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ORIGINAL ARTICLE

Modification of Casein by Grafting Phthalic Anhydride and Loaded with Different Drugs

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KEYWORDS

Casein;
Graft copolymerization;
Phthalic anhydride

ABSTRACT: Ring-opening polymerization was used in this study to intelligent new novel drug natural polymer which has been prepared from grafted copolymerization of Casein with phthalic anhydride (A1)by using ring-opening polymerization. Then the prepared (A1)was bonded with different such as procaine, salbutamol, amoxicillin, and paracetamol through the ring opening of phthalic anhydride to produce a copolymer of casein –g-phthalic anhydride compound [A1] and reacted with SOCl2 in acetone. compound [A1] reaction with different drugs salbutamol, amoxicillin, paracetol, and procaine. Spectral evidence established the structure of synthesized compounds: UV-Visble, FT-IR, and 1HNMR, Thermo gravimetric analysis was carried out y TGA and DSC. The controlled drug release was studied by different pH values at 37°C. The sustained release was observed as pH-sensitive in specific sites with controlling release with suitable concentration. it was found high in basic medium about 90%. antibacterial activity against several bacterial strains i.e. gram-positive bacteria: Staphylococcus aureus and Bacillus subtitles gram-negative bacteria: Pseudomonas aeruginosa and *Escherichia coli*.

INTRODUCTION

Normal polymers have potential pharmaceutical uses because of their low toxicity, bio-compatibility [1, 2], and bio-degradability, and unregulated proteins are special kinds of polyamino acid repeating units. Casein in dairy products is an unregulated protein, and its main properties can be called via the common theory of casein [3], which is a predominant phosphorus protein that makes up about 80 percent of naturally occurring low-protein proteins. Cytotoxic.[4, 5] Biopolymers are usually considered a more important source than synthetic polymers because they are likely to be loaded with both non-hydrophobic and hydrophilic utilizing synthetic organic solvents and chemical reagents are desirable for drugs with bio-medical uses [6-8].

Milk proteins like sodium caseinate (SC) are extensively utilized as functional ingredients in the food industry due to their ease of manufacture, excellent value nutritional [9, 10] various useful properties [11, 12]. sodium caseinate and Casein have been combined under the best optimum conditions with monosaccharide, acetone, and polysaccharides having ribose, glucose, fructose, polysaccharides, pectin maltodextrin, and dextran; These glycol pits carry the properties of the emulsifying of the original protein [13, 14].As an active ingredient carrier generally for the continuous delivery of cytostatics [15, 16].

Microscopic casein bound to glutaraldehyde is resistant to glycolysis of the urinary tract for more than 24 hours and has been suggested to be used as a matrix for orally controlled drug delivery [17, 18]. Casein appears to be the preferred carrier for the sustained release of many biomass aspiration regimens for oral and intravenous drugs, and these synthetic systems of naturally occurring proteins have been considered for several years as a

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matrix for the steady and controlled delivery of several medicines [19, 20] Primary procaine was made just after amylocaine was made and is the oldest limited synthetic anesthetic still in medical use. Procaine is a local anesthetic of the amino ester group. Procaine is mainly used to relieve the pain of intramuscular penicillin injection and is too utilized in dentistry. In some regions, procaine is commonly referred to as novocaine. It acts primarily as a blocker of sodium channels[21].

MATERIALS AND METHODS

Alpha casein was bought from Aldrich, a phthalic anhydride. salbutamol, amoxicillin, and paracetol from Samara company. The FT-IR was measured at (4000-400) cm-1 broken on the spectrophotometer(Shimadzu). The melting points were estimated on the CallencampMF B-600. Electron spectroscopic measurements were made utilizing spectrophotometers CINTRAS UV, TGA, and Differential Scanning Thermometry (DSC), Shimadzu Model 50 WS thermal analyzers were each made, and the sample weight was precisely placed in an airtight aluminum cup lid.

Ring opening polymerization of Casein with phthalic anhydride and substitution of different drugs

In a polymerization bottle covered with screw (3 gm), (0.018 mol) of phthalic anhydride in 10 ml of acetone, 6 gm of casein was dissolved in 25 ml of acetone added to the mixture few drops of dilute H2SO4 was added to the mixture, the bottle was flashed for a few minutes with N2 inside the glove and stopped firmly. The mix was refluxed at 60-70°C for 2h. The graft copolymer was assembled, washed twice with ether, and dried in a vacuum oven at 40°C, and the yield was 90% white in the product. 1gm of this product dissolved in 5 ml of dioxane and5drops of thionyl chloride were added and then refluxed for about 15 min, (0.5gm), (0.02 ml) of procaine, the mixture was refluxed for 1.5h at 60°C with stirring for one hour. Excess solvent was removed by evaporating it, and then the resulting product was washed several times with ether and the other drugs (salbutamol, amoxicillin, paracetamol) used the same procedure to prepare .release of controlled drug in 100 ml buffer solution (buffer phosphate 7.4). or solution acidic like pH1: 1, A 0.1 g of drug-prepared A2 co-polymer, the temporary solution is kept at 37°C, with continuous stirring, and 3 ml of the sample is analyzed via UV.

RESULTS AND DISCUSSION

In the study, casein was inoculated with phthalic anhydride, and the folded polymer having acid anhydride reacted with amino drugs like procaine that produces a phthalamic acid called N-Drug which allows the creation of a functional derivative via opening a ring of phthalic anhydride grafted with a nuclear attack on the nucleus of the casein. The wormy system delivery relies on casein as a bio-degradable system and has the ability to all drug to administer the pharmaceutical application and biomedicine, the reaction shown in Figure 1. [22, 23].

Hydrolysis of drug-polymer

Figure 2 shows that the rate of hydrolysis is better in acidic media, due to the presence of OH- groups in alkaline ones, which act in water as the strongest nucleophile, and that their decomposition in water is faster than their decomposition in acidic media, as a result of the H+ bonding to the oxygen atom of the ester. The spacer effect appeared to improve the hydrolysis of ester or amide groups, Figure 3 When it was compared without spacer, and when it was directly attached to drug-polymer [24]

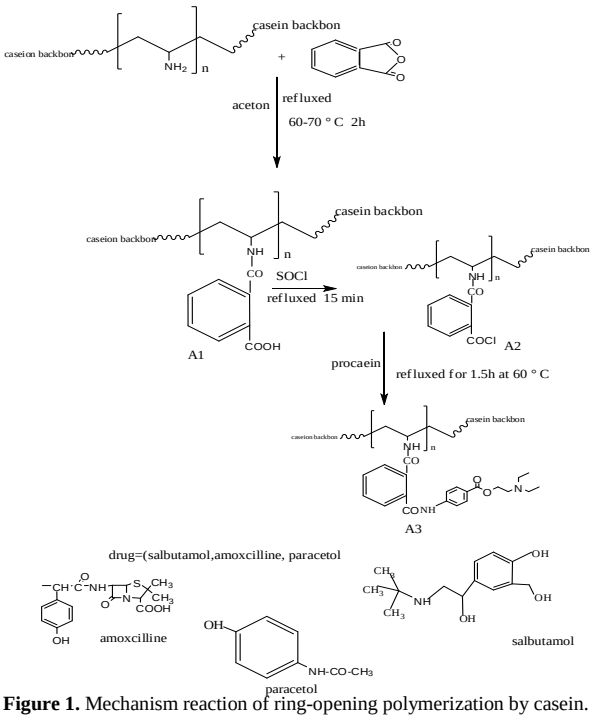

Figure 1. Mechanism reaction of ring-opening polymerization by casein.

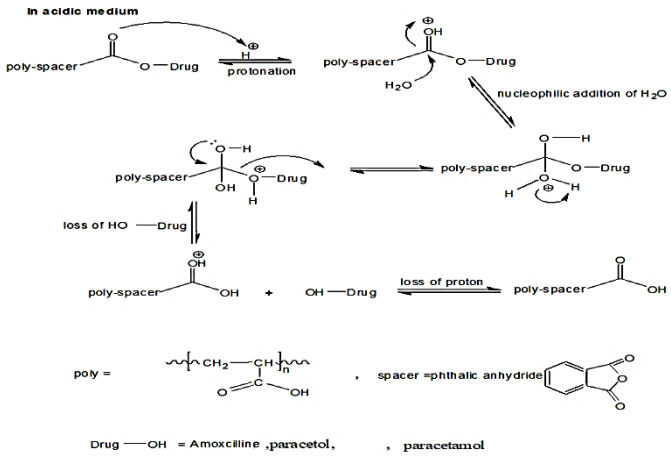

Figure 2. Hydrolysis in an acidic medium.

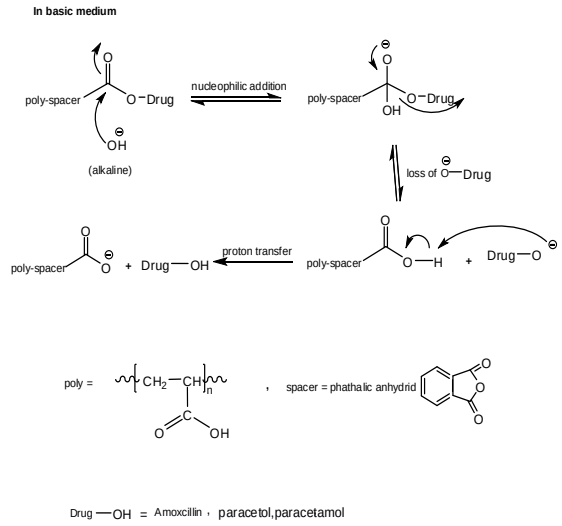

Figure 3. Hydrolysis in basic medium.

FTIR It is a widely used and extremely useful tool for analyzing chemical components and identifying characteristic combinations., the main peak at 1740 cm^{-1} was attributed to C = O expansion vibration, and the main band from 1514 cm^{-1} to 1516 cm^{-1} was assigned to the characteristic vibrations of the positions C = C Aromatic rings other peaks were tabulated in Table 1.

Table 1. FTIR of prepared compounds

Comp. No.	FTIR (cm^{-1})								
	ν(N-H) Amide	ν(C-H) Aliph.	ν(C-H) Arom.	ν(C=C) Arom.	N (C-Cl)	ν(C=O) 2.ester, 3.amide	ν(C-N)	ν(C-S)	Others
1	3292	2961 2932	3034	1616 1514	700	1759 1740	1375	1173	ν(OH) 3448
2	3258	2976 2943	3030	1595 1516	762	1758 1665	1397	1177	ν(OH) 3484
3	3223	2967 2928	3069	1597 1514	762	1754 1657	1387	1171	ν(OH) 3443 ν(-NO_2)1544,1360
4	3250	2963 2932	3059	1596 1514	762	1758 1686	1375	1126	ν(OH) 3522

The ^{1}H- NMR spectrum exhibited signals (singlet) at δ11.02 and 8.03 were assigned for OH and NH protons respectively. Proton of CH. Aromatic protons appeared as multiplets at δ7.95- δ6.6 and CH_3 protons appeared at δ2.1 as singlets as shown in Table 2 and Figures 4-6.

Table 2.^{1}HNMR data cm^{-1} of prepared compounds.

Comp. No.	^{1}HNMR Spectral data(*δ* ppm)
1	6.62-7.65(m,9H,Ar-H); 1.54(s,6H,2-CH_3); 4.65(s,1H,CH-COOH); 4.84(d,1H,CH-CH-S).; 5.13(d.1H,CH-CH-S).; 5.34(s,1H,-OH); 5.37(s,1H,-CH-N=CH); 8.03(s,1H,NH-amide); 8.15(s,1H,CH-N=CH); 11.02(s,1H,-OH --COOH acidic)
2	6.71-7.66(m,8H,Ar-H); 1.55(s,6H,2-CH_3); 4.71(s,1H,CH-COOH); 4.78(d,1H,CH-CH-S) 5.19(d,1H,CH-CH-S) Azet; 5.35(s,1H,-OH) 5.39(s,1H,-CH-N=CH); 8.08(s,NH-amide); 8.19(s,1H,CH=N; 10.95(s,1H,-OH acidic)
3	6.51-7.74(m,10H,Ar-H); 1.76(s,3H,-CH_3); 3,25(s,2H,S-CH_2; 5.01(d,1H,CH-CH-S); 5.38(s,1H,-CH-N=CH); 5.41(s.1H,CH-CH-S); 8.05(s,1H,NH); 8.75(s,1H,CH=N); 11.0(s,1H,-OH acidic)

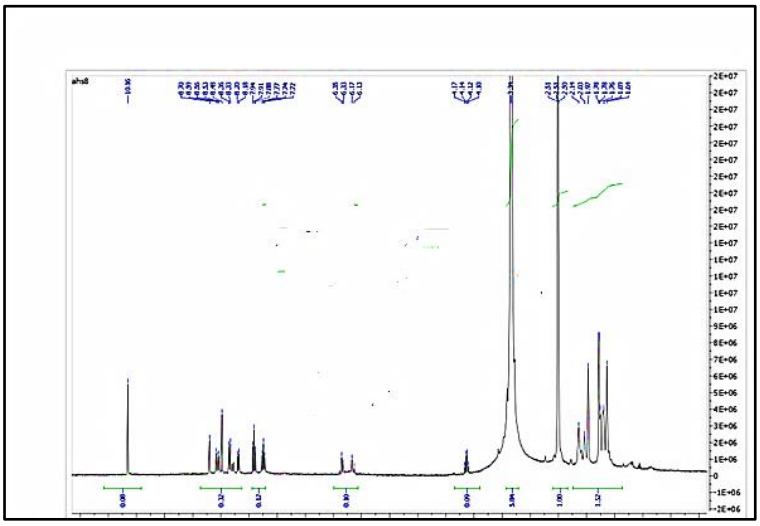

Figure 4. HNMR of casein –co-phathalate

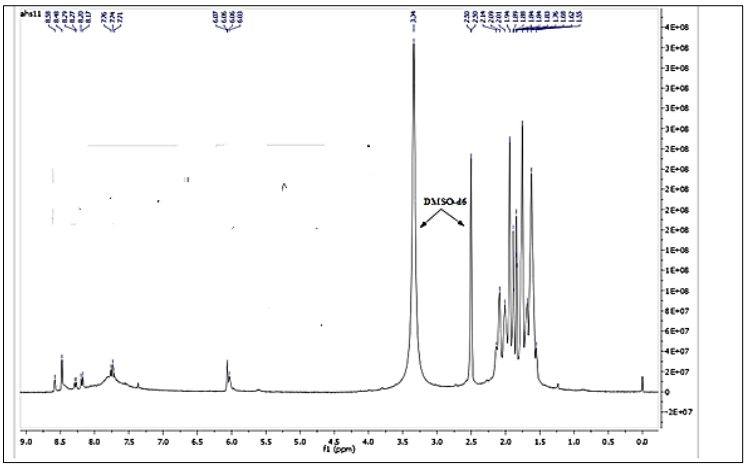

Figure 5. HNMR of casein –co-phathalat-procaien

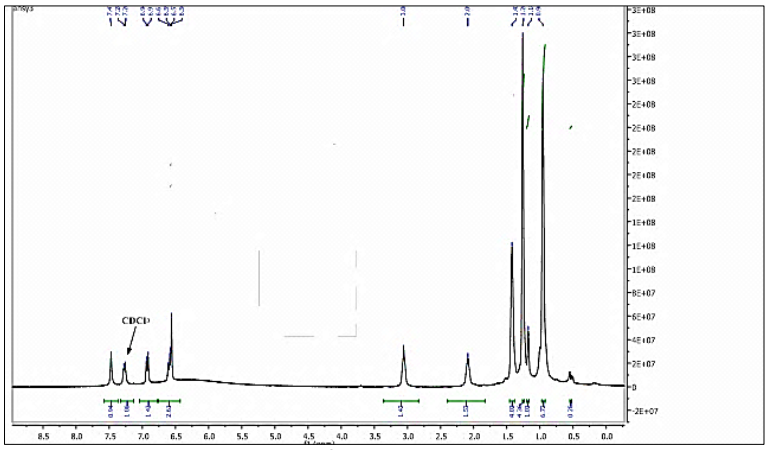

Figure 6. HNMR of casein –co-phathalat-salbutamol.

Thermal analysis

TG-DSCTG analysis is a valuable tool for estimating the content of components' fusion temperatures, and the DSC serves to measure the temperature and energy variability involved in the phase transformations of a compound. In Figures 7 and 8), two peaks of heat at 81.0°C and 211.1°C are allocated to the peak melting of ibuprofen and the peak of hydrolysis.

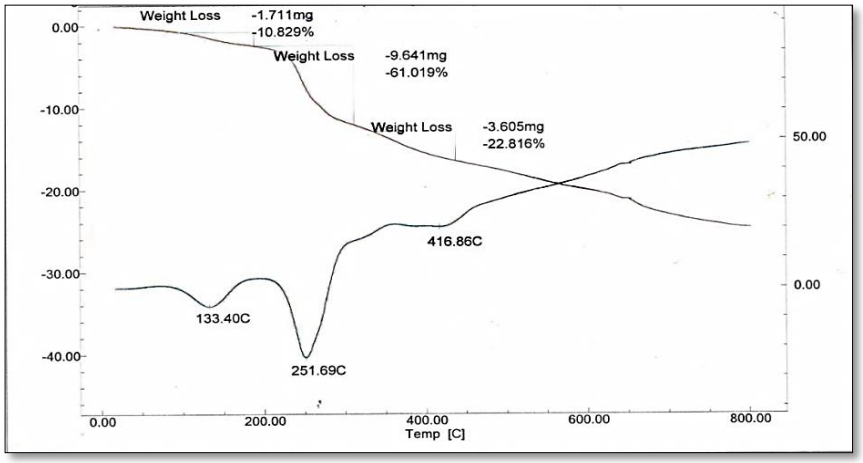

Figure 7. TG-DSC of casein –phthalic anhydride

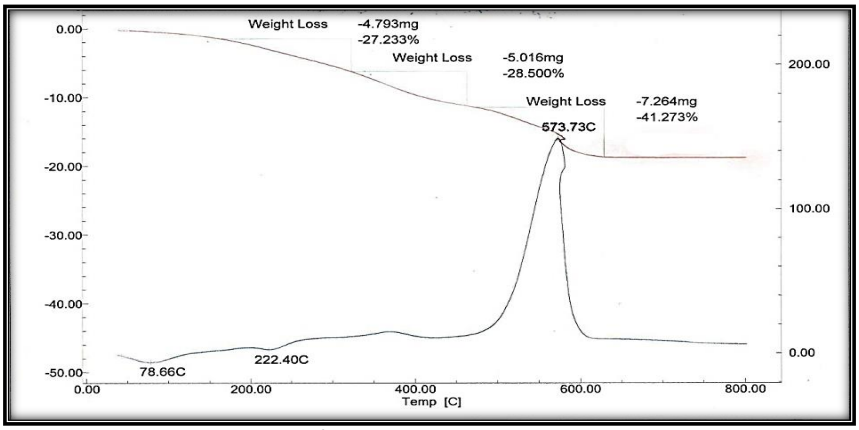

Figure 8. TG-DSC of casein –phthalic anhydride loaded procain.

Drug release

Drug release of casein–phthalic anhydride in different release media: PBS buffer (pH 7.4), SGA, and SGA-PBS. Release curves display the first three, 12 hours, or until they are released, or even do not release, stretching to 48 hours. Within the first 12 hours, 21%,32%, and 22. 33% total procaine were released in PBS, SGA-PBS, and SGA. after, procaine was released to 96. 21% at 48 hr in PBS, whereas in SGA-PBS, there was little command release to 60.55 - 0.43% at 24 hr, followed by a non-release period results shown in Figure 9. As for the release of the drug in SGA, a period of 12 to 48 hours The rise could be caused by adding a new launch medium. You may set it up in PBS and SGA-PBS; that is, you can set it up at the speed of its release of significant casein-phthalate-procaine. [25]

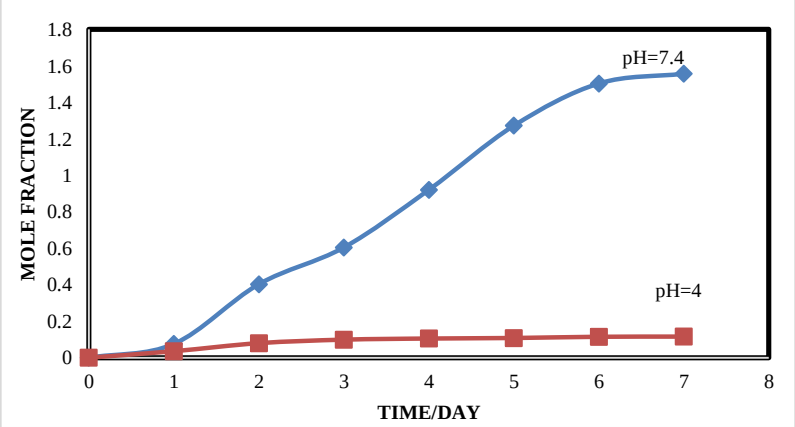

Figure 9. Drug release of casein-phthalate-procaine.

Antibacterial activity and antibacterial screening

Antibacterial activity and antibacterial screenin The antibacterial activity was determined for some synthesized compounds using diffusion agar procedure at (1mg) concentration, control DMSO assisted because there was no visible change in the growth of bacteria, Amoxicillin and Cephalexin were used a standard medicine, and the plates, incubated for 24 hrs at 37℃. The inhibition zone is determined in (mm). Antibacterial screening for some synthesized compounds was assessed for anti-bacterial activity against several bacterial strains i.e. G.P bacteria: B Staphylococcus aurous and acillus subtitles G.N bacteria: Pseudomonas aeruginosa and *Escherichia coli* at conc. (1 mg ml^{-1}).

inhibition zones caused by these compounds are evaluated and recorded in Table 3. Figure 10 shows the display effect of particular compounds on some kinds of bacteria [26].

Table 3. Anti-bacterial activity of synthesized compounds.

Comp. No.	Inhibition zone diameter(mm)			
	Stap. aurous	***Bacillus subtitles'***	***E.coli***	***Pse. Aeruginosa***
1	15	11	9	5
2	14	12	8	-
3	15	11	9	4
4	14	12	8	-
[A]AMX	14	11	5	-
[C]Cep	12	9	5	-
DMSO	-	-	-	-

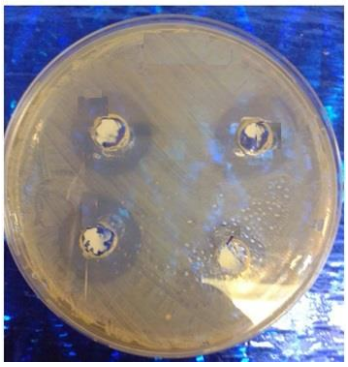

Figure 10. Effect of compounds on *Staphylococcus aureus.*

CONCLUSIONS

The main objective of this study is based on modifying casein by grafting with phthalic anhydride and loading it with different drugs 205 lifferent antibiotics and are replaced with different drugs. The properties of the prepared compound were studied using several FT-IR and 1H-NMR techniques, and it became clear that they agree with the results reported in the literature. The release of the drug from the prepared urine was estimated, as it turned out to have a medical application. It was applied to two types of bacteria to determine the antibacterial activity against different bacterial strains, namely Gram-positive bacteria: B Staphylococcus aurous and Bacillus subtitles bacteria Gram-negative: E. coli and Pseudomonas aeruginosa in conc. (1mg /ml). The inhibition zones causing these compounds are evaluated.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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