

# Application of Factorial Experimental Design to the Study of the Suspension Polymerization of $\beta$ -Cyclodextrin and Epichlorohydrin

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**ABSTRACT:** The suspension polymerization of  $\beta$ -cyclodextrin ( $\beta$ -CD) with epichlorohydrin (EP) has been studied using a fractionated factorial design with two levels and six variables ( $\beta$ -CD:EP molar ratio, NaOH concentration, temperature, stirring speed, delay time before paraffin addition, and  $\beta$ -CD concentration). Different variables, such as the amount of  $\beta$ -CD in the product, particle size, degree of swelling, and sorption capacity, have been analyzed as the

responses for the synthesized polymers. The experimental design approach permitted to select the optimal conditions of synthesis depending on the desired features of the product. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 3393–3402, 2006

**Key words:** copolymerization; hydrogels; crosslinking; polysaccharides

## INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, or eight glucopyranose units ( $\alpha$ ,  $\beta$ ,  $\gamma$ -CDs, respectively) linked by glycosidic bonds. Their shapes resemble a hollow truncated cone with hydrophilic edges and a relatively hydrophobic cavity when compared with aqueous media. Indeed, it is the non-polar character of their cavities that confers them unique properties, most importantly their ability to form inclusion complexes with different guest molecules.<sup>1</sup> For instance, many chlorinated polluting agents (such as pesticides, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, dibenzofurans (DBF), or dioxins) possess a hydrophobic character and are compatible in size with CD cavities.<sup>2</sup>

CDs are, generally, quite soluble in water. This can represent a problem when used to either separate or eliminate specific components in aqueous media. A feasible solution is to incorporate CDs into polymeric structures. These macromolecular networks can hinder the solubility of the CDs without obstructing the access to their cavities. Two types of procedures have been used to incorporate the CD moieties to

macromolecular structures. On the one hand, the CDs can be linked as substituents to the polymeric main chain; on the other hand, they can be crosslinked with bifunctional agents by means of polycondensation reactions.<sup>3</sup> The crosslinking procedure yields polymers in which the CDs are part of the network, with the possibility of obtaining highly branched structures. It becomes evident that if the conditions of either the polymerization of CD derivatives or the crosslinking of the CDs are carefully controlled, products with different arrangements can be obtained. Therefore, the properties of the resin can be varied to fit the desired application.

Several methods of integrating CD molecules into macromolecular structures, by interconnecting them with suitable linking agents, have been proposed.<sup>4–10</sup> One of the first and most frequently used crosslinkers is epichlorohydrin (EP).<sup>11</sup> EP is an example of a chemical reagent that contains two reactive functional groups: an epoxide group and a chloroalkyl moiety. EP can react with the hydroxyl groups of CDs in basic media to yield ethers. The resulting polymer will be constituted by different size segments of 2-hydroxypropyl ether linked to CD molecules. With the aid of a factorial experimental design, the procedure of  $\beta$ -CD polymer synthesis (CDP) has been studied to use the produced material in adsorption columns. Experimental design is a statistical tool of fundamental importance to improve a given process, since higher reliability conditions and lower costs can be delineated.<sup>12</sup> For instance, this technique has been used for the study of polymerization reactions.<sup>13–17</sup>

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TABLE I  
Variables and Levels of the Experimental Design Considered

Notation	Variables	Low level	High level
A	$\beta$ -CD:EP (mole ratio)	1:11	1:16
B	NaOH solution (% mass)	32	40
C	Reaction temperature ( $^{\circ}$ C)	35	50
D	Stirring speed (rpm)	200	300
E	Time before paraffin addition (min)	15	30
F	Water initially added (mL)	58	118

The analysis of the system responses to modifications in the studied variables leads us to identify their influences. Thus, the optimal values of these variables can be established to obtain the required response, diminishing the variability of the response and the effects of the noncontrolled variables.

A factorial design with more than three variables significantly increases the complexity of the experiment. In these cases, it is more advisable to employ fractionated factorial designs. Its main appeal has to do with the possibility of allowing the simultaneous inclusion of five or more variables in a single experiment of a reasonable size, in such a way that a fast determination of the variables with an important effect on the result is possible.<sup>18</sup> In this work, a fractionated factorial design with two levels and six variables is applied to study the suspension polymerization of  $\beta$ -CD with EP.

## EXPERIMENTAL

### Polymerization conditions

The polymerization of  $\beta$ -CD with EP is carried out in basic aqueous media. A water–oil suspension is used to obtain the polymer in the form of spherical beads. The polymer must be able to swell in aqueous media and to form inclusion complexes with aromatic compounds.<sup>5–9</sup>

A preliminary study was carried out to determine the lowest  $\beta$ -CD:EP ratio required to yield insoluble polymers. The variables considered important in the synthesis were kept constant, and the molar ratio  $\beta$ -CD:EP was varied between 1 : 2 and 1 : 11 (i.e., 12.56 and 44.13% weight of EP).  $\beta$ -CD was kindly provided by Roquette-Laisa, Spain, and the EP was supplied by Aldrich. Both were used without further purification. In a batch stirred tank reactor, 58 g of  $\beta$ -CD, 30 mg of  $\text{NaBH}_4$  (Probus, Spain), and 58 mL of water were introduced. After stirring for several minutes, a paste is produced. Then, the corresponding volume of EP is slowly added. Finally, 65 mL of aqueous NaOH (40 wt %) are added dropwise to the mixture. After a defined time interval has elapsed (15 min), 1 L of liquid paraffin (from Panreac, Spain) is added. Throughout the reaction time (20 h), the stirring speed of the reacting

bulk (300 rpm) and the temperature (50 $^{\circ}$ C) are kept constant. Once the reaction has been completed, the stirring is interrupted and the polymer beads are decanted from the paraffin. The polymer is neutralized with hydrochloric acid (6 mol/L), washed several times with water and ethanol, filtered, and dried at 60 $^{\circ}$ C. The products were purified by Soxhlet extraction using *n*-hexane and ethanol and dried at 60 $^{\circ}$ C.

### Experimental design

Table I shows the variables considered in this study, as well as the values assigned to both levels selected for each one. Several reaction parameters, which had been kept constant in previous works,<sup>9,10,19</sup> have been included in our design, such as the stirring speed and the time interval before the addition of paraffin. Other parameters however remain fixed, since they are considered important for a reproducible synthesis: the size of the reactor and the total reaction volume that practically remain constant.<sup>10</sup>

A complete replica of a  $2^6$  design requires 64 experiments. In this case, only 6 of the 63 degrees of freedom correspond to the main effects, and another 15 correspond to the interactions of two variables. The rest are related to interactions of three or more variables. If the interactions of higher order could be disregarded, the information on the main effects and the interactions of smaller order would be obtained performing just a fraction of the complete factorial experiment.<sup>12</sup> To investigate the best conditions of synthesis to yield  $\beta$ -CD insoluble resins, a fractionated experimental design of two levels with six variables of resolution IV is proposed ( $2_{\text{IV}}^{6-2}$ ). It assumes that no main effect is alias of either another main effect, or of some interaction of two variables. Besides, the interactions of two variables are aliases to each other (AB = CE, AC = BE, AD = EF, AE = BC, AF = DE, BD = CF, BF = CD).<sup>12</sup> The structure of this fractionated experimental design with the conditions used in each test is shown in Table II. Symbol “+” indicates that a variable is in its high level, while the symbol “–” refers to low levels. As can be seen, half of the experiments of each variable are made at high level and other half at low level.

TABLE II  
Experimental Design 2<sub>IV</sub><sup>6-2</sup>

Run	A	B	C	D	E	F	Nomenclature
1	-	+	+	-	-	-	bc
2	-	-	-	+	-	+	df
3	+	-	-	+	+	+	ade
4	+	+	-	+	-	-	abd
5	-	+	+	+	-	+	bcd
6	-	+	-	-	+	+	bef
7	-	-	+	+	+	-	cde
8	+	+	-	-	-	+	abf
9	+	-	+	+	-	-	acd
10	+	-	+	-	-	+	acf
11	-	-	-	-	-	-	1
12	-	-	+	-	+	+	cef
13	-	+	-	+	+	-	bde
14	+	-	-	-	+	-	ae
15	+	+	+	+	+	+	abcdef
16	+	+	+	-	+	-	abce

### Response variables

The variables analyzed as the responses for the synthesized polymers are: (1) solubility in water; (2)  $\beta$ -CD content; (3) sorption capacities for phenol and DBF; (4) particle size distribution; and (5) swelling capacity in water. All these variables are quantitative, except the solubility in water of the polymer that is a binary variable (soluble or insoluble). The determination of the water solubility of the polymer (i.e., whether the gel point has been reached or not) is easily ascertained when interrupting the agitation, after the reaction time has elapsed. The polymer (aqueous phase) and paraffin are separated and a small portion of water is added to confirm the result. Once the polymer has been purified, the percentage of  $\beta$ -CD is determined. For soluble polymers, this determination is made by means of magnetic nuclear proton resonance (<sup>1</sup>H NMR), using a simple method described by different authors.<sup>10,20</sup> A solution of  $5 \times 10^{-4}$  mol/L concentration is prepared, and measured in a Bruker Advance 400 Ultrashield (9.36T) spectrometer (100 accumulations) after a presaturation to eliminate the solvent signal. The amount of  $\beta$ -CD in the polymer is quantified considering that the spectrum presents two types of signals: a wide peak at  $\sim 5$  ppm assigned to the anomeric proton of the glucose C1 carbon of  $\beta$ -CD, and two other peaks between 3 and 4 ppm corresponding to hydrogen atoms of both the glucopyranose ring and EP (i.e., to the hydroxypropyl ether fragment resulting from the condensation reaction). For the insoluble polymers, the amount of  $\beta$ -CD is determined by means of CHN elemental analysis.<sup>21</sup> The polymer is pulverized and measured in a LECO CHN 2000 analyzer. The obtained results must be corrected considering the intrinsic humidity of each polymer, which is obtained by thermogravimetric analysis (TGA) (Mettler Toledo TGA-SDTA 851e).

Knowing the values of %C, %H, %O, and %H<sub>2</sub>O, a mass balance permits to obtain the contents of  $\beta$ -CD and EP of the polymer.

The size determinations are also different for water soluble and nonsoluble (gel particles)  $\beta$ -CDP. For soluble polymers, the molecular sizes are measured by gel permeation chromatography (GPC) and dynamic light scattering (DLS). To obtain the chromatograms, aqueous solutions of the polymers (0.5 wt %) are prepared and injected in a system constituted by a pump (Waters 600E), connected to an autosampler (Waters 717 plus) and a refractive index detector (Waters 2414). The samples run through a PL aquagel-OH column with a 30 pore and a water flow of 1 mL/min. For the DLS measurements (DynaPro-MS/X), aqueous solutions of 25 g/L concentration are prepared. In these conditions, some polymers display a slight turbidity, and so those samples were filtered through 0.2 mm pores. The calculations assume spherical shape for the macromolecules. Monomeric  $\beta$ -CD is also measured by this technique for comparative purposes.

To evaluate the size as a response in the case of the insoluble polymers, the samples are submerged into water until the equilibrium swelling is reached, and then the gel beads are wet sieved. A set of sieves is used ranging between 0.250 and 4.00 mm (0.250, 0.315, 0.400, 0.500, 0.630, 0.800, 1.00, 1.25, 1.60, 2.00, 2.50, 3.15, and 4.00). It has to be noticed that the size and distribution measured for the beads with this procedure are not those of the beads obtained at the end of the reaction time, but those resulting of the swelling at equilibrium. The mean diameter for each sample is calculated considering the percentage of the total mass of polymer particles in each fraction and the reciprocal mean of the mesh sizes for the corresponding sieves.

The phenol and DBF sorption capacities are measured placing 180 mg of polymer and 50 mL of either 50 mg/L phenol or 4 mg/L DBF aqueous solutions, and shaking them for 150 min in a thermostatic bath at 25°C. Then, the polymer is decanted and a 3 mL sample of the supernatant solution is measured by UV-visible spectrometry (Hewlett-Packard 8452A). The wavelengths to quantify the remaining solutes are 270 and 280 nm for phenol and DBF, respectively.

The swelling capacities of the insoluble CDP have been studied using several procedures: TGA, centrifugation, and determination of apparent volumes.<sup>22</sup> Concordant results were obtained by the different techniques. First, the gel bead samples are weighed after centrifugation (Orto-Alresa Digicen) until a stable weight value is reached. The TGA is performed on samples placed in 40  $\mu$ L aluminum crucibles, after the excess of water is eliminated with filter paper; the TGA scans are registered from 25 to 200°C at a 10°C/min heating rate with a nitrogen purge (20 mL/min). For the apparent volume method, a weighed amount of dry polymer is placed into a graduated tube ( $\pm 0.1$

**TABLE III**  
Results of the Response Variables

Run	Solubility in water	Mean diameter *	$\beta$ -CD content (% mass)	Sorption capacity (mg solute/g CDP)		Swelling capacity (g water/g CDP)
				Phenol	DBF	
1	No	1.396	58.05	4.005	0.944	5.93
2	Yes	8.50	54.94	—	—	—
3	Yes	8.70	47.66	—	—	—
4	No	0.413	68.23	3.664	0.963	2.26
5	Yes	8.64	46.33	—	—	—
6	Yes	13.12	47.60	—	—	—
7	No	0.840	63.16	4.780	0.968	3.95
8	Yes	37.46	34.22	—	—	—
9	No	0.742	66.63	4.373	0.961	3.65
10	Yes	4.04	50.70	—	—	—
11	No	0.718	63.37	4.034	0.965	4.92
12	Yes	10.78	47.28	—	—	—
13	No	0.890	64.95	4.466	0.977	5.05
14	No	0.806	67.22	4.234	0.964	2.95
15	Yes	17.52	38.52	—	—	—
16	No	1.764	53.98	4.787	0.971	4.44

\*nm for soluble CDP; mm for insoluble CDP.

mL) with an excess of water (10 mL) and the evolution of the swelling gel volume is registered.

## RESULTS AND DISCUSSION

The results corresponding to the analyzed responses are shown in Table III. For those polymers that are soluble in water, only the contents of  $\beta$ -CD and the average molecular sizes are given. The fact that the end product of the crosslinking reaction of  $\beta$ -CD is either soluble or insoluble (i.e., the attainment of the gel point) depends on several parameters such as the concentration of  $\beta$ -CD, the concentration of the NaOH solution, and the ratio  $\beta$ -CD : EP.<sup>19</sup> As it was indicated previously, the water solubility has a binary response. Two results are possible, the polymer is either soluble in water or insoluble; these two alternatives should refer to the gel point. Binary variables are difficult to evaluate, and frequently, special techniques are required.<sup>23</sup>

Prior to the experiments corresponding to the factorial design, the minimum CD:EP molar ratio required to yield insoluble polymers was investigated. Previous results showed that for mole ratios of 1 : 6 and above, gel points were reached.<sup>8</sup> Nevertheless, with the experimental system described, 1 : 11 ratios were needed to yield insoluble resins. Even though we used 1 : 11 as the low level for this variable, half of the  $\beta$ -CDP produced in our set of experiments were water soluble resins (see Table III). It is evident that the amount of water initially added to the  $\beta$ -CD/NaBH<sub>4</sub> mixture (experimental variable F) must play a determinant role: soluble polymers are yielded for the high level of variable F. The same conclusion was pointed

out by Yudiarto et al.<sup>19</sup> Besides a high  $\beta$ -CD:EP mole ratio, a high concentration of  $\beta$ -CD in water must be used to obtain insoluble polymers. The fact that the first stages of the crosslinking reaction occur in a heterogeneous system (EP is immiscible with water) can be responsible for the observed results. EP self-polymerization diminishes the effective ratio of crosslinker to  $\beta$ -CD units, and so the gel point cannot be reached. A concentrated aqueous phase facilitates the contact between  $\beta$ -CD and EP.

The main objective of this study is to ascertain the best conditions of polymerization to produce  $\beta$ -CD polymers used as support for fixed beds to treat contaminated waters by adsorption. Therefore, the experimental variable F will not be considered and the products will be analyzed separately according to their solubility in water. If variable F was suppressed from the design, we could work with these two sets of experiments: those yielding soluble polymers and those corresponding to the insoluble ones. The fractionated factorial designs obtained when this variable is eliminated are somewhat peculiar. One could think that a design with two levels and six variables has been reduced to two designs with two levels and five variables, i.e., from a  $2^{6-2}$  design we get two  $2^{5-2}$  designs. To fulfill this premise, at least the set based on those experiments yielding insoluble polymers should contain the combination in which the five variables are at their respective low levels (represented by the unity in Table II). When a  $2^{5-2}$  design is prepared, first we write the combinations of high and low levels corresponding to variables A, B, and C. Then, D and E are added associating their high and low levels to the positive and negative signs of the interactions of two

**TABLE IV**  
Effect of the Main Variables for Water Soluble Polymers

	A	B	C	D	E
$\beta$ -CD content	-6.26	-8.48	-0.398	1.91	-1.28
Polymer size	3.31	5.57	-3.37	-2.78	-1.09

of the initial variables, for instance  $D = AB$  and  $E = BC$ . Because of the fact that D and E are interactions between two variables, the combination 1 (i.e., low levels for all the variables as that in Table II) cannot be obtained, since the result of the product of two low levels for two variables is a high level for their combination variable (D or E in this case). Consequently, a  $2^{6-2}$  fractionated design cannot be reduced to two independent  $2^{5-2}$  designs. Therefore, to calculate the effects of the interactions, all of them must be calculated without considering the initial aliases.

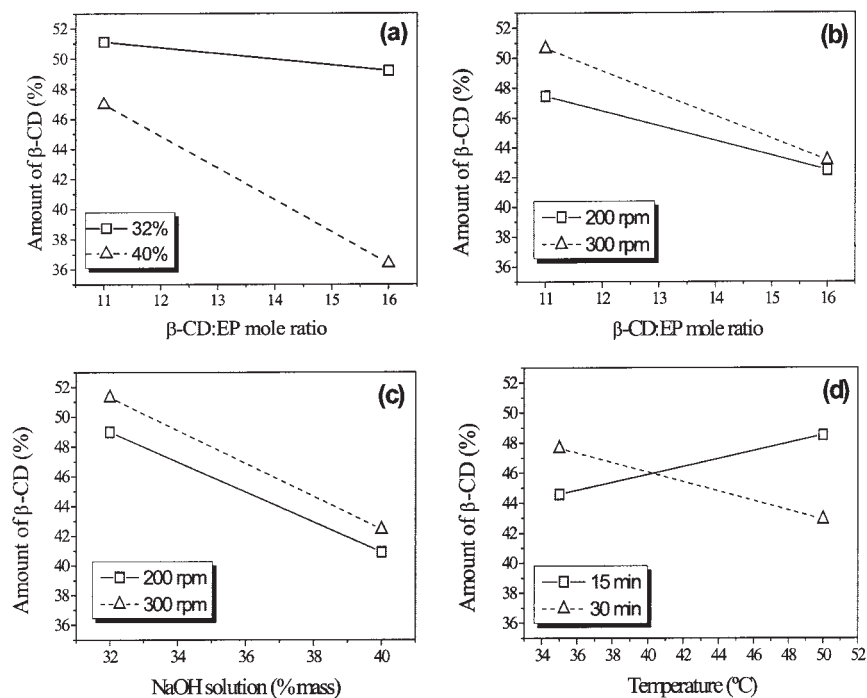
### Soluble polymers

#### Amount of $\beta$ -CD

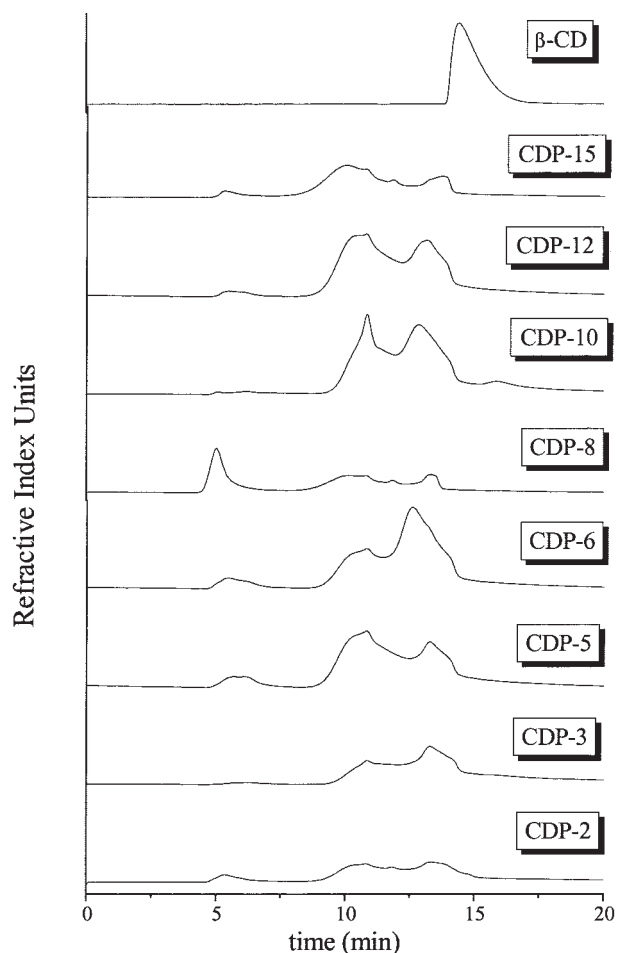
Table III collects the percentages of  $\beta$ -CD measured for the polymers. The averaged response for each variable is estimated with the values of the amounts of CD obtained for the two levels. This average response is used to determine the effect of each variable on the polymer sizes. Higher absolute values always indicate more important effects of the corresponding variable. Table IV shows the main

effects of all the variables under study, calculated as reported in the literature.<sup>12,18</sup> The results of the statistical tests (variance F-tests) showed that all the effects and interactions were non significant, and so we decided not to include them. Both the  $\beta$ -CD : EP mole ratio (variable A) and NaOH concentration (variable B) present negative effects; i.e., low levels for each one produce an increase in the amount of CD in the produced polymer. The temperature of reaction (variable C) and the time before adding paraffin (variable E) also have a negative effect, though much smaller. However, the stirring speed (variable D) has a positive effect, indicating that high levels have to be used when trying to increase the amount of  $\beta$ -CD in the polymer. The value of the effect of variable F is not included in the table because, for the soluble polymers, this variable is always in its high level and its quantitative effect cannot be determined.

Figure 1 plots the corresponding interactions between two variables. Out of the 10 possible graphs of interactions, four of them suffice to justify the conditions in which the polymerization of  $\beta$ -CD yields higher molecular weights for the soluble polymers. Figure 1(a-c) allow to deduce that the amount of CD increases when working at low levels for CD:EP ratio and NaOH concentration, whereas the stirring speed must be at its high level. As can be seen in Figure 1(d), 35°C and a time of 30 min before adding the paraffin produce high percentage of  $\beta$ -CD, although somewhat lower than those obtained when working at 50°C with



**Figure 1** Effect of the interaction between variables on the  $\beta$ -CD contents for the soluble polymers.



**Figure 2** Size exclusion chromatograms of  $\beta$ -CD and soluble CDPs.

a delay of 15 min. Parallel lines indicate no interaction between variables, as occurs in Figure 1(c).

#### Polymer sizes

The size exclusion chromatograms for the soluble polymers are shown in Figure 2. The retention times are lower for bigger particles. These chromatograms suggest that the products are mixtures of fractions of different molecular weights, as was observed by Renard et al.<sup>10</sup> As mentioned in the experimental section,

the hydrodynamic radii have been obtained by DLS. The presence of large particles, high molecular weight fractions or aggregates, is not significant as deduced from their low amounts. For instance, taking into account that the radius of  $\beta$ -CD single molecules is 0.7 nm, DLS results for CDP-10 indicate that an important fraction of unreacted  $\beta$ -CD is present for this sample, a fact confirmed by its GPC pattern. For other samples, the chromatographic peaks at high retention times correspond to larger hydrodynamic radii, a fact attributable to possible residues of EP linked to the CD structure. It can be affirmed that CDP-8 is the one closer to the gel point, since it presents a greater radius, and an intense peak for a low retention time is observed in its chromatogram.

Table III also displays the average radius of the polymer samples, calculated from the values obtained by DLS considering the mass percentage of each fraction. These values are taken as the responses used to calculate the main effects collected in Table IV. Both the concentration of NaOH and the  $\beta$ -CD:EP molar ratio present positive effects. These facts were also observed by some authors. Thus, it has been proposed that to obtain polymers of high molecular weight, the NaOH concentration must be high. This condition favors a single site substitution of  $\beta$ -CD producing a long segment of 2-hydroxypropyl ether; therefore, high molecular weight products are yielded.<sup>10</sup> Besides, crosslinked polymers were obtained for high  $\beta$ -CD:EP ratios, whereas for low mole ratios, dimers and  $\beta$ -CD ethers were produced.<sup>19</sup>

For the rest of variables, the effect is negative, i.e., low levels favor polymers of greater sizes. As it was done previously, the plots of interaction for this response (not shown; the values of interaction have been compiled in Table V) also give an idea of the best conditions to obtain soluble polymers of higher molecular weights.

#### Insoluble polymers

##### Amount of $\beta$ -CD

All the insoluble polymers present an amount of  $\beta$ -CD above 300 mg/g (see Table III), i.e., a high content of

**TABLE V**  
Interactions of Two Variables on Polymer Sizes for the Soluble Polymers

Interaction	AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
LL	4.82	5.41	5.98	4.29	4.30	3.71	3.14	12.6	11.5	10.4
HL	3.19	11.5	10.4	10.4	12.6	12.6	11.5	3.71	3.17	4.29
LH	5.44	4.86	4.29	5.98	3.71	4.30	4.87	4.30	5.46	5.98
HH	13.7	5.35	6.51	6.51	6.50	6.50	7.62	6.50	7.03	6.51

LL, low level for both variables; HL, high level for 1st variable; LH, high level for 2nd variable; HH, high level for both variables.

TABLE VI  
Effect of the Main Variables for the Water Insoluble Polymers

	A	B	C	D	E
$\beta$ -CD content	1.63	-3.79	-5.49	5.09	-1.74
Particle size	-0.029	0.339	0.479	-0.450	0.258
Sorption capacity for phenol	-0.057	-0.125	0.387	0.056	0.548
Sorption capacity for DBF	0.0013	-0.0008	-0.0063	0.0063	0.0118
Swelling capacity	-1.64	0.553	0.698	-0.833	-0.093

CD according to the classification given by Crini et al.<sup>5</sup> Our results using suspension polymerization are similar to those obtained by those authors using emulsion polymerization. Thus, for example, they find that a polymer synthesized with a ratio CD : EP of 1 : 17, at 50°C and with NaOH 23%, yield a  $\beta$ -CDP with 700 mg  $\beta$ -CD/g, whereas, our CDP-9 (CD : EP 1 : 16, 50°C, and NaOH 32%) has 670 mg/g.

The effects of the main variables on the percentage of  $\beta$ -CD are shown in Table VI. These have been calculated in the same way as the soluble polymers. The main effects correspond to temperature (negative) and stirring speed (positive). These observations agree with those found in the literature.<sup>5,10,24</sup> Renard et al.<sup>10</sup> suggest that self-polymerization of EP is favored at high temperatures, so the amount of EP in the poly-

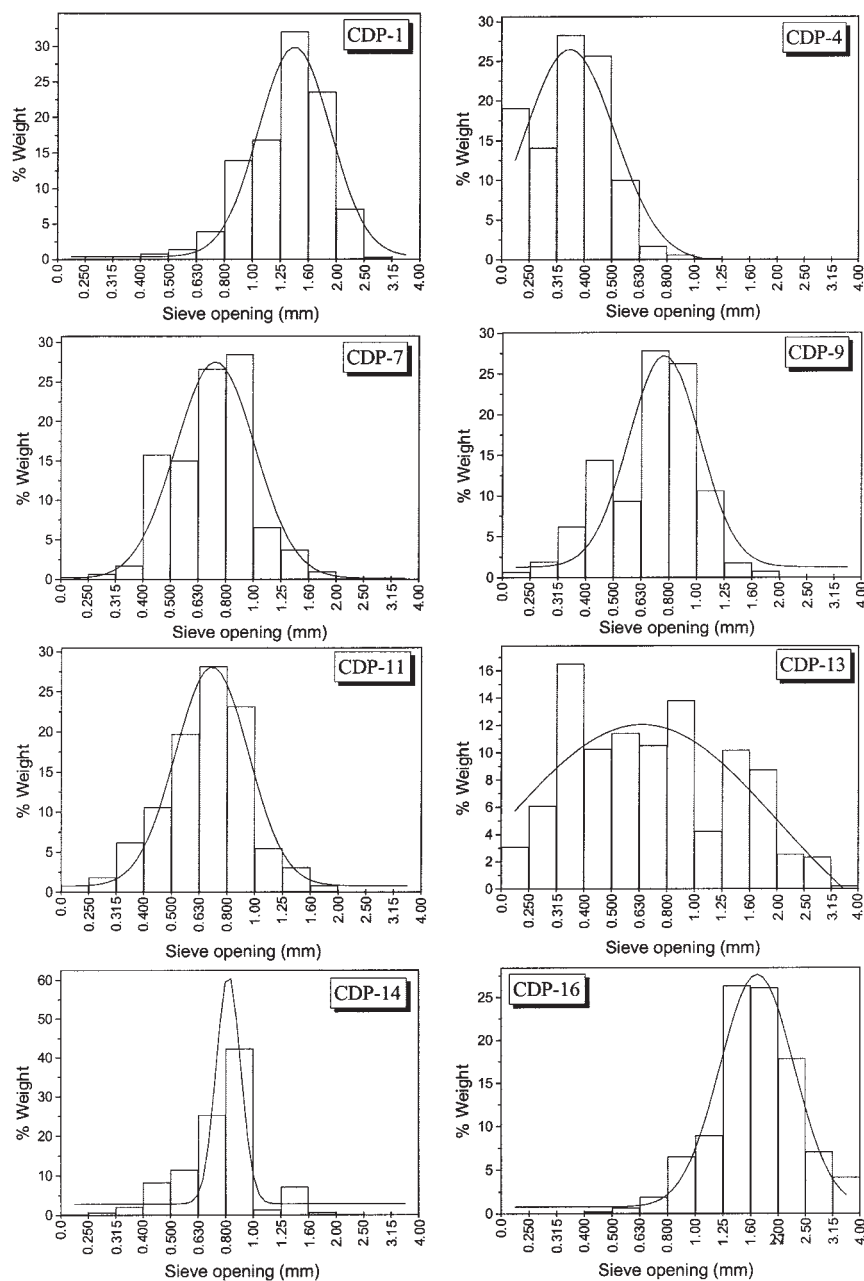
mer increases. The negative effect of the concentration of the NaOH solution can also be emphasized. As discussed in the literature,<sup>19</sup> for low NaOH concentrations, the CD:EP ratio in the product is closer to the feed ratio. The amount of EP increases for high NaOH concentrations.

Table VII collects the data obtained for the interactions of two variables. Some differences are observed between soluble and insoluble polymers with respect to the best conditions to achieve high  $\beta$ -CD contents. In both cases, the stirring speed must be 300 rpm (high level), while the concentration of NaOH solution must be 32% in weight (low level). Thus, for the rest of variables (A, C, and E), an interaction with variable F (water initially added, i.e., initial  $\beta$ -CD concentration) is deduced.

TABLE VII  
Interactions of Two Variables for the Insoluble Polymers

Interaction	AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
$\beta$ -CD content										
LL	63.3	64.2	60.7	60.7	65.3	65.3	65.0	65.3	65.8	60.7
HL	66.9	67.7	60.6	67.4	66.6	56.0	63.1	56.0	62.3	67.4
LH	61.5	60.6	64.1	64.1	64.9	64.9	65.2	66.6	66.1	60.6
HH	61.1	60.3	67.4	60.6	56.0	66.6	59.5	64.9	58.6	64.1
Particle size										
LL	0.779	0.804	1.06	1.06	0.762	0.762	0.730	0.762	0.565	1.06
HL	0.774	0.610	1.29	0.577	0.651	1.58	0.904	1.58	1.07	1.29
LH	1.14	1.12	0.865	0.865	0.791	0.791	0.823	0.651	0.848	0.577
HH	1.09	1.25	0.557	1.29	1.58	0.651	1.33	0.791	1.30	0.865
Sorption capacity for phenol										
LL	4.41	4.25	4.02	4.02	4.13	4.13	4.20	4.13	3.85	4.02
HL	4.30	3.95	4.51	4.02	4.07	4.40	3.83	4.40	4.20	4.02
LH	4.24	4.39	4.62	4.62	4.58	4.58	4.51	4.07	4.35	4.51
HH	4.23	4.58	4.02	4.51	4.40	4.07	4.63	4.58	4.78	4.62
Sorption capacity for DBF										
LL	0.966	0.971	0.954	0.954	0.964	0.964	0.963	0.964	0.964	0.954
HL	0.962	0.963	0.967	0.962	0.970	0.957	0.953	0.957	0.952	0.967
LH	0.960	0.956	0.972	0.972	0.964	0.964	0.966	0.970	0.970	0.962
HH	0.967	0.966	0.962	0.967	0.957	0.970	0.974	0.964	0.969	0.972
Swelling capacity										
LL	4.44	4.99	5.43	5.43	3.94	3.94	4.29	3.94	3.59	5.43
HL	3.30	2.61	3.70	3.70	3.66	5.19	4.10	5.19	4.79	2.96
LH	5.49	4.94	4.50	4.50	3.80	3.80	3.45	3.66	4.00	3.70
HH	3.35	4.05	2.96	2.96	5.19	3.66	4.75	3.80	4.20	4.50

LL, low level for both variables; HL, high level for 1st variable; LH, high level for 2nd variable; HH, high level for both variables.



**Figure 3** Size distribution for the insoluble CDPs.

### Particle sizes and distribution

The size distributions for the different insoluble products are shown in Figure 3, together with the corresponding fitted Gaussian curves. CDP-13 has the broadest distribution, and CDP-14 the narrowest. A high level in the stirring speed seems to represent the main factor responsible for broader distributions. The mean particle sizes, shown in Table III, are used to calculate the effects of the main variables (Table VI) and the two variable interactions (Table VII). It can be noticed that the variables with a greater influence are the temperature, the stirring speed, and the NaOH concentration. The polymer particle sizes increase when the synthesis is carried out

high levels of  $\beta$ -CD:EP mole ratio, NaOH concentration, temperature, and time elapsed, while the level of the stirring speed must be low.

Certain similarities are observed when comparing the data collected for soluble and insoluble polymers. In both cases,  $\beta$ -CD:EP and the NaOH concentration should be in their respective high levels. As mentioned earlier, high temperatures favor longer EP chains.

### Swelling in water

The values obtained for the degrees of swelling (Table III) are used to calculate the effects of the main vari-



ables and their interactions (Tables VI and VII, respectively). The most important effect corresponds to the CD : EP molar ratio, followed by the stirring speed and the concentration of NaOH. Some of the preferred conditions of synthesis to yield high swelling degrees are related to those obtained for the particle sizes. For instance, the NaOH concentration has an analogous effect. Longer hydroxypropyl ether segments between CDs also favor the expansion of the macromolecular network and consequently produce higher degrees of swelling. The polymers with lower percentage of  $\beta$ -CD yield smaller particles and degrees of swelling. A different result is achieved when EP is used as a crosslinking agent of a linear polymer such as a polyamine. Increasing the amount of EP in the gel synthesis causes the resulting gel to be more tightly crosslinked. This reduces its swelling in water, thus improving its mechanical integrity.<sup>25</sup>

#### Sorption capacities for phenol and DBF

Prior to the determinations of the sorption capacities for phenol and DBF, the possible effect of the particle size on the sorption capacity was tested using nine fractions of CDP-9, their diameters ranging between 0.250 and 2.00 mm. No significant differences were found. The swollen particle fractions seem to be chemically homogeneous, in agreement with a previous work by Zhu and Brizard.<sup>8</sup>

In the case of phenol, the results of Table III are comparable to those obtained by Crini et al.<sup>5</sup> All the polymers synthesized in this work have either similar or higher sorption capacities when compared with those of high content in  $\beta$ -CD obtained by them. In general, an increase in the amount of  $\beta$ -CD corresponds to an increase in the sorption capacity. This corroborates the idea that inclusion phenomena play a most important role in the sorption mechanism.<sup>26</sup>

The effects of the main variables for phenol and DBF sorption capacities are shown in Table VI. The data of the interactions of two variables appear in Table VII. Notice that only relative comparisons of the effects for each sorbate are significant. The absolute values depend on the magnitude of the measurements in each case. Nevertheless, some interesting differences are found for both sorbates. The time elapsed before adding the paraffin seems to have a similar influence for phenol and DBF sorptions. NaOH concentration has a negative influence, though quantitatively small for DBF. On the other hand, temperature and  $\beta$ -CD : EP ratio show opposite effects.

These differences between both aromatic compounds can be related to their different structures and polarities. DBF is more susceptible to be trapped in the hydrophobic  $\beta$ -CD cavity than phenol, producing a less stable inclusion complex.<sup>5</sup> The inclusion complex constant with  $\beta$ -CD is about 20 times higher for

DBF.<sup>27,28</sup> The adsorption of phenol in the network structure, by means of hydrophobic and/or hydrogen bonds, can be more important than that of DBF. As shown previously, high temperatures favor EP self-polymerization, creating favorable sites for the interaction with phenol but not with DBF, which occupies the CD cavities. It has to be added that the sorption capacities of DBF correspond to values ranging between 85 and 88% of the total amount of sorbate in the solution, while for phenol, they have values between 26 and 35%. Because of the extremely low solubility of DBF, this test could not be accurate enough. Most of the sorbate molecules can be included within CD cavities, so the latter are far from saturation. In the case of phenol, the higher concentrations used allow a distribution of the sorbate among sites of different interacting energies.

#### CONCLUSIONS

The aim of this work was to select the optimal conditions to obtain crosslinked CD hydrogel particles. In this system, the concentration of the  $\beta$ -CD solution cannot be as low as 33%, since the reaction would not reach the gel point. This shows the importance of variable F. A more rigorous analysis would require a new design considering factor F as a constant, to improve the evaluation of the other variables. Nevertheless, our experimental design approach permits to select the optimal conditions of synthesis depending on the desired features of the product.

The amount of  $\beta$ -CD of the soluble polymers is higher when all the variables, except the stirring speed, are at their low levels. These polymers present greater sizes when the synthesis is carried out using high CD:EP mole ratios and NaOH concentration, a fact also observed for the insoluble products.

For insoluble polymers, high values for the response variables, with the exception of the swelling degree, have been found when the delay time between the additions of NaOH and paraffin was 30 min. High polymerization temperatures favor the phenol sorption capacity, as well as greater particle sizes and degrees of swelling. On the other hand, the synthesis should be carried out at 35°C when looking for a high capacity of DBF sorption, or higher  $\beta$ -CD contents in the polymer. This must be related to the fact that EP reacts faster as the temperature increases, so more units are incorporated to the product. Nevertheless, it has to be added that all the insoluble polymers synthesized in this work present high  $\beta$ -CD contents. There is a certain parallelism between the conditions of synthesis necessary to obtain a high  $\beta$ -CD content and the capacity of sorption of DBF. This can be due to the fact that the DBF molecule has both the size and the polarity compatible with the  $\beta$ -CD cavity to form stable complexes. The situation changes when phenol

is the analyzed sorbate. The adsorption of phenol is greatly determined by its interaction with the polymer network. The inclusion complexes of phenol with  $\beta$ -CD are possible but not as stable as those of DBF. Last, the polymers with greater swelling degrees and particle sizes are those with lower amounts of  $\beta$ -CD. These polymers have linking segments of 2-hydroxypropyl ether of greater lengths, so the swelling in water is facilitated, yielding bigger particle sizes.

## References

- Szejtli, J. Cyclodextrins and Their Inclusion Complexes; Kluwer Academic: Dordrecht, 1988.
- Fenyvesi, E.; Szeman, J.; Szejtli, J. *J Inclusion Phenom Mol Recognit Chem* 1996, 25, 229.
- Elias, H. G. *Macromolecules*, 2nd ed.; Plenum: New York, 1984.
- Edward, V. U.S. Pat 5,075,732 (1991).
- Crini, G.; Bertini, S.; Torri, G.; Naggi, A.; Sforzini, D.; Vecchi, C.; Janus, L.; Lekchiri, Y.; Morcellet, M. *J Appl Polym Sci* 1998, 68, 1973.
- Shao, Y.; Martel, B.; Morcellet, M.; Weltrowski, M.; Crini, G. *Proceedings of the 8th International Symposium on Cyclodextrins*, Budapest, Hungary, 1996; p 571.
- Murai, S.; Imajo, S.; Maki, Y.; Takahashi, K.; Hattori, K. *J Colloid Interface Sci* 1996, 183, 118.
- Zhu, X. X.; Brizard, F. *Pure Appl Chem* 1997, A34, 335.
- Bertini, S.; Crini, G.; Naggi, A. M.; Suardi, R.; Torri, G.; Vecchi, C.; Janus, L.; Martel, B.; Morcellet, M. *Proceedings of the 9th International Symposium on Cyclodextrins*, Santiago de Compostela, Spain, 1998; p 175.
- Renard, E.; Deratani, A.; Volet, G.; Seville, B. *Eur Polym J* 1997, 33, 49.
- Wiedenhof, N.; Lamers, J. N. J. J.; Van Panthaleon Van Eck, C. L. *Die Stärke* 1969, 21, 119.
- Montgomery, D. C. *Design and Analysis of Experiments*, 5th ed.; Wiley: New York, 2000.
- Shan, C. L. P.; Soares, J. B. P.; Pendilis, A. *J Polym Sci Part A: Polym Chem* 2002, 40, 4426.
- Chen, C. H. *J Appl Polym Sci* 2002, 85, 1571.
- De Wet-Roos, D.; Knoetze, J. H. *J Appl Polym Sci* 1999, 71, 1347.
- De Wet-Roos, D.; Knoetze, J. H.; Cooray, B.; Sanderson, R. D. *J Appl Polym Sci* 2000, 76, 368.
- Vivaldo-Lima, E.; Wood, P. E.; Hamielec, A. E.; Pendilis, A. *J Polym Sci Part A: Polym Chem* 1998, 36, 2081.
- Cochran, W. G.; Cox, G. M. *Diseños Experimentales*; Trillas: México, 1974.
- Yudiarto, A.; Kashiwabara, S.; Tashiro, Y.; Kokugan, T. *Sep Pur Tech* 2001, 24, 243.
- Crini, G.; Cosentino, C.; Bertini, S.; Naggi, A.; Torri, G.; Vecchi, C.; Janus, L.; Morcellet, M. *Carbohydr Res* 1998, 308, 37.
- Gao, Z.; Zhao, X. *Polymer* 2004, 45, 1609.
- Isasi, J. R.; Romo, A.; Seville, X.; González-Gaitano, G.; Peñas, J.; *Proceedings of the 11th International Symposium on Cyclodextrins*, Reykjavik, Iceland, 2002.
- Cox, D. R. *Planning of Experiments*; Wiley: New York, 1958.
- Renard, E.; Barnathan, G.; Deratani, A.; Seville, B. *Macromol Symp* 1997, 122, 229.
- Kofinas, P.; Kiuoussis, D. R. *Environ Sci Technol* 2003, 37, 423.
- Crini, G. *Bioresource Technol* 2003, 90, 193.
- Rekharsky, M.V.; Inoue, Y. *Chem Rev* 1998, 98, 1875.
- Rodríguez, P.; Sánchez, M.; Isasi, J. R.; González-Gaitano, G. *Appl Spectrosc* 2002, 56, 1490.