Egypt.J.Chem. **Vol. 62**, Special Issue (Part 2), pp. 549- 562 (2019)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Evaluation of Synthesized Starch Cellulose Acetate Coacrylate and Nanoclay Holding Drugs

Fahima M. Helaly[1], Aman I. Khalaf[1], Doaa E. El. Nashar[1*], Samaa R. Salem, Nehad N. Rozik[1], Adelmohsen M. Soliman[2]

[1]Polymers and Pigments Department, National Research Centre (NRC), Cairo, Egypt
[2]Therapeutic Chemistry Department, National Research Centre(NRC), Dokki, Giza, Egypt

5-FLUOROURACIL (5-FU), doxorubicin (DOX), and chlorambucil (CA) are functional drugs to destroy cancer cells in individuals with different carcinoma. The slow release profile is an optimistic technique and controls the drug release over a long time. This study is based on the incorporation of the drug on the nano-sized clay into polymeric host-carrier. The polymeric material based on potato starch cellulose acetate compound copolymerized with the acrylic acid monomer (SCACA) by a free-radical mechanism. The main aim of the represented study is to increase the drug efficiency for long period stability and simultaneously modifying drug delivery technologies with nano-clay (modified sodium bentonite (MSB)). The prepared drug delivery carrier was characterized by Fourier transform infrared spectroscopy (FT-IR). The evaluation of equilibrium swelling ratios (Q) for the SCACA without and with modified bentonite (0.05 and 2%) depict that greater modified clay content gave the lower Q value of the prepared polymer. The release rate was investigated in aqueous media of different pH values and measured spectrophotometrically. It was found that the release rate depends on the pH of the aqueous media in addition to the content of clay used in the polymeric modification.

The modified polymer/ nano-clay drug carrier has been also evaluated as an antiproliferative agent against human liver cancer cell line (HEPG2). The results show that, 5-FU gave the highest growth inhibition potency from 54:48% followed by CA which gave 37:46% , while DOX showed the lowest growth inhibition donated 41:29% for all days exposure . This may due to the release of 5- FU is more than CA and Dox., but all drugs have a promising results.

Key Words: Drug delivery, Slow release,5-fluorouracil, doxorubicin, chlorambucil

Introduction

The development of the drug delivery carriers based on biomaterials hasreceived great attention for the treatment of different types of carcinoma. Diverse type of clays minerals has a significant role in the domain of health products. They can be considered as raw pharmaceutical materials that can be modified in order to improve pharmaceutical requirements and manufacture of medical products.[1] The clay minerals are mainly utilized in the treatment of skin diseases and in simple gastrointestinal ailments such as diarrhea [2, 3]. Sodium bentonite clay (SB) is used due to its specific featurs; it has large layer space and different characteristics including good water absorption, swelling, adsorbability, cation exchange, and drug-carrying conduct. [4] Moreover, medical SB can issue the mucoadhesive conduction order to cross the gastrointestinal barrier [5]. The polymer/silicate

*Corresponding author e-mail: doaaelnashar@yahoo.com
Received 23/6/2019; Accepted 5/8/2019
DOI: 10.21608/ejchem.2019.13963.1863

nanocomposite often have improved thermal, mechanical, and barrier properties as compared to the pure polymer component, even at a low weight percentage of silicate.[6, 7] In essences, this represents a great challenge in drug delivery and its success could become a revolution in the world of pharmaceuticals.[8-10]

The slow release technique is a good mean for controlling the active agent. The technology of sustained release is used to reveal the disadvantage of the conventional methods and it relies on the organization of the release rate of the bioactive compound seluted out of the drug carrier system over a long have time period.[11,12]

The controlled release system creates a considerable challenge in the world of chemotherapy. It can easily maintain an adequate concentration of the drug in the blood circulation in order to perform an elongated subjection of cancerous cell to the drug. [8,13] There are various advantages to oral administration including patient's convenience and relative cheap costs associated with drug preparation and administration. Moreover, patients would excel an oral agent rather than other treatments for drug administration. In this research, it is intensively attempted to investigate and find possible solutions for the oral delivery of different anticancer drugs. The main aim of this study is to synthesize sustained release nano-sized clay/ starch cellulose acetate co-acrylate holding some investigated drugs such as 5-FU, DOX, and CA, as effective antitumor agents, and study of the release rate of the drug molecules in aqueous media of different pH's values.In addition, examine the antibacterial and antiproliferative properties of the drug release against human liver cancer cell line (HEPG2). Furthermore, the objective is focused on the impact of the non-ionic surfactants, Tween-80, to enhance and ease formulation process as disc shape …etc.

1. Experimental

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, St. Louis, Missouri , USA.
- 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, Jascow, JEOL, BioTek Instruments, Germany.
- Sulfuric acid with density 1.84 g cm^{-3} and boiling point 337 °C was supplied by Aldrich Company, Germany.
- Sodium hydroxide pellets are odorless, white, solid hemispheres of uniform diameter and thickness with melting point 318.4 °C was supplied by Sigma-Aldrich.
- Dicumylperoxide (DCP), pure grade, melting point (39-41°C) Mwt= 270.37g/mol, Sigma-Aldrich product.
- Sodium bentonite clay powder (mesh size 300 mm), with a cationic exchange capacity (CEC) of 90 mequiv per 100 g.
- Tween-80 (polyethylene sorbitol ester) non-ionic viscous liquid and average molecular weight 1,310 Da purchased from Sigma-Aldrich.
- 5-fluorouracil (5-FU),2,4-Dihydroxy-5-fluoropyrimidine, assay is 99% with melting point of 282-286 °C by Sigma-Aldrich.
- Doxorubicin hydrochloride (DOX), Mwt= 579.98 g/mol , mp= 216 °C and empirical formula: $C_{27}H_{29}NO_{11} \cdot HCl$, Sigma-Aldrich product.
- Chlorambucil (CA), 4-(4-[Bis(2-chloroethyl) amino]phenyl)butyric acid, 4-[Bis (2-chloroethyl)amino] benzenebutyric acid, Molecular Weight 304.21

Instruments and 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.

Ultra Violet Spectroscopy and Leaching Rate

The leaching rate technique used was similar to that described by Marson.[14] The obtained starch cellulose acetate co-acrylate (SCAA) polymer was used in the form of cylinder discs of radius 0.75 cm and height 0.5 cm and total surface area of about5.89 cm^2. Samples were each immersed in 50 mL of different aqueous media with different PH 7.5, 4, 9, at room temperature. The leachable medium was changed daily during the period of study. The amount of drug released was determined spectrophotometrically [15] at wavelength measured in distilled water 222, 480 and 258 nm for 5-FU, DOX and CA drugs

respectively. The spectrophotometer used was a UV-240 1PC Visible VIS.

Transmission electron microscopy (TEM)

Micrographs of pure clay as well as nano-clay were detected using a (TEM, JEOL JEM-2100, Japan) with an accelerating voltage up to 160 kV and micro-analyzer electron probe.

Method

Preparation of Polymer/Clay nanocomposites loaded with different drugs

Modification of clay

a) Pretreatment with distilled water:

Sodium bentonite (SB) will be mixed with distilled water and stirred vigorously at 80 °C for 12h; this suspension clay is then filtered and dried in an oven at 80 °C for 12 h until constant weight. The dried product was grinded in a mortar into powder.

b) Treatment with non-ionic surfactant (Tween-80):

Tween-80 (0.01wt %) was dissolved in distilled water, then this solution was mixed with 0.05 g of the pretreated grinded SB with constant slow stirring at 70 ^{O}C for 30 min to obtain the modified SB (MSB). This modified clay was then filtered and washed thoroughly with hot water several times and dried in a vacuum oven at room temperature.

Preparation of SCACA/modified nano-clay
Preparation of SCACA/modified nano-clay loaded with different drugs

Starch cellulose acetate co-acrylate was prepared from starch cellulose acetate (90/10) and acrylic acid monomer in the presence of 2 % benzoyl peroxide. 1gram of the obtained matrix was mixed with 5ml ethanol. The previous solution was heat-treated at 70°C under continuous magnetic stirring until the starchy paste was obtained. The finished paste should have the consistency of sticky and a translucent colour. Hence, the paste was left to cool down and finally [16].

The drugs (5-FU, DOX, and CA) were used and presented in Figure 1. DOX was dissolved in 50 ml of ethanol, however, 5-FU was dissolved in 50 ml distilled water and CA was dissolved in 50 ml of THF.

The modified SB with Tween- 80 will be dispersed in the starch cellulose acetate coacrylate (SCACA) polymer using vigorous stirring, then the anticancer drugs [3.9% of polymeric solid (SCACA + modified clay)] will be added using vigorous stirring, the finished products were casted in a cylindrical mould.

Equilibrium Swelling Ratio Measurement

Swelling measurements for the prepared slow release polymeric materials are important to examine in aqueous solution. The dried prepared tablets were immersed in 50 ml of alkaline water [Dissolve NaCl in distilled water untill pH alkaline medium (pH 9.5) by pH paper] at 25 °C and in distilled water until swelling equilibrium were attained. The weight of the dry samples (S_d) were determined after drying. The weight of the wet sample (S_t) was determined after removing the surface water by blotting with filter paper.

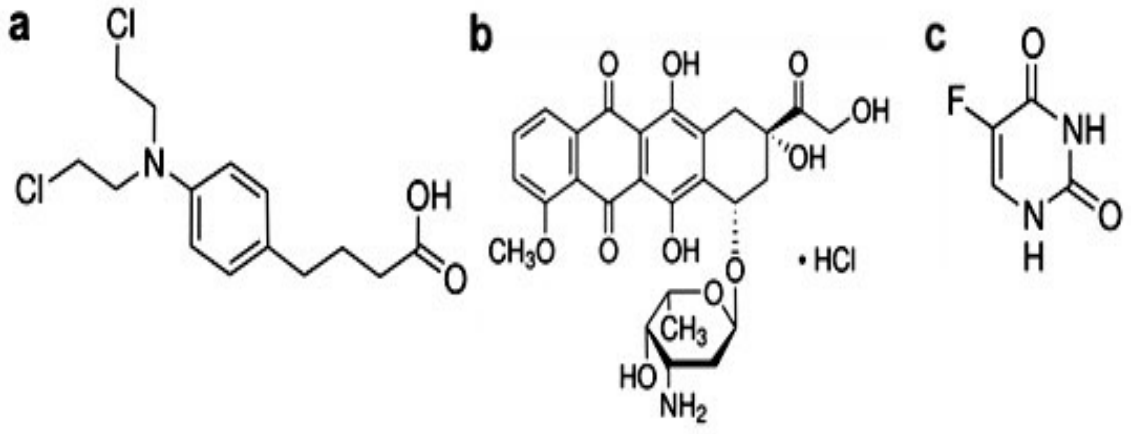


Fig. 1. The chemical structure of (a) CA (b) DOX (c) 5-FU

All swelling measurements were performed in triplicate, with uncertainty indicatingthestandard deviation of measurement data. The equilibrium swelling ratio (Q) of the SCACA/SB-drug composite was calculated using the following equation [10]:

$$Q = (S_w - S_d) / S_d \quad (1)$$

Swelling Kinetics Measurement

The swelling ratio was obtained by weighing the initial and swollen samples at various time intervals. The amount of water absorbed (W_t) was reported as a function of time and the equilibrium absorption at infinite (long time) was reported as ($W_¥$). The following equation will be utilized to calculate the diffusion coefficient D [10]:

$$Wt/ W_¥ = 4 (Dt/pL^2)^{1/2} \quad (2)$$

Where t is the time and L is the initial thickness of the tablet in the immersion solution (NaCl or distilled water), D is the diffusion coefficient of the uploaded molecules or solutes out of the polymer matrix, p is a constant equal 3.14. To investigate the diffusion model of the carrier polymer SCACA/SB, the initial swelling data were fitted to the exponential heuristic equation [17, 18]:

$$Wt/ W_¥ = Kt^n \quad (3)$$

Where K is a characteristic constant of the polymer SCACA /SB and n is a characteristic exponent of the mode transport of the penetrate and n is fitted with the value ½.

In vitrostesting for the toxicity of anticancer drugs released from the prepared polymer towards liver cancer cell line HEPG2.

Cells

Liver cancer cell line HEPG2was obtained from American Type Culture Collection (Rockville, Maryland, USA) and are being maintained in the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (Wrocław, Poland). HEPG2 cells were cultured in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mML-glutamine, 10% fetal bovine serum, 8 ug/mL of insulin and 1% mem non-essential amino acid solution 100x (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The culture media was also supplemented with antibiotics: 100 µg/ml streptomycin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 100 units/ml penicillin (PolfaTarchomin SA, Warsaw, Poland). HEPG2 cells were grown at 37 °C with 5% CO_2 humidified atmosphere.

Released anticancer drugs

Anticancer drugs released from polymers in distilled water

The prepared polymers of starch cellulose acetate coacrylate (SCAAC), loaded with different anticancer drugs such as 5 Florouracil (5FU), doxorubicin (DOX), and chlorambucil (CA), were subjected to distilled water and put in tubes of ultrasonic bath (Ultrasonic Cleaner, SH80, USA) for 30 min. Ice was added to maintain the deionized water at room temperature during the indirect sonication treatments for releasing the active drugs over different time periods.

Antiproliferative assay in vitro

Twenty-four hours before addition of the tested drugs bound by polymers, HEPG2 cells were plated in 96-well plates (Sarstedt, Germany) at density of $1x10^4$ cells per well. The assay was performed after 72 hours' exposure to varying concentrations of the released tested drugs. The in vitro cytotoxic effect of all anticancer drugs was examined using the sulforhodamine B (SRB) assay.

Cytotoxic test: SRB assay

The details of this technique were described by Skehan et al. [19]. The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 hr and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (fife times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCH, Gliwice, Poland) for determination of the optical density (□ = 540 nm) in Synergy H4 multi-mode microplate reader (BioTek Instruments USA).

Results and Discussion

Characterization of modified sodium bentonite

X-ray diffraction

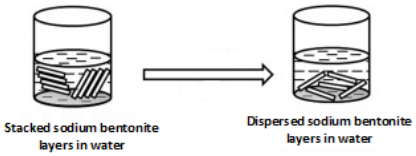
Figure 2 illustrates the XRD patterns of (a) unmodified bentonite (SB), (b) pretreated bentonite (PSB) with distilled water and (c) modified bentonite (MSB) with non-ionic surfactant (Tween-80). The d-spacing of the clay particles was calculated using Bragg's equation

(4) [20]:

$$n\lambda = 2d.\sin\theta \quad (4)$$

where n is the whole number of wavelengths, λ is the wavelength of the X-ray, d is the interspace distance, and θ is the angle of incident radiation.

It is clearly seen that, sodium bentonite showed a characteristic diffraction peak at 2 θ = 5.1° which is corresponding to the inter layer spacing (d-spacing) of 11.7948 (Fig. 2a) but A displacement of this peak towards low angle (2 θ = 4.4°) and high d-spacing (14.9968) can be observed for the pretreated clays with distilled water (Fig. 2b). These results indicate that clays are dispersed in water and there is an immediate increase in viscosity. During this time, water that is imbibed between the clay platelets causes the macroscopic particles to swell and the platelets to exfoliate (Scheme 1) . On the other hand the addition of Tween-80 (nonionic surfactant) (0.01wt %) on 0.05 g of the pretreated grinded SB, the diffraction peak appear at 3.9° and spacing diffraction peak 18.9968A°. This may be attributed to the surfactant polar head-groups present in Tween 80 attach at the tetrahedral sheet surface, leaving the alkyl chains extending away from the edges and faces. Consequently, the alkyl chains undergo hydrophobic interactions that facilitate the association between the platelets and increase the physical structure within the suspension. There is a significant interaction between these Tween 80 and sodium bentonite clays (Fig. 2c) [20].



Scheme 1. pretreated clay with distilled water

Transmission electron microscopy (TEM)

Figure 3 shows the transmission electron microscope photographs analysis at magnification 1000 x for the unmodified bentonite (SB) (Fig. 3a), pretreated bentonite (PSB) with distilled water (Fig. 3b) and modified bentonite (MSB) with non-ionic surfactant (Tween-80) (Fig. 3c). From the figure it is clear that, the particle diameters are found to be 0.1306 µm for SB, 25 nm for PSB and 5nm for MSB .The transmission electron micrographs support the XRD results.

Effect of modified sodium bentonite (MSB) on the prepared polymer (SCACA) nanocomposite properties

The effect of modified sodium bentonite on some properties, such as equilibrium swelling ratio was investigated.

Effect of MSB on the Equilibrium Swelling of SCACA

The equilibrium swelling ratios (Q) for the SCACA without and with modified bentonite (0.05 and 2%) were determined in alkaline solution and distilled water and were shown in Fig. 4.

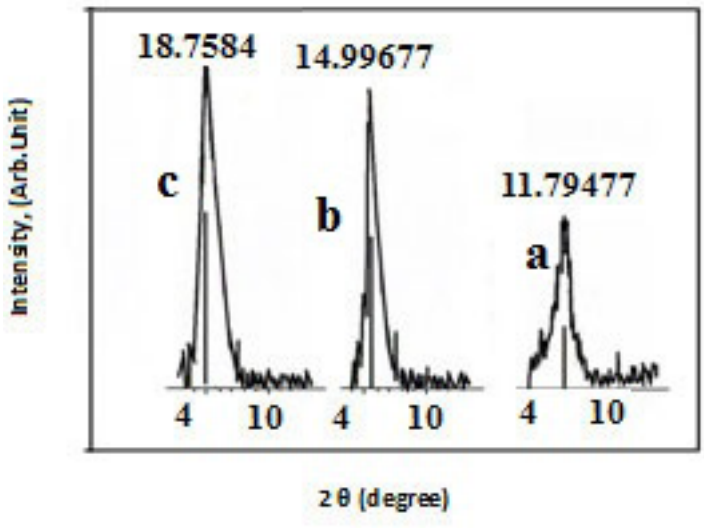


Fig. 2. (a) XRD patterns unmodified bentonite (SB), (b) pretreated bentonite (PSB) with distilled water and (c) modified bentonite (MSB) with non-ionic surfactant (Tween-80)

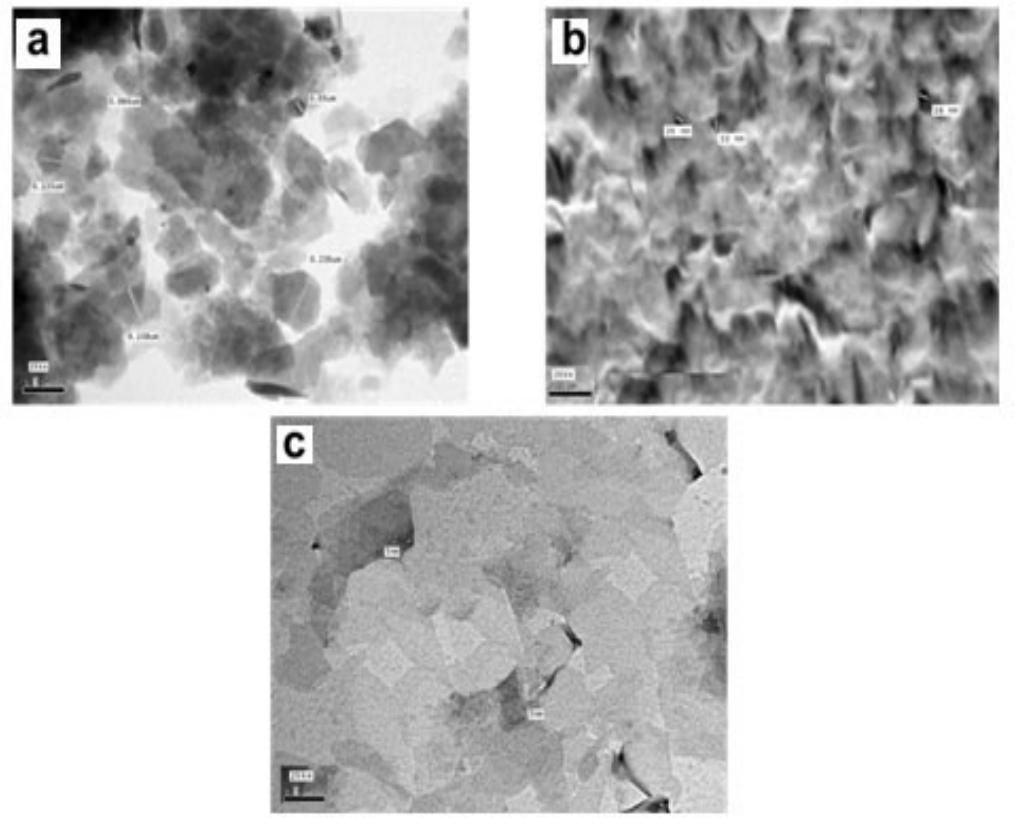


Fig. 3. TEM micrographs of: (a) unmodified bentonite (SB), (b)pretreatedbentonite (PSB) with distilled water and (c)modified bentonite (MSB) with non-ionic surfactant (Tween-80)

It is clear that the greater the modified clay content in prepared polymer, the lower the Q value of the prepared polymer. This is because the original hydrophilic bentonite modified with Tween- 80 becomes a hydrophobic chain: it makes the nanocomposite prepared polymer become more hydrophobic. Therefore, the ratio of equilibrium swelling can be arranged according the concentration of modified bentonite as the following order:

Without modified bentonite> 0.05% modified bentonite> 2% modified bentonite

Also, the effect of pH on equilibrium swelling ratio for the present prepared polymer nanocomposite is shown in Figure 4. The results, shown in Fig. 4, indicate that the Q value of the prepared polymer with modified bentonite increased in alkaline solution (P1-NaCl and P2-NaCl) and decreased in distilled water (P1-H_2O and P2-H_2O). For the prepared polymer without bentonite the Q value decreased in alkaline solution (P0-NaCl) and increased in distilled water (P0-H_2O).

Effect of MSB on the swelling kinetics

The swelling ratios as a function of time for thepresent SCACA in alkaline solutionand distilled water are shown in Table 1. The K, and D values were calculated from eqs. (2) and (3) are listed in Table 1. The results show that the diffusion coefficients for the SCACA in alkaline solutiondecrease with increase in modified bentonite content in the SCACA.

It is clear that the kinetics model is agreement with swelling experiments, since, as depicted in Table 1.

Fourier-transform infrared spectroscopy (FTIR) analysis

Figure 5a-d shows the IR spectra of the prepared SCACA and SCACA/MSB containing different drugs such as doxorubicin, 5-Fluorouracil and chlorambucil nanocomposites. In Fig. 5a, the characteristic absorption bands for the functional groups of the prepared SCACA are found at 3439 cm^{-1} for OH group, 1740 cm^{-1} for acetate group, and 1650 cm^{-1} for acrylate [16].

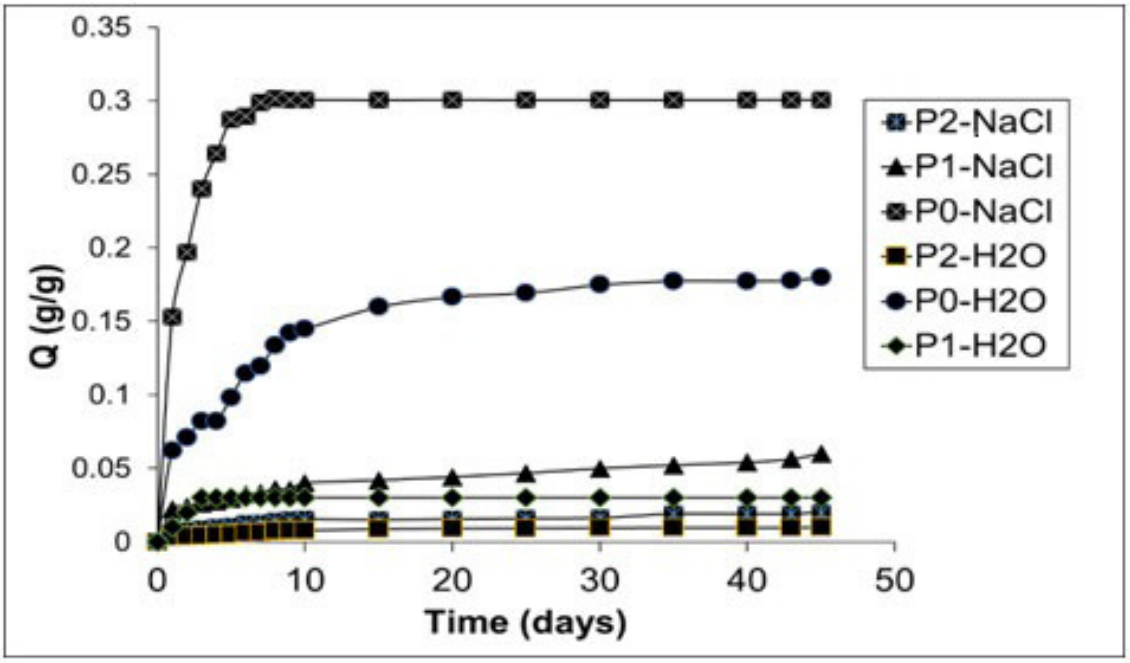


Fig. 4. Swelling ratio as a function of time in alkaline solution and distilled water at 25°C (P0=SCACA without bentonite).
P1 and P2= SCACA with modified bentonite (0.05 and 2%) respectively.

TABLE 1. The equilibrium swelling ratios of SCACA/SB that immersed in alkaline solution and distilled water, all factors were calculated after 45 days.

Sample Code (alkaline solution)	Q (%)	D (cm^2/day)	K
$P0_{NaCl}$	30	0.204	0.189
$P1_{NaCl}$	6	0.194	0.178
$P2_{NaCl}$	2	0.183	0.152
Sample Code (distilled water)	Q (%)	D (cm^2/day)	K
$P0_{H2O}$	18	0.198	0.177
$P1_{H2O}$	3	0.196	0.173
$P2_{H2O}$	1	0.188	0.159

Equilibrium swelling ratio (Q), Diffusion coefficient (D),characteristic constant (K)

An overlay of FTIR of the prepared SCACA / MSB /drugs nanocomposites is shown in Fig. 5 b-d the same characteristic absorption bands of SCACA beside the characteristic absorption bands of clay and drugs. The spectrum of the clay shows a broad absorption band at 3639–3458 cm^{-1} corresponding to the OH stretching vibration of water in the interlayer space of the clay and another band at 1647 cm^{-1} corresponding to the OH bending vibration of water. In addition, a sharp band corresponding to the Si-O stretching vibration of the layered silicate is observed at 1009-1024 cm^{-2} and the Si-O and Al-O bending vibration bands are located at 573 and 444–424 cm^{-1}[17]. Moreover, FTIR spectra of polymer/ clay/drugs nanocomposites showed more additional absorption bands compared with that of the neat clay. The spectra of SCACA/ MSB / DOX nanocomposites (Fig. 3b) show the absorption bands of 3750–3100 cm^{-1} due to N–H stretching vibrations for the primary amine structure and 2924 cm^{-2} for C–H stretching vibrations. Also, the bands observed at 858 cm^{-1} due to the presence of N–H was in DOX. Figure 3c illustrates the characteristic absorption bands for the functional groups which belongs the 5-FU: broad NH for primary amine at about 3443 cm-1, C=O at 1684cm^{-1}, amide group at 1733 cm^{-1} and C–F at 1170 cm^{-1}. On the other hand Figure 3d illustrates the characteristic peaks for the functional groups which belongs the chlorambucil : C=C (aromatic) at 1428 cm^{-1}, C=O (carboxylic) at 1716 cm^{-1} and C–F at 737 cm^{-1}.

Release Profile of SCACA/MSB -Drug

The results for the release of different drugs such as chlorambucil, 5-Fluorouracil and doxorubicin in different media [neutral (pH 6.5), alkaline medium (pH 9.5), and acidic medium (pH 5.5)] using UV-vis absorption and fluorescence emission spectrum versus time are represented in Fig. 6-8. It was found that the sustained release extending to about 45 days and the amount of drug released depended on the pH of the aqueous media.

Figure 6 shows the release rate of chlorambucil inacidic media was much faster than in neutral and acidic media. In acidic media, about 65% of the chlorambucil was released after 45 days.The following order:

Acidic media> alkaline media> neutral

The release pattern of the 5-Fluorouracil compound in aqueous media with different pH values for different time periods up to 45 days (Fig. 7). On the other hand, the release was low in neutral media (pH 7.3), while in alkaline media it was high but in acidic media was moderate. The amount of drug released depended on the pH of the aqueous media as the following order:

Alkaline medium>acidic medium > neutral

Figure 8 presents the release pattern of the DOX in aqueousmedia at different pH values at room temperature (25°C)for different time periods up to 45 days. It was found thatthe amount of doxorubicin released depends on the acidityand basic nature of the aqueous media. The release waslow in neutral media; while it was high inacidic media and was moderated in the alkaline media. The amount ofdrug released depended on the pH of the aqueous [18,21] media as the following order:

Acidic medium >Alkaline medium > Neutral

The release of drugs after 10 days in distilled water according to their wave length at room temperature are presented in Figure 9.

Cytotoxicity Biossay

The cytotoxicity [23] of investigated SCACAcarried active compounds was determined against HEPG2 cell line. Tables 2-4 show the efficiency of drug released from the investigated SCACA on growth inhibition and survival of HEPG2 liver cell line. The cytotoxicity is dependent on the time of exposure, as the longer the time of the sustained release of drug, the higher growth inhibition activity. The bioassay was carried out by subjecting cancer infected liver cell-line to 100µl.ml^{-1} of releasing media at pH 7.5. The results also indicate that 5-FU gave the highest growth inhibition potency followed by CA, while DOX showed the lowest activity. This may due to the release of 5- FU is more than CA and Dox., but all drugs have a promising results and can be used for binding with investigated polymer carrier to controlling their release to obtained release drug for long period of time.

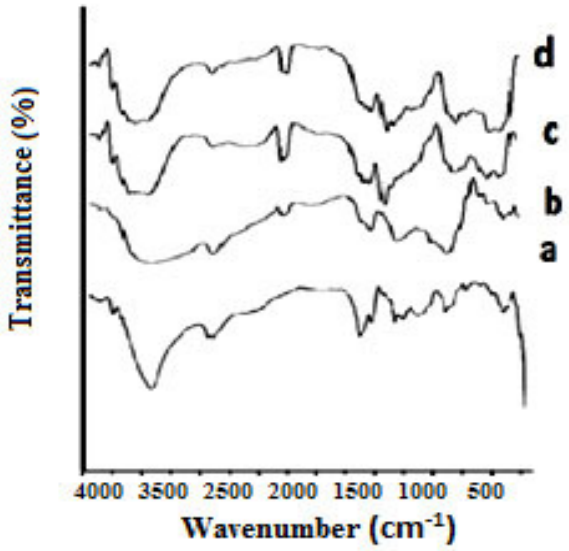


Fig. 5. FTIR spectra of(a) SCACA, (b) SCACA /MSB-DOX, (c) SCACA /MSB-5-FU(d) SCACA /MSB-CA.

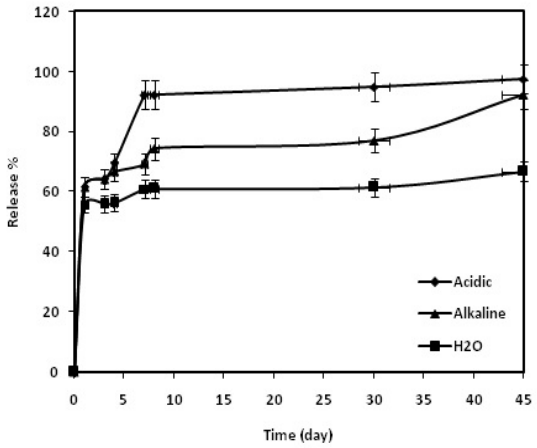


Fig. 6. Release profiles of SCACA/MSB-CA composite at 30°C in aqueous media of different pH values, H_2O (6.5), Acidic (5.5) and Alkaline (9.5).

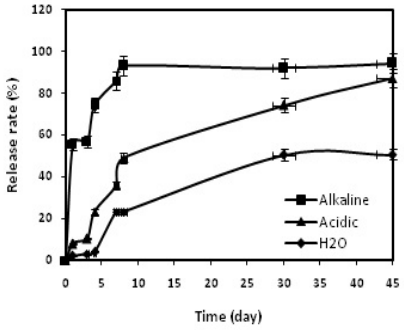


Fig. 7. Release profiles of SCACA /MSB-5-FUcompositeat 30°C in aqueous media of different pH values, H_2O (6.5), Acidic (5.5) and Alkaline (9.5).

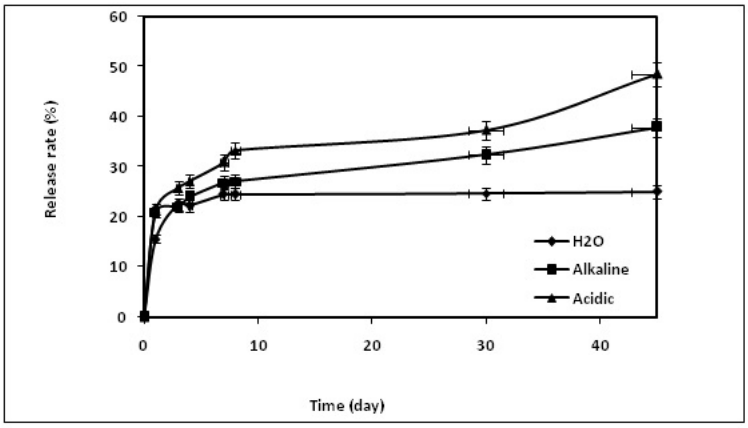


Fig. 8. Release profiles of SCA-co-A /SB- DOX composite at 30°C in aqueous media of different pH values, H_2O (6.5), Acidic (5.5) and Alkaline (9.5).

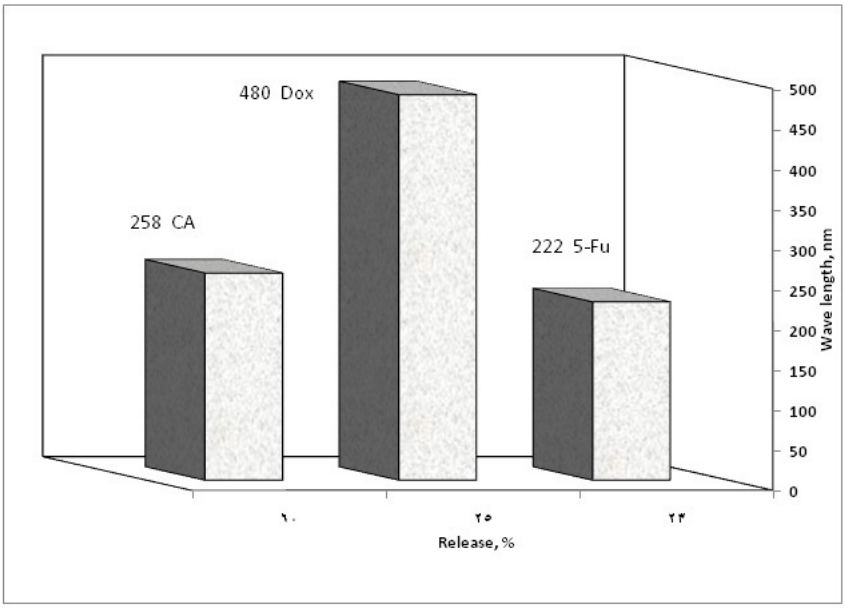


Fig. 9. The release of drugs after 10 days according to wave length measure.

TABLE 2. Effect of released CA from SCACA/MSB towards liver cancer cell line HEPG2. 100µl.ml-1 has been investigated in this study.

Release time days	Conc. µg ml^{-1}	Release µg mm^{-2} day^{-1}	Surviving %	Inhibition %
1	391	2.71	63	37
4	120	2.17	59	41
8	30	1.27	57	43
30	20	0.72	54	46

TABLE 3. Effect of 5-FU released from SCACA /MSBon human liver cancer cell line (HEPG2). 100 µl.ml-1 has been investigated in this study.

Release time days	Conc. µg ml^{-1}	Release µg.mm^{-2}.day^{-1}	Surviving %	Inhibition %
1	391	7.23	46	54
4	400	7.23	48	52
8	300	5.42	49	51
30	100	1.82	52	48

TABLE 4. Effect of DOX released from SCACA /MSB on human liver cancer cell line (HEPG2). 100 µl. ml-1 has been investigated in this study.

Release time days	Conc. µg ml^{-1}	Release µg.mm^{-2}.day^{-1}	Surviving %	Inhibition %
1	391	5.96	59	41
4	304	5.49	64	36
8	295	5.33	69	31
30	295	5.32	71	29

Conclusion

1-Starch cellulose acetate-co-acrylate/nano-clay/ drug nanocomposites were outstand synthesized for controlling the release of the investigated active drug. Modified sodium bentonite was used to enhance the loading of drug into the investigated polymer as well as modify the release rate.

2-The release of the drug was extended to 45 days. The release rate depend on the aqueous media and the type of the drug.

3-The bioassay results of the releasing active compounds on the liver cancer cell-lines showed a promising results for the growth inhibition potency. The sustained release technique is considered an effective strategy for controlling the cancer drug release fromthe polymeric materials over a long period to increase drug efficiency.

Acknowledgement

The author gratefully acknowledges financial support of this research by the National Research Centre (NRC), Cairo, Egypt.

References

1. López-Galindo, A., Viseras, C., Cerezo, P., Compositional, technical and safety specifications of clays to be used as pharmaceutical and cosmetic products. *Applied Clay Science,* **36** (1): p. 51-63 (2007).

2. Williams.L.B.,Haydel. S.E.,Evaluation of the medicinal use of clay minerals as antibacterial agents. *International geology review,* **52** (7-8): p. 745-770 (2010).

3. Ghadiri M., Chrzanowski. W.,Rohanizadeh. R.,Biomedical applications of cationic clay minerals. *RSC Advances,* **5** (37): p. 29467-29481. (2015).

4. McCarter. W., Brousseau. R.,The AC response of hardened cement paste. *Cement and Concrete Research,* . **20** (6): p. 891-900 (1990).

5. Dobrozsi, D.J., Oral liquid mucoadhesive compositions. Google Patents (2003).

6. Guarino. A., Bisceglia. M.,Castellucci. G., Iacono. G., Gobio. Casali. L.,Bruzzese. E., Musetta. A., Greco. L., Smectite in the treatment of acute diarrhea: a nationwide randomized controlled study of the Italian Society of Pediatric Gastroenterology and Hepatology (SIGEP) in collaboration with primary care pediatricians. *Journal of Pediatric Gastroenterology and Nutrition,* **32** (1): p. 71-75. (2001).

7. Cypes. S.H., Saltzman. W.M., Giannelis. E.P., Organosilicate-polymer drug delivery systems: controlled release and enhanced mechanical properties. *Journal of Controlled Release,* **90** (2): p. 163-169 (2003).

8. Lee. W.F., Fu. YT.,Effect of montmorillonite on the swelling behavior and drug-release behavior of nanocomposite hydrogels. *Journal of Applied Polymer Science,* **89** (13): p. 3652-3660 (2003).

9. Fejer. I., Kata. M.,I. Erös. I., Berkesi. O.,I. Dékány. I., Release of cationic drugs from loaded clay minerals. *Colloid and Polymer Science,* **279** (12): p. 1177-1182 (2001).

10. Lee. W.F., Chen. Y.C., Effect of bentonite on the physical properties and drug-release behavior of poly (AA-co-PEGMEA)/bentonite nanocomposite hydrogels for mucoadhesive. *Journal of Applied Polymer Science,* **91** (5): p. 2934-2941 (2004).

11. Krishnaiah.Yellela S.R., V Satyanarayana. V., Dinesh. Kumar. B., Karthikeyan. R. S., In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil. *European Journal of Pharmaceutical Sciences,* **16** (3): p. 185-192 (2002).

12. Dev. R. K., Bali.V.,Pathak. K., Novel microbially triggered colon specific delivery system of 5-Fluorouracil: Statistical optimization, in vitro, in vivo, cytotoxic and stability assessment. *International Journal of Pharmaceutics,* **411** (1): p. 142-151 (2011).

13. Dong. Y., Feng. S. S.,Poly (d, l-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs. *Biomaterials,* **26** (30): p. 6068-6076. (2005).

14. Marson. F. J., Leaching rate determination. Oil Color Chem Assoc. **47**:323 (1964).

15. Yang. J., Lee. C-H., Park. J., Seo. S., Lim. E-K., Song. Y.J., Suh. J-S. , Yoon. H-G., Huh. Y-M., Haam. S., Antibody conjugated magnetic PLGA nanoparticles for diagnosis and treatment of breast cancer. *Journal of Materials Chemistry,* **17** (26): p. 2695-2699 (2007).

16. Helaly. F.M., Khalaf. A.I., El Nashar. D.E., Starch

cellulose acetate co acrylate (SCAA) polymer as a drug carrier. *Res. Chem. Intermed,* **39**: p.3209–3220 (2012).

17. Assema. Y., Khalaf. A.I.,Zidan. T.A., Rabia. A.M., Yehia. A.A., Synthesis and characterization of hybride clay/ poly (N,N-dimethylaminoethyl methacrylate) nanocomposites, Polymer Composites **37** (10): p. 2950–2959 (2016).

18. Helaly, F. M., Khalaf, A.I., El.Nashar, D.E., Abdel Hamid, H.F., Soliman, A. M.,Bioassay and bioactivity of polymer as carrier for some active compounds such as anticancer drugs. Research on Chemical Intermediates, **40** (2): p.749-760 (2014)

19 Skehan, P.A., Storeng, R.,Scudiero, D. A. et al. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **82** (13):1107-12. (1990).

20. Rozik. N.N., El.Nashar. D.E., Younan. A.F., Nanoclay modified with nonionic surfactants as reinforcement for styrene–butadiene rubber, *J Nanoengineering and Nanosystems,* **226** (4) 147–155, (2012).

21. Ross A. Kennedy, R.A., Kennedy. M.L. Effect of Selected Non-Ionic Surfactants on the Flow Behavior of Aqueous Veegum Suspensions, AAPS *PharmSciTech,* **8** (1), (2007).

22. Helaly, F. M., Nashar, D. E. El., Khalaf1, A. I., Abdel Hamid, H. F., Abd El-Moez, S. I. Soliman, A.M.Slow Release of 5-fluorouracil from Natural Polymeric Composites as Controlled Drug Delivery. *Int. J. Pharm. Sci. Rev. Res.,* **30** (1): p. 137-142 (2015).

23. Abdel-Mohsen M. Soliman ; Mohsen Kamel; Ahmed F. Eweas; Joanna Wietrzyk;Magdalena Milczarek. The Antiproliferative Activity and Molecular Docking studies of some sulfonamides against cancer cell lines compared to normal cells. *Egypt. J. Chem.* **61** (3): 330-340. DOI: 10.21608/EJCHEM.2018.2934.1242 (2018).

تقييم المتراكبات البوليمرية المحضرة والمكونة من اسيتات النشا المشارك مع الأكريلات والطفلة النانومترية الحاملة للعقاقير المضادة للسرطان

تستخدم العقاقير التالية فى مقاومة مرض السرطان : 5- فلورويورالسيل ، دكسوروبسن ، الكلورومبيوسيل

يهدف هذا البحث إلى إستخدام تقنيه الإفراز البطىء للعقارات المشار إليها وذلك بالتحكم فى معدل افرازها وإستمرار الأفراز لمدة زمنية طويلة حتى يتسنى ترشيد إستخدام العقار ورفع كفاءته فى مقاومة المرض.

وتعتمد هذه الدراسة على تحضير طفلة نانومترية وتقسيمها إلى ثلاث أقسام وتحميل كل عقار على قسم كما تم تحضير المادة البوليمرية لحفظ العقار المحمل على الطفلة والتحكم فى معدل إفرازة وإستمراره لفترة زمنية طويلة بهدف زيادة تأثير المادة الفعالة والحد من أخطارها وتم تحضير التركيبة البوليمرية من أستيات النشا وحمض الأكريليك وذلك بميكانيكية تفاعل الشقوق أو الشوادر الحرة Free radical mechanism

تم توصيف المتراكبات المحضرة بإستخدام طيف الأشعه تحت الحمراء كما تم تقييم عملية الأنتفاخ (الأنتفاش) للبوليمر المحضر فى وجود الطفلة وعدم وجودها وقد تبين أن معدل الأنتفاخ يقل فى وجود الطفلة وزيادة تركيزها أيضا تم دراسة معدل إفراز المادة الفعالة من كل خلطة فى أوساط مائية مختلفة الأس الهيدروجينى.

ووجد أن معدل إفراز المادة الفعالة يعتمد على درجة الأس الهيدروجينى و تركيز الطفلة المستخدمه حيث أن معدل الأفراز يزداد فى الوسط المائي الحامضي حسب طبيعة المادة الفعالة أيضا تم عمل دراسة بيولوجية على خلايا الكبد السرطانية وقد أعطت نتائج إيجابية.