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A Novel Polymeric Prodrugs Synthesized by Mechanochemical Solid-State Copolymerization of Glucose-Based Polysaccharides and Vinyl Monomers

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Abstract:*We developed the novel polymeric prodrugs synthesized by mechanochemical solid-state copolymerization of glucose-based polysaccharides (dextran orglycogen) and the methacryloyloxy derivative of 5-fluorouracil (5-FU). The copolymerization proceededreadily and each polymeric prodrug was quantitatively obtained within8 h reaction. The number average molecular weight (Mn) and polydispersity (H) of the polymeric prodrug synthesized from dextran was 24,000 g/mol and 5.10, respectively. The number average particle diameter of the polymeric prodrug derived from glycogen was 14.9 nm. The hydrolysis profiles of the polymeric prodrug synthesized from dextranapparently followed the first-order kinetics, and 100% drug release was observed under the experimental condition used. The polymeric prodrug derived from glycogen also continued to release 5-FU at the first-order rate up to 5 h, followed by its rate constant decreased gradually. These results suggest that lower accessibility of water molecules for the synthetic polymer chains inside the glycogen particle might cause the gradual decrease of drug release rate.*

Keywords:*polysaccharide,dextran,glycogen,polymeric prodrugs, mechanochemical solid-state co-polymerization*

I. Introduction

The grafting of synthetic polymers onto polysaccharides has recently attracted much attention as a conventional method to combine the advantages of natural and synthetic macromolecules [1]. Most graft copolymers are prepared through graft polymerization of vinyl or acryl monomers onto the polysaccharide backbone possessing functional groups to initiate the polymerization [2]. Condensation polymerization, however, would not be used for preparing graft copolymers of polysaccharides due to unstability of the polysaccharide backbone to the high temperature and harsh conditions of typical polycondensation reactions [3]. Thus, synthetic polymers consisting of vinyl monomers are typically grafted onto the polysaccharides by radical polymerization [4].

It has been known that mechanolysis of polymers, synthetic and natural, results in the formation of mechanically induced radicals, so-called mechanoradicals, due to scission of the polymer main-chain when mechanolysis is conducted at a temperature below the glass transition temperature (T_g) of the polymers [5]. To elucidate the mechanism of polymer mechanolysis, we systematically conducted the mechanolysis of various synthetic polymers and polysaccharides at room temperature under strictly anaerobic conditions and studied mechanoradical formation, including kinetic analyses by electron spin resonance (ESR) coupled with systematic computer simulations [6][7]. A series of such studies on polysaccharides such as amylose [8], and cellulose/celluloses derivatives [9], reported that 1,4-glucosidic bond scission of the polysaccharide can formally give two pairs of end-chain radicals, and that these radicals participate in subsequent reactions such as hydrogen abstraction to form other types of radicals.

We reported the nature of mechanoradical formation in the course of mechanolysis ofHydroxyethylcellulose (HEC) [10] substituted from hydroxy group of cellulose to hydroxylethyl group [9]. Recently, we have demonstrated the mechanochemical solid-state copolymerization of HEC mechanoradicals and 1-methacryloyloxymethyl-5-fluorouracil (MA-5-FU) in a non-metallic container [11]. The novel block or graft-type polymeric prodrugs, in which 5-FU was covalently attached to a polymer backbone and slowly released *via* hydrolysis under pH 7.4 phosphate buffer solution at 37 ºC, was quantitatively produced by the mechanochemical graft copolymerization. Cellulose derivatives, however, have been known to be lower biocompatibility and biodegradability than glucose-based polysaccharides (When extracted 1,3-β-glucans are administered to animals or humans, they recruit macrophages and stimulate the immune system through a

similar mechanism [12][13] so that cellulose derivatives would induce the immune responses such as thrombogenesis, hemolysis *in vivo*.). Natural polysaccharides (*e.g.* amylose, amylopectin, dextran) composed of α-glucose units connected by α-glucosidic linkages, whereas, have been focused on drug carriers due to the non-toxicity, biocompatibility, and biodegradability. It is desired to utilize glucose-based polysaccharides for preparing a novel polymeric prodrugs consisting of polysaccharides and vinyl monomers.

We also reported the detailed study of mechanoradical formation of glucose-based polysaccharides such as dextran (Dx) and glycogen (Gly) based on ESR coupled with systematic computer simulations [14]. The observed ESR spectra of mechanically fractured samples by ball milling at room temperature showed the multicomponent spectra and were different from each other in pattern. The component radicals of Dx and Gly could be assigned to glucose-based mid-chain alkoxyalkyl-type radicals at C_1, and Dangling Bind Site(DBS), whose radicals might be a mixture of ring-opened and/or conjugated structures resulting from α-1,4- and/or α-1,6-glucosidic bond cleavage and subsequent complex reactions. Thus, DBS is no structural significance. The generated radicals therefore should be available to synthesize the graft-copolymers consisting of glucose-based polysaccharides and vinyl monomers. Additionally, it is hoped that the graft-type polymeric prodrugs utilizing Dx or Gly might be useful for drug carriers administered *via* intravenous injection due to their outstanding advantages as described above.

We aimed to develop novel polymeric prodrugs consisting of glucose-based polysaccharides and vinyl monomers by mechanochemical solid-state copolymerization. In this paper, we synthesized the graft-type polymeric prodrugs of Dx or Gly and MA-5-FU by mechanochemical solid-state copolymerization as shown in Fig. 1. The physicochemical properties (molecular weight and particle diameter) of the resulting polymers were estimated by gel permeation chromatography or dynamic light scattering measurement. We also provided the detailed kinetic reaction analyses such as monomer consumption and characterization of the generated mechanoradicals, as studied by ^{1}H-NMR and ESR. The nature of drug release from the water-soluble polymeric prodrugs *in vitro* was also studied.

II. Materials and Methods

1. Materials

Powdered Dx (Clinical Grade) purchased from Wako Co., Ltd., was screened through a 200- to 235-mesh sieve, and then dried at 60 ^{o}C for 12 h *in vacuo*. Powdered Gly was purchased from NacalaiTesque Co., Ltd., and treated similar to the procedure of Dx. MA-5-FU was synthesized as reported previously [6].

2. Mechanochemical Solid-State Copolymerization

A mixture of Dx or Gly (96.2 mg) and MA-5-FU (3.8 mg, 3 mol% as monomer ratio) was mechanically fractured under a nitrogen atmosphere in a Type MM 200 mill (Retsch Co., Ltd., Germany) equipped with an agate twin-shell blender (14 mmφ, 65 mm long) and an agate ball (6.0 mm diameter, 190 mg) at room temperature for the prescribed period of time at 30 Hz. Residual oxygen was removed using a Model 1000 Oxygen Trap (Chromatography Research Supplies Inc., US) and the oxygen concentration was monitored using an oxygen analyzer (LC750/PC-120, Toray Engineering Co., Ltd., Japan) and kept below 0.01 ppm. The fractured samples were transferred to an ESR tube. The ESR tube was sealed and subjected to ESR spectra measurement. All sample manipulations were carried out in a vacuum glove box (Sanplatec Corp., Japan).

3. ^{1}H-NMR Spectral Measurements

^{1}H-NMR spectra were recorded on a JEOL ECA500 FT-NMR spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6). Tetramethylsilane was used as an internal standard. The fractured samples were exposed to air to quench the radicals and then the ^{1}H-NMR spectra were measured. The ratio of polymer conversion was estimated based on the area of olefinic proton of vinyl monomer against that of proton at C_6 position of 5-FU.

4. ESR Spectral Measurements

ESR spectra were recorded on a JES-RE1X (JEOL Ltd., Japan) spectrometer with X-band and 100-kHz field modulation. Care was taken to ensure that no saturation occurred and that the line shape was not distorted by excessive modulation amplitude. The square root of the microwave power versus the signal peak height was plotted, so that a microwave power level of 0.04 mW was chosen. The ESR spectral intensity was determined by double integration. The radical concentration (spin numbers per gram of sample) was calculated from the spectral intensity of a poly (methyl methacrylate) sample impregnating with 2,2-diphenyl- picrylhydrazyl.

5. Molecular Weight Measurements

The molecular weight of each resulting polymer was measured by gel-permeation chromatography (GPC) using a PU 610 HPLC Pump (GL Sciences Inc., Japan) equipped with an RI 504R refractive index detector (GL Sciences Inc., Japan), a Model 556 LC column oven (GL Sciences Inc., Japan), gel column (GF-

1G 7B and GF-7M HQ, Shodex, Japan), and data analyzer (Runtime Instruments Chromato-PRO, Runtime Instruments Ltd., Japan) under the following conditions: elution solvent, distilled water containing 0.05 w/v% NaCl; flow rate, 0.7 mL/min; column temperature, 40 ºC. Calibration was carried out with pullulan standards (peak top molecular weight [Mp] = 5,900, 9,600, 21,100, 47,100, 109,000, 200,000, 344,000, and 708,000 g/mol).

6. Dynamic Light Scattering Measurements

Dynamic light scattering was measured using a DLS-5500G Photal dynamic light scattering spectrophotometer (Otsuka Electronics Co., Ltd., Japan) equipped with a He/Ne laser. A scattering angle of 90^{o} was used in this study. The hydrodynamic diameter and the polydispersity factor of the polymers, represented as μ_2/Γ^2, were calculated using the Stokes-Einstein equation and the cumulant method. The number-average particle diameter and weighaverage particle diameter were determined by histogram method with Marquardt calculation.

7. Hydrolysis Method

Each polymeric prodrug containing 5-FU (10.1 mg) was dissolved in pH 7.4 phosphate buffer (100 mL) and dialyzed using a pre-swollen semi-permeable membrane (Spectra/Por® 4, Spectrum Laboratories, Inc., US; molecular weight cut-off 12,000-14,000 g/mol). Drug release from the polymeric prodrugs was examined using the Paddle Method described in the Japanese Pharmacopoeia, 16^{th} ed.: Toyama NTR-3000 dissolution tester; eluate, pH 7.4 phosphate buffer (800 mL); temperature, 37±0.5 ºC; revolution speed of paddle, 100 rpm. The amount of 5-FU released was determined by removing 5.0 mL of eluate from the tester at prescribed times and assaying by UV absorption at a wavelength of 265 nm.

III. Resultsand Discussion

1. Observed ESR Spectra

Mechanochemical solid-state copolymerization of Dx (Mn = 30,500 g/mol) and MA-5-FU was performed in a vibratory ball mill using an agate twin shell blender and ball at 30 Hz to obtain the polymeric prodrug of Dx (Dx-5-FU). Dx was also mechanically fractured for comparison (F-Dx). Figure 2 shows the progressive changes in the ESR spectra of Dx-5-FU and F-Dx.

It can be seen from Fig. 2 that the spectral features of Dx-5-FU and F-Dx differed appreciably at 2 h: the spectral pattern of Dx-5-FU gradually exchanged to a single broad line whereas that of F-Dx essentially remained nearly unchanged during the course of vibratory milling. We separately confirmed that MA-5-FU did not polymerize under this experimental condition (an agate vessel and ball), although mechanochemical polymerization of MA-5-FU utilizing a stainless steel vessel and ball proceeded readily. The spectral feature of Dx-5-FU at 2 h was characterized by five major lines with four shoulders ("nine-line spectrum") and was similar to that observed in the mechanochemical polymerization of MA-5-FU using a metallic vessel and ball.

On the other hand, we reported in the previous paper that the mechanoradicals generated by fracture of Dxcould be assigned to mid-chain alkoxyalkyl-type radicals and DBS [14]. Therefore, the mechanoradicals generated by milling Dx served as initiators of the mechanochemical copolymerization with MA-5-FU to form graft copolymers.

Furthermore, we carried out the mechanochemical solid-state copolymerization of Gly and MA-5-FU (Gly-5-FU), and the polymeric prodrug was obtained as well as Dx-5-FU. Gly was also mechanically fractured for comparison(F-Gly).Figure 3 shows the progressive changes in the ESR spectra of Gly-5-FU and F-Gly. Since the nine-line spectrum was observed in earlier stage, the mechanoradicals generated by milling Gly also played the role as initiator in the mechanochemical copolymerization with MA-5-FU.

2. Progressive Changes in Radical Concentration

Figure 4 shows the progressive changes in radical concentration during the course of Dx-5-FU and F-Dx. The progressive changes in radical concentration of Dx-5-FU was larger than those of F-Dx, and the radical concentration of Dx-5-FU gradually decreased after reaching the maximum concentration at 2 h. However, the resulting mechanoradicals of Dx-5-FU did not disappear even after 12 h. These results suggested that the mechanoradicals of Dx-5-FU in earlier stage, in which the mechanochemical polymerization of MA-5-FU preferentially progressed, were more stable than those produced in disproportionation process after that. Thus, these long lifetime radicals existing at 12 h vibratory milling might be ascribed to the DBS with steric hindrance of ring-opened and/or conjugated structures of Dx.

Figure 5 also shows the progressive changes in radical concentration during the course of Gly-5-FU and F-Gly. The radical concentration of Gly-5-FU indicated the maximum concentration at 2 h, and the progressive changes in radical concentration of Gly-5-FU were also larger than those of F-Gly. The longer

vibratory milling of Gly-5-FU caused a decrease of the radical concentrations after reaching the maximum concentration. These results should be similar to those of Dx-5-FU as mentioned above.

On the other hand, the progressive changes in radical concentration of F-Dx and F-Gly were increasing with time in this experimental condition. We previously reported that the progressive changes in radical concentration of fracture of Dx and Gly utilizing stainless steel vessel and a stainless steel ball at 60 Hz indicated the maximum radical concentration during the course of mechanolysis, due to the disproportionation process such as hydrogen abstraction and/or radical-radical coupling reaction after the main-chain scission. It is notable that the progressive changes in radical concentration of F-Dx and F-Gly using agate vessel and an agate ball at 30 Hz did not indicate the maximum radical concentration up to 12 h. This result might be caused by the disproportional process proceeded gradually, or the longer vibratory milling more than 14 h could show the maximum radical concentration of F-Dx and F-Gly.

3. Progressive Changes in Molecular Weight of Dx-5-FU and F-Dx

A mechanoradical is formed by polymer main-chain scission during the course of mechanolysis[6-9], and thus the progressive changes in radical concentration would likely relate to the change in molecular weight. Figure 6 shows the progressive changes in number average molecular weight of Dx-5-FU and F-Dx.

It is seen from Fig. 6 that comparable amounts of mechanoradicals based on Dx main-chain scission were produced in Dx-5-FU and F-Dx, because the progressive changes in the number average molecular weight (Mn) exhibited the similar behavior each other. The Mn and polydispersity (H = Mw/Mn, where Mw is weight average molecular weight) of Dx-5-FU after 12 h reaction were 23,400 g/mol and 5.62, respectively, and those of F-Dx were 23,100 g/mol and 5.47. These results suggested that the progressive changes in the radical concentration of Dx-5-FU might be similar to those of F-Dx, but the progressive changes in the radical concentration of observed radicals in F-Dx were apparently smaller than those of Dx-5-FU (*vide supra*).

4. Progressive Changes in particle diameter in Gly-5-FU and F-Gly

The molecular weight parameter is not suitable for describing changes in a hyper-branched polysaccharide such as Gly. In general, the particle diameter could be used for the size evaluation of branched polymers, and actually it should be determined by Dynamic light scattering (DLS), which has been critically and comprehensively reviewed by Burchard [15].However, DLS requires large molecular weights and sizes (radius of gyration larger than 12 nm) [16], so that it is difficult for current apparatus to measure the particle diameter of fractured Gly.

GPC is a size-exclusion technique in which molecules in solution are separated based on their size, and in some cases, molecular weight. The hydrodynamic radius (R_H) is utilized as an index of the spread of a polymer. It is well-known that the R_H of a spherical polymer dissolved in a solvent is also correlated with the weightaverage molecular weight. Pullulan, a linear polysaccharide, can be used as a standard sample in GPC analyses of polymers. The R_H of pullulan is reportedly proportional to the square of the weight average molecular weight [17]. To evaluate the progressive changes in particle diameter of Gly in the course of vibratory milling, we examined the R_H of Gly from the weight average molecular weight by using GPC of standard pullulan according to previous paper [14].

Figure 7 shows the progressive changes in particle diameter of Gly-5-FU and F-Gly during the course of vibratory milling. It can be seen from Fig. 7 that the progressive changes in the particle diameter showed similar behavior between Gly-5-FU and F-Gly, as in the case of Dx. The result suggested that the Gly main-chain scission would occur in Gly-5-FU and F-Gly by the similar way and frequency. The progressive changes in the particle diameter of Gly also gradually decreased to the limiting value during the course of vibratory milling, and the particle diameter of Gly-5-FU and F-Gly indicated approximately 10 nm at 14 h.

5. Polymer Conversion

Figure 8 shows the amount of monomer consumed with time in mechanochemical copolymerization, determined by monitoring the decay of the olefinic protons of MA-5-FU in Dx-5-FU and Gly-5-FUwith^{1}H-NMR spectroscopy.

It can be seen from Fig. 8 that the kinetics of monomer consumption followed an exponential curve: 88% of the monomer in Dx-5-FU including 6 mol% MA-5-FU was consumed within2 h, and no monomer remained after 8 h reaction. The monomer consumption rate in Dx-5-FU including 3 mol% MA-5-FU was also similar to the case of 6 mol% MA-5-FU.

On the other hand, the monomer in Gly-5-FU including 6 mol% MA-5-FU was completely consumed at 6 h. It is apparent that the monomer consumption rate in Gly-5-FU was more rapid than that of Dx-5-FU. This result might reflect differences in the amount of produced mechanoradicals during the course of mechanolysis between Dx and Gly as shown in Figs. 4 and 5. Therefore, increased amount of mechanoradicals generated by

polymer main-chain scission would allow the mechanochemical copolymerization of a glucose-based polysaccharide and monomers to improve efficiency of the monomer consumption.

The monomer consumption rate in the mechanochemical copolymerization was closely related to the radical concentration participating in the polymerization. Thus, Figs. 4 and 5show that the progressive changes in the radical concentrations after 2 h of Dx-5-FU and Gly-5-FU were approaching to those of F-Dx and F-Gly, since the polymerization of MA-5-FU in Dx-5-FU and Gly-5-FU was almost complete until 2 h. The radical concentration in Gly-5-FU after 6 h that the monomer was completely consumed, however, was obviously different from that of F-Gly, because the mechanoradicals generated by the polymerization of MA-5-FU in Gly-5-FU could not readily undergo the subsequent reactions such as radical recombination and/or hydrogen abstraction due to the stabilization of DBS. In a previous study of the mechanolysis of Gly [14], the DBS in fracture of Gly undergoing vibratory ball milling with a stainless steel vessel and a stainless steel ball at 60 Hz did not dissipate even at 2 h due to steric hindrance of ring-opened and/or hyper-branched structures of Gly. Eventually, the radical concentration in Gly-5-FU at 14 h was similar to that of F-Gly, so that the mechanoradicals based on the polymerization of MA-5-FU in Gly-5-FU would disappear through the longer vibratory milling more than 14 h.

6. Rate of Drug Release from Polymeric Prodrugs

Figure 9 shows the cumulative amount of drug released from the each polymeric prodrug of Dx-5-FU and Gly-5-FU with time in pH 7.4 phosphate buffer at 37±0.5 ºC. Released 5-FU was periodically assayed by UV absorption at 265 nm.

Table 1 shows the number average molecular weight (Mn), polydispersity (Mw/Mn) and hydrolysis rate constant of the polymeric prodrugs in Dx-5-FU, particle diameter and hydrolysis rate constant of the polymeric prodrugs in Gly-5-FU. Drug release from the each polymeric prodrug in Dx-5-FU exhibited the pseudo first-order reaction rate, and 5-FU was completely released until 12 h. The drug release from the polymeric prodrugs in Dx-5-FU including 3-5 mol% MA-5-FU also could not observe a remarkable difference among them. Additionally, the resulting polymers in Dx-5-FU consisting of more than 6 mol% MA-5-FU did not entirely dissolve in the water in this experimental condition. Even though the drug release from the polymeric prodrugs in Gly-5-FU indicated the pseudo first-order reaction until 5 h, the rate of drug release was gradually decreasing and the drug release profile clearly strayed out of exponential curve as represented by the dotted line.

Then, a question may arise as to why the drug releases from the polymeric prodrugs in Gly-5-FU turned away after 5 h, unlike those of Dx-5-FU (*vide supra*). We discussed as follows. The synthetic polymer chains adjacent to the surface of hyper-branched Gly could be hydrolyzed first, and then the synthetic polymer chains inside the Gly particle might be hydrolyzed. Therefore, the gradual decrease of the drug release rate after 5 h was caused by lower accessibility of water.

IV. Conclusion

We have fabricated the novel hybrid-type polymeric prodrugs consisting of a glucose-based polysaccharide and synthetic polymer chains, using mechanochemical solid-state copolymerization of Dx or Gly and MA-5-FU by vibratory ball-milling with an agate vessel and ball at 30 Hz.

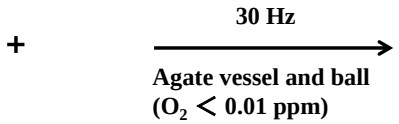
It has been confirmed that the ESR spectrum at 2 h in the mechanochemical copolymerization of Dx and MA-5-FU was similar to that observed in the polymerization of MA-5-FU. Although the progressive changes in the radical concentration of Dx-5-FU during course of vibratory milling were higher than those of F-Dx, the progressive changes in the number average molecular weight of Dx-5-FU and F-Dx were similar to each other. Therefore, the difference of the amount of produced mechanoradicals between Dx-5-FU and F-Dx should be attributed to the thermal stability of generated mechanoradicals. The polymerization of MA-5-FU in Dx-5-FU also quantitatively proceeded within 8 h. The number average molecular weight and polydispersity of the resulting polymeric prodrugs at 8 h in Dx-5-FU including 3-5mol% monomer contents was approximately 23,000 g/mol and 5.0, respectively. It was also shown that the hydrolysis rate constants of each polymeric prodrug were similar values, and all of the drug releases obeyed the pseudo first-order reaction.

Furthermore, the progressive changes in the ESR spectra and the radical concentration in F-Gly were higher than those of F-Dx, so that the mechanolysis of Gly could produce more mechanoradicals than Dx. Thus, the monomer consumption rate in Gly-5-FU was more rapid than that of Dx-5-FU, and the solid monomers in Gly-5-FU were completely consumed at 6 h. The particle diameter of the resulting polymeric prodrug at 6 h in Gly-5-FU was approximately 15 nm, and the drug release showed the pseudo first-order reaction up to 5 h. The drug release of the polymeric prodrug in Gly-5-FU gradually decreased after 5 h, suggesting that the ester moieties of synthetic polymer chains inside the Gly particle cannot be hydrolyzed due to lower accessibility of water molecules.

These findings indicate that novel polymeric prodrugs composed of a glucose-based polysaccharide and synthetic polymers possessing drugs could be provided by mechanochemical solid-state copolymerization under anaerobic conditions. The polymeric prodrugs are promised as a drug carrier possessing biocompatibility and biodegradability. We will discuss on the cellular uptake and cytotoxicity of the polymeric prodrugs against cancer cell lines in the forthcoming paper.

Table 1: Number Average Molecular Weight, Polydispersity, Hydrolysis Rate Constant of Dx-5-FU, and Particle diameter, Hydrolysis Rate Constant of Gly-5-FU

	Mn (g/mol)	Mw/Mn	Particle diameter (nm)	Hydrolysis rate constant ($\times 10^{-1}$) (h^{-1})
Dx-5-FU (5 mol%) (8 h)	24,000	5.1	—	4.31
Dx-5-FU (4 mol%) (8 h)	24,000	5.1	—	3.94
Dx-5-FU (3 mol%) (8 h)	23,000	5.2	—	3.53
Gly-5-FU (1.5 mol%) (6 h)	—	—	14.9	3.4



Dextran (Dx) **MA-5-FU** **Grafted copolymer comprising Dx and MA-5-FU**

Figure1: Reactionscheme of polymeric prodrugs synthesis by mechanochemical solid-state copolymerization of Dx and MA-5-FU.

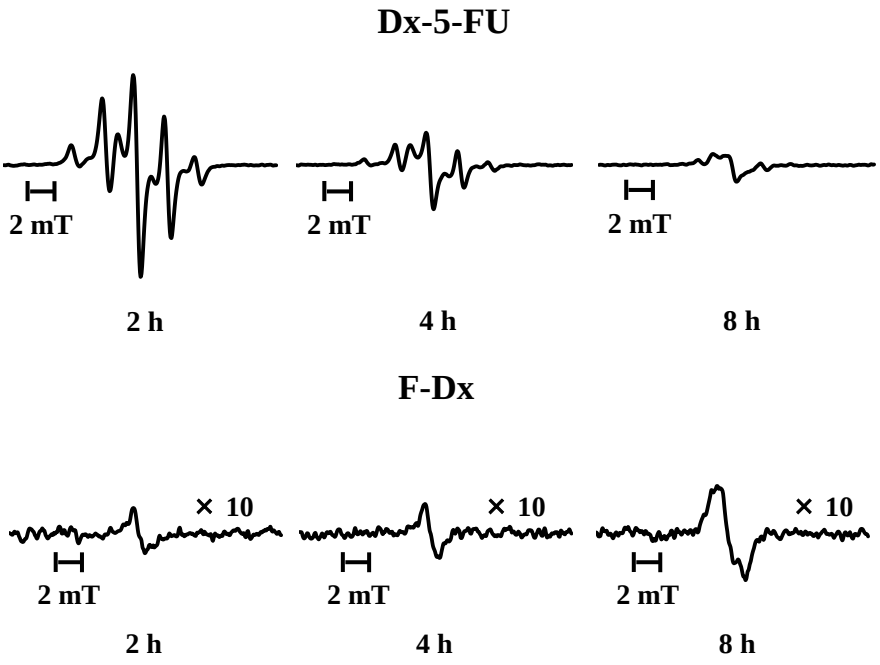


Figure2: Progressive changes in ESR spectra in the course ofvibratory milling of Dx-5-FU and F-Dx.

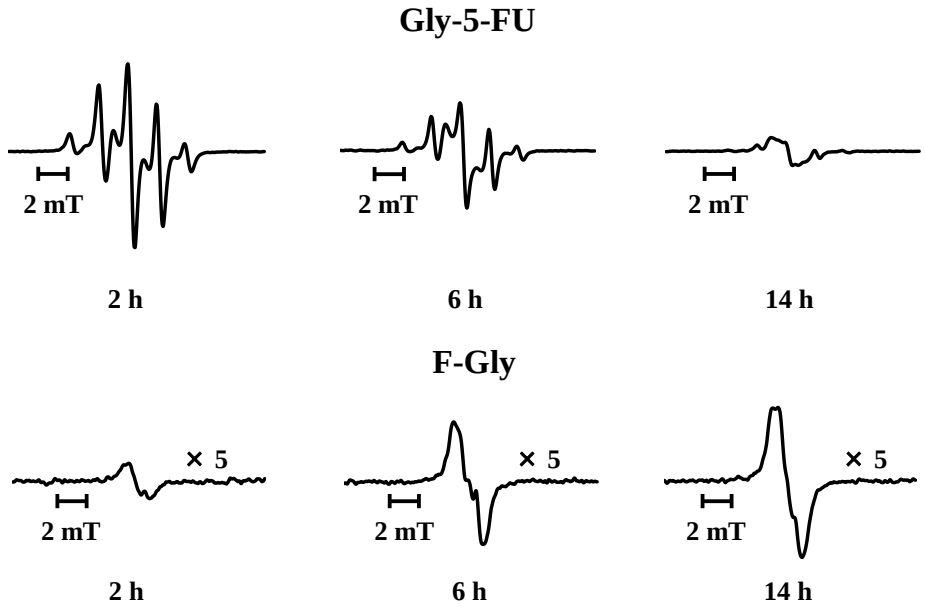


Figure3: Progressive changes in ESR spectra in the course ofvibratory milling of Gly-5-FU and F-Gly.

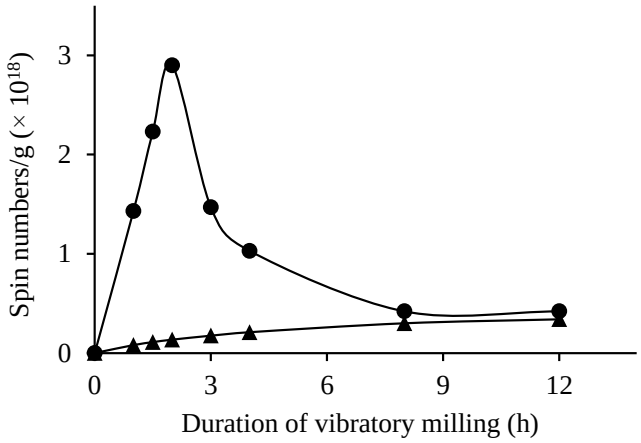


Figure4: Progressive changes in radical concentration in the course ofDx-5-FU (●) and F-Dx (▲).

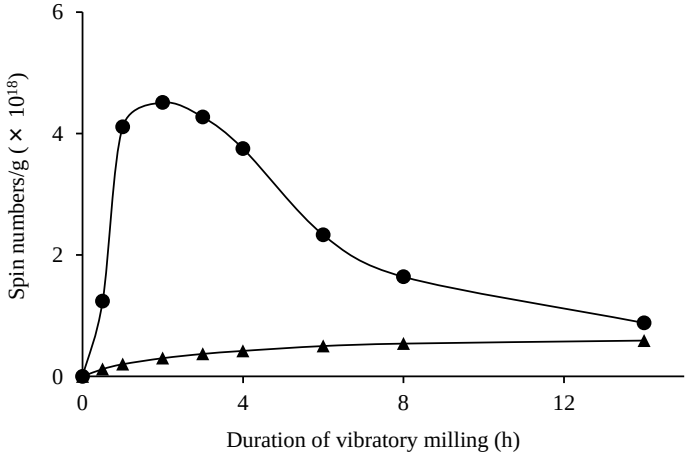


Figure5: Progressive changes in radical concentration in the course ofGly-5-FU (●) and F-Gly (▲).

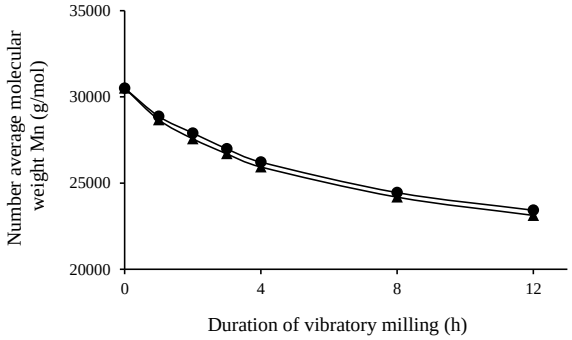


Figure6: Progressive changes in number average molecular weight (Mn)in the course of Dx-5-FU (●) and F-Dx (▲).

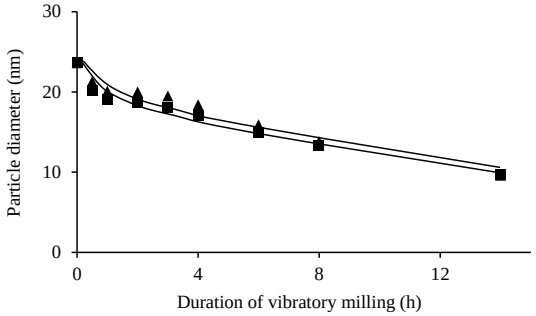


Figure7: Progressive changes in particle diameter of Gly-5-FU (●)and F-Gly (▲)in the course of vibratory milling.

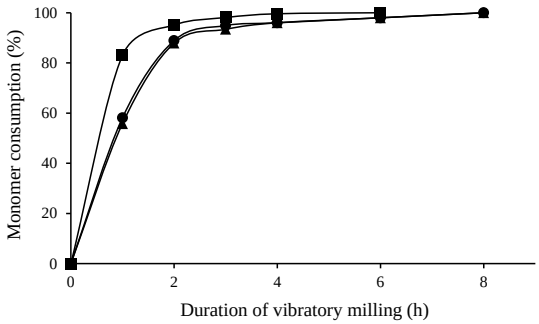


Figure8: Progressive changes in monomer consumption in the course of Gly-5-FU(6 mol%) (■) Dx-5-FU (6 mol%) (▲) and Dx-5-FU (3 mol%) (●).

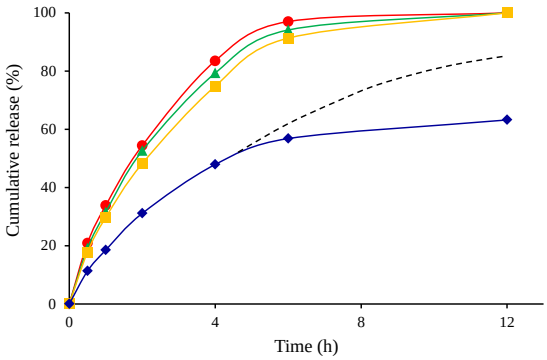


Figure9: Nature of drug release from the resulting polymeric prodrugs of Dx-5-FU (5 mol%) (●) Dx-5-FU (4 mol%) (▲) Dx-5-FU (3 mol%) (■) and Gly-5-FU (1.5 mol%) (◆).

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