

# Preparation and Characterization of Bioadhesive Grafted Starch Copolymers as Platforms for Controlled Drug Delivery

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**ABSTRACT:** <sup>60</sup>Co-gamma-radiation and ceric ammonium nitrate (CAN) redox-induced graft polymerization of acrylic monomers (acrylonitrile, acrylic acid, and methyl acrylate) to starch was performed to produce drug-delivery systems. The grafted starches obtained mainly by redox-induced polymerization were characterized by chemical and physico-chemical methods, and the nature and size of the polyacrylic moiety grafted to the starch were determined. Although the choice of grafting method depended in part on the particular monomer, only copolymers obtained by gamma radiation

proved to be satisfactory drug delivery systems. Changes of parameters in the gamma radiation-induced polymerization, such as radiation times and changes in the amount of starch and/or acrylic monomers, were tested in terms of the release rate of model drug compounds. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 86: 1157–1162, 2002

**Key words:** starch; graft copolymers; drug delivery systems; gamma-ray initiation; biocompatibility

## INTRODUCTION

A bioadhesive material designed to perform as an oral drug-delivery system has to fulfill a series of requirements; in particular, the material must be nontoxic and nonirritant, and it must adhere to the mucosal surface. A broad range of polymers have been examined for their binding affinity to such surfaces.<sup>1,2</sup> It was found that the most suitable polymers are poly-anionic electrolytes that behave as hydrogels in aqueous solution. Carbopol 934P and polycarbophil, both polyacrylic acid-based polymers, are often used as polymer bases for controlled-release drug-delivery systems: both are anionic polymers with a high charge density and swelling characteristics that depend on both the pH and the ionic strength of aqueous solutions. The group of Robinson<sup>3</sup> synthesized a series of crosslinked, swellable polymers from acrylate monomers and found that the *in vitro* bioadhesion to rabbit mucous tissue of some polymers was comparable to that of polycarbophil and significantly higher than that of poly(2-hydroxyethyl-methacrylate) [poly-(HEMA)] or gelatin.

Because of their biocompatibility and high hydrophilicity, biopolymers such as starch, cellulose, and cellulose derivatives have also been used for obtain-

ing controlled-release systems.<sup>4–8</sup> Moreover, it has been shown that physical mixtures of thermally modified starches and polyacrylic acid yield nonirritant drug-delivery systems with good bioadhesion capability.<sup>9–11</sup> The incorporation of polyacrylates into biopolymers and, specifically, the grafting of acrylic monomers onto starch could result in combined properties such as biocompatibility, nontoxicity, and higher bioadhesion, which would confer attractive characteristics on the newly prepared composite materials.

Formation of radicals in the starch or polysaccharide backbone as the initial stage in grafting polymerization of acrylic monomers has been achieved by chemical redox initiation systems,<sup>12–17</sup> UV irradiation,<sup>18</sup> electron beam radiation,<sup>19</sup> and gamma-ray radiation.<sup>17,20,21</sup> In our previous study,<sup>12</sup> grafting of polyacrylonitrile into potato or rice starches was achieved by a redox-initiation-induced polymerization; the starch-g-polyacrylonitrile copolymers were characterized, and the extent of swelling (hydration) was determined.<sup>12</sup>

Grafting of acrylic monomers onto starches or other biopolymers is usually performed to prepare materials with high absorbency for water.<sup>21,22</sup> The current study is the first report of the preparation of graft copolymers (of various starches with acrylic monomers) with the purpose of testing their use as platforms for oral drug-delivery systems. The study constitutes the first stage in setting the basis for an integrated approach to

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the design of bioadhesive drug-delivery systems. Graft polymerization was achieved by two methods of initiation: chemical [with ceric ammonium nitrate (CAN)] or  $^{60}\text{Co}$  gamma radiation. Although the choice of method depended, in part, on the particular monomer (acrylonitrile, acrylic acid, and methyl acrylate), the  $^{60}\text{Co}$  source was preferable: it not only gave pure products and enabled low-temperature polymerizations, but it also afforded materials suitable for use as slow-release matrices for drugs. The grafted starches were characterized by chemical and spectroscopic analyses, and by molecular weight determination of the polyacrylic chains grafted onto the starches from various sources.

## EXPERIMENTAL

### General

Potato (S-4251), corn (S-4126), and rice (S-7260) starches were purchased from Sigma Chemicals (St. Louis, MO). Salicylic acid, acrylonitrile, methyl acrylate, polyacrylonitrile, acrylic acid, and polyacrylic acid were obtained from Aldrich (Milwaukee, WI). Acrylonitrile was freshly distilled before use. Maltodextroses (various oligomers) and theophylline were obtained from Eridania Cerestar (Belgium) and Ludeco (Brussels, Belgium), respectively. CAN, ACS grade, was purchased from Fisher Scientific (Pittsburgh, PA) or Aldrich.

Radiation ( $\gamma$ -rays from a  $^{60}\text{Co}$  source) at a maximum intensity of 1300 rad/min was performed at the Nuclear Engineering Department of Ben-Gurion University. FTIR spectra were obtained on a Nicolet 410 FTIR spectrometer (Nicolet Instruments, Madison, WI). Nitrogen was determined by the Kjeldahl method for organic nitrogen.

The kinetics of drug release were followed in a Hanson Research tablet dissolution system (Gloucester, England) connected to a Spectronic 1201 spectrophotometer (Wootton, England) operated with Dissolution software 10F1, version 1.01. A sample of weighed dry graft copolymer was added to saturated solutions of the model drug (10 mg/mL for sodium salicylate, and 1 mg/mL for theophylline) in sodium phosphate buffer 0.03–0.05M, pH 7.4, and kept for 24 h to ensure maximum swelling. Thereafter, it was dried between two pieces of filter paper until no further changes in weight were measured. The weight of the incorporated drug was calculated as follows:

$$w_{\text{drug}} = \left( \frac{w_1 - w_0}{d} \right) \times c$$

where  $w_0$  is the weight of dry graft copolymer,  $w_1$  is the weight of swollen graft copolymer,  $d$  is the density of solution (g/mL), and  $c$  is the concentration of drug (g/mL).

The swollen graft copolymer containing the drug was then immersed in 0.02M sodium phosphate buffer (900 mL), and the mixture was stirred (65 rpm) at 37–38°C. Samples were withdrawn and absorbance was read on a UV–vis spectrophotometer ( $\lambda_{\text{max}} = 297$  nm for sodium salicylate;  $\lambda_{\text{max}} = 272$  nm for theophylline). The percentage of drug released was then calculated.

### Gamma-radiation-induced polymerization

Radiation-induced polymerization of acrylic acid on the three types of starch was performed at room temperature by exposing pregelatinized starches (2% or 5% w/v) mixed with various amounts of acrylic acid to a dose of 1300 rad/min, which is the maximum intensity of the source at Ben-Gurion University. The gels so formed were cut into small pieces and dried in air, followed by milling to powder with an IKA Labortechnik type A10 Mill (Staufen, Germany).

### Preparation and characterization of chemically induced grafted polymers

Preparation of grafted starches with acrylonitrile and methyl acrylate by CAN initiation was performed as previously described by Fanta et al.<sup>13,14</sup> and by Denenberg and Abbot.<sup>15</sup> Crude graft copolymers were subjected to repeated extractions at room temperature to remove the homopolymer. Dimethylformamide (DMF) was used to remove the polyacrylonitrile (PAN) homopolymer, and toluene to remove poly(methyl acrylate). The nitrogen content was determined before and after DMF treatment of starch-g-PAN. Saponification of PAN-containing grafted starch was performed as previously described,<sup>16</sup> and the disappearance of the nitrile group was followed by IR spectroscopy.

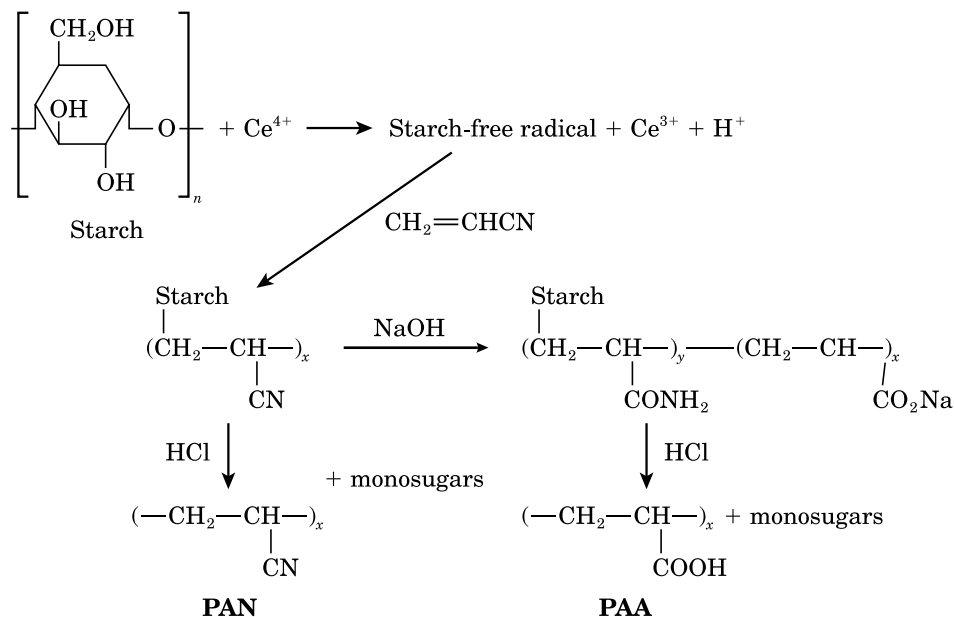
Acid hydrolysis of saponified grafted starches obtained by chemical and radiation induction was performed at 100°C with 1N HCl<sup>16</sup> or in the presence of glacial acetic acid and perchloric acid.<sup>15</sup> The presence or absence of starch after hydrolysis was tested colorimetrically by the addition of a solution of  $\text{I}_2/\text{KI}$ .<sup>23</sup> In parallel, the presence of sugar moieties in the product mixture was determined by the dinitrosalicylic acid method.<sup>24</sup>

## RESULTS AND DISCUSSION

### Starch grafted copolymers obtained by redox initiation

Grafting of acrylonitrile and methyl acrylate onto starches followed by liberation of homopolymer side chains

The general grafting procedure is presented in **Scheme 1**. Potato and rice starches were grafted with



**Scheme 1** CAN initiated graft polymerization of acrylonitrile onto starch (top) and alkaline and acidic hydrolyses of the starch-g-polyacrylonitrile (bottom).

acrylonitrile by a previously described CAN initiation method,<sup>13</sup> whereas corn starch was grafted with methyl acrylate according to Dennenberg et al.<sup>25</sup> For the acrylonitrile grafting, the weight ratios of the various starches to acrylonitrile were varied (1 : 1, 1 : 1.5, 1 : 5) to determine the extent of polymerization of acrylonitrile.<sup>12</sup> As can be seen from Table I, the nitrogen content was somewhat higher for a weight ratio of starch to acrylonitrile of 1 : 5, although the polymerization of the monomer was lower (a large amount of acrylonitrile monomer was thus wasted). Further characterization of the starches grafted with PAN was continued mainly with materials in which the weight ratio of starch to acrylonitrile was 1 : 1.5 or 1 : 5. After removal of the homopolymer of PAN formed as a by-product, the presence of PAN grafted onto the starch was ascertained by FTIR spectroscopy and nitrogen analysis (Table I). The PAN add-on could be

defined as the weight percentage of PAN in the grafted starch.<sup>12</sup>

After basic hydrolysis (NaOH, 1N) as described in Scheme 1, the percentage of nitrogen was determined. The presence of nitrogen in the grafted copolymer indicated that some amide groups had been obtained in addition to the carboxylic groups. This finding was confirmed by FTIR spectroscopy.

Acid hydrolysis (HCl, 1N) of starch-g-polyacrylonitrile was undertaken to degrade the starch backbone and produce PAN chains for molecular weight determination (Scheme 1). The nitrogen content of the chains was 24% versus the expected content of 27.8% (theoretical nitrogen content of PAN homopolymer). This result reflects the efficiency of the acid-catalyzed hydrolysis; that is, most of the starch was degraded by the process. Similarly, acid hydrolysis was performed on the product (starch-g-polyacrylic acid sodium salt)

**TABLE I**  
Partial Characterization of Grafted Copolymer from Starch and Acrylonitrile Obtained by Cerium Ammonium Nitrate Initiation

Starch source	Ratio of reagents starch : acrylonitrile	Yield (%)	Nitrogen content in crude product (%)	Nitrogen content after DMF (%)	PAN-homopolymer (%)	Add-on <sup>a</sup> of PAN (%)	Nitrogen content <sup>b</sup> (%)
Potato	1 : 1	58.8	6.2	4.6	—	17	0.9
Potato	1 : 1.5	61.5	10.0	8.8	0.2	36	1.7
Potato	1 : 5	27.6	12.2	11.9	1.1	43	0.8
Rice	1 : 1	41.0	7.2	5.4	4.2	26	1.1
Rice	1 : 1.5	61.0	11.5	10.2	—	37	2.6
Rice	1 : 5	42.6	16.7	15.7	3.8	57	0.7

<sup>a</sup> Add-on was defined as weight percentage of PAN in the grafted starch.

<sup>b</sup> Nitrogen content (%) after alkaline hydrolysis.

TABLE II  
Molecular Weights of Acrylic Chains Grafted to Starch

Material	Solvent	$K$ (dL/g)	$a$	Temperature (°C)	Reference	$M_n$
PAN <sup>a</sup>	DMF	$3.92 \times 10^{-4}$	0.75	25	26	427,000
PAA <sup>b</sup>	2 M NaOH	42.2	0.64	25	27	252,500
PMA <sup>c</sup>	Acetone	$7.4 \times 10^{-5}$	0.76	20	25	876,000
PMA <sup>d</sup>	Acetone	$7.4 \times 10^{-5}$	0.76	20	25	768,700

<sup>a</sup> Sample obtained from a rice starch-*g*-PAN (1 : 5) after hydrolysis with HCl.

<sup>b</sup> Sample obtained from a rice starch-*g*-PAN (1 : 5) after base-catalyzed hydrolysis followed by acidic hydrolysis with HCl.

<sup>c</sup> Sample obtained from a corn starch-*g*-PMA (1 : 5) after hydrolysis with HCl.

<sup>d</sup> Sample obtained from a corn starch-*g*-PMA (1 : 5) after hydrolysis with acetic acid and HClO<sub>4</sub>.

obtained from the base-catalyzed hydrolysis of starch-*g*-polyacrylonitrile copolymer, and free polyacrylic acid (PAA) chains were obtained (Scheme 1). For both PAN and PAA chains, molecular weight measurements were performed.

For the grafting of methyl acrylate into corn starch, Dennenberg et al.<sup>15</sup> found a strong dependency of the molecular weight of the grafted side chains on the ceric ion concentration used for initiation. In this study, the CAN concentration was kept constant, and the amount used was similar to that used in the grafting polymerization of acrylonitrile into starch. To a suspension of 1.75 g of corn starch in 25 mL of water was added 4.0 g of freshly distilled methyl acrylate, followed by a nitric acid solution of CAN (0.0054 mol). The homopolymer of poly(methyl acrylate) was separated from the grafted starch by repeated washings with toluene.<sup>25</sup> After evaporation, about 2% homopolymer was obtained. Thereafter, the starch-*g*-polymethyl acrylate was subjected to acid hydrolysis in 1N HCl or in a mixture of perchloric acid and acetic acid<sup>15</sup> to facilitate degradation of the starch. The poly(methyl acrylate) chains thus obtained were subjected to molecular weight determination.

#### Molecular weight determination of polymeric chains grafted into starch

Molecular weights of the side chains were determined by measuring intrinsic viscosity. The intrinsic viscosities of PAN, PAA obtained after base-catalyzed hydrolysis of PAN, and polymethyl acrylate were determined by measuring the viscosity of solutions at various dilutions (PAN in DMF, PAA in 2M NaOH, and polymethyl acrylate in acetone) by means of an Ubbelohde viscometer. The molecular weight ( $M_n$ ) of the polymeric residues (Table II) was calculated using the relationship between the intrinsic viscosity and the molecular weight, given by the Mark-Houwink equation:

$$[\eta] = KM_n^a$$

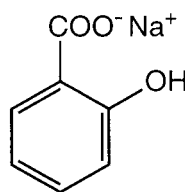
The results obtained for PAA are intriguing. The molecular weight of PAA obtained after base-cata-

lyzed hydrolysis of PAN (Table II) was lower than expected. During the base-catalyzed hydrolysis, only the -CN and/or -CONH<sub>2</sub> groups are hydrolyzed into carboxyl groups, without significant changes in molecular weight. The molecular weights of both PAN and PAA side chains were determined on grafted starches after hydrolysis with HCl.<sup>12,16</sup> The PMA side chains were obtained after hydrolysis in a HClO<sub>4</sub>-glacial acetic acid mixture.<sup>15</sup> For purposes of comparison, the molecular weights of polymethyl acrylate obtained through hydrolysis of starch-*g*-PMA both in HCl and perchloric acid were determined (Table II). Higher molecular weights were found for side chains of PMA obtained after HCl-catalyzed hydrolysis.

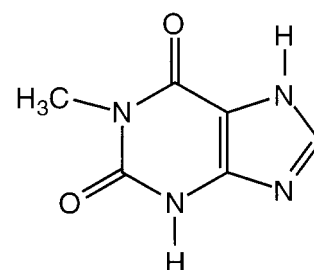
The molecular weight of PAN side chains was also measured by an additional independent method, that is, with multiangle laser light-scattering photometer (MALLS), a DAWN DSP (Wyatt Technology, Santa Barbara, CA) fitted with a He-Ne laser (632.8 nm), and a differential refractive index detector (DRI). The refractive index increment  $dn/dc$  of PAN in THF was found to be 0.11 mL/g, and the molecular weight was  $4.57 \times 10^5$  Da, a value similar to the molecular weight obtained from measurements of intrinsic viscosity (Table II).

#### Release of model drugs from grafted starch copolymers obtained by redox initiation

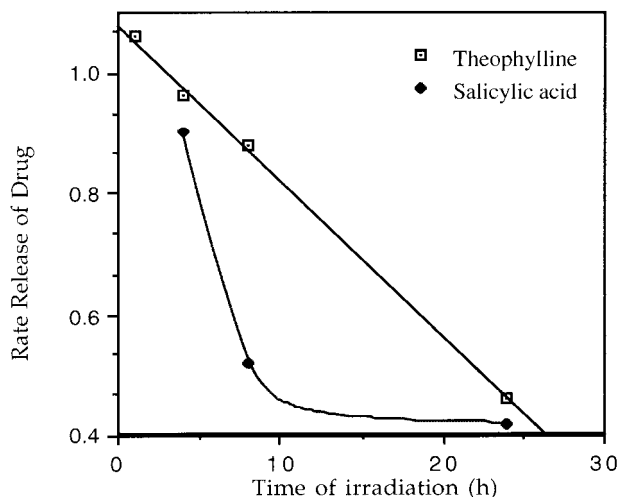
The graft copolymers were then tested to determine whether they could be used as matrixes for drug-delivery systems. Sodium salicylate and theophylline were chosen as model drugs.



Na salicylate



Theophylline



**Figure 1** Effect of radiation time on the rate of drug release.

However, the tablets prepared from starch-g-polyacrylic acid, which was obtained from base-hydrolyzed starch-g-polyacrylonitrile, disintegrated in the dissolution medium, and the drugs were released quickly. The behavior of the grafted starches prepared by exposure to <sup>60</sup>Co was more suitable, as described below.

**Starches grafted with acrylic acid by gamma-ray radiation**

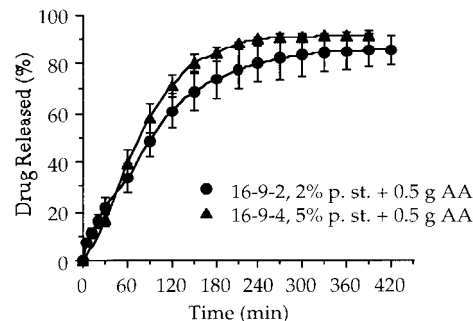
**Characterization**

Acid hydrolysis of the grafted copolymers of starch and acrylic acid obtained by gamma-ray initiation gave PAA side chains that were insoluble in water or in sodium hydroxide solutions over a wide range of pH values. This finding together with the fact that the polyacrylate chains formed weak gels in water indicated that some crosslinking had occurred during the gamma-radiation-induced polymerization; this crosslinking reduced the solubility of the PAA chains in aqueous solutions. Further characterization of the PAA branches and determination of molecular weights was thus not possible.

**Correlation of model drug release to changes in parameters of the preparation method**

Because the gamma-radiation-induced grafting polymerization was undertaken with the purpose of preparing a new class of drug-delivery systems, it was necessary to correlate drug release from the grafted starches to various parameters.

Grafting by <sup>60</sup>Co radiation was performed for various periods of time. After stopping the radiation, drugs were incorporated in the starch-grafted copolymers (as described in the Experimental section), and the kinetics of drug release were followed. The behav-

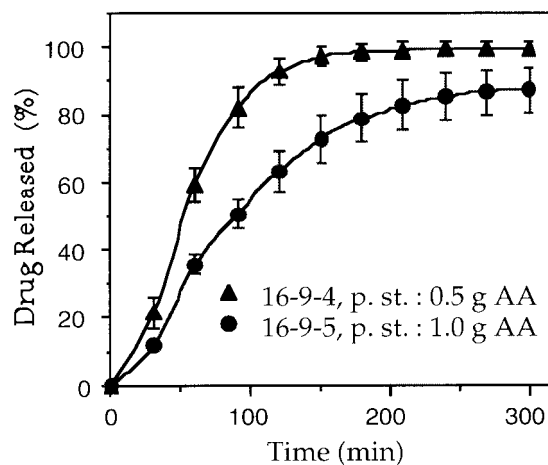


**Figure 2** Release of sodium salicylate from graft copolymers of acrylic acid with various amounts of potato starch.

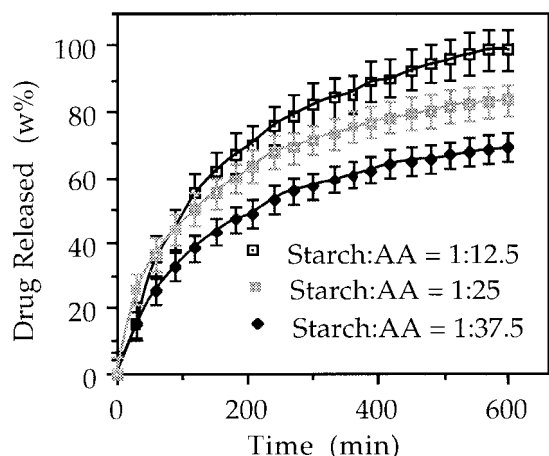
ior of each model drug is better understood from the correlation of the rate of drug release with radiation time, as presented in Figure 1. The release rate of sodium salicylate, the more polar of the two molecules, was retarded only after long periods of radiation (more than 8 h), whereas the release of theophylline seemed to be indirectly proportional to the irradiation time. Given that <sup>60</sup>Co induction produced graft polymers that significantly retarded drug release, all experiments were run for 24 h.

To determine the effect of increasing the amount of starch relative to that of the acrylic monomer, the release of salicylic acid from potato starch-g-acrylic acid copolymer obtained with 2 or 5 wt % starch was compared (Fig. 2). The release curves were similar, but the more rapid release with higher amounts of starch is somewhat puzzling.

When the amount of acrylic acid was increased, the release of salicylic acid was slower (Fig. 3). There is no doubt that the amount of acrylic acid in the grafted starch dictated the behavior of the grafted copolymer as a drug-delivery system. Thus, the weight ratio of the acrylic monomer was further increased to prolong the release time.



**Figure 3** Release of sodium salicylate from a grafted potato starch (5 wt %) and two different amounts of acrylic acid.



**Figure 4** Rate of release of theophylline from graft copolymers obtained from grafting starch with acrylic acid in weight ratios of 1 : 12.5; 1 : 25, and 1 : 37.5.

The slow release of theophylline from starch-g-acrylic acid copolymers obtained from 2 wt % starch and higher amounts of acrylic acid was also investigated (Fig. 4). The release of theophylline was significantly delayed as the amount of acrylic acid in the graft copolymer was increased. When the ratio of starch to acrylic acid was 1 : 12.5, total release of the model drug was obtained in about 10 h. However, when the ratio was tripled by increasing the amount of acrylic acid, only about 60% of the drug was released in the same period of time.

In conclusion, it seems that grafted starches with acrylic acid obtained by gamma-radiation-induced polymerization of the monomer into the starch are indeed suitable matrixes for drug-delivery systems. The evaluation of bioadhesion will determine whether these materials can be used as new platforms for drug-delivery systems.

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