Original Paper

Excretion of Nitrogenous Waste Products from the Intestinal Fluid Simulator Using Super Absorbent Polymer: A New Generation Dialysis

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Introduction. End stage kidney disease (ESKD) is a life-threatening disorder, which results from loss of function of more than 75% of renal tissue. Many treatment modalities have been attempted for this disease, but only renal transplantation, hemodialysis and peritoneal dialysis have been practically accepted. Each of these methods have certain disadvantages, therefore other treatment modalities are needed for better management of these patients. Colonic dialysis (CD) has been proposed as one of the appropriate candidate methods for the removal of electrolytes, nitrogen waste products and excess fluid, using intestinal fluid environment.
Methods. Super Absorbent Polymer (SAP) were synthesized to be used in CD. The intestinal fluid was simulated in terms of concentrations of nitrogenous waste products, electrolytes, temperature and pressure. The simulated environment was treated with 1 g of synthesized polymer at 37 °C. Concentrations of urea, creatinine and uric acid were measured before and after polymer treating.
Results. Intestinal fluid simulator contained 40g urea, 0.3g creatinine, and 0.25g uric acid. SAP adsorbed up to 4000 to 4400% of its weight in the intestinal fluid simulator (1g polymer can absorb 40g fluid). The amount of urea, creatinine and uric acid decreased to 25g, 0.16g and 0.1g, respectively, in the intestinal fluid simulator.
Conclusion. The present study showed that CD is an appropriate method for removal of electrolytes, nitrogenous waste products and excess fluid from an intestinal fluid simulator. Creatinine is absorbed appropriately in SAP, as a neutral molecule. In contrast, urea and uric acid, as weak acids, are absorbed weakly in polymer network.

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INTRODUCTION

Chronic kidney disease (CKD) is a general term, used for a heterogeneous group of disorders, that affect the kidneys, structurally and functionally.[1] Of the main clinical issues in CKD patients, are volume overload, and retention of nitrogenous waste products.[2] In addition, CKD imposes notable financial burden on patients and health care system.[3,4] Ant anger *et al.*[5] showed that, before the advent of hemodialysis, 39% of CKD patients were suffering from significant fluid overload. Therefore, effective and low-cost dialysis method is necessary for the proper treatment of these patients.[6]

There are high levels of uric acid, urea, creatinine and electrolytes in bowel fluid. According to the literatures, intestinal fluid of uremic patients contains approximately 70g of urea, 2.9g of creatinine, and 2.5g of uric acid in a day, which is much more than intestinal solutes of normal individuals.[7] In these patients, the main pathway for entry of the electrolytes, fluid and nitrogenous products into the blood circulation, is gastrointestinal (GI) tract. Therefore, in CKD patients, a number of the functions of the kidneys such as clearance of toxins can be performed by the intestines.[8] These properties, make the GI tract, an appropriate candidate for removal of electrolytes and nitrogen waste products, by using the excretory function of the intestine in dialysis patients.[2] This approach has been overlooked in the treatment of CKD patients. However, recent studies have provided a new interest, in using colonic dialysis (CD) in the treatment of ESKD, and there has been a trend towards the possibility of substitution of some functions of the kidneys by the intestine.

In the recent years, developments of novel chemical materials lead to production of super absorbent polymer (SAP), which has a high absorbing capacity and can absorb fluid and some solutes in the intestinal fluid. Cross-linked polyelectrolyte polymer is a type of SAP polymer, that bounds to another polymer by covalent or ionic binds. SAP is a non-toxic polymer, which can be used orally and absorb excess electrolytes, nitrogenous waste products and fluid in the GI tract.[9] This property makes it an appropriate candidate for removal of these fluids from GI tract. In our previous *in vitro* study,[6] we evaluated the efficiency of SAP, in removal of excess nitrogenous waste products, and in the present study, we will describe the absorption behavior of SAP and excretion of nitrogenous waste products from the intestinal fluid simulator using super absorbent polymer.

MATERIALS AND METHODS

SAP Synthesizing

The polymer, used in this study, is a cross-linked acrylamide/acrylic acid copolymer. It was synthesized using the technique that was proposed by Mahkam and Zohuriaan-Mehr.[10,11] In the process of synthesis of this polymer, acrylic acid (AA)/acrylamide (AM), N, N′-methylenebisacrylamide and Ammonium persulfate have been used as monomer, water-soluble cross-linker and primer, respectably. These polymers are able to absorb distilled water as high as 100 to 1000 g/g after incubation at 37 °C for 4 hours (Figure 1).[11]

Intestinal Fluid Simulator

In the present study, intestinal fluid simulator has been used to examine the absorbing capacity of synthesized polymer. The simulator intestinal fluid was prepared by the following technique: potassium phosphate monobasic (6.8g/L) and sodium dodecyl sulphate (SDS) (2.5g/L) were dissolved in a 1000 mL of deionized water and then pH considered 6.8 ± 0.1. The volume of fluid increased into 1.5L. The pH and transit time were

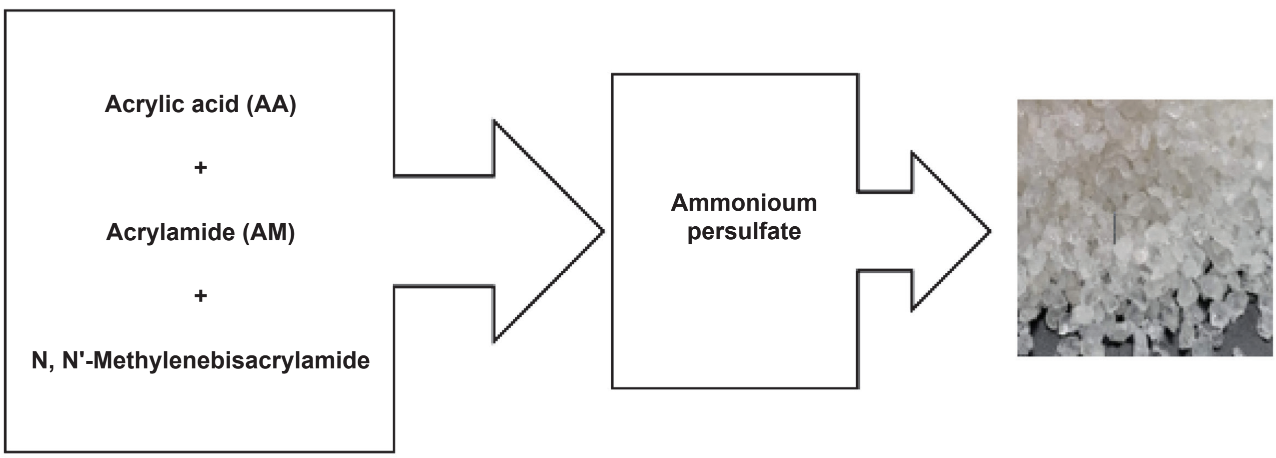

Figure 1. Method of Synthesis of SAP

determined according to previous studies.[12] In addition, the designed system contained 26.6g/L urea, 0.2g/L creatinine, and 0.16g/L uric acid[7] and the temperature was adjusted at 37°C.[10] The intra colonic pressure is considered to be high[13] therefore, all absorbing tests were done under 100 Newton force.

Measurement of Near Infrared (IR) Spectra

A light spectrum close to the infrared light spectrum was recorded, on a PerkinElmer Lambda 9 model spectrophotometer, over the range of 870 to 2600 nm, operated at 60 nm/min. Solid polymer samples were prepared by casting from 2% aqueous solutions of the corresponding polymers, followed by thermal treatment.

Study Protocol

The amount of urea, creatinine and uric acid were measured in the intestinal fluid simulator system, before treatment of polymer. In the next step, 1g of synthesized SAP was passed from intestinal fluid simulator.[6] The amount of urea, creatinine and uric acid were measured in the remaining fluid. The block diagram of the proposed method is shown in Figure 2.

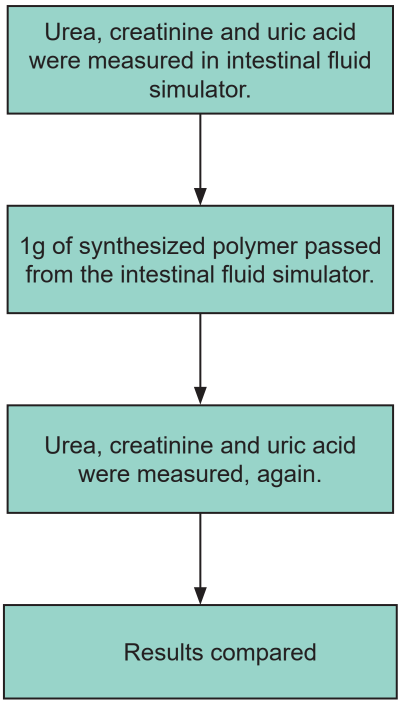

Figure 2. Block Diagram of the Proposed Method

RESULTS

Evaluation of Intestinal Fluid Simulator System

The average transit time was 4 hours for the small intestine, and 6.5 hours for the colon. The pH was approximately 6.2. The temperature was considered 37 °C, in all length of the tract.

SAP Toxicity

SAP property depends on many factors including: the cross-linker concentration, initiator concentration, monomer concentration, reaction temperature, particles porosity and surface cross-linking. The formation of acrylamide and acrylic acid copolymer can be confirmed by observing absorption peaks in the area of 3200 to 3400 cm^{-1}. According to the IR test, the synthesized SAP is a nontoxic polymer, which implicates that all of the monomers have been converted into this polymer and it could be taken oral route, Figure 3.

Polymer Characterization

The absorptive capacity of polymer was 4000 to 4400% in the simulator. Intestinal fluid simulator contained 40g urea, 0.3g creatinine, and 0.25g uric acid. SAP adsorbed up to 4000 to 4400% of its weight in intestinal fluid simulator. After polymer treating, the amount of urea, creatinine and uric acid decreased to 25g, 0.16g, and 0.1g; respectively. As it was mentioned before, with incubation at 37 °C for 4 hours, 1g of synthesized SAP is able to absorb 100 to 1000g distilled water. Surprisingly, it has been shown that the mean amount of potassium increased after SAP treating. This is due to the fact that, K^+ is not only absorbed by ASP, but is also effectively transferred from SAP into the intestinal fluid simulator system. Our investigation also showed that, the low pH environment could significantly decrease the absorptive capacity of SAP (Figure 4).

DISCUSSION

Chronic kidney disease and consequently uremic syndrome can be caused by different pathologic conditions, including diabetic nephropathy, hypertensive nephropathy and glomerular disease, which might finally progress to end-stage kidney disease.[14] Patients with ESKD need at least one of the methods of renal replacement therapy, such as dialysis or transplantation, to keep alive. The use of intestinal fluid is a well-known approach in the

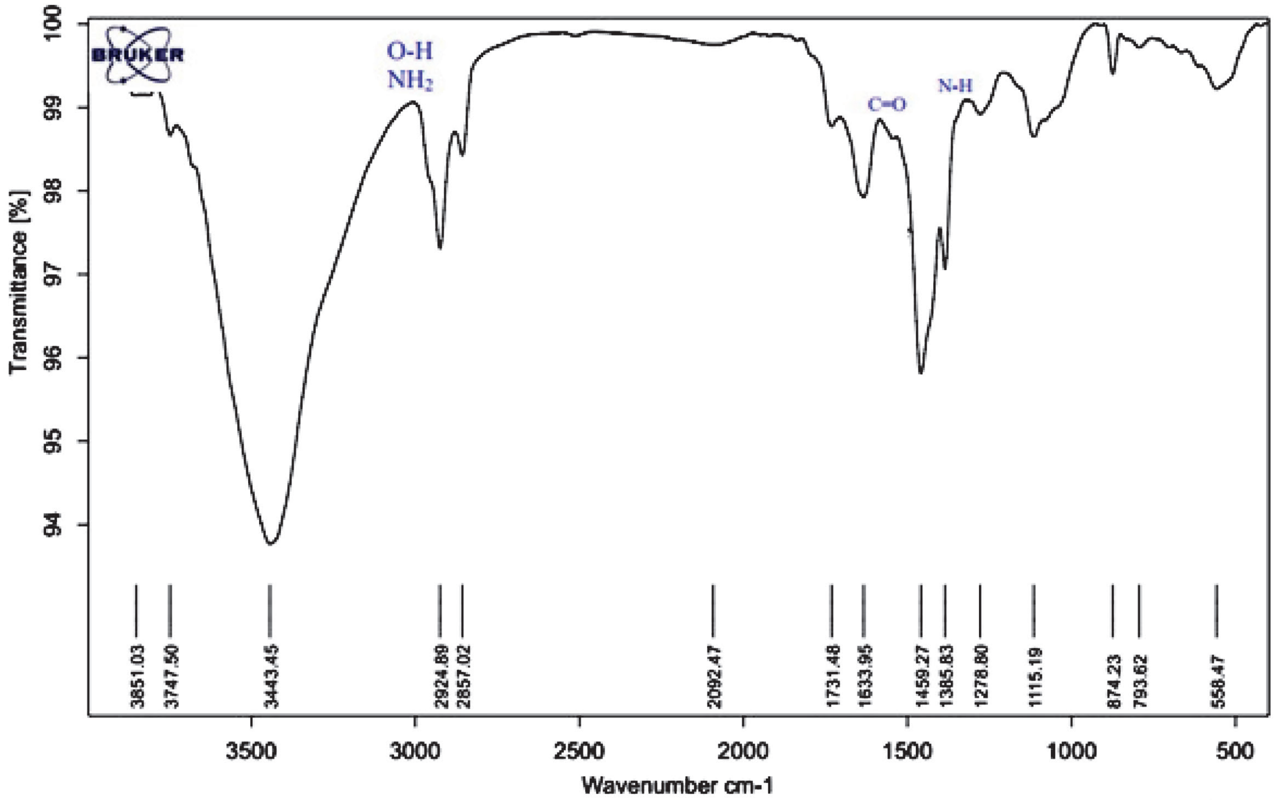

Figure 3. IR Spectra of Synthesized SAP

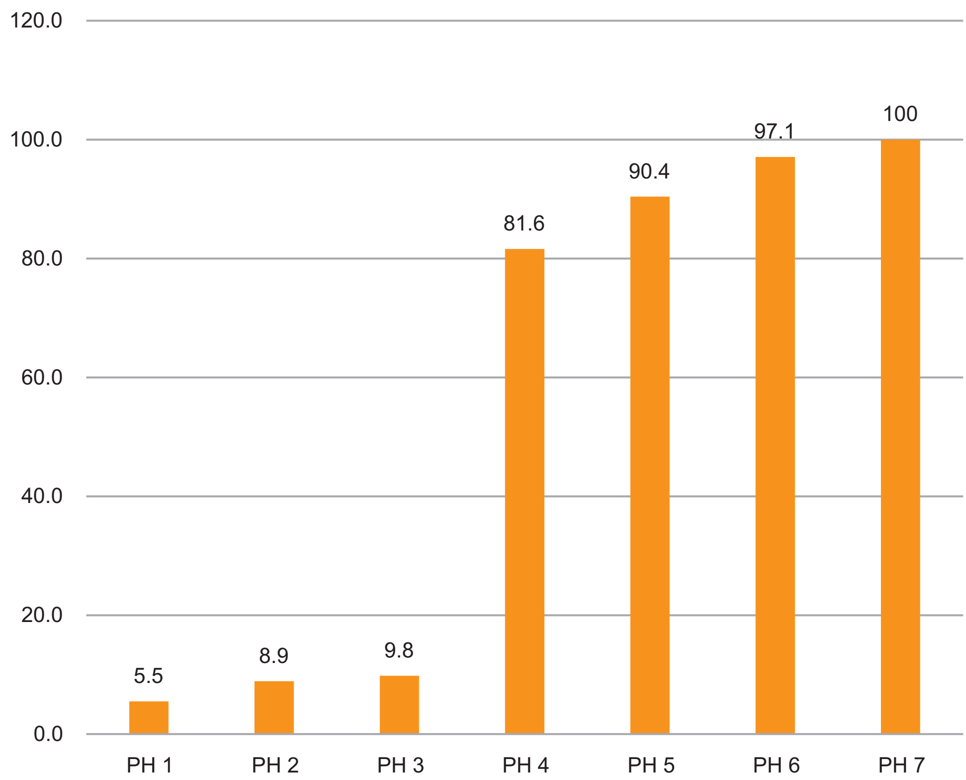

Figure 4. The Absorbing Capacity (%) of SAP at Different pH Values

Table 1. The Values of Urea, Creatinine, Uric Acid and Na in 24 Urine Test, Intestine

	Urea (mg / 24 hours)	Creatinine (mg / 24 hours)	Uric Acid (mg / 24 hours)
24 hours Urine Test	12000 to 20000	800 to 2000	250 to 750
Intestine	70000	2900	2500

treatment of CKD patients. By this technique, the excess nitrogenous waste products and electrolytes, which cannot be removed through the renal route, can be excreted through the intestine. The values of urea, creatinine and uric acid in 24 hours urine sample of healthy men, and GI tract of CKD patients has been shown in Table 1. As is clear, in CKD patients some functions of the kidneys, such as clearance of toxins can be performed by the intestine.

Over the past decades, most studies in CD have emphasized on the use of different materials such as activated charcoal.[6] All of the proposed materials have several limitations, including insufficient absorptive rate of fluid, nitrogenous waste products and electrolytes. It seems that, volume overload and retention of waste products, cannot be resolved by using these materials in ESKD patients.

It has been established that special polymers can improve the absorptive ability of the intestinal environment. The role of SAP in CD has been investigated in limited number of studies. We considered the possibility of using SAP, to absorb excess volume and nitrogenous waste products, from the colon simulated environment. This is the first study reporting advantages and limitations of SAP in removing the nitrogen waste products and electrolytes, from the simulated colon environment. In this study, we tried to simulate the intestinal fluid in terms of concentrations of nitrogenous waste, concentrations of electrolytes, temperature and pressure. In the first step, the simulated environment was treated with 1 g of synthesized polymer at 37 °C. Concentrations of urea, creatinine and uric acid were measured before and after polymer treating. It is clear that creatinine, as a neutral molecule, is absorbed appropriately. In contrast, urea is absorbed weakly in polymer network. These findings confirm the previous findings, and contributes to the evidence that considers uric acid, a weak acid with negative charge. These findings could be explained in terms of electrostatic repulsive force, existing between urea and polymer network. This repulsive force removes the uric acid from the hydrogel polymer, and as a result, urea concentration increases slightly in the intestinal simulated environment. Urea tends to be in aqueous media, instead of being in the SAP structure. The polymer has a negative charge, and prefers the cationic compounds.

Surprisingly, it has been shown that the mean amount of potassium increased after SAP treatment. This phenomenon can be attributed to the types of salts, which are added in the synthesizing process of SAP. In the present study potassium hydroxide has been used for neutralization of acrylic acid. Therefore, after treating, sodium ions migrate from the dialysate effluent fluid to hydrogel structure and exchange with the potassium ions.[6]

Finally, administration of SAP could not remove a wide spectrum of toxic metabolites from the GI tract. Therefore, low protein and phosphorus diet should be recommended to the patients, by using lesser amounts of meat, poultry, fish, milk, cheese, yogurt, and legumes.

LIMITATIONS

Examining the absorptive capacity of the synthesized polymer needs detailed investigation, using complete simulation of GI environment, regarding the concentrations of enzymes and other materials. In addition, this method may be effective only in CKD patients with stage 3 to 4 and before ESKD, and may reduce inflammation and CKD progression as well. Therefore, a case-control or prospective study in CKD patients, seems to be necessary. Finally, similar to PD, this method is not effective in emergency uremic or catabolic conditions.

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