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Formulation and examination of a new urine alkalizing tablet for the symptomatic treatment of bladder pain syndrome

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ABSTRACT

The bladder pain syndrome is a condition with frequent urinary complaints and significant bladder pain. The problem is caused by the loss of the barrier function of the GAG-layer against the irritant substance in the urine, resulting a chronic inflammation of non-bacterial origin.

Our aim was to create a potassium-free, prolonged-release tablet that provides a pH of about 7 by alkalizing the urine, reducing pain and provides proper patient adherence.

Powder blends were formulated, their powder flow was tested and tablets were prepared by direct compression of the powder mixtures with the best flow properties. Dissolution characteristics of the hydrophilic matrix tablets were evaluated, appropriate candidate was selected for a clinical study involving 20 adult patients, previously diagnosed with bladder pain syndrome.

Summarizing the results, an effective alkalizing tablet, causing fewer side effects has been developed for interstitial cystitis/bladder pain syndrome patients. We can conclude that our objectives have been achieved, because the prolonged-release and potassium-free tablet, effectively increases urinary pH, does not irritate the wall of the stomach, and the bladder, which is especially sensitive to potassium.

1. Introduction

Interstitial cystitis (IC) also called bladder pain syndrome (BPS) or painful bladder syndrome (PBS) is a chronic nonbacterial inflammatory disease. In 2002, the International Society for Continence adopted the term painful bladder syndrome. Later, in 2004, the name was changed to bladder pain syndrome because the international consultation on incontinence argued the previous name. After all, the name did not focus on the complexity of the actual symptom. Recently, the name and definition have been refined based on newer findings [1]. The American Urological Association (AUA) also uses the IC/BPS terminology of the Society for Urodynamics and Women's Urology (SUFU) [2], which is precisely defined as "An unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of more than six weeks duration, in the absence of infection or other identifiable causes" [3]. This special disease causes bladder pain and lower urinary tract symptoms, such as pain with or without urgency, frequency, nocturia, and sometimes bloody urine. Symptoms and severity can vary according to stages. Some patients experience only mild discomfort and pressure in the bladder with more frequent urination. However, in advanced stages, people can suffer from extreme pain, sometimes even permanent accompanied by a constant urge to urinate day and night. It is well documented that IC/BPS severely impacts quality of life. It very often leads to depression and impaired sexual function of the patients. Although there are several theories on the etiology of IC/BPS, the cause is not known exactly, and therefore, no definite cure is available either [4–6]. The only background for the development of complaints, the insufficiency of the superficial mucus layer of the bladder, has been proven. This layer consists of glycosaminoglycan (GAG) (Fig. 1) [7]. The proteoglycans and glycoproteins in the GAG layer form a dense layer that protects the pain receptors in the submucosa from urinary excitatory substances such as

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salts, acids, degradation products. This barrier layer is not completely impermeable, but in its intact, undamaged state, it maintains a balance between in and out streaming of fluids and ions. The problem is caused by the loss of the barrier function of the GAG-layer against the irritant substances in the urine. This results in a chronic chemical irritation and an inflammation of nonbacterial origin (sterile inflammation) in the bladder wall [8,9]. The injury of the bladder mucosa is supported by the analysis of biopsy specimens taken from patients with IC/BPS. Loss of the barrier function causes histopathological and molecular changes [10]. An example of them is the appearance of Hunner lesions that show diffuse and intense inflammation due to overexpression of pro-inflammatory genes [11,12].

Treatment strategies consist of oral-, and/or topical (intravesical) treatments, furthermore surgery, or combinations thereof. We studied the main guidelines, the Guideline of the American Urological Association (AUA) and the Canadian Urological Association (CUA), European Urological Association (EUA), and Japanese Urological Association (JUA). All of them contains pain management and medication recommendation:

- Orally: pentosan polysulfate, amitriptyline, cimetidine, hydroxyzine, cyclosporine A
- Intravesically: Heparin, Dimethyl sulfoxide, Lidocaine

Intravesically, in addition to the aforementioned treatments, the CUA, EUA, and JUA recommend hyaluronic acid, chondroitin sulfate and oxybutynin [13–16]. Some articles suggest other intravesical therapy for IC/BPS; containing heparin, hyaluronic acid, chondroitin sulfate, pentosan polysulfate, dimethyl sulfoxide, and botulinum toxin [17].

In the clinical practice GAG replenishing substances (supplements) can be used. Some of such products are already available to patients, like ialuril®containing chondroitin sulfate, hyaluronic acid, calcium chloride, Cystistat®; Hyacyst® containing sodium hyaluronate, and Elmiron® containing pentosan polysulfate, which forms a gel-like layer on the apical cell membrane. Therefore, they help the permeation barrier to regenerate. These active components are used alone or in combination and significant success rates have been reported in patients with IC/BPS [18,19].

It is essential to know that potassium sensitivity differentiates patients with interstitial cystitis from the other sensory disorders of the bladder. Therefore, it was initially recommended to perform a potassium sensitivity test. However, it is not routinely used any longer as it is also ethically questionable because of causing extra pain to the patient. Therefore, therapy aims to avoid increased urine potassium levels to prevent worsening of symptoms [20]. The urine pH is also important because the acidic urine irritates the bladder and increases the pain [14,21]. Urinary alkalinity can be influenced by proper diet, forced fluids intake, and the use of citrates or sodium bicarbonate [22,23]. Citrate salts alkalize urine. For alkalization, a weak acid salt of a strong base is formed. Citrate ions of alkaline citrates are converted to carbon dioxide by oxidative metabolism. They are converted to bicarbonate. The excess base due to the remaining alkali ions is excreted in the urine and increases the pH of the urine. Oral administration of alkaline citrates leads to neutralization or alkalization of urine, depending on the dosage. Serum bicarbonate concentration (negative base excess) is a regulatory factor for citrate secretion. With a negative base excess, the metabolic process moves in an alkaline direction by shifting the value of intracellular pH. This leads to alkalosis-induced inhibition of renal tubular citrate metabolism and decreased citrate resorption as well as increased citrate secretion. Alkalization also affects renal calcium transport in such a way that urinary calcium excretion is significantly reduced. Increased diuresis, and citrate excretion, as well as decreased calcium excretion reduce the potential for calcium oxalate formation, as citrate forms a stable complex with calcium in a weakly alkaline medium. The bioavailability of citrate salts is 100%, they are perfectly degradable. Moreover, citrate salts do not accumulate in the body. As an example, citrate-containing product Blemaren-N® can be mentioned. However, this formulation could be disadvantageous in several aspects: It

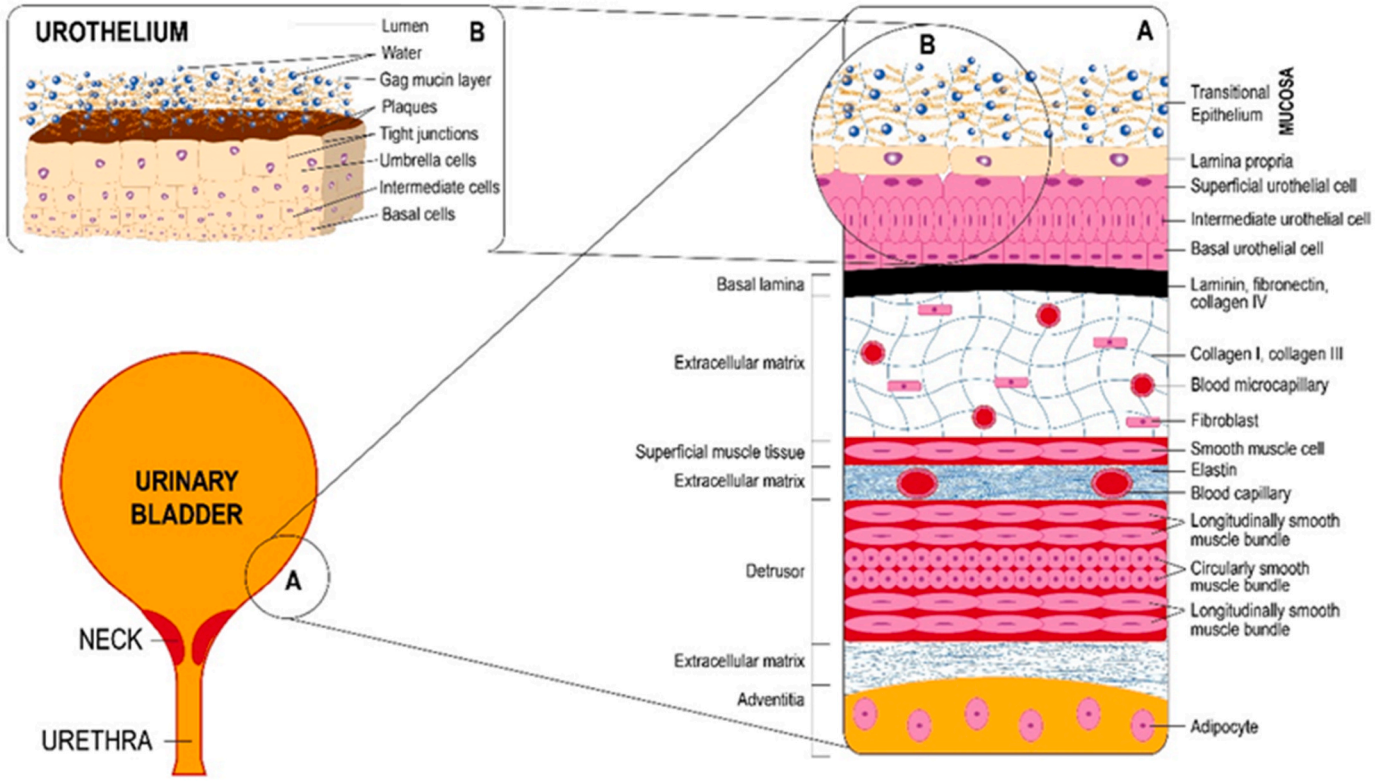

Fig. 1. The bladder structure.

increases urinary potassium levels and therefore severely irritates the inner wall of the bladder in IC/BPS patients.

Taking into account the pharmacokinetic parameters, the amount of sodium and magnesium ingested in one day is entirely eliminated by the kidneys within 24–48 h. During long-term treatment, the daily amounts of excreted sodium and magnesium correspond to the daily intake. Gas and electrolyte levels in blood and serum do not change significantly. This means that by renal regulation of alkalization, the acid-base balance is maintained in the body, and the possibility of accumulation of sodium and magnesium is ruled out in the case of proper renal function [24].

Matrix tablets are solid oral dosage forms. This system is very popular for modified release formulations. The definition of modified release by EMA: "Preparations where the rate and/or place of release of the active substance(s) is different from that of the conventional dosage form administered by the same route. This deliberate modification is achieved by special formulation design and/or manufacturing method. Modified release dosage forms include prolonged release, delayed release, pulsatile release and accelerated release dosage forms" [25]. The active substance may be homogeneously dispersed or dissolved in hydrophilic or hydrophobic polymers in matrices. The preparation of sustained release matrix tablets involves the direct compression of powder mixture of active subtances, retardant material and other additives [24].

We aimed to develop a potassium-free prolonged-release tablet that significantly alkalizes the urine. Important aspect was that the treatment is effective locally, reduce persistent pain, eliminates side effects e.g. gastro-oesophageal reflux, and provides proper patient adherence by a convenient dosage of twice a day. Another aim was to test the efficacy of the new formulation in a randomized double-blind, placebo-controlled clinical pilot study.

2. Materials and method

Materials: Citric acid, sodium citrate, magnesium citrate, colloidal silicon dioxide and magnesium stearate were purchased from Hungaropharma Ltd. (Budapest, Hungary). A high water-binding excipient was used Avicel DG (IMCD Group B.V.,Vienna, Austria) and Benecel K4M PH DC, Benecel K15 M PH DC, and Bencel K100 M PH DC (Ashland Inc., Kentucky, USA) are high viscosity HPMCs were applied. The Benecel™ hypermellose consists of a metoxy content of 20–24% and a hydroxypropil content of 7–12%. These conform to US Pharmacopoeia requeriment for type 2208 subtitution of hypermellose [26]. Hydrophilic matrix may be formulated by direct compression of the blended mixture of active subtances and certain hydrophilic carriers. Such as a hydrophilic carriers is the hydroxipropylmethylcellulose (HPMC). The HPMC is the most widely used semi-synthetic polymer in hydrophilic matrix systems. The best grades to use for sustained release formulations are K4M and K100 M due to their high tensile strength [27]. The "PH" mark indicates that it meets Pharmacopoeia quality and the "DC" mark indicates that it is suitable for direct compression. Avicel DG is a compound of microcrystalline cellulose and anhydrous dibasic calcium phosphate. Avicel DG is an excipient used in dry granulation processes. In addition, this excipient facilitates the compression of the solid pharmaceutical form by improving the robustness of the process, the hardness of the tablet. Furthermore, Avicel DG was chosen because it successfully improves the rheological properties of the formulation for hygroscopic substances.

2.1. Powder blend for direct compression

The first phase of the formulation required special excipients due to the hygroscopic character of magnesium citrate (Ph. Eur.10.). Each active ingredient was powdered separately in a mortar and after sieved until a particle size of 160 μm was obtained, in accordance with the European Pharmacopoeia 10. Then a total of 0.5% anhydrous colloidal silicon dioxide was added to each component in portions with continuous mixing. After homogenization of the active ingredients, the desired lubricating effect is ensured with 0.5% magnesium stearate excipient. A high water-binding excipient called Avicel DG was used to optimize the rheological properties of the blend. The compositions of the blends are shown in Table 2. In all cases the active subtances were the same amount (60%), because the basic immediate release citrate tablet containing 60% by active subtances was efficient previously. . The active substances of the tablets were determined based on the empirical experience of Sándor Lovász, MD, PhD, urologist. Based on the literature [27,29,30], high viscosity HPMC is one of the best options for the preparation of prolonged-release tablets. Three pre-selected Benecel types, namely Benecel K4M PH DC, Benecel K15 M PH DC, and Bencel K100 M PH DC, were used as release rate controlling agents [28]. Powder blends were prepared with five different Avicel DG/Benecel PH DC ratios, using the three different Benecel grades (Table 1). The blends listed in Table 1 were tested to determine their flow properties.

2.2. Powder flow

Flow properties of the 15 different compositions (Hausner ratio and Carr's compressibility index) were determined according to Ph. Eur.10. chapter 2.9.16. ASTM funnel was used for the outflow time determination and for calculating the angle of repose, using the following equation (Eq. (1)). [31].

$$tg\alpha = \frac{h}{d/2} \tag{1}$$

where α is the angle of repose, h and d is the height and the diameter of the powder cone, respectively.

2.3. Tablet compression and physical properties

Based on the rheological results (Hausner ratio and Carr's compressibility index, outflow time) and preliminary dissolution tests three mixtures, namely A/3, B/3, C/3 were selected for direct compression. A Korsch single-punch tablet press equipped (Korsch, Berlin, Germany) with oval shape puches (dye: 7.6 mm, tablet height: 16.2 mm). Compression forces were 10 kN in all cases (4000 tablets/min).

For comparison study the immediate-release tablets with 60% of the active agents (citric acid, sodium citrate, magnesium citrate) were compressed. Average weight of the tablet was 643 ± 2.44 mg.

Uniformity of mass of single-dose preparation (AP224W analytical balance, 220 g/0.1 mg), friability of uncoated tablets (Erweka TAR220 friability tester; ERWEKA GmbH, Langen, Germany) and resistance to crushing of tablets (Erweka TBH 125 tablet hardness tester) was performed according to Ph. Eur.10.

2.4. Dissolution test

Rotating paddle method was used (ERWEKA DT700 and Microette plus 6 autosampler and content uniformity test (Dionex UltiMate 3000 UHPLC + focused with UV detection; Thermo Fisher Scientific Inc., Budapest, Hungary) with the following test parameters: temperature 37 °C, 75 rpm, dissolution media: 900 mL of pH 6.8 phosphate buffer and 2.4 mL of sample volume. HPLC analyses of the active substance content was performed as follows: 1 mL of the sample was acidified with 20 μL of HPLC grade phosphoric acid. A Lichosper 18 °C 250 × 6 mm column was used for the analysis. The determinations were carried out in isocratic conditions at 26 °C. The flow rate of the mobile phase was 1.2 mL min-1. The injection volume was 10 μL. The eluent was 0.05% H_3PO_4/H_2O; (10% H_2O/MeOH column wash only). The limit of detection (LOD) was 0.0607 mg/L. Three parallel dissolution studies were performed, $n = 6$ was in all cases.

Table 1
Different viscosity of Benecel™ grades (from Ashland [28]).

Grade	Weight Average Molecular Weight	Solution Concentration	Nominal Viscosity(mPa*s)[a]	Application	Avareage partical size(μm); D_{90}
Benecel™ K4M PH DC	400.000	2%	2.700–5.040	Controlled released	184 ± 0.2
Benecel™ K15 M PH DC	575.000	2%	13.500–25.200	Controlled released	191 ± 0.5
Benecel™ K100 M PH DC	1.000.000	2%	75.000–140.000	Controlled released	182 ± 0.7

*'K' identifies type of HPMC.; 'PH' Pharmacopoiea; 'DC' direct compression.
[a] NF/EP/JP viscosity method.

Table 2
Composition of the powder blends.

Sample ID	1	2	3	4	5
Active substances	60%	60%	60%	60%	60%
Magnesium- stearate	0.5%	0.5%	0.5%	0.5%	0.5%
Colloidal silicon dioxide	0.5%	0.5%	0.5%	0.5%	0.5%
Benecel [a]	30%	25%	20%	15%	10%
Avicel DG	9%	14%	19%	24%	29%

[a] Type of Benecels

2.5. Dissolution data analysis

A model-independent analysis was performed to determine the comparability of the dissolution profiles of the tested tablet composition and the value of the similarity and difference between them. Dissolution efficacy (*DE*) was also calculated for the average dissolution profile of all compositions (Eq. (2)).

$$DE = \frac{\int_0^t y dt}{y_{100} t} \cdot 100\% \quad (2)$$

where *y* is the cumulative percentage dissolution at time *t* [32].

By calculating the difference, f_1 factor, and similarity f_2 factor, we compared composition C/3 vs. composition A/3, and B/3, respectively. Difference and similarity factors were calculated using the following equations:

$$f_1 = \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} R_j} \times 100$$

$$f_2 = 50 \times \log\left\{ \left[1 + (1/n) \sum_{j=1}^{n} w_j |R_j - T_j|^2 \right]^{-0,5} \times 100 \right\}$$

where n is the sampling number, R_j and T_j are the percent dissolved of the reference and the test products at each time point *j* and where w_j is an optional weight factor.

For kinetic modeling of drug release, dissolution data of composition C/3 were performed by non-linear fitting with zero-order (Eq. (3)), first-order (Eq. (4)), and Korsmeyer-Peppas models (Eq. (5)) using Microsoft Excel.

$$Q = Q_0 + k_0 t \quad (3)$$

$$Q_t = Q_0 e^{-k_1 t} \quad (4)$$

$$\frac{Q_t}{Q_\infty} = k_{kp} t^n \quad (5)$$

where *Q* is the amount of drug release at time *t*, Q_0 is the initial amount of drug, Q_t Qt is the amount of drug remaining at time *t*, and where $\frac{Q_t}{Q_\infty}$ Qt/Q∞ is a fraction of drug released at time *t*. k_0, k_1, and k_{kp} are the kinetic constants for zero order, first order, and Korsmeyer-Peppas models, respectively and *n* is the release exponent, indicative of the drug release mechanism. For Korsmeyer-Peppas model, only release data points were used in the analysis up to 60% drug release [33].

2.6. Stability test of C/3 tablet

After 24 months, we examined the stability of the C/3 tablets that we formulated and tested. It was stored in a dry place, protected from light. We examined the parameters of the tablet again, such as length, width, mass and breaking strength. In addition, the amount of active components was checked by 6 parallel dissolution tests. Finally, the release kinetics were defined.

2.7. A clinical pilot study to examine efficacy and patients' compliance of the prolonged-release tablet

The trial was conducted at a single location; at Rózsakert Medical Center (RMC, Hungary, Budapest). According to the inclusion criteria, twenty adult patients, four men, and sixteen women, previously diagnosed IC/BPS patients on maintenance therapy were involved on a voluntary basis. The trial was carried out under a licence of the Local Committee of Research Ethics (permit number:7074–2/2020).

The endpoints of the clinical trial were the following: to estimate the average urine pH fluctuation depending on the time of the day; to determine whether the conventional (immediate-release dosage formulation, without modified-release) citrate tablet (Table 3) mitigates the pH fluctuation; to estimate the pH raising effect on the average of administering 2 × conventional citrate tablet daily; to estimate the raising effect on the average of administering 2 × 1 of prolonged-release tablet (composition C/3) and determining whether the prolonged-release tablet mitigates the daily pH fluctuation.

During the clinical pilot trial, on the first week, the patients did not take any tablets that may alter the pH of the urine. In the following three weeks, they took either placebo (Table 4), or potassium free immediate-release citrate blend tablet, or prolonged-release citrate tablet, which has a long-lasting (10 h) release effect containing the same compounds as the normal tablet – for one week, 2 tablets per day. Every patient receives every treatment once, one week long, in a randomized

Table 3
Compositions of Prolonged-release citrate tablet and Immediate release citrate tablet [34].

Prolonged-release citrate tablet		Immediate release citrate tablet
53.04 mg	Citric acid	68 mg
142.74 mg	Sodium citrate	183 mg
189.54 mg	Magnesium citrate	243 mg
3.215 mg	Magnesium stearate	16 mg
3.215 mg	Colloidal silicon dioxide	3.50 mg
–	Stearic acid	12.50 mg
–	Sorbitol	300 mg
128.6 mg	Benecel K100 M PH DC	–
122.17 mg	Avicel DG	–

Table 4
Composition of Placebo tablet.

Placebo tablet	–
Sorbitol	743.4 mg
Magnesium stearate	41.3 mg
Talcum	41.3 mg

sequence.

Due to the requirements of a placebo-controlled, double-blind clinical pilot trial, the three different tablets had to be of the same appearance and mass. (Fig. 2). According to this, the prolonged-release tablet contained 22% less active compounds, which was taken into consideration in the discussion of the results.

The patients were given a freshly calibrated, digital Testo-type portable laboratory quality urine pH meter and were given an accurate pH measurement protocol including step-by-step instructions. Patients were asked to measure the pH of the urine, at least five times during the day and twice at night. These data were asked to be registered on a webpage that had been designed especially for the clinical trial, for registering and storing all measured data. These data were exported for the statistical evaluation at the end of the trial period. Moreover, patients were allowed to add any text containing comments, remarks, suggestions, or side effects. In case of urgent issues, we provided a contact to the doctor who was leading the clinical trial.

All twenty patients involved were able to comply with the trial requirements, including recording of the data. The patient compliance, thus, was excellent. No patient reported any side effects or worsening of their condition in connection with the tablets taken.

The statistical evaluation of the data was performed with the program named Statistica (TIBCO Software Inc. (2018). Statistica (Data Analysis Software System), version 13. http://tibco.com).

3. Results and discussion

3.1. Powder flow

Based on the preliminary examination the rheological results of 15 samples are shown in Table 5. Based on preliminary rheological results (Hausner ratio, Carr's compressibility index, etc.) and due to the small variance, we found 3 promising mixtures with rheological 15 grains: A/3; B/3; C/3 mixtures were chosen. These mixtures have the same ratio composition, differing only in the Benecel™ type variety.

3.2. Physical parameters of tablets A/3, B/3, C/3

The rheology results are summarised. The A/3 results: Hausner ratio 1.15, Carr index 13.72 and the tilt angle was 43.83°. The B/3 rheological results were 1.17, 15.34 and 45.83°.The rheological results of C/3 powder mixtures are as follows: Hausner ratio, Carr's index, and angle of repose were 1.17, 15.28, and 44.77°, respectively. Based on the Ph. Eur. 10. Hausner ratio (1.12–1.18) and CI (11–15) can also be classified as good flow category and 44.77° in the acceptable category (41–45).

The results obtained for the determination of the individual and average weight comply with the Pharmacopoeia specifications. For N = 30 tablets, the maximum deviation was 5%, up to a maximum of ±10%.

Table 5
Powder flow results of the powder blends.

Sample	Hausner ratio	Carr's index	Bulk density average (g/65 ml)	Flow rate (sec) average	Angle of repose (degree)
A/1	1.19 ± 0.13	16.57 ± 0.18	38.13 ± 0.31	15.69 ± 0.27	38.66
A/2	1.16 ± 0.19	13.64 ± 0.21	38.13 ± 0.36	18.53 ± 0.85	43.23
A/3	1.15 ± 0.10	13.72 ± 0.11	38.97 ± 0.15	15.75 ± 0.34	43.83
A/4	1.13 ± 0.15	11.64 ± 0.23	39.07 ± 0.26	19.30 ± 0.88	43.23
A/5	1.14 ± 0.22	12.02 ± 0.34	37.93 ± 0.35	13.59 ± 0.45	44.13
B/1	1.19 ± 0.17	15.99 ± 0.15	39.10 ± 0.12	15.55 ± 0.33	43.83
B/2	1.16 ± 0.31	15.96 ± 0.22	39.50 ± 0.27	13.73 ± 0.11	43.83
B/3	1.17 ± 0.13	14.34 ± 0.09	39.97 ± 0.06	10.88 ± 0.08	45.23
B/4	1.18 ± 0.17	15.31 ± 0.14	39.37 ± 0.42	10.12 ± 0.20	43.83
B/5	1.18 ± 0.25	14.95 ± 0.31	39.60 ± 0.35	10.56 ± 0.12	44.42
C/1	1.19 ± 0.35	15.31 ± 0.24	38.43 ± 0.16	17.23 ± 0.21	44.71
C/2	1.18 ± 0.11	14.95 ± 0.35	39.07 ± 0.44	18.65 ± 0.32	44.42
C/3	1.17 ± 0.07	14.16 ± 0.05	39.28 ± 0.05	15.37 ± 0.04	44.71
C/4	1.15 ± 0.20	12.66 ± 0.26	37.93 ± 0.56	10.76 ± 0.23	46.67
C/5	1.14 ± 0.15	12.33 ± 0.19	38.35 ± 0.27	17.47 ± 0.17	47.47

*A samples contain: Benecel K4M PH DC.
B samples contain: Benecel K15 M PH DC.
C samples contain: Benecel K100 M PH DC.

Active substances content uniformity: min 372.4 mg; max 422.6 mg; average of 6 tablets: 397.83 ± 2.99 mg.

Friability is also adequate. For N = 20 tablets, it was 0.88% ± 0.01, fulfilling the pharmacopoeial critera. Mean value of crushing strength (n = 20 tablets): 182 N ± 4.3.

3.3. In vitro drug release: A/3, B/3, C/3

When the in vitro dissolution data of all compositions were compared, composition C/3 were found to release its API content in the most balanced manner. An average of 28.33% of the citrate salts dissolves in 1 h. Subsequently, 54.64% of the active ingredient is dissolved in 3 h, and after 6 h, 80.83% of the active ingredient is released from the matrix tablet (Fig. 3). On the other hand, composition B/3 has released its citrate salt content in the fastest manner, An average of 34.25%, 62.48% and 84.59% dissolved in 1 h, 3 h and 6 h, respectively. The dissolution efficiencies and determination coefficients of model fittings are presented in Table 5. Composition C/3 was compared in a pair-wise way to composition A/3 and B/3, difference and similarity factors are presented in Table 6. When the mechanism of drug release is elucidated, the coefficient of determination of the fit for the First-order model was found with the highest value.

Fig. 2. Patients received placebo, potassium-free prolonged-release and potassium-free normal citrate tablets of the same appearance.

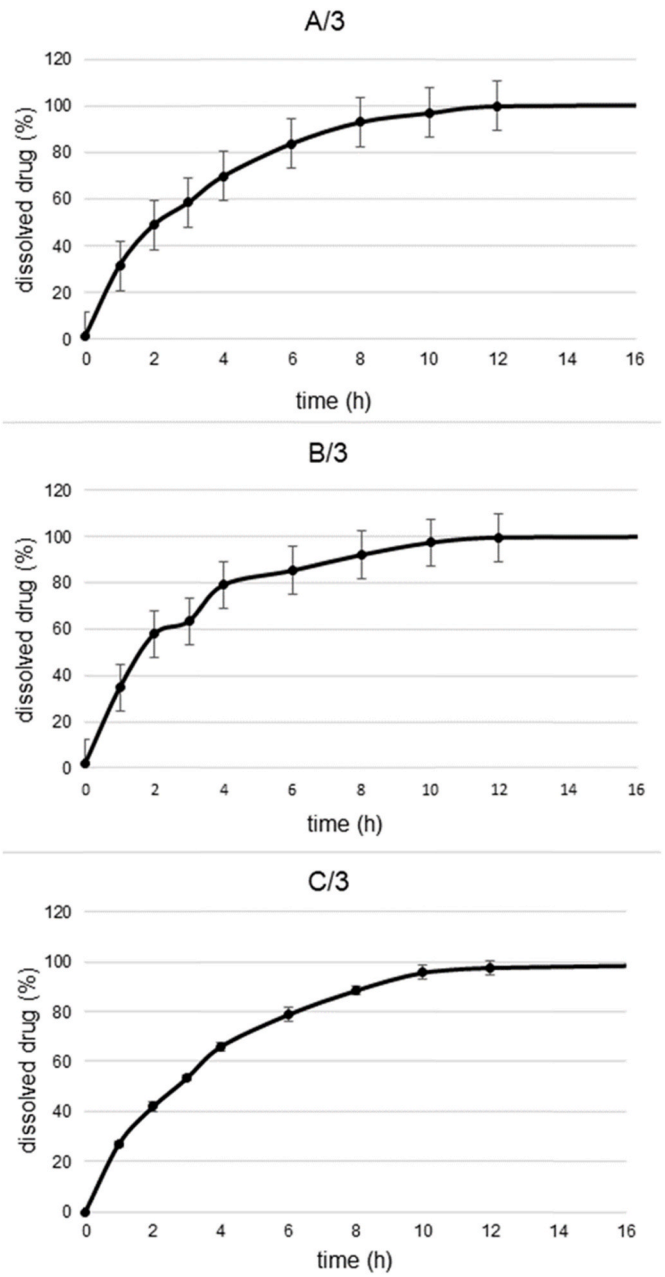

Fig. 3. The amount of prolonged-release tablet dissolved per unit of time (n = 6).

Table 6
Dissolution efficacies of the compositions and model fitting results.

-	A/3	B/3	C/3
DE (%)	87.51%	90.50%	84.35%
Zero order	0.8503	0.7898	0.8850
First order	0.9951	0.9752	0.9962
Korsmeyer-Peppas	0.9313	0.9589	0.9226

The immediate release tablet was also tested, to determine the drug release. The immediate-release tablet disintegrates quickly and complete dissolution occurs after 3 min (Fig. 4). In support of first-order kinetics, compared to the immediate-release tablet, the release rate of the prolonged-release tablet (C/3) is slower and more uniform.

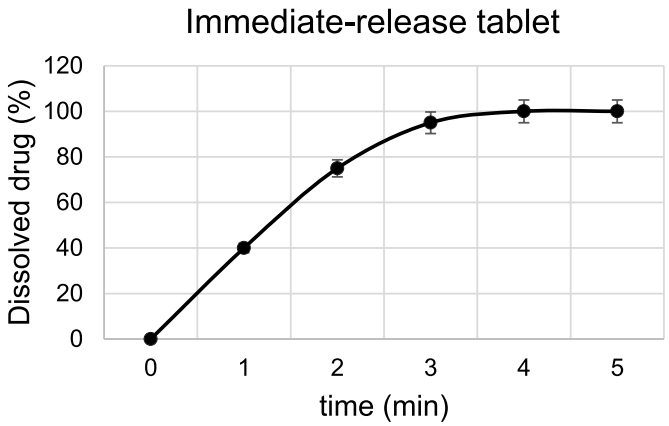

Fig. 4. The amount of immediate-release tablet dissolved per unit of time (n = 6).

The pair-wise comparison have confirmed that the dissolution profile of A/3 is more similar to C/3 than B/3. Concerning the rate of citrate dissolution form their matrix, this is not surprising, drug dissolution was the slowest in case of C/3, while the fastest for B/3. Based on the dissolution properties of composition C/3 tablet, it was selected for a human clinical pilot study.

3.4. Stability test of C/3 tablet

The parameters of the tablet after 2 years were: length 16.2 mm, oblong tablet width: 7.6 mm. Mean value of crushing strength (n = 20 tablets): 167.9 N ± 3.3. The average mass was 625.2 ± 1,4 mg. Friability was 1.02% ± 0.02.

Based on these results, the average weight of a tablet decreased by 2.78% and the crushing strength by 7.75%. C/3 tablets stored for 24 months were analysed and compared to the initial dissolution data. Dissolution efficacy was found to be 89.41%. Model fitting results showed no alteration even after 24 month, First-order release model was found with the highest value for determination coefficient, namely R_2 = 0.9743.

Difference and similarity was also assessed for C/3 tablets regarding their dissolution. Value of difference and similarity factor was 8.34 and 59.94, respectively (Supplementary Fig. 1).

3.5. Clinical study and patients compliance

The weekly average of the urine pH showed no significant difference between the washout period (first week) and the placebo. (pH = 6.10 and pH = 6.13, respectively.) Thus, the placebo effect is minuscule.

Both the immediate release citrate tablet and prolonged-release citrate tablet caused a significant rise in the urine pH (weekly average) compared to the weeks when no active alkalizing compounds were administered (washout and placebo).

The weekly average of the urine pH showed no significant difference between the two alkalizing tablets (potassium-free conventional tablet pH = 6.34, potassium-free prolonged-release tablet pH = 6.29) (Fig. 5). Considering that prolonged-release tablet contained 22% less active ingredients, this result can refer to the fact that the biological utilization of the prolonged-released tablet was more effective.

Another endpoint of the clinical trial was to confirm the hypothesis that the retard tablet mitigates the daily pH fluctuation of the urine, compared to the fast-absorbed alkalizing tablet, which may cause sudden, uncontrollable pH peaks. The smaller fluctuation may result in less pa in and fewer urinary symptoms. According to the statistical evaluation, the clinical trial did not confirm this hypothesis.

The suspected pH peaks might have been detected if the patients, most preferably, had voided and measured the urine in every hour; or at

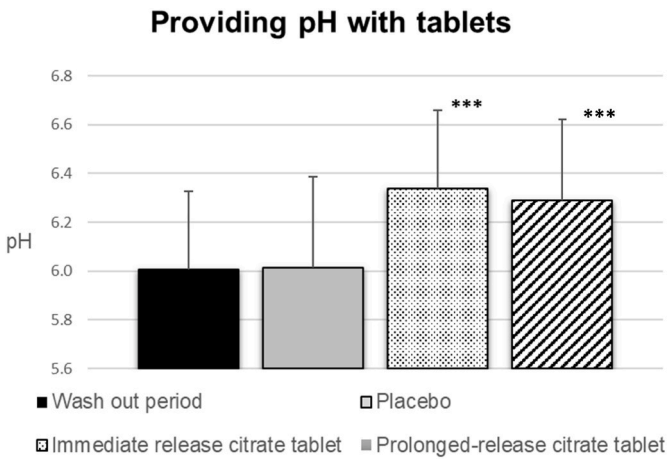

Fig. 5. The patient's mean pH after 1-1 week treatment. Asterisks indicate $p < 0.05$ compared to the wash out period.

a unified amount of time after having taken the tablet. These criteria could not be fulfilled in practice. There are plenty of factors affecting the urine pH, for example, the patient's diet, lifestyle, metabolism, comorbidities, and any medication administered during the treatment. Monitoring or affecting all these factors could not be executed during the trial; standardizing these parameters would have been impossible. The relatively low patient number (20 persons), which is normal for a pilot trial, did not help confirm our hypothesis either.

Nevertheless, the statistical evaluation of the data did not deny our hypotheses either.

Summarizing the results, we successfully developed a prolonged-release tablet, which is suitable as a treatment of patients with BPS. This matrix tablets are solid oral dosage forms. This system is very popular for modified release formulations. The definition of modified release by EMA: "Preparations where the rate and/or place of release of the active substance(s) is different from that of the conventional dosage form administered by the same route. This deliberate modification is achieved by special formulation design and/or manufacturing method. Modified release dosage forms include prolonged release, delayed release, pulsatile release and accelerated release dosage forms" [25].

Based on the measured rheological values, the tested granulate has good flow properties. The choice of excipients had to take into account that polymers and carbomer matrix systems could not be used due to the high Mg^{2+} and Na^{+} content. Because the reason for this, the osmotic pressure induced by the cations would have divided the texture of the matrix. Therefore, the best choice was high viscosity Benecel hypromellose (HPMC, microcrystalline cellulose). Because, the HPMC is the most widely used semi-synthetic polymer in hydrophilic matrix systems. The preparation of sustained release matrix tablets involves the direct compression of powder mixture of active subtances, retardant material and other additives [24]. Hydrophilic matrix may be formulated by direct compression of the blended mixture of active subtances and certain hydrophilic carriers. Such as a hydrophilic carriers is the hydroxipropylmethylcellulose (HPMC). The sensitivity of HPMC to cation-induced osmotic pressure is significantly lower than that of Carbomer matrix systems [32] and the best grades to use for sustained release formulations are K4M and K100 M due to their high tensile strength [26]. Our data have supported this statement, that high viscosity HPMC is the best solution for the preparation of a prolonged-release tablet.

An alternative could be pellets coated with semipermeable membranes, such as Aquacoat ECD. Pellets are then filled into hard gelatin capsules.

According to FDA guidelines [35], based on similarity and difference factors, tablet samples can be considered similar according to their in vitro dissolution kinetics, within the limit (for f_1: 0–15 and f_2: 50–100). The model fit confirmed the First-order kinetics; the drug is released diffusely out of the matrix. According to the First order drug release model, as the remaining drug in the matrix decreases, the released amount of drug by time drops, as well. This finding is not surprising, considering the fact of a relatively high drug loading (60%) with a 20% of dissolution controlling polymer content. Hydrophilic matrices control drug release via the diffusion of the drug through the gel layer of the matrix. Initially, the polymer should transit from the glassy state to the rubbery state during hydration. If the thickness of gel layer is not large enough, highly soluble drugs (in our case, the citrates) could dissolve quickly in an uncontrolled manner [36]. Sustained release of potassium citrate was achived when hydrophobic matrix was formulated with carnauba wax, stearyl alcohol and stearic acid with the drug: release retardant ratio of 2.84:1 [37]. In our case, the C/3 composition formulation, the ratio of citrates to the Benecel polymer was slighty higher, namely 3:1. Interestingly, this resulted a more balanced release compared to the hydrophopbic matrix. Slight increase in the dissolution rate was found up on storage for C/3 tablets. According to the difference and similarity factor calculations the dissolution profiles can be considered to be similar to each other even after 24 months of storage.

It is confirmed that prolonged-release effectively raises the urine pH. To reach the optimal pH range (6.5–7.2) its dose can be increased according to the needs. It appears to be more effective than the standard alkalizing tablet, although the difference may be insignificant (Table 7).

The dosing of twice per day, considering the 10-h release (which was confirmed), is beneficial because patients do not have to be remembered to take their third dose in the middle of the day. This beneficial aspect results in high patient compliance.

Due to the prolonged-release property, this tablet does not irritate the stomach, does not cause substantial acid production since the active ingredients are mostly absorbed in the intestines. Thus, it can securely be administered in case of hyperacidity and gastroesophageal reflux, as well.

Since prolonged-release tablet is potassium-free, its administration significantly lowers the risk of cardiac symptoms, which is present in other alkalizing tablets. It is not contraindicated even in case of hyperkalemia caused by impaired kidney function, and it does not irritate the bladder wall, which is especially sensitive to potassium.

However, the new tablet minimally increased urinary pH compared with placebo. Thus, based on the results, 2 × 2 dosing would be more efficient, and the urine pH could get even closer to 7. Furthermore, a longer duration of the study and a higher patient number would be required for more accurate results.

Our results could not be compared with other results in IC/BPS indication. Citrate preparations have been used in other indications such as hypersensitive bladder syndrome [14], but these preparations contained potassium and did not possess a prolonged-release.

4. Conclusion

Considering the release kinetics of the drug, it can be clearly stated that the newly developed tablet provides a uniform therapeutic concentration in vivo at a frequency of 2 × 1. According to the results of the prospective, placebo-controlled, randomized, double-blind clinical pilot trial the potassium-free, prolonged-release alkalizing tablet effectively mitigates the acidity of the urine, causes fewer side effects. Due to the long-lasting and stable release of the active compounds, it is to be

Table 7
Difference and similarity factors of dissolution data of composition C/3 compared to the other compositions.

-	A/3	B/3
f_1	4.56	9.34
f_2	75.37	58.59

administered twice a day, which results in positive patient compliance.

An easy-to-take, effective alkalizing tablet causing fewer side effects has been developed for interstitial cystitis/bladder pain syndrome patients.

Based on the stability tests performed, we can state that our preparation is stable even after 2 years and meets the requirements.

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Author statement

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Declaration of competing interest

We declare that no conflict of interest exists.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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