Research Article

Slow Release of 5-fluorouracil from Natural Polymeric Composites as Controlled Drug Delivery

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ABSTRACT

Natural polymer has received more attention in the field of controlled drug delivery. The potato starch cellulose acetate was reacted with acrylic acid to produce starch cellulose acetate coacrylate (SSCA) as synthesized polymeric material. The 5-fluorouracil (5-FU) as a traditional anticancer drug was loaded into the prepared polymer. It is expected that, prolonged circulation of anticancer drugs will increase their anticancer activity while decreasing their toxic side effects. The prepared polymer with and without the 5-fluorouracil drug were characterized by Fourier-transform infrared spectroscopy and Gel permeation chromatography (GPC). The polymer-carried 5-fluorouracil drug was tested for slow release drug delivery through subjecting to different aqueous media for different time periods and also, examining it as an anti-proliferative agent against human liver cancer cell line (HEPG2) as well as antimicrobial against both Gram positive and Gram negative bacteria. The release rate of the drug was evaluated in aqueous media at different pHs. The release was measured spectrophotometrically. It was found that, the release rate depends on the pH of the aqueous media. The release of the drug in the acidic media was found to be high compared with the other medias. Also, the sustained release of the drug was extended to about 60 days. The determined diffusion coefficient (D) of the released 5-fluorouracil compound from the polymer carrier was found to be $0.215x10^{-4}$, $0.805x10^{-4}$ and $0.437x10^{-4}$ in neutral, acidic and basic solution respectively according to Higuch's equation. Results of the *in vitro* cytotoxic activity of the released 5-fluorouracil showed a sustained antiproliferative potency against the liver cancer cell line which extended to 36 days. Moreover, results of the antimicrobial study revealed that the most potent inhibitory activity was against S. aureus which show hindrance zone of about 40 mm against the tested ref strain.

Keywords: Natural polymer, 5-fluorouracil, Sustained release, anticancer activity, antimicrobial activity, kinetics and permeability.

INTRODUCTION

Natural polysaccharides and their derivatives represent a group of polymers widely used in the pharmaceutical and biomedical fields for the controlled release of drugs. The advantages of controlled drug delivery systems are mainly the achievement of an optimum concentration, usually for prolonged times, the enhancement of the activity of labile drugs, due to their protection against hostile environments, and the diminishing of side effects due to the reduction of high initial blood concentrations. The polysaccharides hold several advantages over the synthetic polymers, generally because they are nontoxic, less expensive, biodegradable, and freely available, compared to their synthetic counterparts[1]. Starch shows poor flow property and enormous swelling owing to their hydrophilic nature, which results in premature release of drug in the stomach/upper intestine, and therefore, they should be protected while gaining entry into stomach and small intestine.

These problems can be rectified by the modification of starch in order to improve their rheological behavior and stability in acidic environment with a variety of useful hydrophobic monomeric and polymeric products by physical and chemical means[2]. Cellulose acetate (CA) is a starch derivative in which some of the hydroxyl groups are replaced by acetate groups to reduce the hydrogen bonding in order to facilitate processability, leading to high melt strength[3,4]. The preliminary study on the development of a new polymer, based on maize starch/cellulose acetate (SCA) blends, produced by free radical polymerization, for use as a biodegradable drug delivery carrier.

Hydrophilic-hydrophobic diblock copolymers have great potential as vehicles for the delivery of anticancer drugs[5-7]. A hydrophobic block forms the inner core, which acts as a drug reservoir, and a hydrophilic block forms the hydrated outer shell, which impedes uptake by the reticuloendothelial system (RES). The advantages of these copolymers includes solubilization of hydrophobic drugs, sustained release and selective targeting of drugs, and reduced drug interaction with the RES[8,9].

5-Fluorouracil (5-FU) is a next-generation antiviral drug, administered as vaginal topical treatment of vaginal lesions associated with the Human papilloma virus[10,11], including Condylomata acuminata[12] and cervical intraepithelial neoplasia[13]. From the chemical point of view, 5-FU is a fluoropyrimidine derivative, namely 5-fluoro-1 H-pyrimidine-2,4-dione. A large body of cancer research has been devoted to the development of targeted anti-neoplastic drugs that are selectively taken up by tumor tissues. Toward this end, researchers have

recently developed anti-cancer drugs that are incorporated into polymeric micelles, surface-modified particles, liposomes, or nanoparticles[14-17].

However, there are problems with this general approach, including limited biodistribution, toxic side effects, rapid clearance by the reticuloendothelial system (RES), and limited distribution in the circulation. In order to prolong the circulation time of 5-FU and increase its efficacy, numerous researchers have attempted to modify its delivery by use of polymer conjugates or by incorporation of 5-FU into particulate carriers[18-22].

The aim of the present work is to create a system of slow release techniques using 5-FU loaded with potato starch/cellulose acetate (SCA) co-acrylate polymer as a binding matrix for the drugs to develop and validate a new method for the quantitative determination of 5-FU by UV spectrophotometry. This method will be used to assess the in vitro release profile of 5-FU in various modern pharmaceutical dosage forms with vaginal administration that are under research in our department.

Also, study the cytotoxicity of the released drug against liver cell line and as antimicrobial activity.

MATERIALS AND METHODS

Materials

- Potato starch was supplied as neutral white powder by El Nasr Pharma Central Chemical Company, Abu Zaabal, Egypt.
- Cellulose acetate containing 40 % acetyl group was supplied by Sigma-Aldrich.
- Acrylic acid with molecular weight of 72, freezing point of 13 °C, boiling point of 141 °C, density at 20 °C of 1.046 g cm^{-3}, and relative index n_D at 20 °C of 1.42–1.421 was supplied by Sigma-Aldrich.
- Ethyl alcohol with density of 0.789 g cm^{-3} and boiling point of 78 °C was supplied by Aldrich Company, Germany.
- Hydrochloric acid with density of 1.2 g cm^{-3} and boiling point of 100 °C was supplied by Aldrich Company, Germany.
- Potassium hydroxide was supplied as white deliquescent pellets with melting point of 361 °C by Sigma-Aldrich.
- Dimethyl Sulfoxide (DMSO): density 1.101 g/mL and boiling range 189–191 °C was supplied by Sigma-Aldrich.
- Dicumyl proxide (DCP), pure grade, melting point (39-41 °C) Mwt=270.37 g/mol, Aldrich product.
- 5-Fluorouracil (5-FU),2,4-Dihydroxy-5-fluoropyrimidine, assay is 99% with melting point of 282-286 °C by Sigma-Aldrich.

Characterization Techniques

Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of samples were obtained using a Jascow (Japan) FTIR 430 series infrared spectrophotometer equipped with KBr discs.

Leaching Rate

The leaching rate technique used was similar to that described by Marson[23]. The obtained starch cellulose acetate co-acrylate (SCAA) polymer was used in the form of cubes discs of 1 mm side length and total surface area of about 6 mm^2. Samples were each immersed in 50 ml of different media of distilled water, acidic and alkaline solutions, at room temperature. The leachable medium was changed daily during the period of study. The amount of drug released was determined spectrophotometrically[24] at wavelength of 222, 224 and 238 nm for the drug compound in distilled water, acidic and alkali solutions respectively. The spectrophotometer used was a UV-240 1PC Visible VIS. The calibration standard is shown in Figure 1.

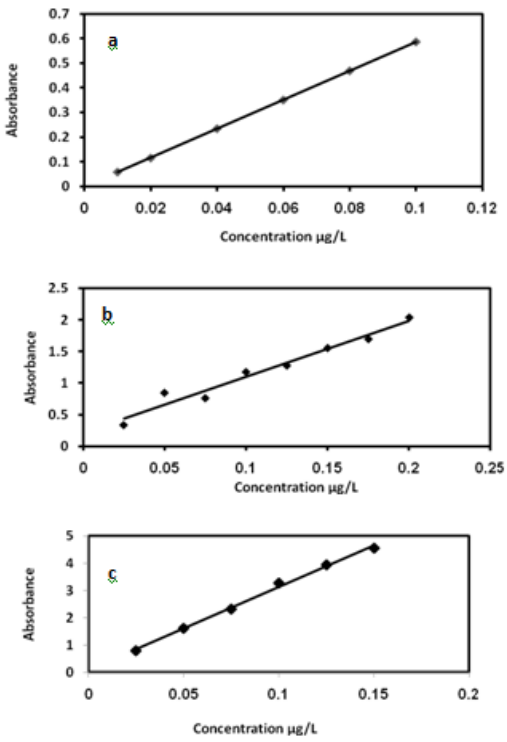

Figure 1: Calibration curve for 5-Fluorouracil in a) neutral medium b) acidic medium c) alkaline medium

Gel Permeation Chromatography (GPC)

The average molecular weight of the synthesis material was determined by gel permeation chromatography (GPC) using Agilent Technologies Walden Bonn, Germany 1100 series equipped with two Styragel columns (10^2 and 10^3Á) and Refractive Index detector (Agilent G 1362).

THF was used as eluent at flow rate of 1ml.min^{-1}. The columns were calibrated by polystyrene as internal standard.

Biological Activities

Anticancer Testing

Measurement of potential antiproliferative activity was done using SRB assay[25]. The timely released drug (5-flurouracil) was subjected to a screening system for evaluation of its antitumor activity against HEPG2 liver cancer.

Antibacterial Testing

Antibacterial activity of the timely released drug (5-Flurouracil) was *in vitro* evaluated using agar well diffusion test[26] against the following tested stains; Gram negative bacterial reference strain; E. coli O157 ATCC 700728, Gram positive bacterial reference stains; Strept. mutans ATCC 25175, Cl. perfringens ATCC 13124 and Enterotoxigenic S.aureus ATCC 13565 and mycotic reference strain; C. albicans EMCC 105 as control positive Antibiotic (AMC_{30}) (30µg/ml) was used as standard antibacterial while Antimycotic (AMB_{30}) (30µg/ml) was used as standard antifungal while dimethyl sulphoxide (DMSO) was used as control negative[27].

RESULTS AND DISCUSSION

Polymer Synthesis and Characterization

Starch cellulose acetate coacrylate polymer was prepared from potato starch/cellulose acetate thermoplastic blend of 90 % starch content and acrylic acid monomer in the presence of 2 % benzoyl peroxide as initiator; the reaction was carried out at 60 °C for 10 min, then the active cancer drug 5-Fluorouracil (5-FU) (5% from the solid content), was added gradually under stirring for 15 min. The reaction mixture was poured into a Petri dish and dried to constant weight in an oven at 70 °C. The obtained polymer was found to be a solid, white, and homogeneous material[3,28]. The prepared polymer/drug was characterized by FTIR.

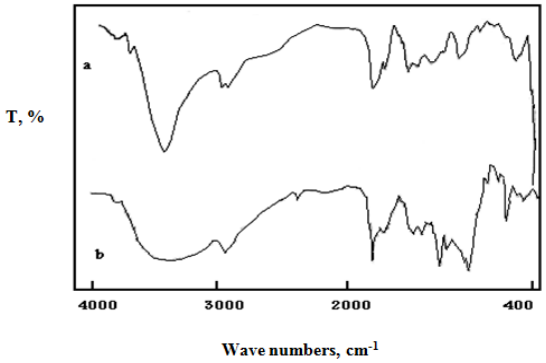

Figure 2: FTIR of the prepared polymer containing 5-fluorouracil compound. a) Pure polymer b) polymer loaded with5-Fluorouracil compound

The compounded polymeric materials contained the anticancer drug 5-fluorouracil compound characterized by FTIR. Figure 2a–b shows the IR spectra of the prepared polymer starch cellulose acetate co-acrylate alone and after being loaded with anticancer organic compound 5-Fluorouracil. It can be seen that the characteristic peaks for the functional group of the prepared polymer are found at 3439 cm^{-1} for the OH group, 1740 cm^{-1} for the acetate group, and 1650 cm^{-1} for acrylate Figure 2a. Figure 2b illustrates the characteristic peaks for the functional groups which belongs the 5-fluorouracil compound: broad NH at about 3406 cm^{-1}, C=O at 1692 cm^{-1}, amide group at 1758 cm^{-1} and C-F at 1177 cm^{-1}.

The average molecular weight (Mw) was determined using Gel permeation chromatography (GPC) for starch, cellulose acetate, acrylic acid and the obtained polymer loaded with 5-fluorouracil compound. The average molecular weight (Mw) obtained are $1.9x10^5$, 30,000,72.06 and $8.2087x10^7$ g/mole, respectively.

The Release rate of the 5-Fluorouracil Compound from the Investigated Polymer in Distilled Water

The release was measured spectrophotometrically and its rate expressed as µg day^{-1}. The results for the release of the 5-Fluorouracil compound (drug) in distilled water versus time are presented in Figure 3a. It was found that the release rate was extended to about 60 days. The release increased gradually according the time produced the highest value was observed at about 36th day, after that the amount of release decreased and steady sustained release was obtained up to the end of the period of study (60 days).

The release was observed through diffusion-dissolution mechanism, diffusion of water inside the polymeric system, and dissolution outside, upon exposure to water. The 5-Fluorouracil compound which dispersed in the synthesis polymer was gained through dissolution mechanism. As the surface layers of the investigated discs depleted, the pore structures were formed also they provided the 5-Fluorouracil compound release to be sufficiently rapid, these leads to the penetration of water to the pore structures. The process was continued, although the rate of lose usually varies according the growing of tortoises path of the pore structure and other the factors[28,29].

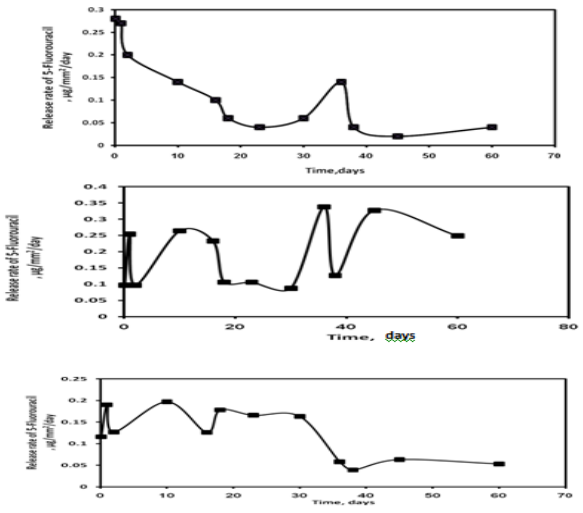

Figure 3: The release rate of 5-Fluorouracil in a) distilled water b) acidic solution of HCl c) alkaline solution of KOH

Table 1: The diffusion coefficient (D) of the prepared polymer containing 5-Fluorouracil compound (drug) in different media

Mediums	The Diffusion coefficient (D)
H_2O, pH 7.3	0.215 x 10-4
Acid solution HCl, pH 4	0.805 x 10-4
Basic solution KOH, pH 9	0. 437 x 10-4

Effect of pH of the Aqueous Media on the Release rate of Fluorouracil Compound

It is interesting to study the effect of the environmental pH of the medium on the release of the 5-Fluorouracil compound, to regulate the drug delivery for anticancer cell targeting. Figures 3b, c presents the release pattern of the 5-Fluorouracil compound in aqueous media with different pH values at room temperature (25 °C) for different time periods up to 60 days. It was found that the amount released active ingredient depends on the nature of acid and base in the aqueous medium. The release was low in neutral medium (pH 7.3) as shown in Figure 3a, while in acidic media it was high but in basic media was moderate as shown in Figure 3b, c respectively. The basic media were adjusted to (pH 9) using potassium hydroxide, while in the acidic media (pH 4) using HCl; the release was high and increased compared with that obtained in the neutral media. The sustained release extending to about 60 days. The amount of drug released depended on the pH of the aqueous media as the following order:

Acidic medium> basic medium > neutral

Study of the Release Kinetics of the 5-Fluorouracil

The release kinetics of the drugs was studied according to the Higuchi equation. Higuchi developed[28,30] models to study the release of soluble and low solubility compounds incorporated in solid matrices from the planer system having a homogeneous matrix, and the following equation (1) was used:

$$M_t = A\,(2DTC_sC_o) \qquad (1)$$

Where, M_t, accumulated amount of release 5-fluorouracil at time T; A, the surface area; D, Diffusion coefficient; C_s, solubility of 5-fluorouracil (10 mg/ml of water by weight); C_o, initial drug; T, the time.

The release of the 5-fluorouracil compound was measured in different environmental media and the diffusion coefficient was calculated and is tabulated in Table 2. The results show that the diffusion coefficient (D) of the released 5-fluorouracil compound from the polymer carrier can be arranged in the following order:

Acidic medium> basic medium > neutral

Also, it was observed that, the diffusion coefficient (D) was increased as the release rate increased, therefore higher solubility compounds enhanced its diffusion from the matrix[30], and, consequently, the amount of release of 5-fluorouracil increased[29]. The larger degree of solubility of the drug facilitates the drug molecules to be released.

The diffusion coefficient (D) was strongly dependent on the nature of compounds as well as the type of environmental media.

Table 2: Antiproliferative Activity of the released Drug (5-Flurouracil) against HEPG2 cell line

Time, days	SURVIVING, %	INHIBITION, %
0.014	62.54826	37.45174
1	62.16216	37.83784
2	62.16216	37.83784
10	69.49807	30.50193
16	71.04247	28.95753
18	76.06178	23.93822
23	74.90347	25.09653
36	73.74517	26.25483

The Release Kinetics of the Investigated Polymeric System containing 5-fluorouracil

It is interesting to calculate the release kinetics to show the behavior of the 5-fluorouracil released. The release data from the polymeric system containing 5-fluorouracil compound at 25 °C for different time ranged from 1 to 60 days and different media were followed the following equation (2):

$$Q = K\,t\,n \qquad (2)$$

Where, Q is the fractional amount released at time t, K is the kinetic constant, n is the release exponent indicate Fickian diffusion[31], values of n ~ 0.5. Figure 4 indicates the release kinetics of 5-Fluorouracil compound. It was found that, the kinetic constant (K) decrease as the time increased and consequently the release increased.

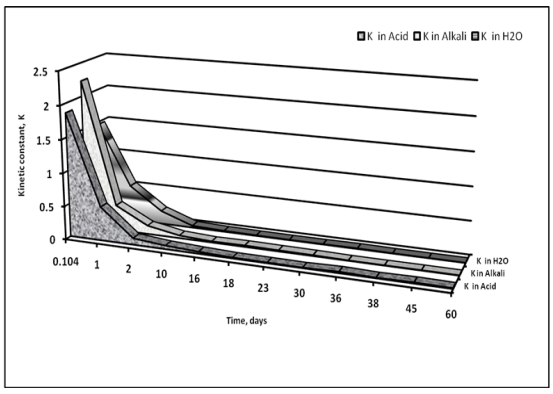

Figure 4: The release kinetic constant [K]of the prepared polymer containing 5-Fluorouracil

Permeability of the prepared Polymer loaded with 5-fluorouracil Compound

Study of leaching was carried out to evaluate and compare the release in different media (KOH, HCl, and H2O) by determination of the permeation coefficient, P, of the investigated polymer carrier containing 5-

fluorouracil compound. The permeation coefficient P was calculated using the following relationship (3):[3,29,32,33]

$$J = P C_d \quad (3)$$

Where J is the amount of release at different times and C_d is the drug donor concentration. The permeability of the polymer containing the 5-Fluorouracil compound was studied based on the diffusion–dissolution mechanism.

Figure 5 represent the release profiles of drug. The permeation coefficient was calculated from the release profile using the above equation. From the obtained results, it was observed that the permeation coefficient of the prepared polymer (P) depends on the medium type and the nature of the investigated polymer.

Therefore, it is easy to study the release of drug using conventional release experiments as described by Marson.

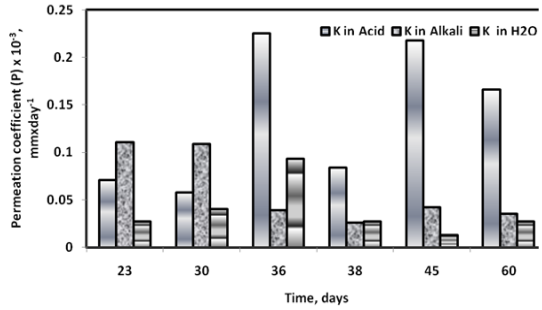

Figure 5: Permeation Curve of the prepared Polymer containing 5-Fluorouracil

Biology Results

In vitro Cytotoxicity of the Released Drug from the Polymer

The antiproliferative activity of the investigated SCAA-polymer carried the anticancer drug (5-flurouracil) against HEPG2 cell line was illustrated on Table 2. The data of the table shows the efficiency of the drug released from the prepared polymer on growth inhibition and survival of HEPG2 liver cell line. It was found that the potency of the drug was controlled and sustained up to 36 days.

Antibacterial Activity of the Released Drug from the Polymer

The antibacterial activity of the investigated SCAA-polymer loaded with 5-Flurouracil were evaluated against Gram Positive bacterial reference stains; Strept. mutans ATCC 25175, Cl. perfringens ATCC 13124 and Enterotoxigenic S.aureus ATCC 13565. As control positive Antibiotic (AMC_{30}) (30µg/ml), while dimethyl sulphoxide (DMSO) was used as negative control. Results revealed that the released drug gave the highest antibacterial activity against the Gram positive bacterial reference strains; Enterotoxigenic S.aureus ATCC 13565 strain with a mean zone of inhibition about 40 mm. On the contrary that the released drug had very weak antibacterial activities against Cl. perfringens ATCC 13124 and had no inhibitory effect against Strept. mutans ATCC 25175, as shown in Table 3.

Table 3: The Antimicrobial activity of the released drug (5-Flurouracil) against Gram Positive bacterial reference strains

Time, days	Strept. mutans ATCC 25175	Cl.perferingens ATCC 13124	S.aureus Enterotoxigenic ATCC 13565
0.014	-ve	30s	40
1	-ve	20s	30
2	-ve	22s	36
10	-ve	12s	34
16	-ve	24s	42
18	-ve	24s	40
23	-ve	-ve	32
36	-ve	-ve	28
DMSO	-ve	-ve	-ve
ANTIBIOTIC (AMC_{30})	15	13	30

CONCLUSION

- SCAA was prepared from potato starch cellulose acetate and acrylic acid monomer.
- SCAA could be used as a polymer carrier for the 5-fluorouracil compound and applied as controlled release drug delivery system because it is a good technique used for controlling the release of drug delivery.
- The release of drug from the investigated polymer loaded with 5-fluorouracil compound as well as release kinetics and Permeability depend on the aqueous media.
- The sustained release of 5-fluorouracil compound was extended to ~ 60 days.
- The prepared investigated natural polymer had promising effect on liver cell line and also had antibacterial activity.

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