

## Chelating Performance Evaluation of Ion Exchange Resin Chelex-100

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**Abstract**—This study aims to answer the question about the chelating capacity of Chelex-100 resin according to the size of grains obtained after different grindings of a 200–400 mesh powder. Optical microscopy was used for size characterization of different new sub-types of resin obtained after grinding. The copper chelating capacity was evaluated using inductively coupled plasma–mass spectrometry. Grinding enables copper to be chelated by all iminodiacetate groups of the resin. Thus, the copper chelating capacity increases by 17% compared to unground resin and is more repeatable from one aliquot to another (1% RSD with optimal grinding versus 3% without grinding).

**Keywords:** Chelex-100 chelating capacity grinding copper

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Chelex is an ion exchange resin consisting in iminodiacetate (IDA) groups incorporated in a styrene-divinylbenzene matrix [1–3]. Polyvalent (typically divalent and trivalent) metal ions have a strong affinity for IDA groups that act as chelating sites [1, 4]. Chelex is widely used for the removal of metal elements in effluents and the preconcentration of metal elements originally present at very low concentrations in aqueous samples [3, 5–7]. There are two types of Chelex, generally supplied as a wet white bead powder: Chelex-20 and Chelex-100 corresponding to technical and analytical grade respectively, i.e. raw and treated resin respectively. The resin treatment consists in a chemical purification and a size-based sorting [1]. Among commercial analytical resins, there are three sub-types of Chelex-100 with three ranges of bead sizes, 50–100, 100–200 and 200–400 mesh respectively [1, 3]. Chelex-100 beads have a microporous structure, with an average pore size of 1.5 nm [8–10]. Their external surface contribute little to the total chelating capacity, suggesting that IDA groups are mainly located at the interior of the beads [11, 12].

Whatever the Chelex-100 sub-type, suppliers usually indicate a metal chelating capacity value of ~0.6 or ~0.7 meq/g (wet resin) resin. These values are often directly quoted in literature and used without any prior verification [1, 2, 13, 14]. However, there is no sense to provide the value of capacity of the wet resin without specifying the humidity percentage. Therefore, some

suppliers sometimes indicate an unique metal chelating capacity value of 2.9 meq/g (dry resin) [15–19]. In fact, this value is theoretical, and obtained by calculation using the reference capacity value of ~0.6 meq/g (wet resin) and considering a humidity percentage of ~80%. However, the humidity percentage may differ from one batch to another, and also in the same batch over time. In addition, quoting such values implies that the capacity would be absolute and identical for all metals considered, regardless the Chelex-100 sub-type, i.e. the bead size range. Few authors determined the humidity percentage and the capacity of the resin by performing acido-basic titration. They reported a capacity value of 2.0 mmol (active groups)/g (dry resin), corresponding to all IDA groups (IDA) contained in the resin [10, 20–22]. Pesavento et al. [23] and Alberti et al. [24] indicated 2.0 mmol (IDA)/g (dry resin) and 1.6 mmol (Cu<sup>2+</sup>)/g (dry resin), corresponding to the maximal capacity of the resin and the capacity relative to copper chelation respectively.

Regarding recent analytical applications, Diffusive MilliGels (DMG) were Chelex-100 based samplers developed in our laboratory using a millifluidic system. They were used for evaluating labile copper in environmental waters [25–27]. Indeed, copper is of high environmental interest because of its duality essentiality-toxicity. Especially, it is used as biocide in various activities including agriculture. The millifluidic system being composed of tubing with an internal

diameter close to bead size, Chelex-100 had to be ground before introduction in the system to avoid clogging. However, there is no study dealing with the effect of grinding on Chelex-100 performances.

In the present paper, an original approach was developed to answer the question about the effective chelating capacity of Chelex-100 according to its size. For that, a commercial Chelex-100 was ground to obtain new sub-types of resin with different size ranges. Sizes were determined using optical microscopy and copper chelating capacities by inductively coupled plasma–mass spectrometry (ICP–MS). Copper was selected because of its harmlessness and strong affinity with Chelex-100 at pH  $\sim$  4–5 [1]; pH 4.50 was selected both because there is information in literature about the affinity of metal ions with Chelex-100 at this pH and also because there is no risk of copper precipitation [1, 23, 28].

## EXPERIMENTAL

**Reagents and materials.** Chelex-100 was purchased from Sigma-Aldrich in sodium form with a range of size of 200–400 mesh. Chelex beads were expected to have size between 75 and 150  $\mu\text{m}$ . The supplier indicated a metal chelating capacity of  $\sim$ 0.6 meq/g (wet resin) and a humidity percentage of  $\sim$ 80%. This percentage was precisely determined by drying a known amount in an oven at 110°C to constant weight; the result is presented in the section “Results and discussion.”

Acetic acid ( $\text{CH}_3\text{COOH}$ ; 99.99%) and sodium hydroxide monohydrate ( $\text{NaOH}$ ,  $\text{H}_2\text{O}$ ; 99.99%) were purchased from Sigma-Aldrich to prepare acetate buffer (pH 4.50). Solution of concentrated nitric acid ( $\text{HNO}_3$ , 69–70%) was purchased from Atlantic laboratory and used to prepare 5%  $\text{HNO}_3$  solutions by dilution with ultra-pure water (18  $\text{M}\Omega$  cm, Milli-Q system).

Copper(II) nitrate trihydrate ( $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , 99.5%) was purchased from Merck and used to determine the copper chelating capacity of all Chelex-100 sub-types considered (ground or not; see below). Standard solution of copper at 1000 mg/L (dissolved Cu in 4%  $\text{HNO}_3$ , 99%) was purchased from SCP Sciences and used for the calibration of elemental analysis. Standard solution of yttrium at 1000 mg/L (dissolved Y in 4%  $\text{HNO}_3$ , 99%) was purchased from SCP Sciences and used as internal standard for ICP–MS analysis.

**Chelex-100 grinding.** Grinding was performed in triplicate with a Retsch MM 200 model mixer mill using Teflon jars and Teflon beads with a diameter of 10 mm. Depending on the grinding conditions, from the Chelex-100, several powders with different characteristics were obtained, which were named hereinafter “new sub-types” with reference to those defined according to the grain sizes (see below). As for

unground Chelex-100, humidity percentages were determined for all sub-types and obtained values are presented in the section “Results and discussion.”

**Strategy for Chelex-100 capacity evaluation.** The study focussing on the effect of Chelex-100 grinding on its copper chelating capacity, the operating parameters (temperature, copper and Chelex-100 amounts, pH and composition of the medium, mixing conditions) were fixed. All the experiments were performed at room temperature.

First, a reference copper solution consisting in a solution of  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  was prepared in 0.01 M acetate buffer at pH 4.50. Then, for both unground and ground Chelex-100, an aliquot of about 25 mg of Chelex-100 and 10 mL of reference copper solution were introduced in a 60 mL polypropylene flask. In these proportions, copper amount was in small excess compared to IDA amount (based on the published maximal capacity value of 2 mmol (IDA)/g (dry resin) [10, 20–22]). All mixtures were gently shaken with an orbital shaker (Edmund Bühler GmbH) for 12 h. The shaking duration was chosen to ensure the chelation equilibrium of copper by Chelex-100 was achieved [2, 23]. Then, the mixtures were filtered at 0.20  $\mu\text{m}$  to remove Chelex-100. Only filtrates, named thereafter “samples,” were considered, after verification of the total removing of Chelex-100 and the absence of significant sintering (see below the part dedicated to additional mixtures). Finally, the amount of copper in samples (which corresponding to non-chelated copper i.e. the copper excess) was determined, and the capacity of Chelex-100 deduced by indirect quantification. Blanks, consisting in 25 mg of Chelex-100 (unground or ground) in 10 mL of Milli-Q water, were prepared and analyzed in the same manner as samples. All the blanks and samples were prepared in triplicate.

From unground and ground Chelex-100, 3 additional mixtures were considered. These mixtures were prepared by introducing about 25 mg Chelex-100 in 10 mL of solutions with quantities of copper equal to about 90% of the published copper chelating capacity, i.e.  $\sim$ 1.4 mmol ( $\text{Cu}^{2+}$ )/g (dry resin) [23, 24]. After equilibration step, these mixtures were filtered at 0.20  $\mu\text{m}$  and all the samples were analyzed. The copper signal obtained for all samples was inferior to the limit of detection. This proved that all the copper was chelated with Chelex-100, and all ground Chelex-100 was in the form of grains quantitatively retained in the filter during the filtration step.

**Optical microscopy.** Unground and ground Chelex-100 were characterized by optical microscopy (Leica microscope). For each, 3 aliquots were taken and within each aliquot, 3 images from different places were taken for microscopy measurements.

**Elemental analysis.** Total copper analysis was performed by an Agilent 7900 quadrupole ICP–MS equipped with an octopole collision/reaction cell (CRC). Both stable isotopes of copper were moni-

**Table 1.** Chelex-100 grinding conditions

Condition	A*	B	C
Duration, min	3 series of 10	3 series of 10	3 series of 10
Frequency, Hz	30	30	30
Unground Chelex-100 mass, g	1.5	1.5	<b>0.5</b>
Number of Teflon grinding beads	2	<b>4</b>	<b>4</b>

\* Chelex-100 grinding conditions derived from Perez et al. [25, 26].

tored, i.e.  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$ . The operating parameters were used as follows: sample and skimmer cones, nickel; plasma and make up gas, argon; plasma gas flow rate, 0.9 L/min; make up gas flow rate, 0.3 L/min. The CRC was used in helium mode with a flow rate of 4.3 mL/min in order to eliminate potential polyatomic interferences. All these parameters were optimized using a solution of 1 mg/L Li, Y, Tl, Ce in 2 wt %  $\text{HNO}_3$ . The limits of detection were 17 and 21 ng/L for  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  respectively. The reference copper solution without Chelex-100 addition was also analyzed to validate the accuracy of the analysis. The ICP-MS analytical performances were evaluated by the analysis of a certified reference water (TMDA-64.3, batch 0316 made with water from the Lake Ontario). The analysis was proved to be accurate: trueness evaluated by a recovery  $>99.1\%$  and repeatability precision estimated by a RSD  $<1.2\%$ .

## RESULTS AND DISCUSSION

**Preparation of new sub-types of Chelex-100.** First, the resin grinding protocol was investigated to obtain new sub-types of Chelex-100. For that, four samples were considered, that is unground Chelex-100, and three Chelex-100 ground under the conditions presented in Table 1. The conditions A were selected in this paper because they are based on those by Perez et al. [25, 26]. In their work, a grinding oscillation frequency of 30 Hz corresponding to the maximum frequency that the mixer mill could reach was used; each grinding series did not exceed 20 min to avoid heating which could alter the chelating properties of the resin [25, 26]. Therefore, in the present study, 3 series of 10 min of grinding were carried out and between each series, a step of manual soft mixing of Chelex-100 using a wooden spatula was added.

From conditions A described above, two other grinding conditions were proposed: conditions B for which the number of Teflon grinding beads in each jar was increased to 4; conditions C for which 4 beads into each jar were also used and the Chelex-100 amount was reduced to 0.5 g.

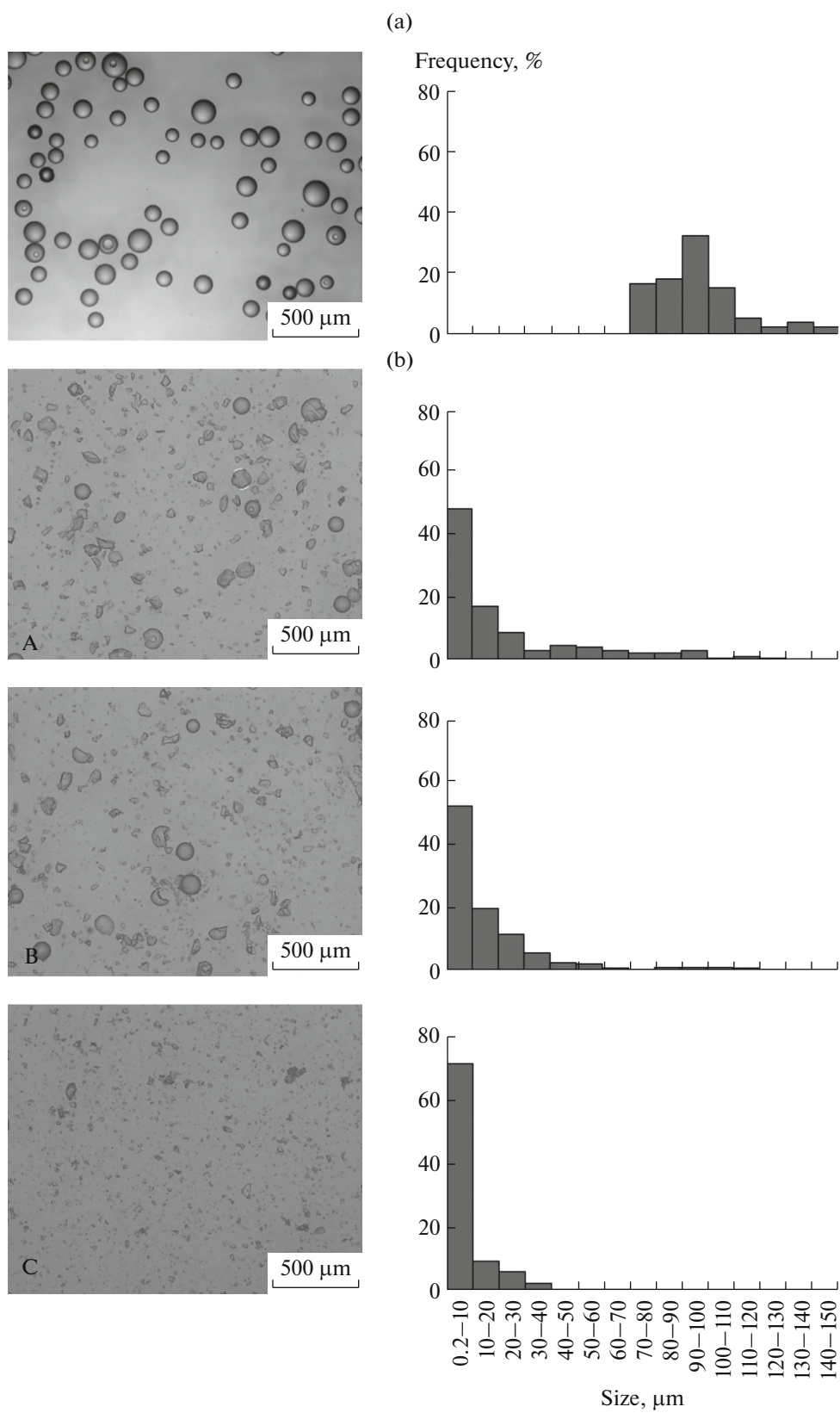
The humidity, determined in triplicates, was found to be  $74.1 \pm 0.7\%$  for all sub-types. This value showed that the humidity was not modified whatever the grinding conditions used and it was significantly lower

than humidity value indicated by the supplier. Therefore, it is important to measure it regularly.

**Optical characterization of Chelex-100.** Unground Chelex-100 was first size characterized as the reference in this investigation. The corresponding image is presented in Fig. 1a. It displays a size distribution from  $76 \pm 2$  to  $149 \pm 5 \mu\text{m}$  with a maximum number of beads at about  $100 \mu\text{m}$ . This range of size is in agreement with the supplier specifications concerning the commercial Chelex-100, 200–400 mesh.

Images and graphs presented in Fig. 1b provide data on Chelex-100 new sub-types resulting from grinding A, B and C respectively. The sub-types A and B contain grains with very different sizes as well as some original beads resulting from incomplete grinding. The corresponding size distributions show a minimum size class from 0.2 to  $10 \mu\text{m}$ ; this class is majority in number (49 and 53% respectively for A and B). Compared to the original unground Chelex-100, the maximum size decreased to  $122 \pm 2 \mu\text{m}$ , and the two largest size classes (i.e. 130–140 and 140–150  $\mu\text{m}$ ) were no longer present. It means that the largest Chelex-100 beads were all broken. Some grains in the new sub-types A and B were larger than  $76 \pm 2 \mu\text{m}$  (corresponding to the minimum size of original beads (see image Fig. 1a)). They may be either original unground or partially ground Chelex-100. In summary, after grinding with conditions A and B, the mean size of Chelex-100 decreased significantly as expected but the size distribution widths and so polydispersities increased due to incomplete grinding. However, the B size distribution appears narrower than the A one, with less than 10% in number superior to  $40 \mu\text{m}$ . Considering also the smallest size, class 0.2– $10 \mu\text{m}$  was more numerous in number for B than A. All of this suggests that using four Teflon grinding beads (B) was slightly but significantly more efficient than only two ones (A).

Unlike A and B, the C new sub-type appears to be the result of a complete grinding with no intact original bead remaining (Fig. 1b, sub-type C). This is confirmed by the maximum size inferior to  $70 \mu\text{m}$ , which is also inferior to the minimum size of unground resin. In addition, the polydispersity was low, with more than 70% in number between 0.2 and  $10 \mu\text{m}$ , and less than 2% between 40 and  $70 \mu\text{m}$ . In summary, in conditions C using four Teflon grinding beads and decreasing the mass of resin from 1.5 to 0.5 g, the most



**Fig. 1.** Selected microscopy images and associated size distributions of unground Chelex-100 (a) and ground Chelex-100 according to grinding protocols A, B and C (b).

efficient grinding was achieved in terms of size and polydispersity.

**Evaluation of the copper chelating performance of Chelex-100.** The chemical influence of Chelex-100 grinding was investigated by determining the copper chelating capacity. The corresponding values are reported in Table 2. For unground Chelex-100, this capacity is in accordance with the value determined by Pesavento et al. [23] and by Alberti et al. [24], i.e., 1.6 mmol (Cu<sup>2+</sup>)/g (dry resin), for which no uncertainty was associated. However, if the same value of RSD obtained in the present study (3%) is considered, both chelating capacities values would not be significantly different. Nevertheless, by repeating this measurement several times, the copper chelating capacity obtained for unground Chelex-100 were consistently greater than 1.6 mmol (Cu<sup>2+</sup>)/g (dry resin). This suggests a tendency that can not be explained by the random error. In addition, the value of capacity published by Pesavento et al. and Alberti et al. referred to Chelex-100, 100–200 mesh whereas the value obtained in the present work referred to Chelex-100, 200–400 mesh. Since Chelex-100, 200–400 mesh is smaller than Chelex-100, 100–200 mesh, this suggests copper chelating capacity may increase slightly as Chelex-100 size decreases.

At first glance, the new sub-type A and unground Chelex-100 (Table 2) also do not appear to exhibit significantly different copper chelating capacities. This is due to the important RSD associated to the A capacity (7% against only 3% for unground resin).

The copper chelating capacity determined for Chelex-100 ground with conditions B is higher than with conditions A and is significantly higher than for unground Chelex-100. The copper chelating capacity determined for Chelex-100 ground with conditions C is maximal and significantly higher than all the other values.

In summary, the copper chelating capacity of Chelex-100 increased significantly while its size decreased (correlation coefficient  $r = -0.68$ ; significant in a 95% confidence interval). With the C grinding which leads to the smallest sizes of grains, the copper chelating capacity of Chelex-100 is improved by 17% compared to the one of original commercial Chelex-100 (unground). Moreover, this improved copper capacity corresponds to the maximal capacity of Chelex-100, i.e. the capacity obtained by acid-basic titration [23, 24]. This could be explained by the microporosity of Chelex-100; consequently, some IDA groups deep within the resin were not accessible for copper so that this element could not be chelated. Thus, grinding the resin increased accessibility to these IDA groups and made them all functional, while they are still all accessible for H<sub>(aq)</sub><sup>+</sup> even without Chelex-100 grinding. Thus, the C grinding of Chelex-100 increases optimally its copper chelating capacity.

**Table 2.** Copper chelating capacity ((mmol (Cu<sup>2+</sup>)/g (dry resin)) of unground and ground Chelex-100

Unground	Ground		
	A	B	C
1.68 ± 0.05 (3)*	1.8 ± 0.1 (7)	1.82 ± 0.09 (5)	1.97 ± 0.02 (1)

\* RSD, %.

RSDs associated with the determination of these capacities appear to depend on grinding conditions. The tendency observed suggests RSD could be related to polydispersity, larger for A and B and smaller for C than for unground Chelex-100. Considering all the new sub-types of Chelex-100, a correlation ( $r = 0.99$ ; significant in a 95% confidence interval) can be established between RSDs and the relative size range (i.e., the ratio between size range and median). Indeed, copper chelating capacity depending on size as explained above, a polydispersity in size induces a polydispersity in chelating capacities.

Note that the reproducibility in terms of size and polydispersity decreased from 3 to 1% between the original unground Chelex-100 and grinding C. If grinding is not efficient enough, the polydispersity and the irreproducibility it induces lead to a lack of accuracy in the determination of the chelated amounts. Therefore, conditions A and B are not recommended for future uses.

## CONCLUSIONS

This work demonstrated that Chelex-100 grinding with condition C resulted in a new sub-type of Chelex-100 with unprecedented chelating performances. Indeed, this enables IDA groups of the resin to be totally accessible for copper, leading to both higher copper chelating capacity and reproducibility. The optimal grinding determined in this study enables Chelex-100 to be dispersed more efficiently than usual due to its smaller sizes. Consequently, the exchange surface between the resin grains and the surrounding medium is increased, which contributes to optimal chelating performances. For future application with Chelex-100-based samplers, this new sub-type should also enable samplers to be more efficient with a better accuracy, i.e. trueness and precision, due to its better robustness and reliability.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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