0$ with stirring for 1hr .the solvent was evaporated under vacuum .

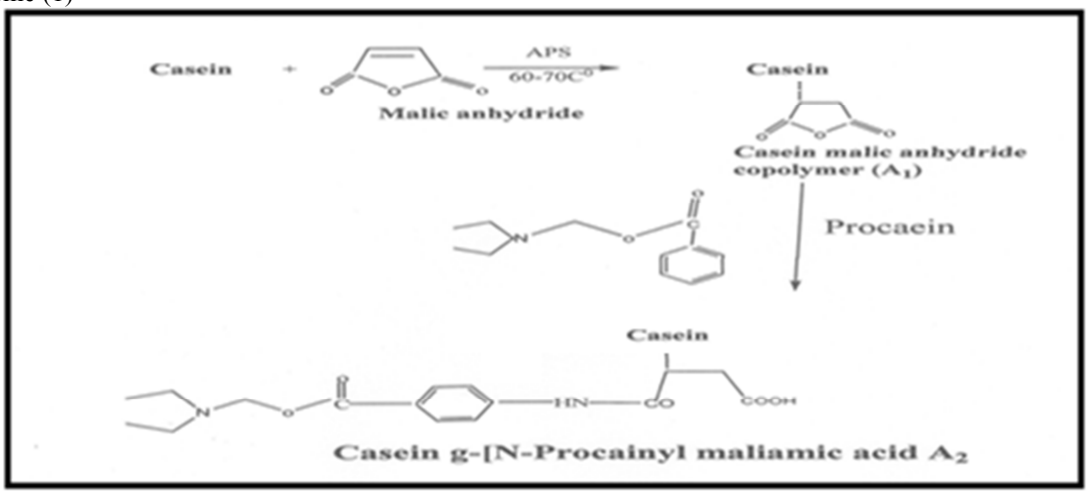
The yellowish white product was washed three times with ether and dried, drug copolymer (A2) was obtained with 70%.

**Controlled Drug Release[24]**

In 100ml of aqueous buffer solution such as (phosphate buffer pH 7.4) or acidic solution pH1:1 ,it was added 0.1g of prepared drug copolymer A2,the buffer solution kept at 37C$^0$,with continuously stirred and 3ml of sample was analyzed by UV.Spectrophotometer and compared many samples in different periods .

**Result and Dissections :-**

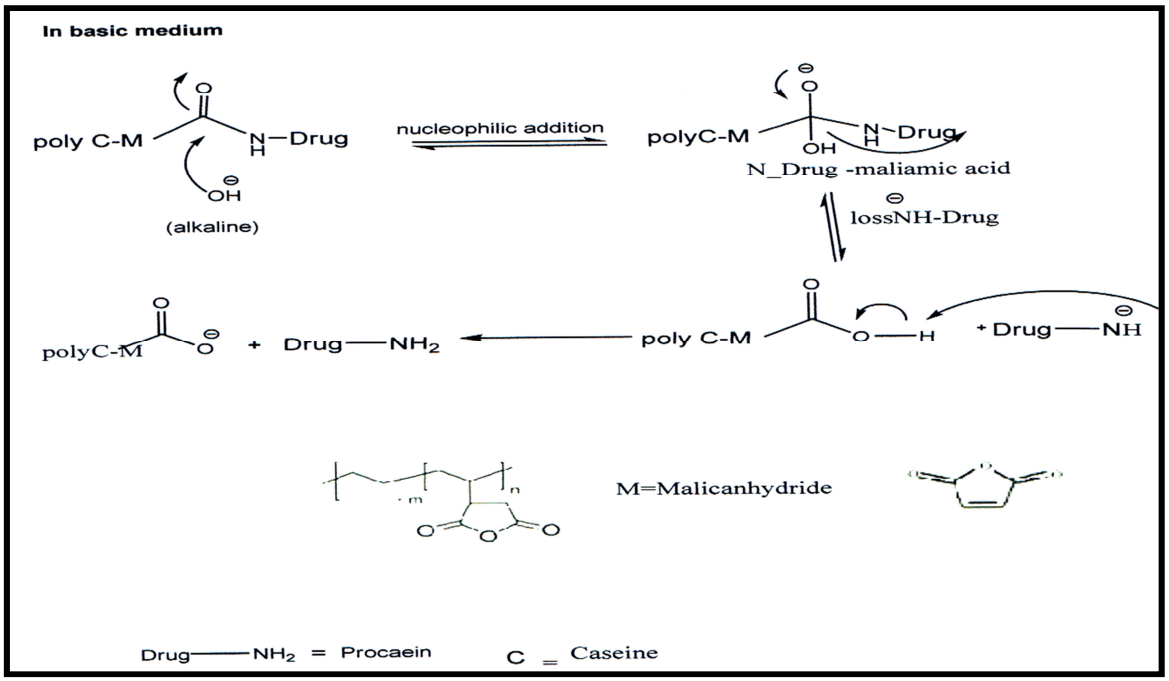
In this work Casein was grafted with maleic anhydride ,the grafted polymer containing acid anhydride was reacted with amino drug such as procaine producing grafted N-Drug maleamic acid which allows the formation of functional derivative by ring opening of grafted malic anhydride by nucleophilic attack on Casein back bone .Drug delivery system based on Casein as a biodegradable system which has the ability to release the drug for the administration of pharmaceutical and biomedical application ,The reaction was showed as Scheme (1)



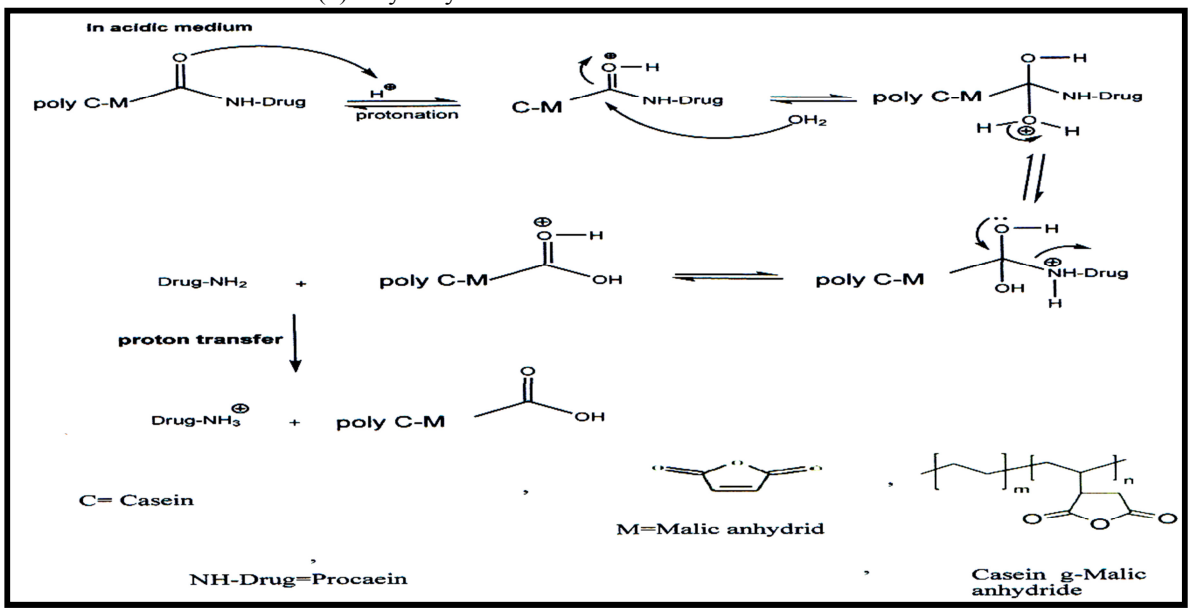

Scheme (1) :**The synthesis Route of The** copolymer (A2)

Fig (1) and Fig (2) showed FTIR spectra of Casein and A2 respectively. Casein graft maleic anhydride indicated the absorption of (C=O) of anhydride at (1780 and 1750)cm$^{-1}$due to grafted copolymer A2,Fig (3) ,FTIR spectrum of drug carrier Casein grafted polymer showed the absorption band at 3500-3000 cm$^{-1}$ attributed to the formation of (OH) stretching of carboxylic acid ,3250cm$^{-1}$ assignment to NH amide of amic acid in A2 structure .in addition to disappearance of carboxyl group ( -COO$^-$) of anhydride to (C=O)amic at 1650cm$^{-1}$and 1720cm$^{-1}$of carboxylic acid.

Fig(4) showed UV spectrum of controlled drug release at $\lambda_{max}$320 nm showed the sustain release due to hydrolysis of amide bond in different pH values, the following mechanism illustrated the hydrolysis at 37C$^0$.See Scheme (2) and (3) respectively.




Scheme (2):Hhydrolysis of Casein N-Maleamic acid in basic medium




Scheme (3):Hydrolysis of Casein N-drug maleamic in acidic medium

This study is included modification the Casein as a natural polymer to grafted maleic copolymer then substituted to its corresponding N-drug maleamic acid copolymer to enhanced the sustained delivery system through chemical bonds and slowly release under appropriate medium conditions such as pH 7.4 and 1.1at $37C^0$.Fig(4) UV spectrum which indicated the hydrolysis of $A_2$ drug polymer.

We concluded that the hydrolysis in basic medium is higher than acidic medium this attributed to $OH^-$ is more nucleophlic attack on carbonyl of amide than $H^-$ and water molecule. Fig (5) TGA and DSC of Casein -g- maleic anhydride ($A_1$)and ($A_2$) exhibit thermal stability ,which recorded a function of temperature with weight loss of polymer samples as listed in Table (1)

Table (1)ThermalDecomposition Temperature for Casein- g-maleic anhydride copolymer

| Polymer No. | Temp.$C^0$ | Weight loss % | Mass change | Enthalpy J/g | $T_mC^0$ |
|---|---|---|---|---|---|
| $A_1$ | 219.3<br>229.1 | -2.032 | -5.40<br>-2.032 | -209.4 | 300 |
| A2 | 240<br>400 | -2.75 | -10.28<br>-2.754 | -8.84 | 320 |

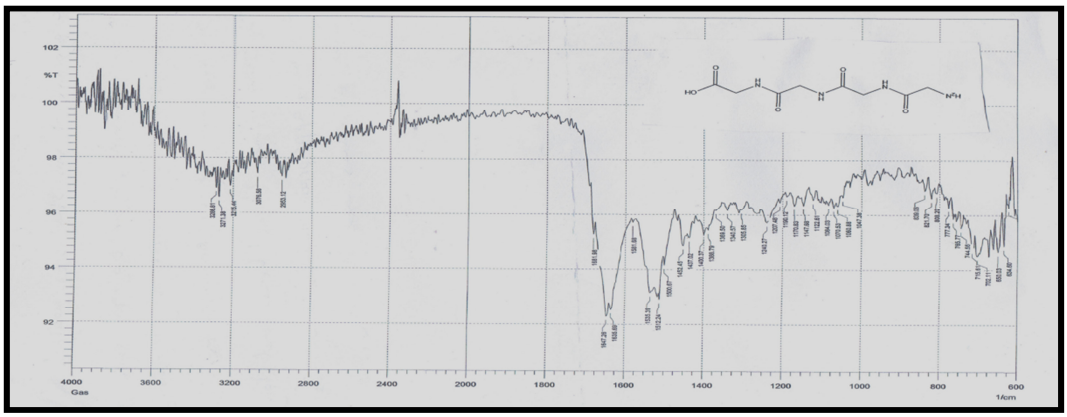
Grafted copolymer $A_1$ indicated high thermal resistance due to incorporated Maleic anhydride copolymer at $300C^0$ in an enthalpy -209.4 J/g with weight loss 50% .

After ring opening of grafted maleic anhydride to N-procaenylmaleamic acid grafted on Casein gave higher thermal resistance at $320C^0$ with 50% weight loss%.in an enthalpy -8.84J/g .The elemental analysis values of N% agreed quite well with the exhibited for the proposed structure of Casein g- N-procaeinyl maleamic acid copolymer $A_2$.

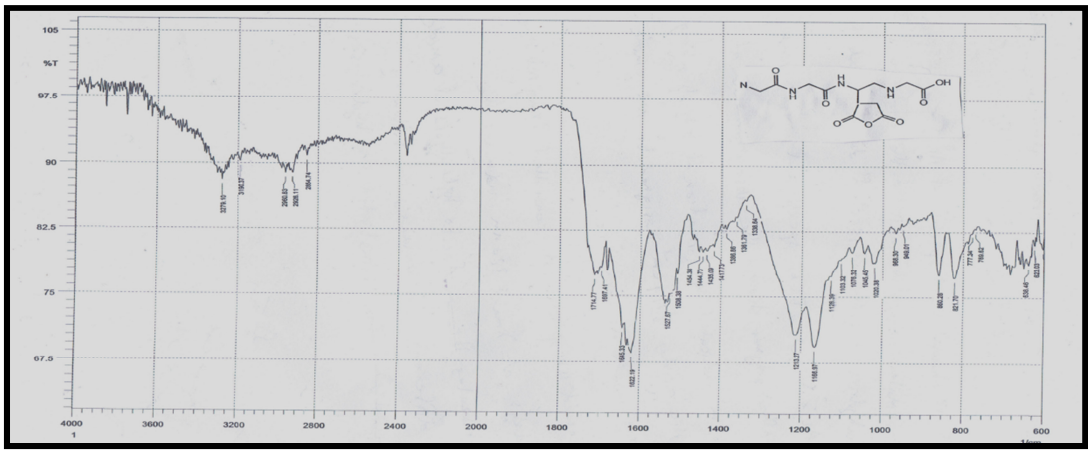
Swelling percentage was measured in water ,it was found that high swelling% of Casein g-N- procainyl maleamic acid $A_2$about 60% indicated high absorption ratio of $A_2$.

A novel drug copolymer which concluded as natural local anesthetic action andother uses due to high absorption ratios of water through one day .and sustained drug release for prolong time.

In this work new properties in final copolymer were obtained and the pendent carboxylic groups of maleamic acid could be converted to Na salt through back bone of Casein with more interesting features ,such as swelling% and sustained drug release with low toxicity ,biodegradability with high potential pharmaceutical application due to its potentials loading for both hydrophilic and hydrophobic system.

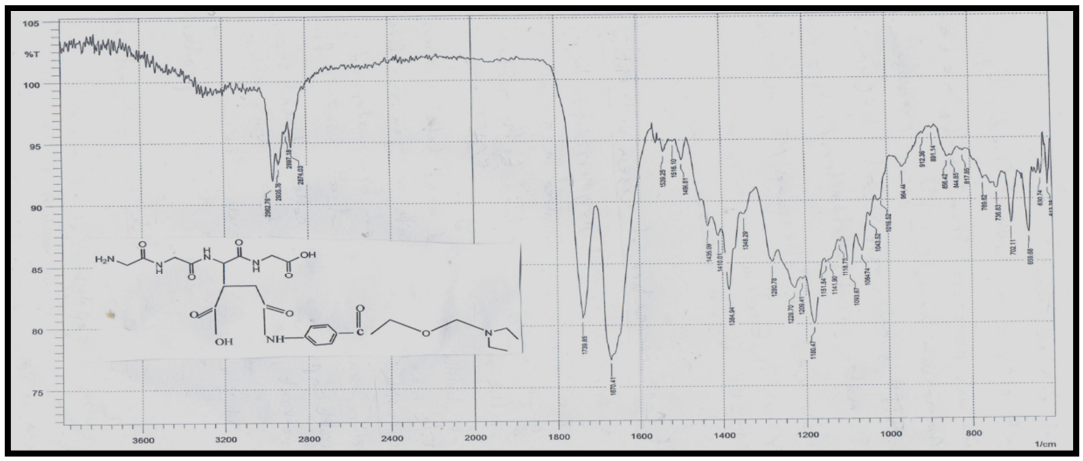



Figure(1): FTIR Spectrum of Casein

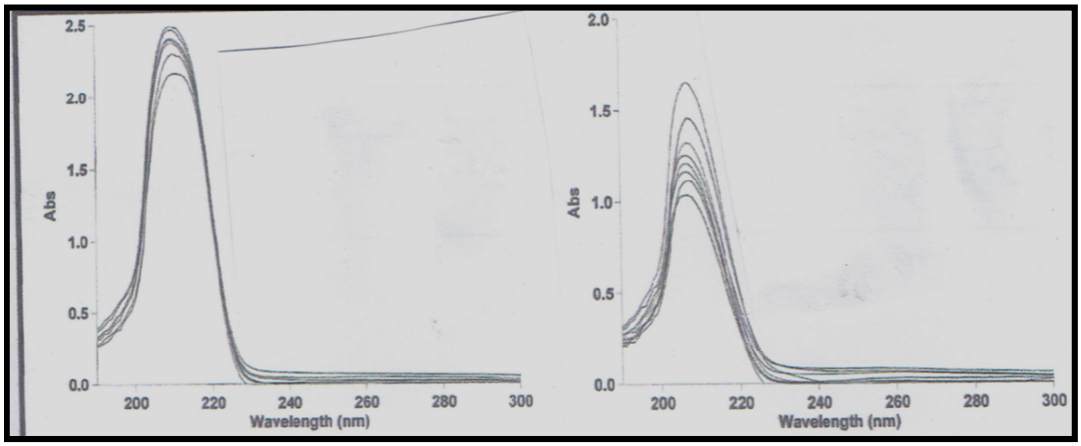



Figure(2):FTIR Spectrum of Casein grafted maleic anhydridecopolymer($A_1$)

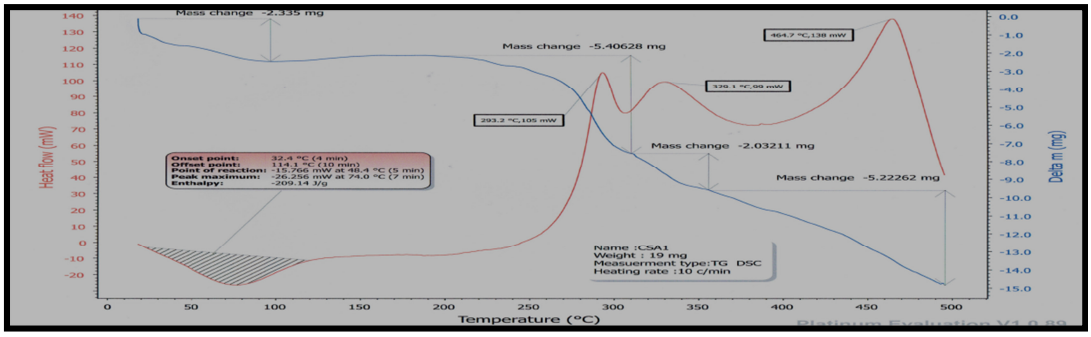
 



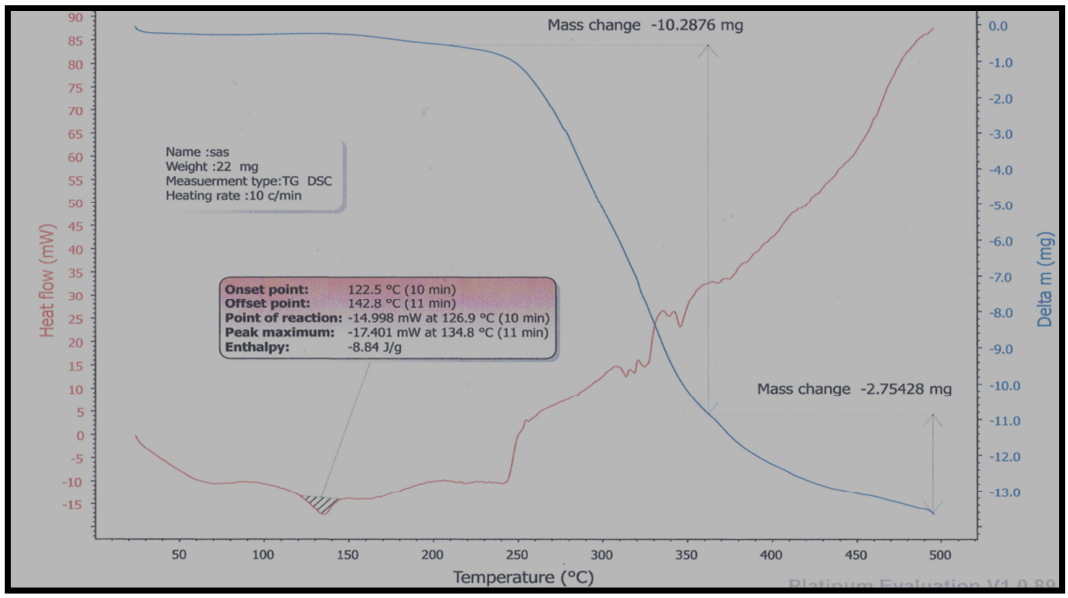

Figure(3): FTIR Spectrum of Casein (N- Procanylmaliamic acid)copolymer($A_2$)




Figure(4) UV spectra of controlled release of drug copolymer $A_2$ in pH 7.4and 2.1




Figure(5) TGA and DSC of copolymer of Casein g- maleic anhydride




Figure(6) TGA and DSC of Casein g –[N- procainylMaleamic$A_2$]

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