Carbohydrate Polymers 81 (2010) 372–376

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and characterization of superparamagnetic chitosan–dextran sulfate hydrogels as nano carriers for colon-specific drug delivery

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ARTICLE INFO

Article history:
Received 8 February 2010
Received in revised form 12 February 2010
Accepted 19 February 2010
Available online 4 March 2010

Keywords:
Chitosan
Dextran sulfate
Targeted drug delivery
Hydrogels, Iron oxide nanoparticles

ABSTRACT

The purpose of this study was to examine chitosan (CS)–dextran sulfate (DS) nanoparticles coated iron oxide as drug carriers detectable using magnetic resonance imaging (MRI) technique. The 5-aminosalicylic acid (5-ASA) was chosen as model drug molecule. CS–DS hydrogels were formulated by a complex coacervation process under mild conditions. The influence of process variables, including the two ionic polymers, on particle size, and hydrogel entrapment of 5-ASA was studied. The in vitro release of 5-ASA were also evaluated, and the integrity of 5-ASA in the release fraction was assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The release of 5-ASA from hydrogel was based on the ion-exchange mechanism. The CS–DS hydrogel developed based on the modulation of ratio show promise as a system for controlled delivery of drug detectable using magnetic resonance imaging (MRI) technique.

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1. Introduction

Targeting of drugs specifically to the colon is advantageous in the treatment of diseases such as amoebiasis, Crohn's disease, ulcerative colitis, and colorectal cancer. In addition, it has shown great potential in the oral delivery of therapeutic peptides and proteins, which are unstable in the upper part of the gastrointestinal (GI) tract. The colonic region is recognized as having less diversity and intensity of enzymatic activities than stomach and small intestine (Davis, 1990). Various strategies are available for targeting drug release selectively to the colon (Chourasia & Jain, 2003). The designing of prodrugs is based on the concept of preventing the release of drugs in the stomach and small intestine and drug release is triggered by the use of specific property at the target site such as altered pH or high activity of certain enzymes in comparison to nontarget tissues (Davaran, Hanaee, & Khosravi, 1999; Schacht et al., 1996). Since it is known that azo function can be reduced in the colon (Chung, Stevens, & Cerniglia, 1992), many novel polymers containing azo groups either in the polymeric backbone (Yamaoka, Makita, Sasatani, Kim, & Kimura, 2000) or in the cross-links (Shantha, Ravichandran, & Rao, 1995; Van den Mooter, Samyn, & Kinget, 1992) have been synthesized. To promote further selective degradation in the vicinity of the colonic environment, delivery systems have been designed that contain both pH-sensitive acidic monomers and degradable azo aromatic cross-links (Ghandehari, Kopeckova, & Kopecek, 1997; Kakoulides, Smart, & Tsibouklis, 1998). Chitosan is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes (Furda, 1983; Ormrod, Holmes, & Miller, 1998). Chtosan is a copolymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units links with β-(1–4) bonds. It should be susceptible to glycosidic hydrolysis by microbial enzymes in the colon because it possesses glycosidic linkages similar to those of other enzymatically depolymerized polysaccharides. The polysaccharide, on reaching the colon, undergoes assimilation by microorganisms or degradation by enzymes or break down of the polymer back bone leading to a subsequent reduction in molecular weight and thereby loss of mechanical strength and is unable to hold the drug any longer (Yamamoto, Tozaki, Okada, & Fujita, 2000). Chitosan has drawn attention for its potential to achieve site-specific delivery to the colon. Chitosan, a natural linear polyamine with a high ratio of glucosamine to acetyl-glucosamine units, is a weak base and carriers a positive charge. Its solubility is pH-dependent, and it reacts readily with negatively charged surfaces and materials, including polymers and DNA. Ionic gelation, complex coacervation, emulsion cross-linking, and spray-drying are methods commonly used for the preparation of chitosan nanoparticles (Agnihotri, Mallikarjuna, &

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doi:10.1016/j.carbpol.2010.02.034

Aminabhavi, 2004). Among those methods, ionic gelation and complex coacervation are mild processes occurring in a pure aqueous environment and are ideal for maintaining the in-process stability of drugs. Ionic gelation and complex coacervation are very similar except that the former involves the gelation of chitosan using an electrolyte such as tripolyphosphate (TPP) (Calvo, Remunan-Lopez, Vila-Jato, & Alonso, 1997), whereas the latter employs an oppositely charged ionic polymer such as alginate (De & Robinson, 2003). A new type of chitosan (CS) hydrogel using dextran sulfate (DS) as a polyanionic polymer was developed to achieve complex coacervation for the incorporation and controlled release of an anti-angiogenesis hexapeptide (Chen, Mohanraj, & Parkin, 2003), this was the first report describing the use of DS to formulate CS-based hydrogels. Although there have been investigations of how the properties of CS and formulation variables such as CS molecular weight (MW), concentrations of CS and 5-ASA, and formulation pH affect the formation and encapsulation capability of hydrogels (Ma, Yeoh, & Lim, 2002; Tiyaboonchai, Woiszwillo, Sims, & Middaugh, 2003), to our knowledge, no attempts have been made to study how the ratio of CS to the oppositely charged polymer influences the formulation and properties of hydrogel. In this study, 5-aminosalicylic acid (5-ASA) was chosen as a model drug (Pan et al., 2002).

2. Materials and methods

2.1. Materials

The polymer chitosan (medium MW 400,000 Da, 85% deacetylation) was purchased from Fluka/Sigma–Aldrich. Sodium salt of dextran sulfate (MW 12,750 Da), and 5-aminosalicylic acid were purchased from Sigma Chemical Co. All other solvents and materials were of analytical grade. Deionized water (Milli-Q water) was used in the preparation of buffers and standard solutions. All other chemicals and reagents used in this study were of analytical grade.

2.2. Methods

2.2.1. Preparation of superparamagnetic CS–DS hydrogel with 5-ASA

Chitosan (CS) and dextran sulfate (DS) hydrogels were prepared using the method reported by Zhang et al., with slight modifications. Chitosan–dextran sulfate (at the charge ratio (N:P) of 1.12) and 35 mg $FeCl_3{\cdot}6H_2O$ were solved in 4 mL H_2O and nitrogen was flushed for 1.5 h. 14 mg $FeCl_2{\cdot}4H_2O$ was added, followed by 100 μL aqueous ammonia in two portions while the mixture was kept under nitrogen. The solution turned black and was heated to 80 °C for 100 min. After the mixture cooled to room temperature, the ammonia was removed by flushing the solution with nitrogen over 10 min. Then, the solid mixture was dissolved in 10 mL acetic acid (1%, w/v) and 5-aminosalicylic acid (5-ASA) (5–20 mg) was dissolved separately in dimethyl formamide (DMF). Then, the magnetic chitosan/dextran sulfate (2.5 mL) and the drug solution (7.5 mL) were mixed together to obtain 10 mL of chitosan/dextran sulfate drug solution. The chitosan/dextran sulfate drug solution was added dropwise (using a disposable syringe with a 22-gauge needle) into 40 mL of sodium-saturated Tris–HCl buffer solution containing glutaraldehyde-saturated toluene (GST) in different concentrations (1–3 mL) under magnetic stirring (~200 rpm) at room temperature. The hydrogel suspension was formed spontaneously. The mixture was stirred for a further 15 min. The hydrogel was separated after 1 h of curing time and subsequently decanted, washed twice with 3 mL of 0.05 M Tris–HCl buffer, and the hydrogel was dried in vacuum oven at 40 °C.

2.2.2. Shape and surface morphology

Surface and shape characteristics of chitosan–dextran sulfate hydrogels were evaluated by means of a scanning electron microscope (FEI-Qunta-200 SEM, FEI Company, Hillsboro, OR). The samples for SEM were prepared by lightly sprinkling the hydrogel on a bouble adhesive tape, which stuck to an aluminum stub. The stubs were than coated with gold to a thickness of ~300 Å using a sputter coater and viewed under the scanning electron microscope.

2.2.3. Particle size

After drying at 37 °C for 48 h, the mean diameter of the dried hydrogel was determined by a sieving method using USP standard sieves. Observations are recorded.

2.2.4. Drug content and encapsulation efficiency

Encapsulation efficiency (EE) is the amount of added drug (%) that is encapsulated in the formulation of the hydrogel. The EE of drug from hydrogel can be calculated in terms of the ratio of drug in the final formulation to the amount of added drug. An accurately weighed amount (100 mg) of the formulation of hydrogel was dispersed in 100 mL of Tris–HCl buffer. The sample was ultrasonicated for three consecutive periods of 5 min each, with a resting period of 5 min each. It was left to centrifuged at 3000 rpm for 15 min. The concentration of 5-aminosalicylic acid (5-ASA) in the decanted Tris–HCl buffer and two washing solutions was determined by measuring the absorbance at 235 nm using a GBS Cintra 10-UV–visible spectrophotometer (Shimadzu, Japan). The determinations were made in triplicate, and results were averaged (Table 1).

2.2.5. Equilibrium swelling studies

Chitosan–dextran sulfate hydrogels (100 mg) were placed in phosphate buffered saline (PBS) (pH 7.4) and allowed to swell upto a constant weight. The hydrogels were removed, blotted with filter paper, and changes in weight were measured and recorded in Table 1. The degree of swelling (α) was then calculated from the formula:

$$\frac{W_g - W_o}{W_o}$$

where, W_o is the initial weight of hydrogel and W_g is the weight of hydrogel at equilibrium swelling in the medium.

2.2.6. In vitro drug release study of 5-ASA-loaded hydrogel

In vitro drug release studies were performed according to extraction technique using USP dissolution test apparatus. The dissolution studies were performed in 900 mL of dissolution medium, which was stirred at 100 rpm at 37 ± 0.1 °C.

The scheme of using the simulated fluids at different pH was as follows:

- First hour: simulated gastric fluid of pH 1.2
- Second to third hour: mixture of simulated gastric and intestinal fluid of pH 4.5
- Fourth to Fifth hour: simulated intestinal fluid of pH 7.4
- Sixth to eighth hour: simulated colonic fluid of pH 7.0

In vitro drug release studies were performed as per the scheme in different simulated fluids. Simulation of GI transit conditions was achieved by using different dissolution media. Simulated gastric fluid (SGF) pH 1.2 consisted of NaCl (0.2 g), HCl (7 mL), and pesin (3.2 g); pH was adjusted to 1.2 ± 0.5. Simulated intestinal fluid (SIF) pH 7.4 consisted of KH_2PO_4 (6.8 g), 0.2 N NaOH (190 mL), and pancreatin (10.0 g); pH was adjusted to 7.4 ± 0.1. SIF pH 4.5 was prepared by mixing SGF pH 1.2 and SIF pH 7.4 in a ratio of 36:61. The experiment was performed with a continuous supply

Table 1
Compositions and characteristics of different chitosan–dextran sulfate hydrogel.

Variables	Values	Degree of swelling	Average particle size (mm)	Encapsulation efficiency (%)
5-ASA	0	1.36 ± 0.05	1.20 ± 0.08	–
	5	1.24 ± 0.20	1.68 ± 0.08	74.8 ± 2.34
	10	1.00 ± 0.01	1.70 ± 0.06	75.2 ± 1.98
	15	0.95 ± 0.09	1.92 ± 0.04	80.1 ± 2.54
	20	0.68 ± 0.12	1.20 ± 0.10	85.6 ± 1.63
CS–DS hydrogel (%w/w)	1	1.10 ± 1.00	1.38 ± 0.11	80.8 ± 3.24
	2	1.20 ± 1.21	1.75 ± 0.09	82.3 ± 1.25
	3	1.32 ± 1.35	1.90 ± 0.05	86.7 ± 2.47
	4	1.50 ± 1.40	1.87 ± 0.02	88.7 ± 2.35
Time (h)	10 min	0.50 ± 0.52	1.90 ± 0.12	70.3 ± 1.47
	20 min	0.53 ± 0.14	1.87 ± 0.21	86.4 ± 1.98
	40 min	0.85 ± 0.05	1.70 ± 0.32	76.7 ± 2.45
Drying (°C)	Lyophilization	0.86 ± 0.50	1.32 ± 0.12	69.8 ± 4.35
	45	0.71 ± 0.72	1.00 ± 0.64	65.4 ± 3.98

of carbon dioxide into dissolution media. Aliquots of samples were withdrawn periodically and replaced with an equal amount of fresh dissolution media bubbled with carbon dioxide. The volume was made upto 10 mL and centrifuged. The supernatant was filtered through Whatman filter paper (Dawsonville, GA), and drug content was determined spectrophotometrically at 235 nm (UV 1601, Shimadzu, Japan).

2.2.7. Statistical analysis

Experimental data have been represented as the mean with standard deviation (SD) of different independent determinations. The significance of differences was evaluated by analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.005$.

2.2.8. Conductivity measurement

Conductivity of hydrogel dispersion, 0.1% CS, 0.1%DS with iron oxide nanoparticles in the deionized water was determined using a conductivity meter (Systronics 307) with a conductivity range from 0.1 to 200 mυ at room temperature. All samples were prepared in deionized water.

3. Results and discussion

3.1. Hydrogel characteristics

These hydrogels had good spherical geometry. It is obvious that the surface of the hydrogel shrank and a densely cross-linked gel structure was formed. This may explain the greater retardation

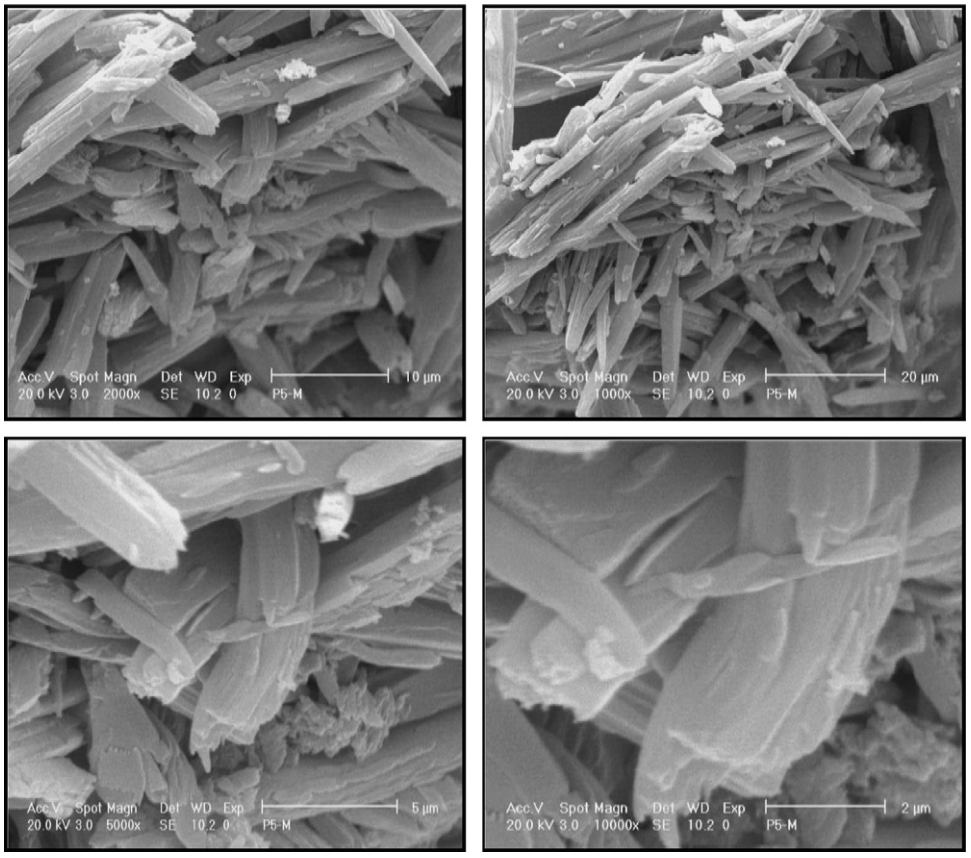

Fig. 1. Morphology of chitosan–dextran sulfate (CS–DS) hydrogel by emission scanning electron microscopy field.

of drug release from matrices of higher cross linker content. The average drug entrapment was found to be 81.21 ± 1.86% in the hydrogel.

3.2. Drug content and encapsulation efficiency

Results of drug content and EE demonstrated that drug content increased from 10.23 ± 0.50 mg/100 mg to 26.24 ± 0.43 mg/100 mg of hydrogel with increasing the amount of drug from 5 to 20% (w/w). No signification increase in drug content was observed on further increasing the amount of drug, i.e., above 15% (w/w), which could be due to the limited solubility of the drug in DMF and that is endorsed from the presence of drug particles on the surface of the hydrogel prepared with 20% of drug concentration. The percent EE was increased upto 86.32 ± 0.20% with increasing polymer concentrations to 4%. Concentration of the cross-linking agent exhibited no significant effect on percent EE.

3.3. Morphology of superparamagnetic CS–DS hydrogel

FESEM images of superparamagnetic CS–DS hydrogel (Fig. 1) show that hydrogel have a solid and near consistent structure. Furthermore, the incorporation of 5-ASA into the hydrogel produced a smooth surface and compact structure. The particle size observed in FESEM is smaller than that measured by the Zetasizer. This is because dried hydrogel was used in FESEM, whereas particles in the liquid dispersion was analyzed by the Zetasizer. CS–DS hydrogel are hydrophilic and would be expected to swell in water, thus producing a large hydrodynamic size when measured by the Zetasizer (Fig. 2).

3.4. Incorporation of 5-ASA into CS–DS hydrogel

5-aminosalicylic acid (5-ASA)-loaded hydrogel was obtained spontaneously upon the mixing of the DS aqueous solution (0.1%, w/v) with the CS solution (0.1%, w/v) under magnetic stirring, with 5-ASA dissolved in CS–DS solution. The incorporation of 5-ASA into the CS–DS hydrogel resulted in a sharp increase in the particle size of the nanoparticle dispersion. The significant increases in particle size give a good induction of the incorporation of 5-ASA into CS–DS hydrogel. A study was undertaken to investigate the effect of the order of 5-ASA mixing with CS and DS. The data obtained show that the order of 5-ASA mixing had no effect on the size, entrapment efficiency, and yield of 5-ASA-loaded hydrogel.

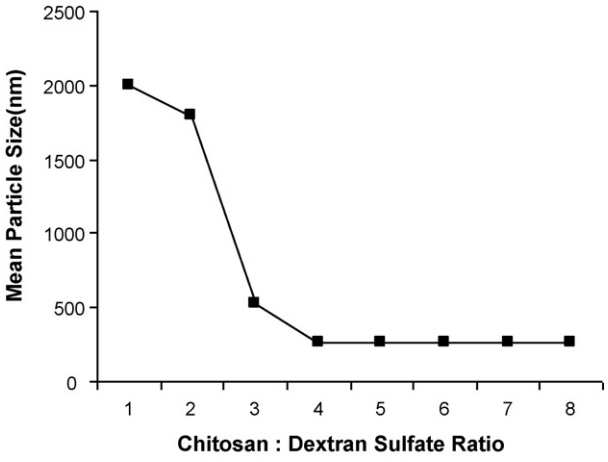

Fig. 2. Influence of the chitosan:dextran sulfate ratio on the particle size.

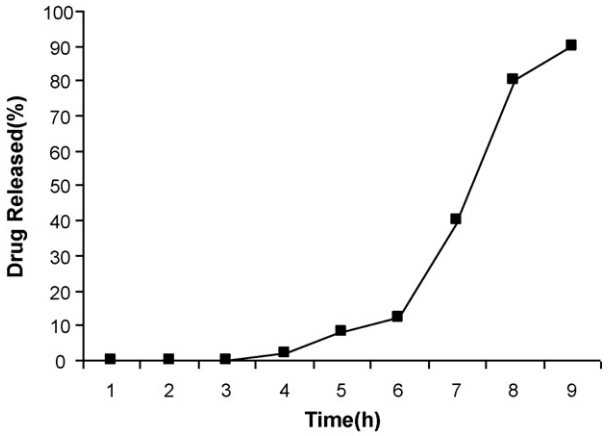

Fig. 3. The in vitro release profile of 5-aminosalicylic acid from chitosan–dextran sulfate hydrogel in various simulated gastrointestinal fluids ($n = 3$).

3.5. In vitro release studies

The effect of drug concentration, chitosan–dextran sulfate concentration, GST concentration, and cross-linking time on in vitro drug release was also observed. In vitro drug release after 5 h was 86.3 ± 4.0% in the case of hydrogel having 15% drug, while it was 88.2 ± 3.1% for hydrogel with 20% drug. The effect of chitosan–dextran sulfate on the release of drug was found to be meager. It is also observed that the amount of drug released from hydrogel decreased on increasing cross-linking time. These properties are probably explained by the promotion of cross-links between chitosan–dextran sulfate chains and GST. Freeze-drying of the samples resulted in larger and more porous hydrogel compared with air-dried hydrogel. Freeze-drying had the advantage of avoiding drug extraction by immediately freezing and removing the water present within the hydrogel and a burst effect during the dissolution study (Fig. 3). Conventional dissolution testing is less likely to accurately predict in vivo performance of colon delivery systems triggered by bacteria residing in the colon (because aspects of the colon's environment, i.e., scarcity of fluid, reduced motility, and presence of microflora, cannot be simulated in conventional dissolution methods). Hence, release studies were performed in an alternate release medium. The release profiles of 5-ASA-loaded hydrogel was evaluated in water and a phosphate buffer, which was either in a different ionic strength or with saline, to study the

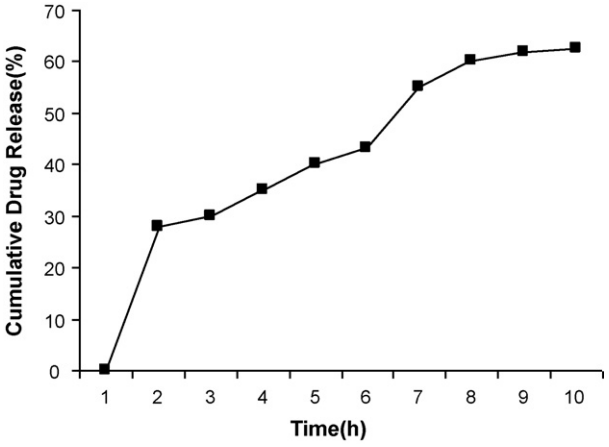

Fig. 4. In vitro drug release from chitosan–dextran sulfate hydrogel in SCF (pH 7.0) in presence 5-ASA.

Table 2
Conductivity of dispersion of hydrogel in the different pH.

Weight ratio of CS:DS	Charge ratio (N:P) of the hydrogel	pH of hydrogel	Conductivity of dispersion (in mυ)	Size (in nm)
5:4.5	1.01	4.0	3.72	1892
5:5	1.12	3.9	3.70	350
5:8.5	1.90	4.1	3.78	240

underprinning mechanisms for drug release. The greatest release for 5-ASA–loaded hydrogel occurred in the release media of a high ionic strength. In contrast, a significantly small portion of 5-ASA was released in water over the release study period. The burst release was observed with hydrogel, and it may have arisen from the desorption of loosely attached 5-ASA from the surface of the matrix polymers (Fig. 4).

3.6. Hydrogel conductivity studies

The opposite charges of CS–DS hydrogel were responsible for the formation of nanoparticles. The charge ration between the negatively charged sulfate groups (N) in DS and the positively charged amine groups (P) in CS was determined. Under the experimental conditions (pH 3–4), DS carries ~74 sulfate groups per mole, equivalent to 5.78×10^{-3} negatively charged groups per gram of DS; CS has ~2073 amino groups per gram of CS. Table 2 shows conductivity of dispersion of hydrogel.

4. Conclusion

Results of release studies indicate that superparamagnetic chitosan–dextran sulfate hydrogel offer a high degree of protection from premature drug release in simulated upper conditions. These hydrogels deliver most of the drug load in the colon, an environment rich in bacterial enzymes that degrade the chitosan–dextran sulfate and allow drug release to occur at the desired site. Thus, spherical superparamagnetic hydrogel is a potential system for colon delivery of 5-ASA. Also, this hydrogel can be detect by magnetic resonance imagining (MRI) technique.

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