# Speciation Studies in Relation to Magnesium Bioavailability. Formation of Mg(II) Complexes with Glutamate, Aspartate, Glycinate, Lactate, Pyroglutamate, Pyridoxine and Citrate, and Appraisal of their Potential Significance Towards Magnesium Gastrointestinal Absorption

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## Abstract

Associations of $Mg^{2+}$ ions with organic ligands are frequently advertised as likely to enhance the bioavailability of magnesium from orally administered commercial preparations. However, no systematic study of the relevant equilibria has been produced so far to substantiate these assertions, and no superiority has yet been demonstrated for any magnesium salt on clinical grounds.

After a review concerning different aspects of magnesium gastrointestinal absorption, in particular with respect to calcium interactions, this paper deals with the determination of formation constants for magnesium complexes with glutamate, aspartate, glycinate, lactate, pyroglutamate, pyridoxine and citrate, under physiological conditions of ionic strength (0.15 mol $dm^{-3}$) and temperature (37 °C).

Corresponding results are then used to assess the potential capacity of each of these ligands to mobilise $Mg^{2+}$ ions into membrane diffusible complexes. At usual therapeutic concentrations, pyroglutamate and pyridoxine do not coordinate magnesium in appreciable amounts, and glycinate and lactate do not form any neutral complex. In contrast, glutamate, aspartate and citrate do form neutral magnesium species; the extent of their expected effects with respect to magnesium uptake by enterocyte membranes is discussed on the basis of relevant computer simulations. Potential calcium interactions are also examined, which required the determination of formation constants for calcium complexes with these three ligands.

## Introduction

Bioavailability has progressively emerged as a major determinant of the nutriture of humans with respect to minerals [1–7]. This notion proceeds from both dietary intake and absorption efficiency of the element involved. Thus, studies on gastrointestinal absorption and transport processes of any nutrient are of central importance for obtaining a better knowledge of its nutritional biochemistry and metabolism [7]. However, while major advances have been recorded in the understanding of the absorption of organic substances, progress relative to mineral nutrition has been uneven. For instance, although magnesium is the second most abundant intracellular element in the human body and activates about 300 enzymes [8], relatively little is known about its gastrointestinal absorption.

### *Site and Intraluminal Factors Affecting Magnesium Absorption*

Magnesium can be absorbed throughout the length of the intestinal tract [9]. Nevertheless, most investigators have concluded that the small intestine plays the dominant role in this process [10–12], even though colonic absorption can occur [13]. As is the case for any mineral nutrient, the freeing of magnesium from original food matrices into a form sufficiently soluble to be taken up across the intestinal membrane is a prerequisite step for its absorption [14]. Yet, the role of intraluminal factors in its bioavailability is poorly defined. For example, the role of physiological variations in gastrointestinal pH, bile acid secretion, and transit time on its absorption in normal populations is not well understood. Nor is the dependence of the efficiency of this absorption on the nature of the source of the dietary element [8]. In particular, the form of supplemental magnesium salts does not appear to be of much nutritional importance in animals [8–15], but, once again, the correlation of these studies with bioavailability in man is poorly defined.

### *Magnesium versus Calcium Absorption*

Considering magnesium absorption in perspective with the better documented calcium absorption may

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help understand some of its aspects. Intestinal calcium absorption proceeds from both passive (linear) and active (saturable) components [6, 16–18], vitamin D being generally considered as primarily affecting the latter [16, 19]. Although passive movement of $Ca^{2+}$ ions across the small intestine does occur and does affect measurements of net absorption *in vitro*, its contribution to calcium absorption in a healthy individual is probably minor [18]. Furthermore, the presence of complexing substances in the diet would not be important, except when accompanied by dietary vitamin D deficiency, marginal calcium intake, or malabsorptive disorders [18].

Like calcium, magnesium is thought to be absorbed through a double process involving both saturable and linear components [20]. Furthermore, given that (i) calcium and magnesium have been shown to interact [21, 22] and (ii) attempts to demonstrate the existence of an active transport process for magnesium have failed [23, 24], it can be concluded that the saturable component of magnesium absorption represents a process of facilitated diffusion [8, 25]. Nevertheless, the very matter of the antagonism of calcium *versus* magnesium absorption in humans is controversial [12, 21, 26]. It has been contended that the interaction between these two cations may occur at the level of a common intestinal transport system [27], whereas experimental results suggest that calcium and magnesium transport systems are separate [12], this being in line with the existence of a congenital malabsorption syndrome specific to magnesium [28]. The elucidation of this problem is still thwarted by the likely involvement of vitamin D in magnesium absorption [12, 22], which would preferentially affect jejunum absorption [29, 30]. Reduced magnesium absorption observed in patients with chronic renal failure [12, 31] tends to substantiate this role. Reciprocally, it has recently been stated that magnesium depletion may impair vitamin D metabolism [32].

*Magnesium Deficiency and Present Needs*

Magnesium deficiency is fairly common in man [33]. It may result from insufficient dietary intake or pathological conditions affecting the absorption of this element, like intestinal mucosal disease, increased secretion, fat malabsorption, renal wastage, etc. [8, 33, 34]. Clinical manifestations and health disorders due to magnesium deficiency have recently been reviewed [35, 36]. More specifically, France is known for the poor availability of magnesium in its soils, and the magnesium deficiency which affects a significant fraction of its population has even been invoked as a possible cause for a greater general incidence of cancers [37]. Indeed, low incidence of cancers has been associated with abundant magnesium in soils and drinking waters in many countries [38].

Commercially available magnesium salts are numerous. Unfortunately, the required dose of magnesium is often greater than the tolerated oral dose due to the cathartic properties of its cation. Thus, as was recently pointed out, 'the development of a carrier that would enhance the bioavailability of magnesium, thereby leading to a reduction in the administered dose, would be invaluable in the long-term clinical management of magnesium malabsorption' [8].

*Magnesium and Bioavailability*

Generally speaking, it is a well accepted principle that the bioavailability of organic drugs depends on the form in which they are present in relevant biological fluids. Clearly, non-ionised molecules pass more readily through membrane barriers than corresponding ionised species [39]. With the restriction of possible active transport and pinocytosis, bioinorganic chemists have extended this principle to metal ion complexes [40–42]: neutral forms, which are lipid soluble, can freely diffuse through phospholipid membranes, whereas electrically charged forms are confined within the compartment in which they have been introduced, or are directly excreted. This rationale holds in gastrointestinal absorption of minerals [37]. On the proviso that the metal ion has been liberated from its original matrix, which, under specific conditions such as in presence of soya proteins, may necessitate the use of powerful chelating agents [43], its subsequent association with anionic substances is then desirable so as to give rise to membrane diffusible complexes. In addition, it is noteworthy that whatever the mode of transport of the metal ion may be, its dissolution into the outer layer of the gastrointestinal membrane constitutes the first essential step of its absorption [37]. Several attempts to apply these principles to the oral administration of various metal ions have already been reported [37, 44–47], most of them being clinically validated [2, 48].

As far as magnesium is concerned, associations of its cation with potential carriers present in various commercial preparations are commonly advertised as likely to enhance its bioavailability. However, no systematic study of Mg(II) complex equilibria with the relevant ligands has been produced to substantiate these assertions and no superiority has yet been demonstrated for any magnesium salt on clinical grounds [35].

*Present Objectives*

The main objective of this paper, thus, is the quantitative study of Mg(II) complex equilibria with a number of these substances, namely glutamate (Glutamag®), aspartate (Mégamag®), glycinate (Mag-

nésium Glycocolle®), lactate (Magnéspasmyl®, Magné B6®), pyroglutamate (Mag2®, Solumag®) and pyridoxine (Magné B6®) under biological conditions. In addition, citrate was recently reported to induce interesting clinical results with respect to magnesium absorption [49]. As its use as a taste additive is widespread in magnesium-containing pharmaceutical preparations, its coordination to this metal was also examined.

In other respects, calcium antagonism *versus* magnesium absorption may result from the competition of both metals for the same carrier in the gastrointestinal fluid. Therefore, calcium complex equilibria were studied for those ligands which gave rise to the formation of significant magnesium neutral species.

The role of the above ligands as potential carriers of $Mg^{2+}$ ions into the intestinal membrane was also investigated. This involved the use of computer simulations of the distribution of magnesium into its corresponding complexes within the gastrointestinal pH range, for usual therapeutic concentrations. Competition with calcium is also discussed when necessary.

## Formation Constant Determinations

### *Materials*

L-glutamic acid, L-aspartic acid and glycine were Merck biochemical grade products. L-pyroglutamic acid, pyridoxin hydrochloride and L(+) lactic acid were supplied by Sigma Chemical Co., whereas citrate was supplied by PROLABO R.P. as a 'Normapur' grade reagent.

Stock solutions of magnesium and calcium in diluted perchloric acid were prepared from perchlorate salts purchased from Fluka as *pro analysi* products. Their respective metal contents were determined by complexometric titrations against EDTA using murexide and Erio T as indicators [50], their mineral acid concentrations being deduced from direct potentiometric readings.

Solutions of Merck sodium perchlorate were prepared as previously described [51]. Carbonate free hydroxide solutions, prepared as before [52], were constantly kept under a nitrogen blanket.

### *Technique*

Potentiometric titrations were carried out using Ingold glass and calomel electrodes fitted in an Ingold thermostatted cell system. Emf's were monitored by means of a Beckman model 4500 mV-meter.

The temperature was maintained at 37 ± 0.02 °C inside the reaction cell, and a constant bubbling of purified nitrogen was set up in the solution throughout the titrations.

An excess of mineral acid was initially imposed to solutions to be titrated so as to reach high protonation degrees for corresponding ligands. Sodium perchlorate 0.15 mol $dm^{-3}$ ensured a constant ionic strength in order to hold activity coefficients constant. Since the electrode system was calibrated in the concentration scale, pH must be understood in terms of $-\log[H]$.

Metal-to-ligand ratios were made to vary to a large extent over the system investigated. Table I gives a short account of the experimental conditions used.

### *Calculation Procedures*

The twofold approach involving both optimisation and simulation steps previously described by one of us [51–53] was developed throughout. The MINIQUAD programme [54] was used to refine formation constant estimates deduced from relevant formation curves. For each system, the final discrimination among the constant sets displaying similar numerical fits was based on graphical comparisons between experimental formation curves and pseudo-formation curves simulated by means of PSEUDOPLOT [55] or ESTA [56] programmes.

Further attention must be paid to citrate coordination studies using this approach: the number of dissociable protons (NDP) of citrate may be considered as 4 or 3, depending on whether or not the dissociation of its hydroxy group is taken into account. For optimisation calculations, both solutions are mathematically equivalent, but it is not the case for simulation ones. To simulate the formation curves on the assumption that NDP = 3, it must be considered that the dissociation of the hydroxy group actually represents the formation of a hydroxo species. Furthermore, this will impose negative values to related protonation numbers, which in turn implies that corresponding complex formation numbers become meaningless. Thus, although (i) the NDP = 3 hypothesis is more satisfactory from a chemical point of view [51, 57, 58] and (ii) the NDP = 4 hypothesis involves the use of a protonation constant determined in a pH range where glass electrode measurements are less reliable, the latter alternative had to be used beforehand for discriminating the 'best' constant set. Once this 'best' set was ascertained, the corresponding constants were refined again in the NDP = 3 hypothesis.

A number of formation constants determined by one of us under identical experimental conditions were included in the calculations. They refer to protonations of glutamate [59], aspartate [59], glycinate [53] and citrate [51], and to the ionic product of water [51].

TABLE I. Summary of the Titration Data Used in Formation Constant Calculations. Initial overall concentrations of metal ($C_M$), ligand ($C_L$), mineral acid ($C_H$) and pH[a] range. All concentrations are expressed in mmol dm$^{-3}$

| System | $C_M$ | $C_L$ | $C_H$ | pH range |
|---|---|---|---|---|
| Magnesium–glutamate | 5.07 | 20.25 | 23.44 | 1.9–9.3 |
| | 5.07 | 20.25 | 23.44 | 1.9–9.7 |
| | 5.07 | 20.00 | 23.44 | 1.9–9.7 |
| | 5.07 | 15.19 | 16.55 | 2.1–10.1 |
| | 10.15 | 20.25 | 23.91 | 1.9–9.6 |
| | 5.07 | 8.10 | 9.66 | 2.2–10.0 |
| | 10.15 | 10.13 | 12.42 | 2.1–9.9 |
| | 8.12 | 4.05 | 5.34 | 2.4–9.6 |
| Magnesium–aspartate | 3.81 | 15.00 | 18.77 | 1.9–10.1 |
| | 5.07 | 15.00 | 18.89 | 1.9–10.3 |
| | 10.15 | 20.00 | 23.96 | 1.9–10.2 |
| | 5.07 | 8.00 | 9.68 | 2.2–10.2 |
| | 10.15 | 10.00 | 12.45 | 2.1–10.0 |
| | 8.12 | 4.00 | 5.35 | 2.4–9.8 |
| Magnesium–glycinate | 5.07 | 20.00 | 26.72 | 1.9–10.1 |
| | 5.07 | 15.00 | 21.47 | 2.0–10.1 |
| | 10.15 | 20.00 | 27.19 | 1.9–9.9 |
| | 5.07 | 7.50 | 10.97 | 2.2–9.9 |
| | 10.15 | 10.00 | 21.94 | 1.8–9.9 |
| | 10.15 | 5.00 | 11.44 | 2.1–9.7 |
| Proton–lactate | | 5.00 | | 3.0–8.6 |
| | | 10.00 | | 2.9–8.4 |
| | | 20.00 | | 2.7–9.1 |
| Magnesium–lactate | 10.15 | 30.00 | | 2.5–8.3 |
| | 10.15 | 20.00 | | 2.6–8.8 |
| | 10.15 | 10.00 | | 2.7–8.6 |
| | 10.15 | 5.00 | | 2.8–7.7 |
| Proton–pyroglutamate | | 10.00 | 5.25 | 2.2–5.4 |
| | | 10.00 | | 2.6–5.5 |
| | | 20.00 | | 2.4–6.3 |
| | | 20.00 | 10.50 | 2.0–6.2 |
| Magnesium–pyroglutamate | 5.07 | 20.00 | 0.47 | 2.4–5.9 |
| | 5.07 | 15.00 | 0.47 | 2.5–6.2 |
| | 5.07 | 10.00 | 0.47 | 2.6–5.2 |
| | 10.15 | 20.00 | 0.94 | 2.4–4.9 |
| | 10.15 | 10.00 | 0.94 | 2.5–5.4 |
| | 10.15 | 5.00 | 0.94 | 2.6–5.5 |
| Proton–pyridoxine | | 5.00 | 5.00 | 3.6–10.1 |
| | | 10.00 | 10.00 | 3.4–10.1 |
| | | 20.00 | 20.00 | 3.2–10.3 |
| Magnesium–pyridoxine | 5.07 | 20.00 | 20.47 | 3.1–10.0 |
| | 10.15 | 20.00 | 20.94 | 2.9–9.9 |
| | 10.15 | 10.00 | 10.94 | 2.9–9.8 |
| Magnesium–citrate | 5.07 | 20.00 | 0.47 | 2.3–11.2 |
| | 5.07 | 15.00 | 0.47 | 2.3–11.1 |
| | 10.15 | 20.00 | 0.94 | 2.2–11.0 |
| | 5.07 | 7.50 | 0.47 | 2.5–10.7 |
| | 10.15 | 10.00 | 0.94 | 2.4–10.6 |
| | 10.15 | 5.00 | 0.94 | 2.5–10.1 |
| Calcium–glutamate | 5.02 | 20.00 | 23.46 | 2.0–10.4 |
| | 5.02 | 15.00 | 16.57 | 2.0–10.4 |
| | 10.05 | 20.25 | 23.96 | 1.9–10.4 |
| | 5.02 | 8.18 | 9.68 | 2.2–10.3 |
| | 10.05 | 10.13 | 12.47 | 2.1–10.2 |
| | 8.04 | 4.00 | 5.38 | 2.4–10.3 |
| Calcium–aspartate | 3.77 | 15.00 | 18.79 | 1.9–10.6 |
| | 5.02 | 15.00 | 17.76 | 2.0–10.6 |
| | 10.05 | 20.00 | 24.00 | 1.9–10.5 |
| | 5.02 | 8.00 | 9.70 | 2.2–10.4 |
| | 10.05 | 10.00 | 12.49 | 2.1–10.5 |
| | 8.04 | 4.00 | 5.39 | 2.3–10.2 |
| Calcium–citrate | 5.02 | 20.00 | 0.49 | 2.3–11.2 |
| | 10.05 | 20.00 | 0.98 | 2.2–11.2 |
| | 5.02 | 7.50 | 0.49 | 2.5–11.0 |
| | 10.05 | 10.00 | 0.98 | 2.4–11.1 |
| | 10.05 | 5.00 | 0.98 | 2.5–10.9 |

[a]See text.

## Results and Discussion

Table II first reports the stability constants for magnesium complexes determined in these studies. Figures 1 and 2 are given as an illustration of the above approach. As pointed out in the introduction, constants relative to calcium equilibria may also be found in Table II for those ligands which gave rise to significant magnesium neutral species.

Ligands involved in magnesium equilibria may actually be classified into three groups:

(i) those (namely pyroglutamate and pyridoxine) for which no complex at all could be found under the present experimental conditions. In such cases, protonation curves determined in the presence of metal were exactly superimposable with the original ones. In other words, the corresponding complex formation degrees remained nil within the whole range of free ligand concentrations investigated. This does not mean that these ligands may not coordinate to magnesium at far higher concentrations, but it *definitely rules out any possibility for their complexes to exist as independent species in the gastrointestinal fluid.* Indeed, ligand concentrations used for the present investigations (Table I) are higher than those resulting in this fluid from usual oral doses of the corresponding drugs;

(ii) those (namely glycinate and lactate) which gave rise to ionised complexes only. These complexes

TABLE II. Formation Constants Obtained from these Studies. The formula of the general complex is $M_qL_pH_r$. $S$ = sum of the squared residuals; $R$ = $R$ factor as defined in ref. 54; $n$ = number of experimental observations

| System | $p$ | $q$ | $r$ | log $\beta$ | $S$ | $R$ | $n$ |
|---|---|---|---|---|---|---|---|
| Magnesium–glutamate | 1 | 1 | 2 | 14.876 ± 0.021 | 4.31 E-06 | 0.0036 | 353 |
| | 1 | 1 | 1 | 11.081 ± 0.018 | | | |
| | 1 | 1 | 0 | 2.196 ± 0.021 | | | |
| | 2 | 1 | −1 | −6.125 ± 0.023 | | | |
| Magnesium–aspartate | 1 | 1 | 2 | 14.074 ± 0.042 | 1.17 E-06 | 0.0031 | 259 |
| | 1 | 1 | 1 | 10.501 ± 0.030 | | | |
| | 1 | 1 | 0 | 2.040 ± 0.029 | | | |
| | 1 | 1 | −1 | −8.666 ± 0.023 | | | |
| | 1 | 2 | 0 | 4.426 ± 0.049 | | | |
| Magnesium–glycinate | 1 | 1 | 1 | 10.879 ± 0.042 | 3.05 E-06 | 0.0052 | 233 |
| | 1 | 1 | 0 | 1.979 ± 0.019 | | | |
| | 2 | 1 | 2 | 21.614 ± 0.103 | | | |
| | 1 | 1 | −1 | −8.735 ± 0.042 | | | |
| Proton–lactate | 1 | 0 | 1 | 3.666 ± 0.001 | 8.13 E-08 | 0.0020 | 77 |
| Magnesium–lactate | 1 | 1 | 0 | 1.235 ± 0.004 | 9.93 E-08 | 0.0012 | 117 |
| Proton–pyroglutamate | 1 | 0 | 1 | 3.090 ± 0.001 | 1.35 E-06 | 0.0054 | 142 |
| Magnesium–pyroglutamate | no complex in evidence | | | | | | |
| Proton–pyridoxine | 1 | 0 | 1 | 8.653 ± 0.001 | 3.19 E-07 | 0.0022 | 149 |
| | 1 | 0 | 2 | 13.463 ± 0.002 | | | |
| Magnesium–pyridoxine | no complex in evidence | | | | | | |
| Magnesium–citrate | 1 | 1 | 2 | 11.008 ± 0.063 | 5.95 E-06 | 0.0054 | 349 |
| | 1 | 1 | 1 | 7.483 ± 0.024 | | | |
| | 1 | 1 | 0 | 3.333 ± 0.009 | | | |
| | 2 | 1 | 1 | 10.411 ± 0.069 | | | |
| | 2 | 1 | 0 | 5.126 ± 0.041 | | | |
| | 2 | 2 | −2 | −12.638 ± 0.033 | | | |
| | 1 | 1 | −2 | −18.468 ± 0.015 | | | |
| Calcium–glutamate | 1 | 1 | 2 | 14.020 ± 0.074 | 2.08 E-06 | 0.0030 | 296 |
| | 1 | 1 | 1 | 10.377 ± 0.040 | | | |
| | 1 | 1 | 0 | 1.474 ± 0.041 | | | |
| | 1 | 1 | −1 | −9.071 ± 0.019 | | | |
| Calcium–aspartate | 1 | 1 | 2 | 14.128 ± 0.042 | 1.43 E-06 | 0.0035 | 274 |
| | 1 | 1 | 1 | 10.590 ± 0.022 | | | |
| | 1 | 1 | 0 | 1.135 ± 0.166 | | | |
| | 1 | 1 | −1 | −9.241 ± 0.022 | | | |
| | 1 | 2 | 0 | 3.855 ± 0.079 | | | |
| Calcium–citrate | 1 | 1 | 2 | 11.005 ± 0.034 | 2.55 E-06 | 0.0042 | 281 |
| | 1 | 1 | 1 | 7.614 ± 0.015 | | | |
| | 1 | 1 | 0 | 3.364 ± 0.006 | | | |
| | 2 | 1 | 0 | 4.965 ± 0.044 | | | |
| | 1 | 1 | −1 | −8.395 ± 0.026 | | | |
| | 2 | 1 | −2 | −16.808 ± 0.023 | | | |

may represent a significant fraction of magnesium in the gastrointestinal fluid, but their electrical charge will prevent them from dissolving into phospholipid membranes. Moreover, it has been suggested that the passive diffusion component of magnesium should rather be considered in terms of solvent drag [60]. Thus, the formation of such ionised species may tend to reduce the extent of this process, since

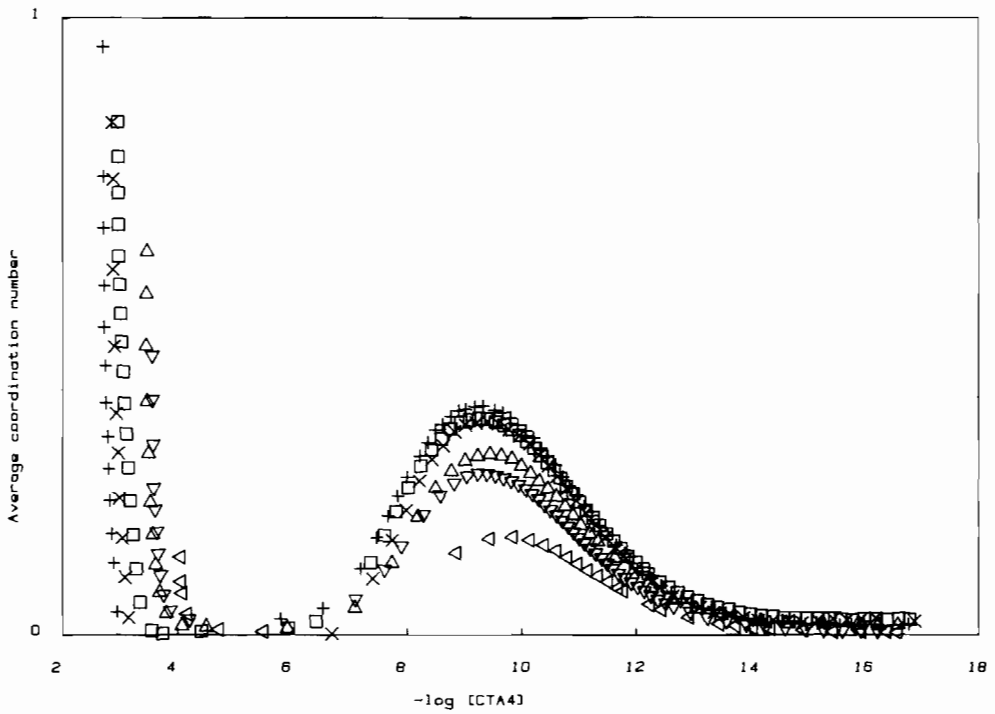


Fig. 1. Experimental formation curve for the magnesium–citrate system (NDP = 4 for citrate). The following symbols +, ×, □, △, ▽, ◁, correspond to the respective order of experiments summarized in Table I.

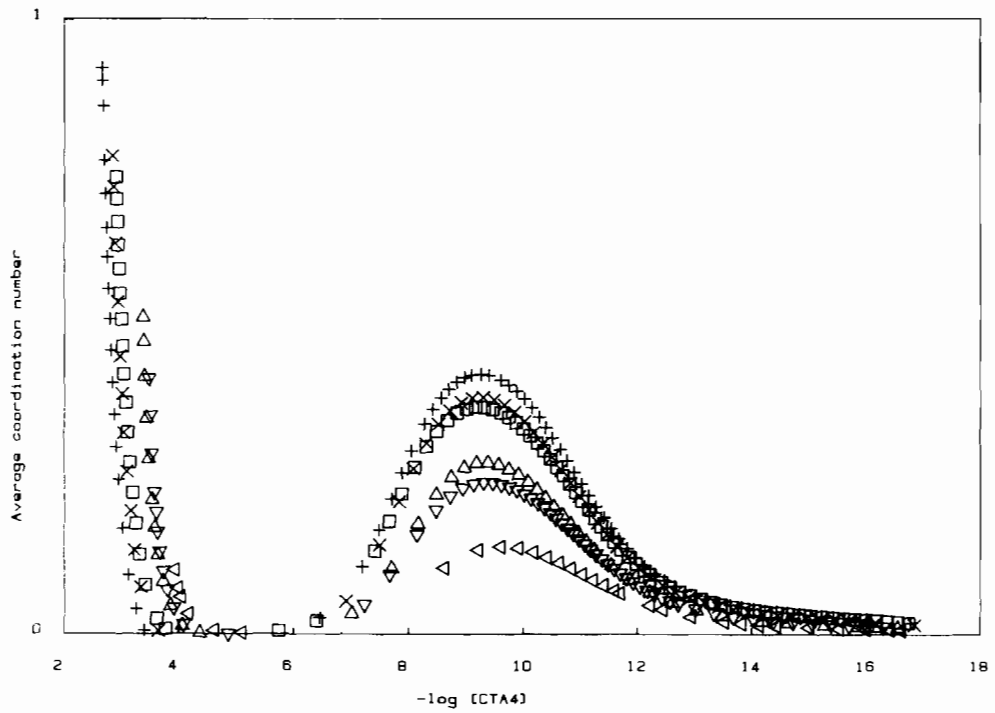


Fig. 2. Simulated formation curve for the magnesium–citrate system as obtained by means of the ESTA programme using results in Table II. Symbols are identical with those in Fig. 1.

solvation of magnesium complexes will not compare favorably with that of free $Mg^{2+}$ ions on size and charge density grounds. By analogy with the case above, it is noteworthy that glycinate and lactate may form neutral $ML_2$ complexes of magnesium when present in much higher concentrations. How-

ever, the present results clearly show that their occurrence as independent species in the gastrointestinal fluid can be definitely excluded;

(iii) those (namely glutamate, aspartate and citrate) with which magnesium can form neutral complexes. The latter species, being lipid soluble, are expected to favour the uptake of magnesium by enterocyte membranes, depending on the extent of their corresponding fraction of metal. This extent can be assessed using computer simulations.

## Computer Simulations

*General Considerations*

To interpret gastrointestinal bioavailability of metal ions administered in the presence of food is not straightforward. As outlined in the introduction, metal ions contained in food must be freed from their original matrices before they can be taken up by the low molecular weight ligands likely to make them lipid soluble through the formation of adequate complexes [37]. Likewise, metal ions administered during meals as mineral or organic salts are subject to a challenge from other ligands present in the chyme. Some competition may also occur towards the administered ligands from other metal ions contained in food.

Such difficulties may be overcome by administering metal ions as their complexes on an empty stomach, and this is actually becoming established practice [37]. This solution offers an additional advantage: gastrointestinal secretions specific to the digestion process can thereby be avoided, and the distribution of the metal ion into its different complexes with the selected ligand can be simulated. This provides a very useful tool, essential in that it can help to reduce the extent of, and to design protocols for, biological screenings.

Basically, such simulations are governed by only three critical factors: the concentrations of the metal ion and of its associated ligand in the gastrointestinal fluid, which are actually under therapist's control, and the pH of this fluid, which is imposed by the physiological sites potentially involved.

Two programmes were thus used in the present studies: (i) COMPLOT – an updated BASIC plotting version of the COMICS programme [61, 62] –; (ii) TRIPLOT – a specifically made BASIC programme – which can plot in three dimensions the percentage of the neutral complexed fraction of metal, as a function of pH and of the concentration of associated ligand [63].

*Results*

In spite of the considerations above [37], it is still often recommended that magnesium-containing drugs referred to in the present study be ingested during meals. To simulate the real distribution of the metal under such circumstances remains elusive. Nevertheless, in order to take account of the variations that may arise from dissolving the same average dose of $Mg^{2+}$ ions (125 mg) in different volumes of gastrointestinal fluid, two concentrations of magnesium (0.005 mol $dm^{-3}$ and 0.025 mol $dm^{-3}$) were considered in the present simulations. For each of them, the concentration of associated ligand was scanned between limits allowing the metal-to-ligand ratio to vary to a large extent (from 1 to 10 as a maximum).

Potential interactions of calcium on the mobilisation of magnesium into neutral species were then investigated. This was primarily done using concentrations of this metal equivalent to those of magnesium, which roughly corresponds to one third of its daily required dietary allowance [18].

*Glutamate*

Figure 3 shows the COMPLOT distribution of the above dose of magnesium, dissolved in 200 $cm^3$ of water together with the smallest glutamate concentration inducing a percentage of ML near its maximum value. It is noteworthy that the dilution of the same dose in 1 $dm^3$ resulted in quite similar profiles.

Other distributions were also simulated in the presence of calcium, which did not significantly modify the ML percentages shown in Fig. 3, even for calcium concentrations amounting to four times that of magnesium.

The present results suggest that glutamate may help $Mg^{2+}$ ions to dissolve into enterocyte membranes within the distal part of the small intestine and possibly the colon. In spite of its limited extent, this effect may be of interest since calcium cannot antagonise it.

*Aspartate*

At first sight it seems that, for any given concentration of aspartate equal to or higher than that of magnesium, the higher the concentration of metal, the lesser the effect to be expected from this ligand, there being a parallel decrease of the percentage of ML. Nevertheless, since this percentage is applied to larger and larger metal concentrations, derived conclusions are not so clearcut.

It can be inferred from the whole of the results obtained that the aspartate mobilisation of magnesium into neutral species is roughly equivalent to that evidenced for glutamate, this being observed in spite of the antagonistic influence of the $M_2L$ complex (see Fig. 4).

As far as potential calcium interactions are concerned, they were insignificant for concentrations of this metal equal to those of magnesium, but this was not true at higher concentrations. Indeed,

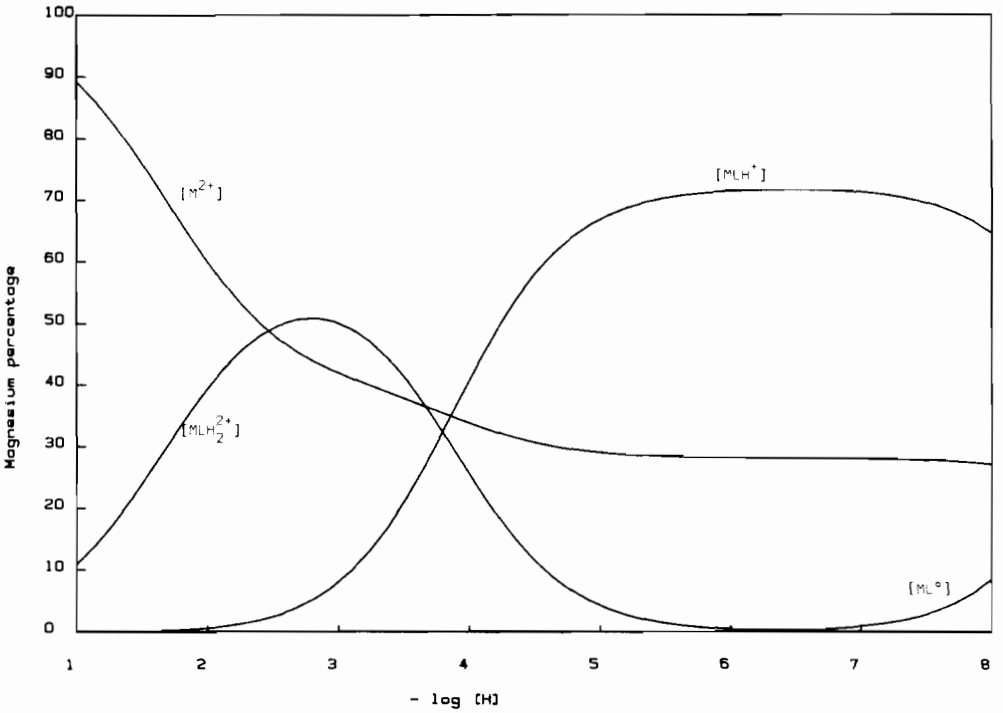


Fig. 3. Distribution of magnesium into its different glutamate complexes ($C_{Mg}$ = 0.025 mol dm$^{-3}$; $C_{Glu}$ = 0.05 mol dm$^{-3}$).

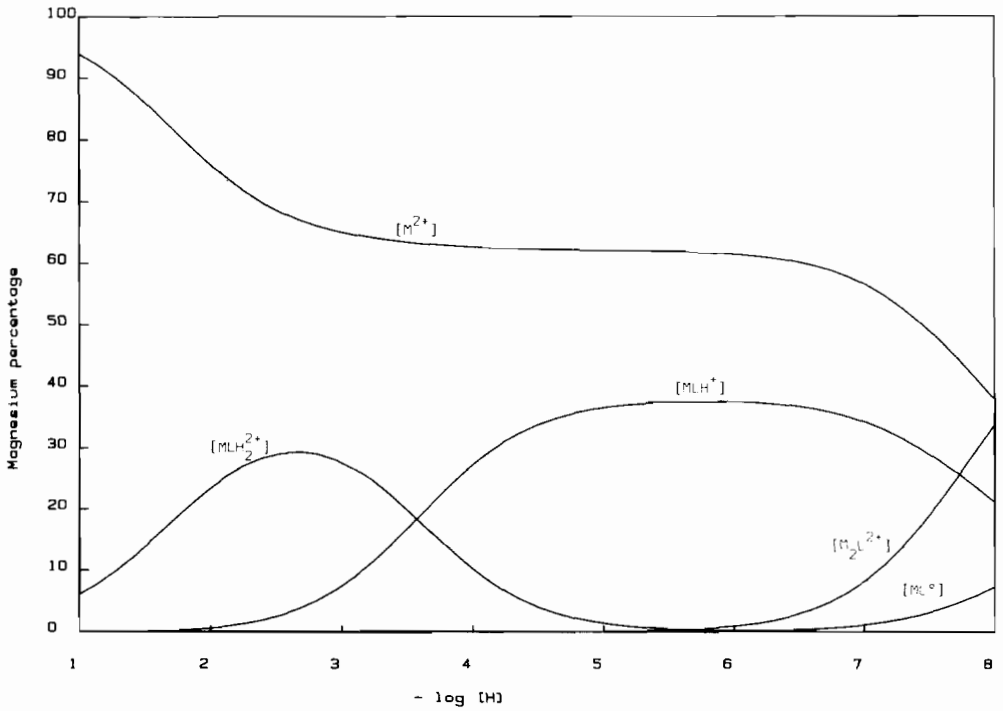


Fig. 4. Distribution of magnesium into its different aspartate complexes ($C_{Mg}$ = 0.025 mol dm$^{-3}$; $C_{Asp}$ = 0.05 mol dm$^{-3}$).

even though calcium aspartate complexes are much less stable than their magnesium homologues, the existence of $M_2L$ for calcium as well as for magnesium (Table II) tends to reduce the percentage of the ML magnesium–aspartate species. Actually, this reduction amounts to 50% for a concentration

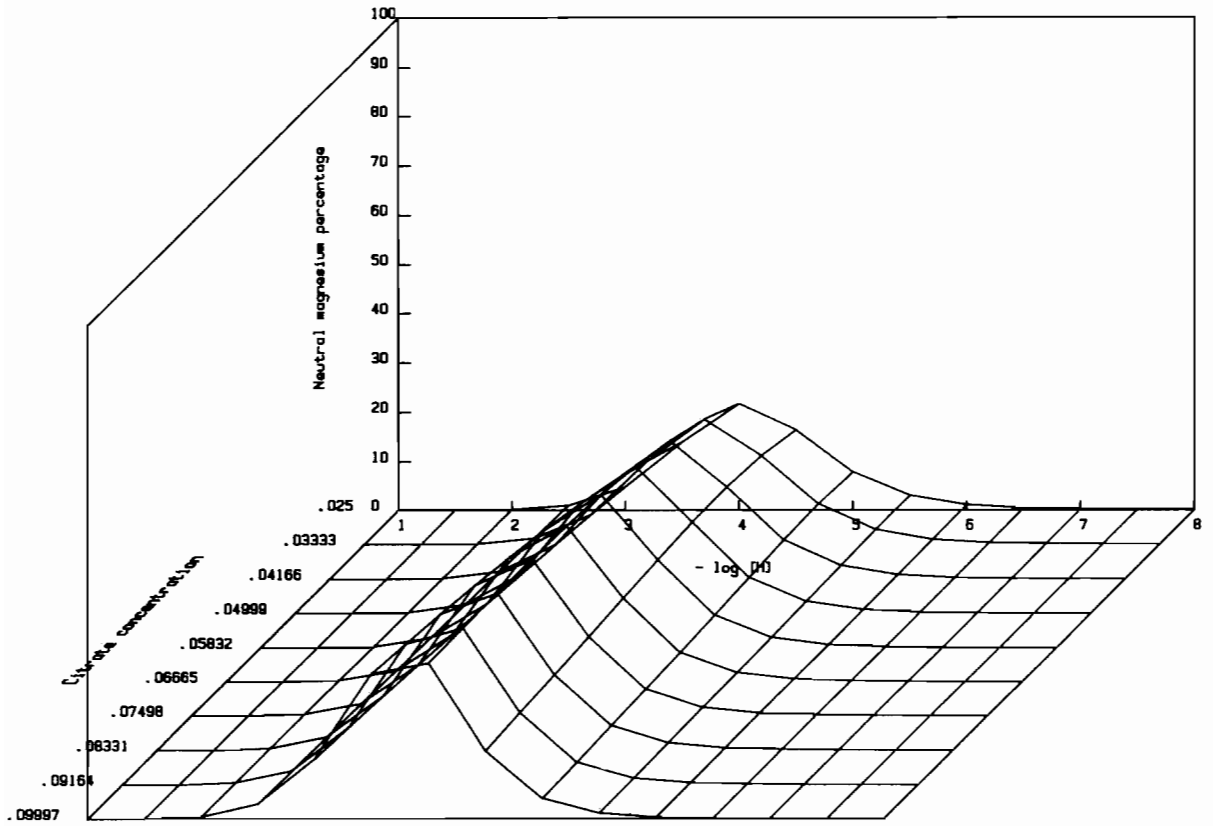


Fig. 5. Distribution of the neutral fraction of magnesium in the presence of different concentrations of citrate ($C_{Mg}$ = 0.025 mol $dm^{-3}$).

of calcium four times that of magnesium under conditions of Fig. 4. From this aspect, aspartate would be still a poorer potential 'carrier' of magnesium than glutamate.

*Citrate*

As shown in Fig. 5, the charge neutralisation of a significant fraction of magnesium occurs in the presence of citrate between pH 2 and 6, this fraction being maximum around pH 4. This result, which is in line with the good therapeutic effects of high doses of magnesium administered in the form of citrate [49], is still observable at higher dilutions of magnesium (simulations not presented here).

Since formation constants of citrate complexes with magnesium and calcium are closely matched (Table II), one would logically expect a strong interference of calcium *versus* the capacity of citrate to mobilise magnesium into a neutral form. Actually, this interference proved to be very dependent on the concentration of citrate. For example, with identical 0.025 mol $dm^{-3}$ concentrations of calcium and magnesium, the fraction of neutral magnesium was reduced by about 30% in the presence of 0.025 mol $dm^{-3}$ of citrate, but it remained essentially unchanged for citrate concentrations over 0.05 mol $dm^{-3}$ (Fig. 6). However, as was the case with aspartate, higher concentrations of calcium lessened this fraction to a more significant extent. Similar effects were noted for lower magnesium concentrations.

From considerations above, citrate appears to be a possible 'carrier' for magnesium. Nevertheless, its use may suffer from a serious limitation. The pH range within which the mobilisation of neutral magnesium is maximum falls between the values relative to stomach (more acidic) and intestine (more basic). One should thus rely on a possible rise of the gastric pH in the presence of food, which is undesirable (see above), or on the buffer capacity of the administered mixture to fit the suitable interval.

To conclude, none of the present ligands can be considered as an ideal 'carrier' likely to help $Mg^{2+}$ ions to dissolve into enterocyte phospholipid membranes. Potentially more promising substances are currently being studied in our laboratory.

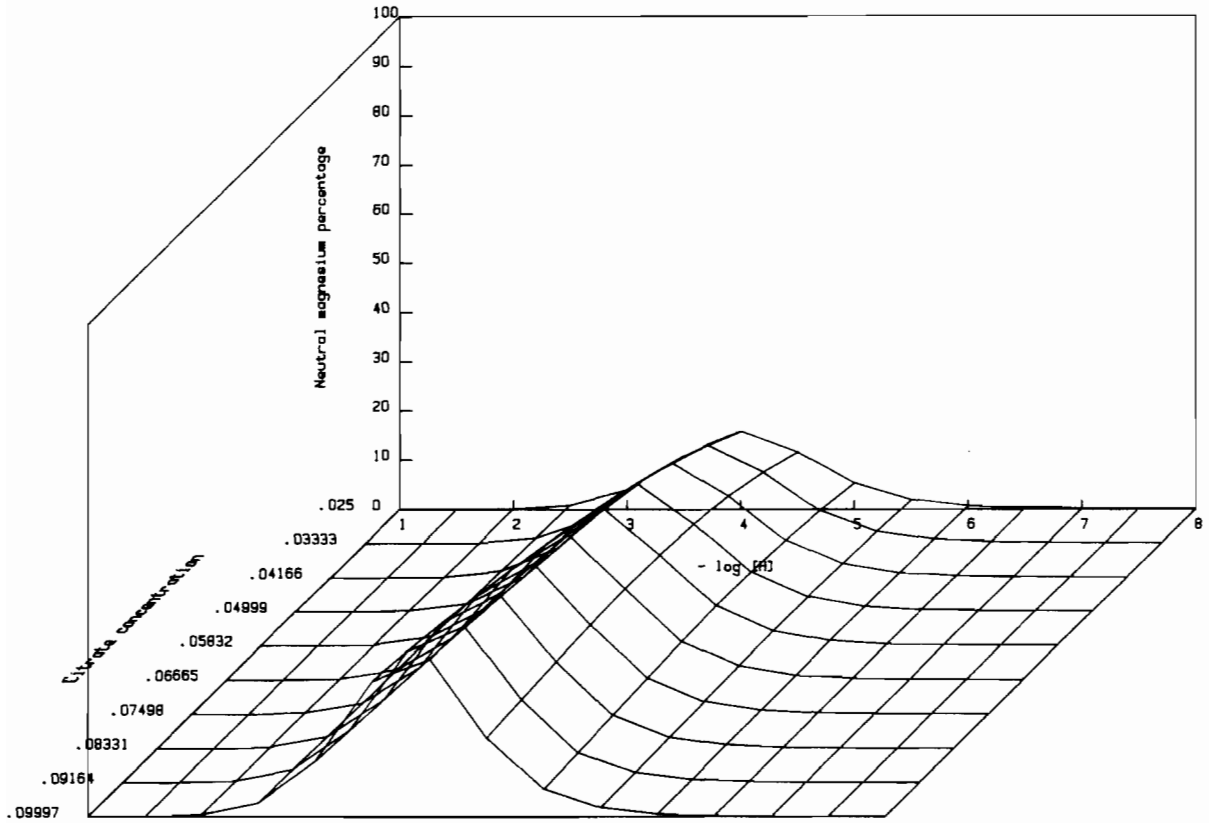


Fig. 6. Distribution of the neutral fraction of magnesium in the presence of 0.025 mol $dm^{-3}$ of calcium and of different concentrations of citrate. Magnesium concentration is the same as in Fig. 5.

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