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Novel oral phosphate binder with nanocrystalline maghemite-phosphate binding capacity and pH effect

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ABSTRACT

Hyperphosphatemia is one of the main risk factors contributing to morbidity and mortality in patients with end stage renal disease. The demand for a new phosphate binder is continuously increasing since the number of patients suffering under hyperphosphatemia is growing. However, side effects and high pill burden of currently available phosphate binders are the main reasons for low compliance and uncontrolled serum phosphate levels. Therefore, the aim of this study was to develop a novel phosphate binder with a high phosphate binding capacity over the entire gastrointestinal (GI) pH range. This novel phosphate binder C-PAM-10 is based on D-mannose coated nanocrystalline maghemite and belongs to the new class of phosphate binders, called the "iron based agents". It was possible to obtain a phosphate binding product that showed very high phosphate binding capacities with the characteristic of being pH independent at relevant pH ranges. The simulation of a GI passage ranging from pH 1.2 to pH 7.5 showed a 2.5 times higher phosphate binding capacity compared to the commonly used phosphate binder sevelamer carbonate. The simulation of a pH sensitive coating that releases the iron based phosphate binder at pH values ≥4.5 still showed a very high phosphate binding capacity combined with very low iron release which might decrease iron related side effects in vivo. Therefore, C-PAM-10 and its variations may be very promising candidates as a superior phosphate binder.

1. Introduction

An estimated 5–10% of the world's population is affected by chronic kidney disease (CKD) which can progress to end stage renal disease (ESRD). Loss of phosphorus homeostasis caused by excretion failure results in an increased serum phosphorous level which is known as hyperphosphatemia. For ESRD patients hyperphosphatemia is one of the main risk factors contributing to morbidity and mortality (Bailie, 2004; Block et al., 2004). Therefore, the mainstay of therapy consists of phosphate binders to bind ingested phosphate in order to reduce phosphate absorption. Studies have shown a significant decrease in mortality in hemodialysis patients with prescribed phosphate binders compared to untreated patients (Isakova et al., 2009; Lopes et al., 2012). These studies also promoted the possibility that phosphate binders have mechanisms contributing to the survival benefit which are beyond controlling the serum phosphorus (Isakova et al., 2009; Lopes et al., 2012). However, further investigations are needed to fully understand these effects.

Although, the demand for a new phosphate binder is increasing, the ideal phosphate binder still does not exist. High pill burden (Table 1) and side effects of the currently available phosphate binders are the main reasons for low compliance and uncontrolled serum phosphate levels. Calcium-based phosphate binders were once used as the standard therapy (Sprague, 2007). However, these agents have been associated with arterial calcification (Goodman et al., 2000; Guerin et al., 2000; London et al., 2003) and therefore are no longer predominantly used in the treatment of hyperphosphatemia. According to the KDOQI (Kidney Disease Outcomes Quality Initiative) clinical practice guidelines for bone metabolism and disease in chronic kidney disease, non-calcium containing phosphate binders such as sevelamer hydrochloride or lanthanum carbonate are preferred in dialysis patients with severe vascular or other soft tissue calcifications (National Kidney Foundation, 2003). Sevelamer hydrochloride (Renagel®, Genzyme) is a non-absorbable polymer of allylamine hydrochloride which is cross-linked with epichlorohydrine (Slatopolsky et al., 1999). It has good

Abbreviations: CKD, chronic kidney disease; ESRD, end stage renal disease; GI, gastrointestinal; KDOQI, Kidney Disease Outcomes Quality Initiative; PCS, photon correlation spectroscopy; ZP, zeta potential.

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Table 1
Daily doses of commonly used phosphate binders.

Active substance and trade name	Range of the daily dose[a]
Calcium carbonate 2500 mg (Calcichew Forte®)	3 tablets
Calcium carbonate 1250 mg (Calcichew®)	3–6 tablets
Calcium carbonate 1500 mg (Adcal®)	3–6 tablets
Sevelamer hydrochloride 800 mg (Renagel®)	3–15 tablets
Sevelamer carbonate 800 mg (Renvela®)	3 –15 tablets
Lanthanum carbonate 500 mg (Fosrenol®)	3–6 tablets

[a] The daily doses are given only for comparison and do not indicate therapeutic equivalence (Scottish Medicines Consortium, 2014).

phosphate binding capabilities (Amin, 2002; Goldsmith et al., 2008) and is well tolerated (Ketteler et al., 2008). Lanthanum carbonate (Fosrenol®, Shire plc) is another non-calcium phosphate binder that is well tolerated if taken with food (Freemont, 2006) and demonstrates good phosphate binding capacity. Like sevelamer hydrochloride, lanthanum carbonate is significantly more expensive than the calcium-based phosphate binders. A further problem of phosphate binders are their pH dependent phosphate binding capacities. For example, calcium carbonate (Calcichew®, Takeda Nycomed) shows a decreased phosphate binding capacity at low pH values (Sheikh et al., 1989) and sevelamer hydrochloride shows optimal binding capacity at approximately pH 7. At values below or above this pH the binding of sevelamer is not as effective (Chertow et al., 1997; Rosenbaum et al., 1997).

Therefore, the aim of this study was to develop a novel phosphate binder with a high phosphate binding capacity that is independent from pH changes in the gastrointestinal tract. This innovative phosphate binder is termed C-PAM-10 and it is based on D-mannose coated maghemite (γ-Fe_2O_3). It belongs to the new class of phosphate binders, known as the "iron based agents". To reduce the risk of iron related side effects, another goal was to achieve low iron release.

2. Materials and methods

Materials used for the synthesis: iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium hydroxide, hydrogen peroxide solution (30% w/w), gum Arabic, sodium phosphate monobasic, and polysorbate 80 (Tween® 80) were purchased from SIGMA Aldrich (Steinheim, Germany). D-mannose was purchased from Acros Organics (New Jersey, USA) and inulin from Spinnrad (Bad Segeberg, Germany). Poloxamer 407 (Pluronic® F-127) was a gift from BASF (Ludwigshafen, Germany).

Materials used for the analytics: sodium phosphate monobasic, ammonium heptamolybdate, sulphuric acid (95.0–98.0%), potassium antimony(III) tartrate hydrate, hydroxylamine hydrochloride, 1,10-phenanthroline hydrochloride monohydrate, glacial acetic acid, sodium acetate trihydrate, phosphate standard for IC 1000 mg/L, and L-ascorbic acid were purchased from SIGMA Aldrich (Steinheim, Germany). Iron ICP standard solution 1000 mg/L Fe was purchased from CARL ROTH (Karlsruhe, Germany). Water used for all experiments was ultra-filtered by a Milli-Q Gradient A10 system (Millipore, Merck KGaA, Darmstadt, Germany).

2.1. Synthesis of D-mannose coated maghemite nanoparticles

The synthesis of coated maghemite nanoparticles as an oral phosphate binding agent was based on the patent DE102011112898A1 (Wagner et al., 2013). First of all, 3.20 g $FeCl_2 \cdot 4H_2O$ and 7.55 g $FeCl_3 \cdot 6H_2O$ were dissolved in 50 mL of aqueous D-mannose solution containing 25 g of D-mannose. The dissolved mixture was then rapidly added under vigorous stirring to 100 mL of a 1.5 M sodium hydroxide solution and stirred for 15 min. Then 3 mL of a hydrogen peroxide solution (30% w/w) was added to oxidize the iron oxide particles to maghemite. The mixture was then heated to 60 °C and stirred for an additional 15 min. To remove excess D-mannose and unreacted water soluble salts, the formed maghemite nanoparticles were washed by dialysis using a molecular porous membrane with a molecular weight cut-off of 3.5 kDa. The dialysis was performed in a water bath filled with approximately 4 L of Milli-Q water. The suspension was dialysed for 7 days and the water was changed three times daily. The suspension was then centrifuged at 3220 g for 10 min to remove large particles. The supernatant containing the maghemite nanoparticles was used for the following steps of the synthesis. Then 3 g inulin and 3 g gum Arabic as pharmaceutical excipients were dissolved. The suspension was dried overnight in the oven at 60 °C. After grinding the dry product with mortar and pestle, a fine brown powder was obtained.

The production was also performed with polysorbate 80 or poloxamer 407 as additional steric stabilizers. After synthesis, these stabilizers were dissolved in the suspension before the dialysis step to form a solution of polysorbate 80 (5% w/v) or poloxamer 407 (1% w/v) concentration. The addition of the steric stabilizers should maintain fine dispersion state and minimize aggregation. The dialysis was performed against an aqueous polysorbate 80 (5% w/v) or poloxamer 407 (1% w/v) solution to prevent washing out the steric stabilizing layers on the particle surface.

2.2. Determination of unbound phosphate

The in vitro test to determine the unbound amount of phosphate was performed according to DIN EN ISO 6878: 2004–2009 (D 11) and is based on the original method of Murphy and Riley (1962).

To 500 µL of blank[1] (Milli-Q water), sample, or standard solution, 10 µL ascorbic acid and 20 µL molybdate reagent were added and filled up with Milli-Q water to 1 mL. Afterwards the mixture was incubated at room temperature (RT) for 15 min. The reduced phosphoantimonylmolybdenum complex was intensely blue colored and photometrically quantified against the blank sample at 880 nm. The unknown phosphate concentration was determined with a standard reference curve.

2.3. Determination of released iron

To 200 µL of blank (Milli-Q water), sample, or standard solution, 100 µL of aqueous hydroxylamine hydrochloride solution 10% (w/v) and 700 µL phenanthroline reagent were added. Afterwards the mixture was incubated at room temperature (RT) for 15 min. The deep red colored tris (1,10-phenanthroline) iron(II) complex subsequently formed was photometrically detected against the blank sample at 510 nm. The unknown Fe^{3+} concentration was determined with the standard reference curve. All photometric measurements were performed by using a UV VIS spectrophotometer Specord® 205 (Analytik Jena AG, Germany).

2.4. Determination of the particle size

To determine the agglomeration tendencies during the dialysis process, the particle sizes of the dialysed and non-dialysed

[1] For all blank solutions pure Milli-Q water was used to assure that no phosphate was present in the blank sample. The blank sample was used to zero the absorbance of all the components excluding the analyte of interest in the sample that was photometrically quantified.