Interaction of Alginic Acid with Organic Diacidic Base Piperazine

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The molar binding ratio (x_{pip}) of piperazine (*i.e.*, piperazinium ion) to a carboxylate group of alginate (Alg) was studied by using the titration/dialyzing method in a salt-free system. The value of x_{pip} at the equivalence point of piperazine to alginic acid was far less than unity, because the dominant ionic species of piperazine at the equivalence point (pH 5.0) was bivalent piperazinium cation. The ratio increased further with the amount of added piperazine, leveling off at unity after adding a sufficient quantity of piperazine, where the dominant species was univalent cation. On the other hand, competition of alkali metal ion with piperazinium ion for Alg was observed when MCl (M = Li^+, Na^+, or K^+) was added. The effect was in the order of $Li^+ > Na^+ > K^+$. The sum of x_{pip} and x_M (binding ratio of M) was almost unity even at low pH where dominant species of piperazine was bivalent piperazinium cation. This fact suggests that a bivalent piperazinium cation is bound to a carboxylate group of Alg through one of two positively charged sites while another site captures chloride anion as another counter ion by electrostatic force.

Keywords polyelectrolyte; alginic acid; alginate; piperazine; piperazinium ion; counter ion binding; competitive binding; alkali metal ion

Alginic acid (HAlg) is an acidic polysaccharide, which is a copolymer of β-D-mannuromic acid (M) and α-L-guluronic acid (G).[1] HAlg is almost insoluble but swells in water, but it becomes soluble with increases in a solution's pH due to dissociation from the alginate ion (Alg). Binding of an inorganic counter ion to Alg and other related polymers, such as arabate, has already been discussed by some authors, taking the Manning's theory into consideration.[2,3] On the other hand, the binding mechanism for organic ions is rather complicated. Surface active cation, for example, is cooperatively bound, showing a sigmoid-type binding isotherm owing to electrostatic interaction together with a hydrophobic effect.[4] It is also reported that some medical drugs such as bacampicillin (penicillin antibiotics), tobramycin, and gentamicin (aminosugar antibiotics) are bound to Alg,[5] probably by the mechanism of ion-ion and/or ion-dipole interaction concomitant with one another, as in hydrogen bonding.

Another significant factor to be considered in the discussion of the organic counter ion binding toward Alg is the dissociation/association constant. The electric charge of a multivalent organic cation, which usually contains nitrogen atoms, increases with a decrease in solution pH and, therefore, the affinity for Alg also changes with pH. However, the discussion taking into consideration the effect of the charge of an organic ion and/or solution pH on the binding ratio of a counter ion has been unsatisfactory to date.

In the present paper, binding of diacidic base piperazine to HAlg in the presence or absence of alkali metal chloride will be discussed by means of titrimetry and the equilibrium dialysis method, taking into account the equilibrium constants. Piperazine behaves as a bivalent cation (H_2pip^{2+}), univalent cation ($Hpip^+$), or free base, depending on the solution pH, where the first and second acid dissociation constants are $10^{-5.57}$ ($=K_{a1}$) and $10^{-9.81}$ ($=K_{a2}$).[6] The nondissociated carboxylate groups of HAlg and H_2pip^{2+} are dominant species in low pH solutions, whereas a dissociated carboxylate group and $Hpip^+$ are dominant in moderate pH solutions. That is, piperazine is expected to be bound to a carboxylate group mainly in the form of H_2pip^{2+} and $Hpip^+$ in low and moderate pH solutions, respectively, consuming a hydrogen ion from HAlg. The titration method or neutralization method used in this report is convenient because it excludes the metal cation effect which may arise when alginate metal salt is used instead of HAlg.

Experimental

Materials All the reagents used in the present study were of analytical grade. These were used without further purification. HAlg was purchased from Nakarai Chemicals Ltd. Its molecular weight, M, was reported as 1.7×10^4 by the manufacturer. Sodium alginate (NaAlg) was kindly provided by Sakai Chemical Industry Co., Ltd. The viscosity average molecular weight, M, of the latter was determined as 4.7×10^5 by means of the following equation,[7]

$$[\eta] = 2.0 \times 10^{-5} M$$

where $[\eta]$ means the intrinsic viscosity in 0.1 M NaCl at 20 °C in dl/g units.

HAlg and NaAlg were analyzed by means of circulater dichroism (spectropolarimeter, JASCO J-600)[8] and ^{1}H-nuclear magnetic resonance (^{1}H-NMR (400 MHz, NMR spectrometer, JEOL GSX-400))[9] after partial hydrolysis by HCl.[10] The gluronate fraction (F_G) and mannuronate fraction (F_M) for HAlg were found to average 0.53 and 0.47, respectively. The fractions for NaAlg were 0.55 and 0.45, while the fraction of the consecutive gluronate block (F_{GG}), that of the consecutive mannuronate block (F_{MM}), and that of the alternative block composed of gluronate and mannuronate (F_{GM}) were determined to be 0.40, 0.31, and 0.29 by ^{1}H-NMR, respectively.

Since HAlg powder usually contains some water, the concentration of HAlg was determined by means of titrimetry with a standard solution of NaOH. The concentration of NaAlg was determined by weighing the residue after drying at 105—110 °C. Henceforth, the concentration of HAlg and NaAlg will be shown by that of the molarity units of carboxylate group on sugar units. As the formula weight of the sugar unit is 176.1 for HAlg and 198.1 for NaAlg, 1 mM of Alg is equivalent to 176.1 mg/l HAlg or to 198.1 mg/l NaAlg.

Titration 9.72 mM HAlg (100 ml) was titrated with 18.7 mM NaOH or 9.29 mM piperazine at room temperature. The titration curves are shown as a function of the molar mixing ratio of added based to the carboxylate group.

Dialysis Equilibrium Dialysis equilibrium was attained with gentle shaking at 7 or 30 °C for 24 h in the following way: Visking cellulose tubing (Union Carbide Co.) containing 6 ml of an aqueous solution of given concentrations of piperazine and 7.95 mM HAlg (the inner solution) was soaked in 6 ml of the outer solution of alkali metal chloride ([MCl] = 0, 34, 68, or 136 mM). It was comfirmed that HAlg was easily dissolved in the presence of piperazine or NaOH, in amounts of at least 80—85% equivalent to HAlg. It was also confirmed that the equilibrium was attained within 24 h with respect to the concentrations of piperazine and added salt.

Equilibrium dialysis to study the effect of solution pH on the binding

ratio of piperazine was done in the following two ways: (1) 6 ml of 7.95 mM HAlg mixed with a given concentration of piperazine (the inner solution) was dialyzed against 6 ml of distilled water (the outer solution) at 7 or 30 °C for 24 h. Equilibrium pH increased with added amounts of piperazine; (2) 6 ml of 7.95 mM HAlg mixed with 9.29 mM piperazine (the inner solution) was dialyzed at 7 °C against 6 ml of water (the outer solution), of which the pH was prepared by HCl or NaOH. That is, according to the latter method (2), the total amount of piperazine was kept constant, but the Na^+ or Cl^- concentrations were changed to adjust the solution pH by means of NaOH or HCl. On the other hand, according to the former method (1), a foreign electrolyte such as HCl or NaOH was not added, but the total amount of added piperazine was varied to adjust the solution pH.

The concentration of piperazine in the outer solution free from Alg, $[pip]_{free}$, was determined after attaining equilibrium by means of a gas chromatograph (Shimadzu GC-6AM) with a Theed column (Shimadzu, 10%, 1 m) at 125 °C. Ethylene glycol was applied as an internal standard. The piperazine concentration, thus determined, is the total concentration including H_2pip^{2+}, $Hpip^+$, and free base piperazine. The binding ratio of piperazine to HAlg, x_{pip}, was obtained in a molar ratio of the bound piperazine (as H_2pip^{2+} plus $Hpip^+$) to a carboxylate group of HAlg as a function of $[pip]_{free}$ or as a function of the mixing ratio of added piperazine to added HAlg, taking into consideration the control experiment without HAlg.

The concentration of Na^+ or K^+ in the outer solution at the equilibrium was determined by means of a flame photometer (Hitachi type 205). The binding ratio of metal ions to carboxylate groups, x_M ($M = K^+$ or Na^+), was obtained by the same procedure as mentioned above.

Donnan equilibrium should be taken into consideration when the binding of a counter ion to a polyelectrolyte or the complex formation of ionic species with a nonionic polymer is discussed by means of dialysis equilibrium method.[11] Neutral salt is often added to the system to preclude the Donnan effect. In the present paper, however, some experiments were done in the absence of salt and the others in the presence of salt to study the effect of alkali metal cation (Li^+, Na^+, or K^+) on the binding ratio of a piperazinium ion.

Measurement of Sodium Ion Activity and Solution pH Sodium ion activity, a_{Na^+}, and solution pH were measured at 35 °C while 500 mM piperazine or piperazinium dichloride (H_2pipCl_2) was added by drops to 47.5 mM NaAlg (100 ml). The results were obtained as a function of the concentration of added piperazine or piperazinium dichloride, $[pip]_{added}$. The solution pH was measured by means of a pH-meter (Yamaco model PH-8A).

Sodium ion activity was determined at 35 °C by using an Orion sodium-sensitive electrode (type 9711BN) connected to an Orion expandable ion analyzer (model EA940). Prior to the measurement on the sample solution, the sodium electrode was calibrated with an aqueous solution of NaCl, taking the activity coefficient into consideration. The electrode exhibited a Nernstian response throughout the concentration range of the present work. According to the thermodynamic definition, activity (*i.e.*, relative activity) is essentially dimensionless. However, sodium ion activity, a_{Na^+}, in the present paper will be shown in mM units by reference to a standard solution of NaCl, assuming that it is an effective concentration free from the polyanion, Alg.

Results and Discussion

Titration Although the degree of dissociation of HAlg was evaluated as 2.6% from the intercept to the ordinate (pH 3.6) in Fig. 1, this was just an apparent value. HAlg suspended in water swelled to a certain extent but the dissolved amount was very small. The dissolved amount increased with the addition of piperazine or NaOH owing to the dissociation/neutralization of carboxylate groups of HAlg. Therefore, piperazine or NaOH was added by drops to attain the solubility equilibrium at each step when the titration of HAlg was carried out. HAlg was not fully dissolved until *ca.* 85% of the equivalence amount of piperazine was added (*ca.* pH 4.5).

Therefore, titration data around and above the equivalence point is reliable. According to the titration curve with piperazine in Fig. 1 (○), the mixing ratio at the equivalence point determined by pH-jump (pH 5.00) was 0.525. Since mole fractions of $Hpip^+$ and H_2pip^{2+} at pH 5.00 were estimated to be 0.212 and 0.788 on the basis of $pK_{a1} = 5.57$ and $pK_{a2} = 9.81$, the total positive charge arising from piperazinium cations is about 6% less than the total negative charge of a carboxylate group of HAlg determined by NaOH titrimetry. Although 6% is rather small, the discrepancy should be considered.

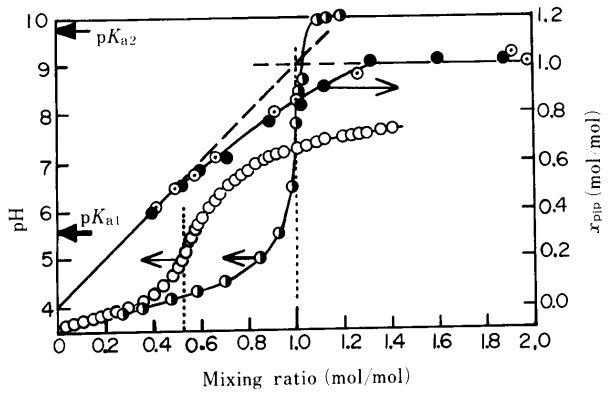

Fig. 1. Solution pH and the Binding Isotherm of Piperazine as a Function of the Mixing Ratio

Titration data at room temperature: ◑, NaOH; ○, piperazine. Binding ratio of piperazine: ●, at 7 °C; ⊙, at 30 °C.

Vertical dotted line shows the place at which the mixing ratio of piperazine or NaOH is at the equivalence point. The straight line with a slope of unity shows the relationship that the mixing ratio $= x_{pip}$; that is, all of the piperazine added to the system is completely bound by Alg. The deviation of the experimental points downward from the straight line indicates that a part of piperazine is unbound to Alg (the mixing ratio $> x_{pip}$, see the text). Saturation ($x_{pip} = 1$) is experimentally attained when the mixing ratio becomes somewhat higher than unity. If $x_{pip} = 1$ is attained at the mixing ratio = 1, this ideal point should be plotted on the cross point of the two broken lines.

Other literature cites that $pK_{a1} = 5.56$ and $pK_{a2} = 9.83$[12]; or $pK_{a2} = 9.81$.[13] These constants, almost the same as those used in the above calculation, derive similar results. Therefore, the constants used in the calculation might be reliable. On the other hand, a carboxylate group of Alg should attract a hydrogen ion to form in part carboxylic acid at a low pH such as pH 5.00.[14] The attraction is, from a different point of view, hydrogen ion condensation around a polyanion[15] and/or concomitant 1:1 binding of a hydrogen ion to a carboxylate group. If the pH in the neighborhood of polyanion is assumed as 4.60 for trial, mole fractions of $Hpip^+$ and H_2pip^{2+} become 0.097 and 0.903. According to these fractions, titrant piperazine (55 ml, 9.29 mM) becomes exactly equivalent to HAlg (100 ml, 9.72 mM). This result suggests that electroneutrality of Alg with piperazinium ion is attained by a pH in the neighborhood of a polymer domain rather than in the bulk phase. In other words, 5.1 and 94.9% of carboxylate groups of Alg bind the uni- and bivalent piperazinium cations at the equivalence point.

Binding Isotherm Binding isotherms of piperazine in the presence and absence of known amounts of alkali metal chloride are shown in Fig. 2A. The binding ratio, x_{pip} in the absence of salt is also shown in Fig. 1 as a function of the mixing ratio of piperazine to a carboxylate group of HAlg (● and ⊙). The binding isotherm 1 for a salt-free system (● and ⊙) in Fig. 2A was of the high affinity type. The point where the isotherm 1 deviates from the ordinate in Fig. 2A corresponded to the point where the x_{pip} deviates from the straight line which shows x_{pip} = the mixing ratio

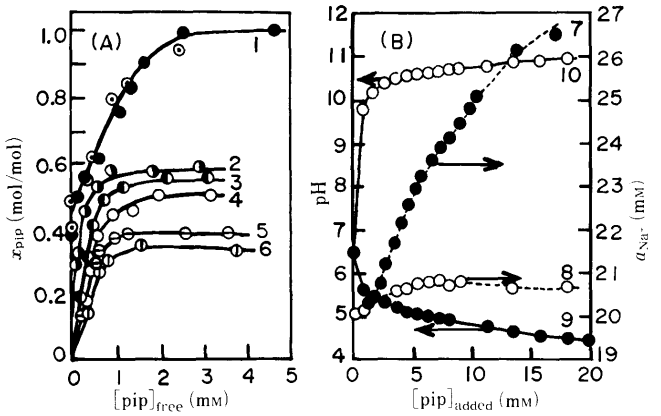

Fig. 2. Competitive Binding of the Piperazinium Ion with an Alkali Metal Ion

(A) Binding isotherm of the piperazinium ion in the presence of given amounts of alkali metal chloride. Additives: curve 1, no salt at 7 (●) or 30 °C (⊙); curve 2, 17 mM KCl (7 °C); curve 3, 34 mM KCl (7 °C); curve 4, 68 mM KCl (7 °C); curve 5, 68 mM NaCl (7 °C); and curve 6, 68 mM LiCl (7 °C). Solution pH at equilibrium was between 5 and 8. The salt concentration cited here is the mean over the outer and inner solutions.

(B) Solution pH and sodium ion activity: curve 7, a_{Na^+} in the presence of H_2pipCl_2; curve 8, a_{Na^+} in the presence of piperazine; curve 9, pH in the presence of H_2pipCl_2; curve 10, pH in the presence of piperazine.

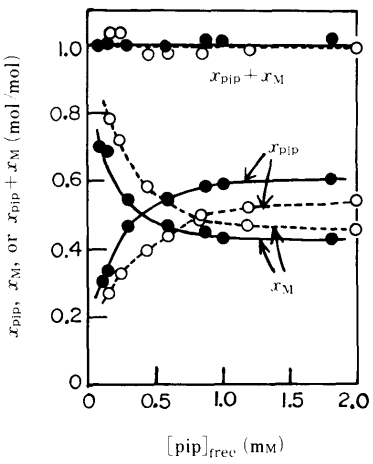

Fig. 3. Binding Ratio of the Piperazinium Ion and an Alkali Metal Ion, and the Sum of the Two at 7 °C

—●—, in the presence of 17 mM KCl (M = K^+). - - -○- - -, in the presence of 17 mM NaCl (M = Na^+).

The salt concentration cited here is the mean over the outer and inner solutions. Solution pH was from 5.1 through 7.2, depending on the concentration of added piperazine.

in Fig. 1. The mixing ratio at the deviating point was 0.52—0.53. This ratio is the same as that at the equivalence point of titration with piperazine (=0.525). That is, almost all of the added piperazine was consumed to neutralize HAlg and was captured in the polymer domain up to the equivalence point. Therefore, piperazine free from Alg was not detected in the outer solution until attaining the equivalence point for HAlg, resulting in a high affinity type isotherm.

The binding ratio increased further with the mixing ratio or with added piperazine. It attained unity and leveled off at *ca.* 1.3 of the mixing ratio (Fig. 1) or at *ca.* 2.5 mM piperazine free from Alg (Fig. 2). This fact indicates that the binding ratio increases up to unity through the transition of bivalent piperazinium cation to a univalent piperazinium cation with increases in pH when excess amounts of piperazine are added. In other words, piperazine behaves almost as a diacidic base at the equivalence point while it behaves as a monoacidic base after attaining saturation of the binding.

If the Donnan effect significantly appeares, the apparent binding ratio might become higher than the true value. However, the binding ratio was equal to the mixing ratio when it was low, less than the mixing ratio when it was intermediate, and attained saturation (=1.0) when an excess amount was added (see Fig. 1). These results are reasonable according to the stoichiometry. Therefore, the Donnan effect seems to be negligible in the present study.

Competitive Binding of the Piperazinium Ion with an Alkali Metal Ion Curves 2—6 in Fig. 2A show the binding isotherms of piperazine in the presence of given amounts of alkali metal chloride. The binding ratio, x_{pip}, decreased with the concentration of KCl, and in the order of KCl, NaCl, and LiCl when the salt concentration was kept at 68 mM. This is due to the competitive binding of metal cation with piperazinium cation for the carboxylate group of HAlg. The latter sequence is the reverse of the affinity of alkali metal cation toward the carboxylate group. That is, the higher the affinity was, the more frequently the exclusion against piperazinium cation from the polymer domain occurred.

Sodium ion activity, a_{Na^+}, of 47.5 mM NaAlg was found to be 20 mM, resulting in 42% of an apparent degree of dissociation of Na^+ from NaAlg. It was almost the same value as that reported by Yonese *et al.* (45%).[3] Sodium ion activity, a_{Na^+}, increased and the solution pH decreased with the concentration of added H_2pipCl_2 (curves 7 and 9), while a_{Na^+} slightly decreased after attaining a maximum level and the solution pH increased monotonously with the concentration of free base piperazine (curves 8 and 10). These results again show the competitive binding of Na^+ with piperazinium cation. Piperazinium cation excluded Na^+ from the polymer domain, resulting in an increase in a_{Na^+}. This effect was more remarkable in H_2pipCl_2 than in free base piperazine. The results of the competition between Na^+ and piperazinium cation, but not free base piperazine, as shown in Fig. 2B, support the idea that metal cation competes with piperazinium cation for the carboxylate group of Alg, as shown in Fig. 2A.

Electroneutrality Synchronous binding of piperazinium and an alkali metal ion was measured in the presence of 17 mM MCl (M = Na^+ or K^+) as shown in Fig. 3. The binding ratio of the alkali metal ion, x_M, decreased with an increase in the binding ratio of the piperazinium ion, x_{pip}. The value of x_{Na^+} (M = Na^+) was higher than that of x_{K^+} (M = K^+), while the value of x_{pip} in the presence of NaCl was lower than that in the presence of KCl over the studied range of concentrations of piperazine, as expected from Fig. 2A. On the other hand, the sum of x_{pip} and x_M was almost identical throughout the concentration range of piperazine studied.

Intuitively, however, the results might sound strange, especially at a low pH such as pH 5.1, because bivalent piperazinium cations still remain in the solution and each of them should be bound to two carboxylate groups. Therefore, $x_{pip}+x_M$ should be less than unity. This expectation is inconsistent with the experimental results.

However, this apparent inconsistency is resolved by taking into consideration the effect of the chloride ion of MCl. It plays the role of counter ion for piperazinium and alkali metal cations in the solution. Therefore, each bivalent piperazinium cation bound to Alg in the presence of MCl does not necessarily require two carboxylate groups of Alg. It could capture a chloride anion through electrostatic force as one of the counter ions, while still remaining bound to the carboxylate group as its other one. The carboxylate group liberated from piperazinium cation captures metal cation (M), instead, as its counter ion. That is, the counter ion exchanges with respect to bivalent piperazinium cation (between Cl^- and carboxylate group) and with respect to carboxylate group (between M and piperazinium cation) occur simultaneously in the presence of MCl. These processes result in the 1 : 1 binding of bivalent piperazinium cation to a carboxylate group in the same manner as that of univalent piperazinium cation, while the electroneutrality of the polymer domain is still maintained. Therefore, it is reasonable that the sum of x_{pip} and x_M became unity in the presence of excess amounts of MCl (17 mM) even though bivalent piperazinium cation still remains in an acidic solution and the competition occurs.

Although the fractions of uni- and bivalent piperazinium ions with respect to total piperazine should decrease with an increase in the total concentration of added piperazine due to synchronous increases in pH, it was experimentally found that the binding ratio, x_{pip}, did not decrease with total concentration of added piperazine but attained a plateau in alkaline pH (see Figs. 1 and 2A). This fact may be explained as follows: H^+ of HAlg is consumed by free base piperazine to form an equivalent amount of univalent piperazinium cation (and an anionic carboxylate group). Ionized carboxylate groups attract protonated univalent piperazinium cations as the counter ion to maintain the electroneutrality of the system. Therefore, x_{pip} is saturated at unity for the salt-free system (see the curve 1 in Fig. 2A), while it decreases from unity to a certain plateau value in the presence of MCl due to the cation competition (see the curves 2—6 in Fig. 2A).

On the other hand, when the alkaline pH was adjusted by NaOH at a given amount of base piperazine, x_{pip} decreased with the increase in pH, as shown Fig. 4. In this case, OH^- of NaOH accelerates the ionization of HAlg while piperazine still remains in the free base form, because OH^- from NaOH suppresses the protonation of piperazine. Therefore, Na^+ becomes the dominant species of counter ion for Alg rather than the piperazinium ion. This is another mode of competitive binding between Na^+ and the piperazinium ion to maintain the electoneutrality of the system in an alkaline solution (see the curves 8 and 10 in Fig. 2b).

The binding mode of bivalent piperazinium cation is considerably different from that of bivalent metal cation; Alg gel, which is formed by mixing NaAlg with $CaCl_2$, $SrCl_2$, or $BaCl_2$, does not readily melt in the presence of NaCl. It is well known that Ca^{2+} and Alg form the egg-box structures, which strengthen the gel network.[1] These facts indicate that the binding of bivalent metal cation with Alg is much stronger than that of Na^+,[1] while the binding of bivalent piperazinium cation to Alg was rather weak, as mentioned above. This difference comes from the fact that piperazine is an organic diacidic base, and the two sites of electric charges on the molecule are apart, located on opposite ends of the molecule from each other.

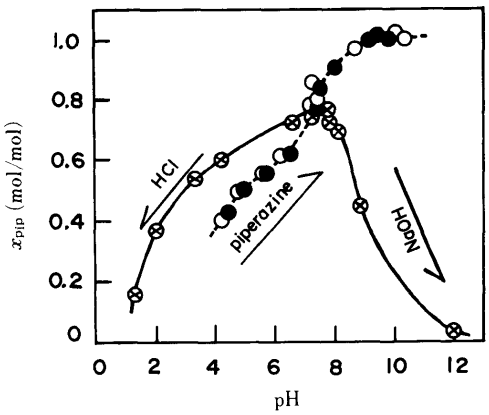

Fig. 4. Binding Ratio of Piperazinium Ion as a Function of Equilibrium pH

● (at 7 °C) and ○ (at 30 °C): pH was adjusted by piperazine. Initial inner and outer solutions were of 6 ml of 7.95 mM HAlg mixed with a given amount of piperazine, and of 6 ml of water, respectively. ⊗ (at 7 °C): pH was adjusted by HCl or NaOH. Initial inner and outer solutions were of 6 ml of 9.29 mM piperazine mixed with 7.95 mM HAlg, and of 6 ml of water mixed with a known amount of HCl or NaOH, respectively. The arrow mark indicates the direction in which the concentration of an annexed reagent increases to prepare the solution pH.

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