Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **37** (6): 1097-1103 (2018)

Received: January 22, 2018
Revised version: April 16, 2018
Accepted: April 17, 2018

In Situ Synthesis, Encapsulation in Arabinoxylan and Release Kinetics of Microcrystalline Copper(II)-Aspirinate

Fozia IRAM [1], Mohammad S. IQBAL [2] *, Abdul H KHAN [3], Riaz AHMAD [4] & Muhammad SHER [5]

[1] *Department of Chemistry, LCW University, Lahore, Pakistan*
[2] *Department of Chemistry, Forman Christian College, Lahore 54600, Pakistan*
[3] *Department of Pharmacy, Forman Christian College, Lahore 54600, Pakistan*
[4] *Centre for Advanced Studies in Physics, GC University, Lahore, Pakistan*
[5] *Department of Chemistry, University of Sargodha, Sargodha, Pakistan*

***SUMMARY*. Microcrystalline copper(II)-aspirinate was synthesized *in situ* by allowing copper(II) acetate monohydrate and aspirin to react in pre-swollen arabinoxylan (AX) from ispaghula *(Plantago ovata)* husk in aqueous medium. The method resulted in formation of microcrystalline (< 40 mm) copper(II)-aspirinate dispersed in AX matrix. The product was characterized by microscopic FT-IR spectroscopy, pXRD, scanning electron microscopy and atomic force microscopy. The AX-encapsulated copper(II)-aspirinate exhibited a smooth release profile of aspirin over 8 h following Korsmeyer-Peppas model for swellable polymer films in alkaline medium. The release was of Fickian type with n = 0.5. The release rate appeared to follow the order: alkaline pH > distilled water > acidic pH suggesting the pH-dependent release from AX. These profiles were highly sustained as compared with those of the naked drug.**

RESUMEN. El aspirinato de cobre(II) microcristalino se sintetizó *in situ* permitiendo que el acetato de cobre(II) monohidrato y la aspirina reaccionen en medio acuoso de arabinoxilano (AX) preinflamado de la cáscara de ispaghula *(Plantago ovata)*. El método dio como resultado la formación de aspirinato de cobre(II) microcristalinoa (<40 mm) dispersadr en una matriz de AX. El producto se caracterizó por espectroscopia de FT-IR microscópica, pXRD, microscopía electrónica de barrido y microscopía de fuerza atómica. La aspirina de cobre(II) encapsulada en AX exhibió un perfil de liberación suave de aspirina durante 8 h siguiendo el modelo de Korsmeyer-Peppas para películas de polímero hinchables en medio alcalino. La liberación fue del tipo Fickiana con n = 0.5. La velocidad de liberación pareció seguir el orden: pH alcalino> agua destilada > pH ácido que sugiere la liberación dependiente del pH de AX. Estos perfiles fueron muy sostenidos en comparación con los de la droga desnuda.

INTRODUCTION

Copper(II)-aspirinate, tetrakis-μ-acetylsalicylato-dicopper(II), is a copper(II) complex of aspirin (**I**), known to possess enhanced anti-inflammatory, anti-ulcer, anti-cancer, anti-thrombotic and anti-oxidant activities [1-6]. Orally it is about ten times more active as an anti-inflammatory and anti-thrombotic agent than aspirin [1,3,7,8]. It is a drug with promise for rheumatoid arthritis, where large doses of aspirin are required [9]. However, clinicians are still skeptic of its use because of the presence of copper in the molecule. Therefore, an enhancement of its bioavailability and thereby reduction in dose to overcome the risks associated with this drug is still a challenge [10,11]. It is a poorly soluble drug as it is only soluble in coordinating solvents like dimethylformamide [2,12]. Bioavailability of poorly soluble drugs is largely dependent on particle size; smaller the particle size (larger surface area) greater is the bioavailability [13]. The other factors which affect the bioavailability are solubility and lipophilicity. These properties are inherent in the molecules and can only be changed by modification of the structure of the drug substance, whereas, reduction in particle size is a smarter technique to improve bioavailability. The particle size can be reduced either by milling or through microcrystallization techniques [13,14]. The milling process generally converts the crystalline phase to an amorphous phase, which results in lower stability of drug

KEY WORDS: arabinoxylan, copper(II)-aspirinate, *in situ* synthesis, microencapsulation.

* Author to whom correspondence should be addressed. *E-mail:* saeediq50@hotmail.com

ISSN 0326 2383 (printed ed.)
ISSN 2362-3853 (on line ed.)

molecules as the crystalline solid is in a thermodynamically more stable state. So, in the present study it was desirable to enhance surface area without losing the crystalline character of a drug. Therefore, it was thought that *in situ* synthesis and encapsulation of microcrystalline copper(II)-aspirinate would be an appropriate way to achieve this objective. Such a product would also provide a sustained targeted delivery of the drug at the site resulting in better efficacy and reduced toxicity.

Usually microenapsulation is carried out by using a drug solution and little attention has been paid to *in situ* synthesis and encapsulation of drug molecules. Also the solution method cannot be applied to sparingly soluble or insoluble drugs. Copper(II)-aspirinate being insoluble in common solvents cannot be encapsulated after synthesis, therefore, *in situ* synthesis and encapsulation strategy was considered suitable for this compound.

Naturally occurring biopolymers from renewable sources especially polysaccharide are ideal matrices for encapsulation of drugs for sustained release formulations due to their abundant availability, cost-effectiveness and non-toxic nature. A variety of natural gums and mucilages have been investigated for this application [15-17]. Among these is a group of materials that can hold huge amounts of water without dissolving. They are generally known as hydrocolloids [18]. Release of drugs from some of these materials has been found to be sensitive to external stimuli including pH and temperature [19-24]. In this regard polysaccharides have shown promise for use as preferred drug carriers, because of their high biocompatibility and pH-dependent behavior. They exhibit diverse physicochemical properties and can be conveniently functionalized to suit an application [25-28].

Recently, arabinoxylan (AX) isolated from ispaghula *(Plantago ovata)* seeds has been shown to provide a good matrix for release of some model drugs [15,29-31]. It is a well characterized highly branched hemicellulosic material soluble in alkaline medium and insoluble in acidic environment [30]. Thus an AX-encapsulated drug is expected to pass through gastro-intestinal tract without being attacked by the aggressive acidic environment as the AX will coagulate under this condition [32]. It would deliver the drug at the colon where the pH is alkaline [33-35]. Thus the objectives of this study were to synthesize copper(II)-aspirinate in microcrystalline state and encapsulate in AX for preparing a product to deliver aspirin in an alkaline environment.

The hypothesis of this research was that AX network will provide voids where the reactants can enter into and react *in situ* to produce microcrystalline copper(II)-aspirinate; from this reaction mixture AX-encapsulated drug can be isolated, which can provide sustained release of the drug.

MATERIALS AND METHODS

Materials

The materials and chemicals used in the present study were: *Plantago ovata* seed husk (Marhaba laboratories, Pakistan), copper(II)-acetate momohydrate, aspirin, acetone, ethanol, methanol, disodium hydrogen phosphate, hydrochloric acid and ether were of analytical grade from E. Merck, Germany and used without further purification. Distilled water was used throughout this study. AX was isolated from *Plantago ovata* seed husk as described below.

Isolation of AX from ispaghula husk

The AX was isolated according to a reported method [36] with slight modification. Ispaghula seed husk (50.0 g) was soaked in distilled water (2.5 L) at 25 ± 2 °C and allowed to swell overnight. The AX mucilage thus obtained was separated from fibrous part of the husk by filtration through a muslin cloth under vacuum. In order to coagulate the AX in the mucilage acetone (500 mL) was added. The wet mass of the coagulated AX was separated out by use of a stainless steel strainer, washed several times with water, acetone and ether. The washed powder was spread in a petri plate and air-dried for 24 h at room temperature. The dried powder was stored in a refrigerator till further use. The product was found to be identical to that reported earlier as indicated by elemental analysis, monosaccharide content and FT-IR spectrum [15,30]. The monosaccharide analysis was performed after acid hydrolysis of the sample [37] by Dionex ICS 3000 HPLC system, consisting of: CarboPacPA20 column (0.4 × 150 mm) and electrochemical detector, according to a reported method using appropriate standards [38].

In situ synthesis and encapsulation of microcrystalline copper (II)-aspirinate

The isolated AX (2.00 g) was suspended in water (50 mL) and allowed to swell overnight.

Swollen mucilage was homogenized by use of mechanical stirrer (Model RZR1 Heidolph). The suspension was chilled by addition of crushed ice in order to minimize hydrolysis of aspirin. To the chilled AX suspension copper (II)-acetate monohydrate (1.5972 g, 8.0 mmol) was added with stirring (the sample was marked as S1). In another beaker sodium bicarbonate (1.1760 g, 14.0 mmol) was dissolved in chilled water (20 mL); to this aspirin (2.5222 g, 14.0 mmol) was added slowly. Thus the aspirin was converted to its soluble sodium salt in solution (marked as S2). The chilled S2 was added drop wise to the chilled S1 under vigorous stirring using the mechanical stirrer. After the complete addition, stirring was continued for 5 h. The microcrystalline copper(II)-aspirinate formed and dispersed in the AX matrix, which was separated by filtration through sintered glass, washed adequately with water, methanol, ether and suck-dried on the funnel. The wet material was spread on a glass surface to form thin film, which was dried in air at room temperature for 24 h to obtain the final product having ~ 6% moisture content.

Characterization

The AX-encapsulated copper(II)-aspirinate was characterized by microscopic FT-IR, powder XRD (pXRD), and microscopic techniques including optical, scanning electron (SEM) and atomic force (AFM) microscopies. The FT-IR spectra of thin films were recorded on IR Prestige-20 (Shimadzu, Japan) by using microscope and diffuse reflectance accessory. The microscope was first focused on the blank part of the AX film for obtaining the background spectrum. Then it was focused on the copper(II)-aspirinate crystals dispersed in the AX film and spectrum was recorded. The spectrum of copper(II)-aspirinate was obtained after subtraction of the blank from the spectrum of the AX-encasulated copper(II)-aspirinate thus elimating the absorptions due to AX.

pXRD

The pXRD spectra of the naked and encapsulated copper(II)-aspirinate were recorded on MPD PAnalytical X'Pert Pro diffractometer (PANalytical B.V., The Netherlands) using monochromatic Cu Kα radiation (λ = 1.5406 Å) operating at 40 kV and 30 mA. The data were collected over a 10-80° 2θ range.

Optical microscopy

In order to observe the shape and morphology of the product, pure AX matrix and encapsulated copper(II)-aspirinate microcrystals were examined under optical microscope DM1750 M (Leica, Germany) with magnification power 200 ×.

Electron microscopy

Thin films of the samples for SEM were prepared by placing a drop of the AX- encapsulated copper(II)-aspirinate suspension in water on a polished aluminum sample holder and air-drying the sample at room temperature in a dust free environment. Images were recorded with SEM S-3700N (Hitachi, Japan). AFM images of thin films (0.5 × 0.5 μm) were obtained with the SPM-9500 J3 scanning probe microscope (Shimadzu, Japan) using the contact mode.

Particle size analysis

Particle size analysis was carried out using the suspension (10 mL) of AX-encapsulated copper(II)-aspirinate in water by the Laser Particle Sizer Analysette 22 Compact (Fritsch, Germany). This technique gives higher values than those by SEM as it measures the hydrodynamic radius of the particle. Thus the AX-coated microcrystals will show up a relatively larger size. Measurements were made in triplicate. Particle size and size distribution were determined from the data obtained.

Release study

The *in vitro* drug release was studied by using AX-encapsulated copper(II)-aspirinate (containing equivalent to 300 mg copper(II)-aspirinate) and dissolution media including water (1000 mL), 0.1M HCl (900 mL) and pH 8 phosphate buffer (1000 mL) in USP paddle dissolution apparatus II at 37 ± 0.1 °C and 60 rpm. Samples (3 mL) were withdrawn after every 15 min during first hour and after every 30 min thereafter up to 8 h. At each sampling time, the volume removed was replaced with fresh medium in order to maintain dilution and sink conditions. The withdrawn samples were filtered, suitably diluted and released aspirin was assayed spectrophotometrically, using UV-1700 spectrophotometer (Shimadzu, Japan), at 265 nm according to the USP assay method [39]. The release was studied for 8 h.

Release kinetics

The release data were fitted to various models including zero order (Eq. [**1**]) [21], first order (Eq. [**2**]) [40], Higuchi equation (Eq. [**3**]) [41], Hixson-Crowell cube root law (Eq. [**4**]) [42] and Korsmeyer-Peppas model (Eq. [**5**]) [43].

$$M = k_0 t \quad [1]$$

where k_0 is the zero order release constant and M is the amount of aspirin released in time t.

$$lnM = -k_1 t + lnM_0 \quad [2]$$

where k_1 is the first order release constant, M is the remaining amount of aspirin in the sample after time t and M_0 is the initial amount of aspirin in AX.

$$M = k_H t^{1/2} \quad [3]$$

where M is the amount of aspirin released in time t, and k_H is the Higuchi release constant.

$$M_0^{1/3} - M^{1/3v} = - k_{HC} t \quad [4]$$

where M is the amount of ASA released in time t, k_{HC} is the Hixson-Crowell release constant and M_0 is the initial amount of amount of aspirin in AX.

$$lnM/M_0 = k_k lnt \quad [5]$$

where k_k is the Korsmeyer-Papaas release constant, M is the amount of aspirin remaining in AX after time t and M_0 is the initial amount of aspirin in AX.

RESULTS AND DISCUSSION

In the first step of the method described in the present work, copper(II)-acetate solution was uniformly absorbed by swollen AX, which was allowed to react with the aspirin solution in the second step. A microcrystalline copper(II)-aspirinate produced therein was encapsulated by the polymer instantaneously as characterized below. The unreacted reagents being soluble in the medium washed out whereas the copper(II)-aspirinate being insoluble is trapped in the polymer matrix.

Characterization

The monosaccharide composition of the water-extractable fraction of AX was found to be: arabinose (21.25 %) and xylose (78.70 %), which was similar to the previously reported values [15]. The percentages of C and H of the AX were 41.43 ±0.01 and 5.60 ± 0.06, respectively, on dry-substance (anhydrous) basis. Nitrogen and sulfur were below the detection limit.

Microscopic FT-IR spectroscopy

The major FT-IR absorptions (cm^{-1}) along with their assignments of pure AX film are: 3360 (νOH), 2930 (aliphatic saturated νCH), 1610 (deformation of absorbed H_2O), 1422 (δCH_2), 1380 (δCH), 1249 ($\delta_{antisym}$ bridging O), 1158 (νC–O–C, νC–C, due to arabinosyl side chain), 1057 (νC–C, νC–O, νC–O–H), 899 (β-glycosidic bond, $\delta_{antisym}$ out of plane), and 618, 525 (polymer backbone). These assignments clearly establish the polysaccharide structure of the material.

The bands in the FT-IR spectrum of the AX-encapsulated copper(II)-aspirinate were similar to those in the spectrum of a standard copper(II)-aspirinate sample (Fig. 1) after subtraction of the spectrum of the AX film. The major FT-IR absorptions (cm^{-1}) long with their assignments are: 3090 and 3080 due to ν(C-H), 1753 (ester νC=O), 1727 (carboxyl νC=O), 1406 (ν_{sym}C-O), 1243 (ν_{asym}C-O), 1200 (ν_{asym}C-O), and 500 (Cu-O); the bands were assigned according to William *et al* [44]. There were no additional bands observed here indicating any chemical interaction of the drug with the polymer.

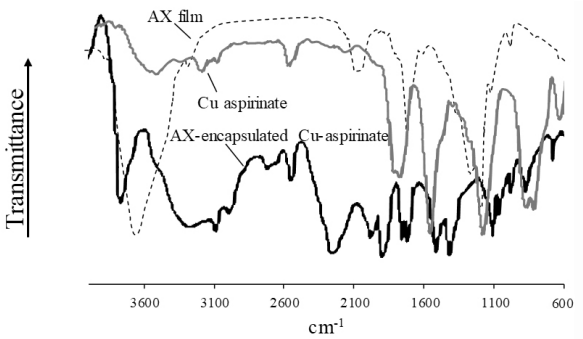

Figure 1. FT-IR spectra of naked and AX-encapsulated copper(II)-aspirinate (after subtraction of the spectrum of blank AX film).

pXRD

The values of diffraction angle (2θ) and relative intensities of the major peaks in the pXRD spectra of pure and encapsulated copper(II)-aspirinate (Fig. 2) were largely identical that confirmed the similarity of crystalline structure of the *in situ* synthesized product to that of the standard copper(II)-aspirinate [45].

Microscopic examination

Optical microscopy depicted blue colored microcrystals uniformly dispersed in the AX matrix (Fig. 3). The image of blank AX shows

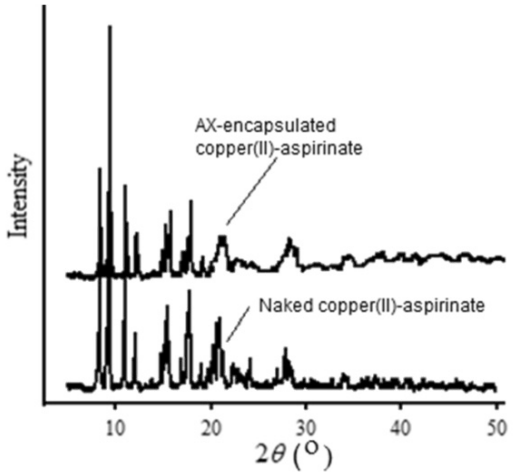

Figure 2. pXRD spectra of naked copper(II)-aspirinate and AX-encapsulated copper(II)-aspirinate.

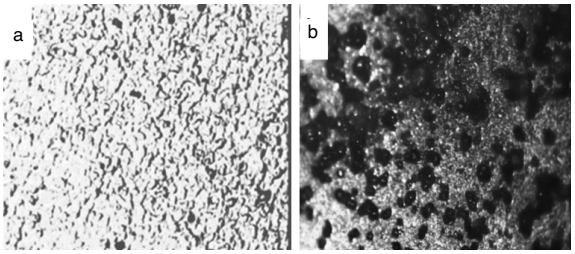

Figure 3. Optical micrographs (magnification 200 ×) of blank (**a**) and AX-encapsulated copper(II)-aspirinate (**b**).

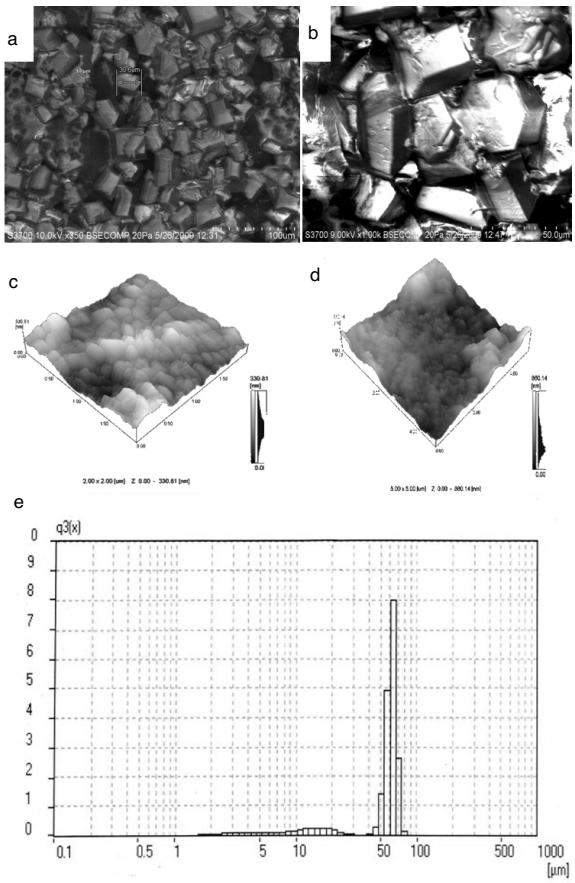

Figure 4 Microscopic images and size distribution histogram of AX and AX-encapsulated copper(II)-aspirinate. SEM images of AX-encapsulated copper(II)-aspirinate: **a**) magnification × 350; **b**) magnification × 1000; **c**) AFM images of blank AX; **d**) AX-encapsulated copper(II)-aspirinate; **e**) particle size distribution of copper(II)-aspirinate crystals in AX film.

voids in the polymer film. The surface morphology and size distribution of encapsulated microcrystals were studied by SEM and AFM analysis. The SEM images were obtained by directly spreading the sample on aluminum stub and observed without sputter coating of gold; this was possible due to the presence of copper (a conducting element) in the drug molecule. The images (Figs. 4a and 4b) depicted typical crystalline copper(II)-aspirinate particles uniformly distributed in the AX matrix. The particle size as determined by SEM was in the range 15-40 µm with a mean value less than 40 µm. A comparative view of the AFM images (Figs. 4c and 4d) of blank AX and AX-encapsulated copper(II)-aspirinate revealed that surface roughness of the AX film increased from 330 µm to 860 µm after encapsulation of the microcrystals. The increase in roughness presents a clear evidence of trapped particles in previously smooth film. A histogram representing particle size distribution of the particles in AX film (Fig. 4e) was obtained from laser particle analyzer. This analysis showed that the mean size of the microcrystals was ~65 µm (> 80 %), that is higher value than that given by SEM as this technique also takes into account the coating on the particles.

Release study

The release profiles of aspirin from AX-encapsulated copper(II)-aspirinate films are presented in Fig. 5.

These profiles were highly sustained as compared with those of the naked drug. It can be seen that highest release occurs in alkaline medium followed by water and acid. This trend can be attributed to different swelling behavior and solubility of AX in different media. AX is slowly soluble in alkaline medium, which resulted in higher sustained release; the release was augmented by rapid hydrolysis of copper(II)-aspirinate to aspirin and subsequently to salicylic

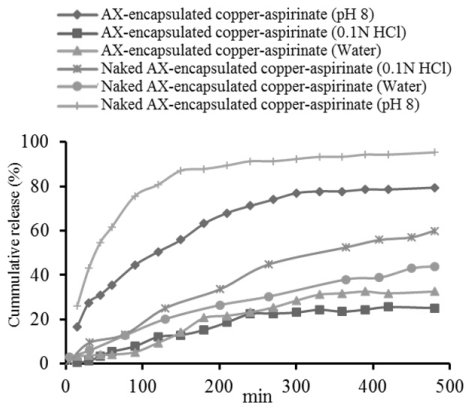

Figure 5. Drug release profiles showing cumulative drug release as a function of time.

acid. On the other hand the AX is insoluble in acidic medium and as such exhibits little swelling, resulting in highly retarded release. In water it swells well, resulting in slow release of the microcrystals, but the insoluble character of copper(II)-aspirinate in water prevents it from rapid hydrolysis. It can be seen that after 8 h approx. 25% aspirin was released in acid, whereas approx. 80% release occurred in 8 pH buffer in that time period from AX-encapsulated copper(II)-aspirinate suggesting that approx. 75% of aspirin will pass on to intestine because of encapsulation by AX.

The release data in the buffer fits well (R^2 > 0.991) in Korsmeyer-Peppas model (Power Law) with the value of diffusional exponent n = 0.5 (Table 1), which means that the release is of Fickian type [46].

These results suggest that AX provides a matrix suitable for targeted drug delivery at the colon. It may be noted that polysaccharides are broken down by the microflora at the colon to smaller saccharides. Thus hydrolysis of the glycosidic linkages on arrival at the colon triggers the release of the entrapped drug particles. It has been demonstrated that most of the polysaccharide-based delivery systems protect the bioactive from the hostile conditions of the upper GIT. The polysaccharide-based delivery systems mostly incorporate cellulosic materials and it is well known that humans cannot digest cellulose [33,47]. The AX being hemicellulose can be digested by humans so it becomes a preferred material for targeted drug delivery at the colon. It is one of the required characteristics of the drug carriers that they should not adversely interact with the immune system; the AX from ispaghula has been shown to be inactive in this respect [29]. Therefore, use of AX in drug delivery systems will be more appropriate.

The sustained release formulations are preferred way of administering toxic drugs. The copper(II)-aspirinate contains copper as a part of the molecule, which though being essential element is among toxic heavy elements. So it will be highly desirable to deliver it at the target in a sustained manner. As shown by the release of aspirin from AX-encapsulated copper(II)-aspirinate in alkaline and acidic media the drug is expected to remain intact to larger extent in the acidic environment in the stomach and delivered in the intestine when administered orally. Thus it will survive the first-pass metabolism and also will not be harmful to the GI tract. This is an anticipation which needs to be verified by an *in vivo* study.

CONCLUSIONS

This study demonstrates that copper(II)-aspirinate can be synthesized *in situ* in microcrystalline form in arabinoxylan matrix. The microcrystalline product is instantaneously encapsulated by arabinoxylan producing a slow-release device. It further demonstrates that the AX-encapsulated copper(II)-aspirinate provides a sustained release of aspirin in alkaline medium over a period of 8 h following the Korsmeyer-Peppas model (Power Law). The release mechanism was found to be of Fickian type (n = 0.5). Approx. 75% aspirin remains intact after passing through 0.1N HCl, a medium simulating the gastric juice. Thus the AX-encapsulated copper(II)-aspirinate is expected to be a good candidate for evaluation as a slow-release device for delivering aspirin in the intestine. The advantages

Medium	Zero order	First order	Higuchi	Korsmeyer-Hixon-Peppas	Hixon-Crowell
Water (pH 6.8)	0.9321	0.8345	0.9874	0.3249	0.2184
HCl (pH 1.0)	0.9248	0.7206	0.9667	0.6762	0.8076
Buffer (pH 8.0)	0.8688	0.7522	0.9688	0.9911	0.7989

Table 1. Drug release data from AX-encapsulated copper(II)-aspirinate in different media. The values are coefficients of determination (R^2) for fitness to different kinetic models.

linked with this product are: the arabinoxylan used in it is more biocompatible than the cellulose-based materials being currently used, and augmented anti-inflammatory effect of copper in combination with aspirin.

REFERENCES

1. Zhiqiang, S., W.Y. Lei, L. Li, Z.H. Chen & W.P. Liu (1998) *Inflammopharmacology* **6**: 357-62.
2. Fujimori, T., S. Yamada, H. Yasui, H. Sakurai, Y. In & T. Ishida (2005) *J. Biol. Inorg. Chem.* **10**: 831-41.
3. Liu, W., H. Xiong, Y. Yang, L. Li, Z. Shen & Z. Chen (1998) *Met. Based Drugs* **5**: 123-6.
4. Fujimori, T., H. Yasui, M. Hiromura & H. Sakurai (2007) *Exp. Dermatol.* **16**: 746-52.
5. Roch-Arveiller, M., D.P. Huy, L. Maman, J.P. Giroud & J.R.J Sorenson (1990) *Biochem. Pharmacol.* **39**: 569-74.
6. Abuhijleh, A.L. (2011) *Inorg. Chem. Commun.* **14**: 759-62.
7. Fujimori, T., S. Yamada, H. Yasui, H. Sakurai, Y. In & T. Ishida (2005) *JBIC J. Biol. Inorg. Chem.* **10**: 831-41.
8. Sorenson, J.R. (1989) *Prog. Med. Chem.* **26**: 437-568.
9. Kishore, V. (1988) *Res. Commun. Chem. Pathol. Pharmacol.* **60**: 257-60.
10. Strecker, D., A. Mierzecki & K. Radomska (2013) *Ann. Agric. Environ. Med.* **20**: 312-6.
11. Iqbal, M.S., M. Sher, H. Pervez & M. Saeed (2008) *Biol. Trace Elem. Res.* **124**: 283-8.
12. Manojlovic-Muir, L. (1967) *Chem. Commun.* 1057-8.
13. Junyaprasert, V.B. & B. Morakul (2015) *Asian J. Pharm. Sci.* **10**: 13-23.
14. Bhakay, A., M. Merwade, E. Bilgili & R.N. Dave (2011) *Drug Dev. Ind. Pharm.* **37**: 963-76.
15. Massey, S., M.S. Iqbal, B. Wolf, I. Mariam & S. Rao (2016) *Lat. Am. J. Pharm.* **35**: 146-55.
16. Bhosale, R.R., R.A.M. Osmani & A. Moin (2014) *Int. J. Pharmacogn. Phytochem. Res.* **6**: 901-12.
17. Sahoo, S., V.K., Singh, K. Uvanesh, D. Biswal, A. Anis, U.A. Rana, *et al.* (2015) *J. Appl. Polym. Sci.* **132**: 1-8.
18. Nishinari, K., H. Zhang & S. Ikeda (2000) *Curr. Opin. Colloid Interface Sci.* **5**: 195-201.
19. Schmaljohann, D. (2006) *Adv. Drug Deliv. Rev.* **58**: 1655-70.
20. Burey, P., B.R. Bhandari, T. Howes & M.J. Gidley (2008) *Crit. Rev. Food Sci. Nutr.* **48**: 361-77.
21. Möckel, J.E. & B.C. Lippold (1993) *Pharm. Res.* **10**: 1066-70.
22. Janaswamy, S. & S.R. Youngren (2012) *Food Funct.* **3**: 503-7.
23. Lee, O.J., J.H. Kim, B.M. Moon, J.R. Chao, J. Yoon, H.W. Ju, *et al.* (2016) *Tissue Eng. Regen. Med.* **13**: 218-26.
24. Jabeen, S., M. Maswal, O.A. Chat, G.M. Rather & A.A. Dar (2016) *Colloids Surfaces B* **139**: 211-8.
25. Debele, T.A., S. L., Mekuria & H.. Tsai (2016) *Mater. Sci. Eng. C.* **68**: 964-81
26. Venkatesan, J., S. Anil, S.-K. Kim & M. Shim (2016) *Polymers.* **8**: 30.
27. Zhao, Y., X. Zhang, Y. Wang, Z. Wu, J. An, Z. Lu, *et al.* (2014) *Carbohydr. Polym.* **105**: 63-9.
28. Luo, Y., K.R. Kirker & G.D. Prestwich (2000) *J. Control. Release* **69**: 169-84.
29. Iqbal, M.S., J. Akbar, M.A. Hussain, S. Saghir & M. Sher (2011) *Carbohydr. Polym.* **83**: 1218-25.
30. Saghir, S., M.S. Iqbal, M.A. Hussain, A. Koschella & T. Heinze (2008) *Carbohydr. Polym.* **74**: 309-17.
31. Nayak, A.K., D. Pal & K. Santra (2013) *J. Pharm.* **79**: 756-60
32. Cummings, J.H. (1984) *Proc. Nutr. Soc.* **43**: 35-44.
33. Sinha, V.R. & R. Kumria (2001) *Int. J. Pharm.* **224**: 19-38.
34. Amidon, S., J.E. Brown & V.S. Dave (2015) *AAPS PharmSciTech* **16**: 731-41.
35. Rao, K.M., S. Nagappan, D.J. Seo & C.S. Ha (2014) *Appl. Clay Sci.* **97**: 33-42.
36. Iram, F., M.S. Iqbal, M.M. Athar, M.Z. Saeed, A. Yasmeen & R Ahmad (2014) *Carbohydr. Polym.* **104**: 29-33.
37. Saeman, J.F., W.E. Moore, R.L. Mitchell & M.A. Millett (1954) *Tappi* **37**: 336-43.
38. Weitzhandler, M., V. Barreto, C. Pohl, P. Jandik, J. Cheng & N. Avdalovic (2004) *J. Biochem. Biophys. Methods* **60**: 309-17.
39. United States Pharmacopoeia 40-NF 35 (2017) The United States Pharmacopeial Convention. Rockville, Maryland.
40. Gibaldi, M. & S. Feldman (1967) *J. Pharm. Sci.* **56**: 1238-42.
41. Higuchi, T. (1961) *J. Pharm. Sci.* **50**: 874-5.
42. Hixson, A.W. & J.H. Crowell (1931) *Ind. Eng. Chem.* **23**: 923-31.
43. Korsmeyer, R.W., R. Gurny, E. Doelker, P. Buri & N.A. Peppas (1983) *Int. J. Pharm.* **15**: 25-35.
44. Williams, D.A., D.T. Walz & W.O. Foye (1975) *J. Pharm. Sci.* **65**: 126-8.
45. Manojlovic-Muir, L. (1973) *Acta Cryst.* **B29**: 2033-7.
46. Brazel, C.S. & N.A. Peppas (2000) *Eur. J. Pharm. Biopharm.* **49**: 47-58.
47. Salyers, A.A., S.E.H. West, J.R. Vercellotti & T.D Wilkins (1977) *Appl. Environ. Microbiol.* **34**: 529-33.