Journal of Pharmaceutical Research International

19(3): 1-9, 2017; Article no.JPRI.36903
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Studies on a Hydrophilic Cellulose Matrix Derived from *Ipomoea batatas* Tubers II: Application as a Filler-disintegrant in Piroxicam Orally Dispersible Tablets

Ugoeze Kenneth C.[1*] and Brown Sinyeofori A.[1]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Nigeria.

Authors' contributions

This research was carried out in collaboration between both the authors. Author UKC conceived the work. Both authors designed the study, wrote the protocol and interpreted the data. Both authors anchored the bench work and managed the literature search, performed the data and statistical analysis. Author UKC produced the initial draft under the supervision of author BSA. Both the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2017/36903
Editor(s):
(1) Mohamed Fathy, Assiut University, Assiut, Egypt.
Reviewers:
(1) Norma Aurea Ragel Vazquez, Mexico.
(2) Safwan Ashour, University of Aleppo, Syria.
(3) Oluyemisi A. Bamiro, Olabisi Onabanjo University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/21751

Original Research Article

Received 22nd September 2017
Accepted 18th October 2017
Published 4th November 2017

ABSTRACT

Aim: In this work, piroxicam orally dispersible tablets (ODTs) was formulated using a hydrophilic cellulose matrix, *I-hydrocel* derived from the tubers of *Ipomoea batatas* as a filler-disintegrant in comparison with avicel PH 101 and lactose.
Methods: The differential scanning calorimetry (DSC) of a mixture of piroxicam and *I-hydrocel* was carried out for compatibility studies. Granules containing piroxicam (10.0% w/w) (20.0 mg per tablet), mannitol, 25.0% w/w, PVP, 2.5% w/w and *I-hydrocel* or avicel PH 101 or lactose (62.0% w/w) were prepared by wet granulation method. Their micromeritic properties were evaluated using standard methods. They were lubricated with 0.5% w/w magnesium stearate and compressed into tablets at 0.75 tons with an 8.5 mm concave punch fitted in a table-top single punch tablet press.

**Corresponding author: E-mail: kenneth.ugoeze@uniport.edu.ng;*

The properties of the tablets were studied using the British Pharmacopoeia standards alongside tablets wetting time, water absorption ratio and the dissolution efficiency (DE).
Results: Piroxicam and *I-hydrocel* were compatible. The granules were compressible and fairly flowable. The uniformity of weight of tablets was within acceptable range. Tablets with the highest mechanical strength were obtained with *I-hydrocel* (P = 0.000) while the tablets containing avicel PH 101 wetted most easily, disintegrated fastest (P = 0.000) and showed the highest water absorption ratio (P = 0.000). However, the respective value of DE was as follows: *I-hydrocel* (89.69%) > avicel PH 101(79.72%) > lactose (59.0%) (P = 0.000).
Conclusions: The high release rate and bioavailability of piroxicam, a poorly water-soluble drug from the ODTs formulated with *I-hydrocel* may be due to its hydrophilic nature which may have enhanced the solubility of the drug. Therefore, *I-hydrocel*, being a new excipient could serve as an economic and efficient filler-disintegrant in the formulation of piroxicam orally dispersible tablets.

Keywords: Hydrophilic cellulose matrix; Ipomoea batatas; piroxicam; orally dispersible tablet.

1. INTRODUCTION

Drug delivery refers to the way a pharmaceutical dosage form is administered to humans and animals. Drugs have always been taken to achieve a therapeutic benefit which is always aimed at alleviating or eliminating a disease state in man or animal [1]. Drugs are designed or formulated for administration in dosage forms. Tablet is a popular example of solid dosage form taken orally and is widely accepted. The oral route of taking medicinal substances has been widely utilized since it is very suitable to many persons especially as it makes it easy for self-use or administration of medicament among other advantages such as its compactness, dosage precision, portability and ease of production. In as much as the tablet has very wide acceptance due to its many advantages, it still suffers some setbacks such as the difficulty experienced in swallowing solids by some group of patients that fall into the class of geriatrics, paediatrics, bedridden patients, users of medicine who may lack access to water at some location, situations where the patient suffers from nausea or vomiting, etc. The implication of this glaring problem is patient poor compliance to the regimented drug routine which leads to poor therapeutic outcome [2-4]. These problems are overcome following the introduction of the orally disintegrating tablets (ODTs) which has helped to improve the patient's compliance especially among the class of medicine users who suffer from dysphagia. A patient may dislike taking some medicinal products orally for several reasons ranging from the product poor aesthetic value, taste or odour. For these reasons, ODTs have been innovated to provide oral solid dosage forms with good taste, flavour and increased acceptability of pharmaceutical products with an unpleasant taste or odour providing acceptable mouth-feel with insignificant residue in the oral cavity after a drug oral dosage form is taken. The ODTs has the advantage of quick attainment of bioavailability for drugs that are not very soluble. This technology enhances their solubilisation leading to high dissolution outcome of such drugs. Several other terms such as the orodispersible tablets, quick disintegrating tablets, mouth dissolving tablets, etc. have been used to describe the ODTs. Irrespective of these terms, the United States Pharmacopoeia (USP) ratified this class of dosage forms as ODTs. They are expected to disintegrate in less than a minute [5-7]. This trend is achievable with superdisintegrants. Examples of disintegrants useful for such formulation include crosscarmellose (a modified cellulose), sodium starch glycolate (a modified starch), etc. Some classes of drugs targeted for the ODTs are the analgesics (e.g. piroxicam, ibuprofen), anti-infectives (e.g. albendazole), antibacterials (e.g. doxycycline), antihypertensives (e.g. amlodipine), etc. Low-dose drugs with agreeable taste, odour, long half-life and stability in saliva and water are preferable and are expected to infuse the oral mucosa (5). The techniques used in producing ODTs include freeze drying [8], direct compression [9], melt granulation [10], etc.

The purpose of this work is to formulate and evaluate an orally dispersible tablets (ODTs) of piroxicam using a hydrophilic cellulose matrix derived from *Ipomoea batatas* tubers (*I-hydrocel*). The processing and evaluation of *I-hydrocel* have been documented [11]. The *I-hydrocel* has an added advantage as a filler-disintegrant hydrophilic matrix. Its use in tabletting or encapsulation will enhance quick water uptake leading to faster disintegration, drug dissolution, quick onset of action and bioavailability, especially of poorly water-soluble

drugs. Utilisation of this new material in tabletting or encapsulation will reduce the cost of production. The ease of wetting of *I-hydrocel* makes it a good excipient for the production of orally dispersible tablets since a mere amount of saliva in the mouth will wet and disperse it. These properties are utilised in this study to formulate a piroxicam orally dispersible tablets using avicel® or lactose for comparison. Piroxicam, from the oxicam group, is a non-steroidal anti-inflammatory medicine known for low water solubility-high permeability and also a long half-life that makes it easy for once a day therapy [12]. It blocks prostaglandin synthesis through the inhibition of enzyme cyclo-oxygenase (prostaglandin synthetase). This inhibition is probably the cause of its analgesic, antipyretic and anti-inflammatory effect [13].

2. MATERIALS AND METHODS

The following materials were used as procured: piroxicam (Trichem Laboratories, India), magnesium stearate (BDH, England), lactose (SureChem, UK), avicel PH 101 (FMC Biopolymer, Norway), alcohol (JHD, China). *I-hydrocel* was processed in the Department of Pharmaceutics and Pharmaceutical Technology laboratory, Faculty of Pharmaceutical of Sciences, University of Port Harcourt, Port Harcourt, Nigeria.

2.1 Compatibility Studies of Piroxicam and *I-hydrocel*

The differential scanning calorimetry (DSC) thermogram of a binary mixture (1:1) of piroxicam and *I-hydrocel* was obtained using a DSC equipment, Mettler Toledo; DSC 822, USA to check the compatibility of the two samples.

2.2 Preparation of Granules Containing Piroxicam

A batch of granules containing *I-hydrocel* (62.0% w/w) as filler-disintegrant, 20.0 mg of piroxicam (10.0% w/w), mannitol (25.0% w/w), polyvinyl pyrrolidone (PVP) (2.5%w/w), magnesium stearate (0.5% w/w) per tablet was prepared by wet granulation method. A blend of piroxicam and *I-hydrocel* was wet-massed in an alcoholic solution of PVP. Sieves 10 (1.7 mm) and 16 (1.00mm) (Retch, Germany) were used for wet and dry screenings respectively. Drying was carried out at 60 ^{o}C in a hot air oven (Memmert, England). Similar batches were prepared with avicel PH 101 and lactose respectively for comparison.

2.3 Properties of the Granules

The assessment of the bulk, tapped, particle densities and flow rate [14] were carried out with 15.0 g of granules. The particle density was studied by the pycnometer method using *n*-hexane as a displacement fluid. The angle of repose was evaluated using the constant height of 4.0 cm [15].

The following equations were used in calculating the various parameters:

$$Bulk\ density = weight\ of\ bulk\ granules / volume\ of\ granules \quad (1)$$

$$Tapped\ density = weight\ of\ granules / tapped\ volume\ of\ granules \quad (2)$$

$$Particle\ density = W2 \times W3 \div V(W3 - W4 + W2 + W1 \quad (3)$$

where, V = 25 mL (volume of pycnometer),
W = weight of empty pycnometer,

W1= weight of pycnometer and n - hexane,
W2 = the difference between the W & W1,
W3 = weight of sample powder,
W4= weight of sample + *n* - hexane + pycnometer.

$$Flow\ Rate = weight\ of\ granule / time\ of\ complete\ flow \quad (4)$$

$$Angle\ of\ repose, \theta = tan^{-1}\ 2h/d \quad (5)$$

Where *h* = constant height of granules heap, *d* = diameter of granules heap.

The Carr's index, Hausner's ratio and the porosity [16,17] were calculated as follows:

$$Carr's\ index = \underline{(Tapped\ density - Bulk\ density)} \div Tapped\ density \times 100 \quad (6)$$

$$Porosity = 1 - (bulk\ density / true\ density) \times 100 \quad (7)$$

$$Hausner's\ ratio = Tapped\ density \div Bulk\ density \quad (8)$$

2.4 Compression of Tablets

The granules were lubricated with 0.5% w/w of magnesium stearate and compressed at 0.75

tons using an 8.5 mm diameter die and flat-faced punches on a table-top single punch tablet press (Erweka, EP-1, Germany). All the compressed tablets were left for 24 h in a desiccator before they were analysed to allow for possible recovery from stress.

2.5 Determination of the Properties of the Tablets

The methods outlined in the British Pharmacopoeia [18] were used in the study of the properties of the tablets. The tablets were also examined organoleptically for colour, odour, shape, taste and texture. Twenty tablets were used in the study of uniformity of weight on an electronic analytical balance (Mettler, Germany). The hardness and thickness of ten tablets per batch were determined using a diametrical digital tablet hardness tester (Veego, India) while friability of ten tablets per batch was determined in a tablet friabilator (Erweka TAR 220, Germany).

The tensile strength [19] of the tablets were calculated from the equation:

$$T = 2P/\pi dt \quad (9)$$

Where T = radial tensile strength,
P = tablet hardness,
t = tablet thickness,
d = tablet diameter.

The disintegration time of 6 tablets per batch was determined using a tablet disintegration apparatus (Erweka, ZT 122, Germany) in 900.0 mL of 0.1 N hydrochloric acid maintained at 37.0 ± 1℃. The dissolution studies were conducted in a dissolution apparatus (Erweka DT600, Germany) using the rotating paddle method in 900 mL of 0.1 N hydrochloric acid at 37.0 ±1℃ at a paddle speed of 50.0 rpm. A 5.0 mL of dissolution sample was withdrawn at predetermined intervals of 5.0 min for a dissolution period of 30.0 min and replacing withdrawn fluids with 0.1 N hydrochloric acid at the same temperature. The absorbance of samples was determined in a UV spectrophotometer (Jenway, model 6405, England) at a wavelength of 324.0 nm. The effect of the filler-disintegrants on the dissolution of piroxicam from the compressed tablets was evaluated using Dissolution Efficiency (DE) parameter.

2.6 The Wetting Time of Tablets

The wetting time of the respective tablets was studied by devising five circular adsorbent papers of 10.0 cm diameter placed in a petri-dish holding 10.0 mL of water coloured with a water-soluble dye (eosin). Each tablet was dropped on top of the adsorbent paper respectively and the time taken for water to reach the upper surface of the tablet was recorded as the wetting time.

2.7 Water Absorption Ratio

A piece of adsorbent paper folded twice was placed in a small petri dish (internal diameter, 6.5 cm) holding 6.0 mL of water. A tablet at a time was weighed and then dropped on the paper. The tablet was reweighed after complete absorption. The water absorption ratio (R) of the tablet was calculated as follows:

$$R = (W2 - W1/W2)\, x\, 100 \quad (10)$$

Where W_2 = weight of tablet after water absorption
W_1 = weight of tablet before water absorption

2.8 Statistical Analysis

All statistical analysis of data was performed using the IBM SPSS Statistics 20 software.

3. RESULTS AND DISCUSSION

3.1 Differential Scanning Calorimetry (DSC)

The DSC thermograms of piroxicam and a binary mixture of piroxicam and *I-hydrocel* are presented in Figs. 1 and 2 respectively. Fig. 2 shows a single endothermic peak of 202℃. The melting point of piroxicam is within the range of 202 – 213℃ [20]. This shows a possible compatibility of *I-hydrocel* and piroxicam.

3.2 Properties of Granules Containing Avicel PH 101, Lactose or *I-hydrocel*

The properties of the granules are shown in Table 1. The interparticulate contacts affect the bulking properties of a powder as they in turn influence the powder flow. In order to understand the relative importance of these interactions in a given powder, the bulk and tapped densities are

compared to confirm the ability of the powder to flow. The compressibility index and Hausner's ratio indicate the ability of a given powder bed to settle, and permit an evaluation of the relative importance of interparticulate interactions. If a powder is free-flowing, these interactions are less significant and the values of bulk and tapped densities will be closer. However, if the powder is poorly flowing, there will be greater interparticulate interactions and a greater difference between the bulk and tapped densities will be observed. These differences are also reflected in the compressibility index and Hausner's ratio. In this work, the values of bulk and tapped densities were not close (P = 0.006) and it shows that the granules flowability was not excellent, though, they were compressible. Values of compressibility index and Hausner's ratio in the ranges of 16 - 20 % and 1.26 -1.34 respectively are indication of fair flow of powder. Values above these show that the flow is poor. From these, the granules were generally compressible and exhibited fair flow properties with those prepared with avicel PH 101 having poorer flow [16,17,21].

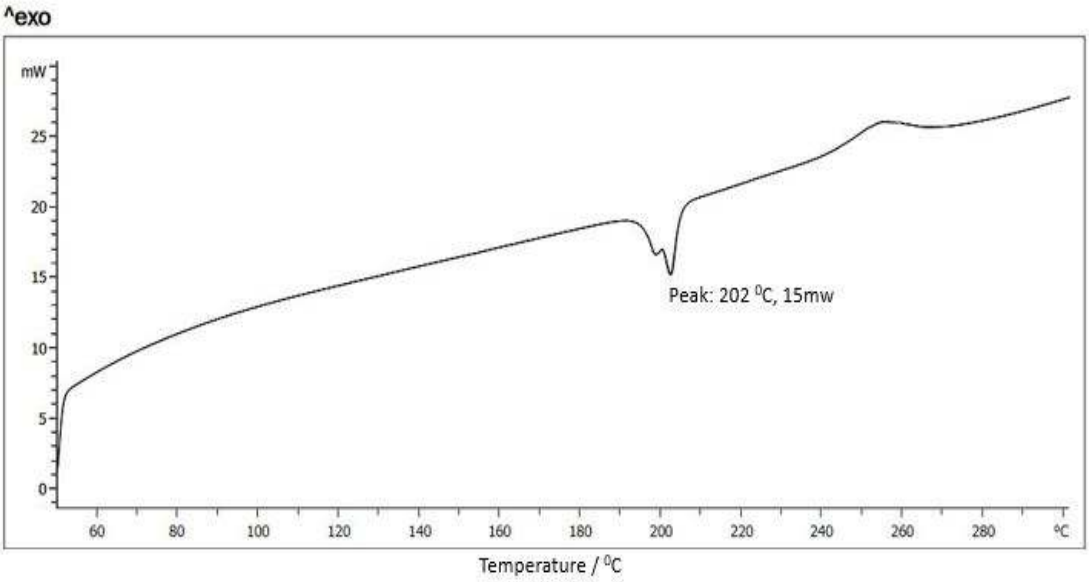

Fig. 1. DSC thermogram of piroxicam

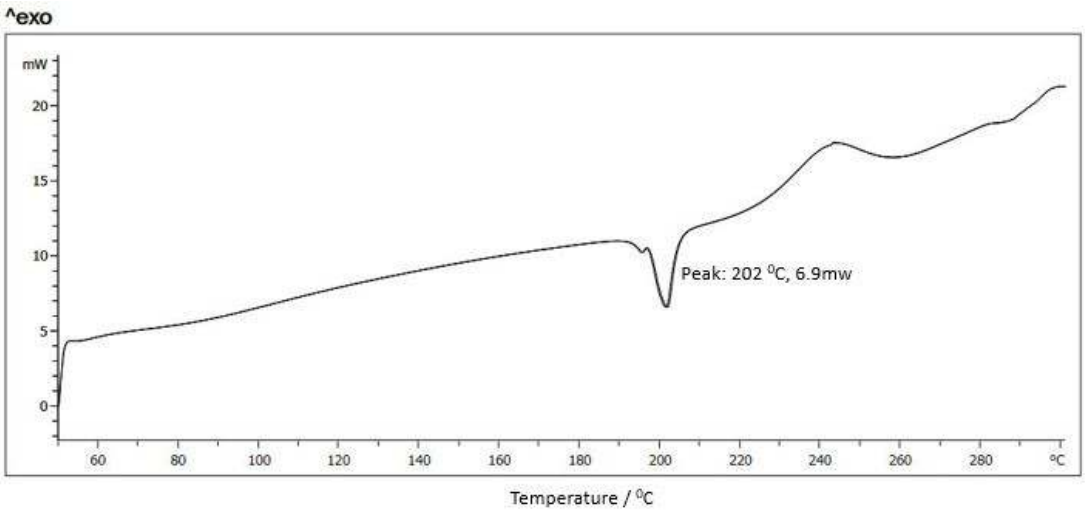

Fig. 2. DSC thermogram of the mixture of piroxicam and *I-hydrocel*

3.3 Properties of the Tablets Prepared with Avicel PH 101, Lactose or *I-hydrocel*

Table 2 shows the properties of the tablets containing either avicel PH 101, lactose or *I-hydrocel.*

The uniformity of weight of the respective batches of the compressed tablets was within acceptable range. The British Pharmacopoeia [18] allows a limit of 7.5% maximum variation in tablet weight for tablets weighing more than 130.0 mg and less than 324.0 mg. In all the formulations in this study, the tablet weight falls within this range and none of the batches had a value of tablet weight variation more than 7.5%.

Generally, the tablets prepared with *I-hydrocel* possessed higher mechanical strength than those of lactose while avicel PH 101 was the least (*I-hydrocel* > lactose > avicel PH 101) (P = 0.000). Oral tablets are normally compressed to have a hardness of 4.0-10.0 kg. However, hypodermic and chewable tablets are usually softer with about 3.0 kg and some sustained-release tablets are harder (10.0-20.0 kg) [22]. The friability of tablets less than 1.0% is acceptable for uncoated and immediate release tablets. However, orally disintegrating tablets are expected to possess lesser value of hardness to ease their break-down in the mouth [23]. Therefore, the range of hardness for the respective batches of tablets studied is acceptable with those prepared using *I-hydrocel* showing the highest mechanical strength. However, the tablets containing avicel PH 101 had low mechanical strength, they were the easiest to be wetted and disintegrated in the shortest time compared to those containing *I-hydrocel* and lactose (avicel PH 101< *I-hydrocel* < lactose) (P = 0.000). It also exhibited the highest water absorption ratio than *I-hydrocel* and lactose (avicel PH 101>*I-hydrocel* > lactose) (P = 0.000). In terms of the dissolution efficiency, DE, the tablets containing *I-hydrocel* ranked highest, followed by those of avicel PH 101, lactose being the lowest [DE: *I-hydrocel* (89.69%) > avicel PH 101(79.72%) > lactose (59.0%)] (P = 0.000). Fig. 3 represents the dissolution profile for the batches of tablets prepared with the respective excipients under study. From this, T_{50} and T_{80} respectively were estimated as shown in Figs. 4 and 5. These

Table 1. The properties of the granules

Parameters	Values		
	I-hydrocel	**Lactose**	**Avicel PH 101**
Flow rate (g/s)	13.60 ± 0.80	13.32 ± 0.45	12.10 ± 1.15
Bulk density (g/mL)	0.38 ± 0.01	0.58 ± 0.02	0.37 ± 0.01
Tapped density (g/mL)	0.48 ± 0.01	0.69 ± 0.01	0.51 ± 0.01
True density (g/mL)	1.51 ± 0.01	1.50 ± 0.01	1.24 ± 0.01
Hausner's ratio	1.24 ± 0.03	1.20 ± 0.03	1.36 ± 0.05
Carr's index (%)	19.27 ± 2.16	16.63 ± 2.31	27.33 ± 2.52
Angle of repose (deg.)	32.63 ± 0.85	32.97 ± 0.3	34.00 ± 0.10
Porosity (%)	70.13 ± 0.01	61.60 ± 0.01	69.90 ± 0.01

Table 2. The properties of the tablets

Parameters	Values		
	I-hydrocel	**Lactose**	**Avicel**
Weight uniformity (mg)	202.05±5.89	202.60±5.15	202.25±3.89
Thickness (mm)	3.81± 0.01	3.72 ± 0.01	3.95 ± 0.02
Hardness (kgf)	3.74 ± 0.37	2.42 ± 0.24	1.76 ± 0.25
Friability (%)	0.05 ± 0.02	0.30 ± 0.04	1.67 ± 0.12
Tensile strength (N/m^2)	73.52 ± 0.21	48.72 ± 0.28	33.37 ± 0.27
Hardness Friability Ratio (HFR)	74.80 ± 0.31	8.07 ± 0.28	1.05 ± 0.29
Disintegration time (min)	2.05 ± 0.48	9.89 ± 1.25	1.05 ± 0.43
Wetting time (s)	30.00 ± 1.41	86.50 ± 6.36	14.00 ± 1.41
Water absorption ratio (%)	23.60 ± 3.24	3.52 ± 0.56	43.80 ± 0.89

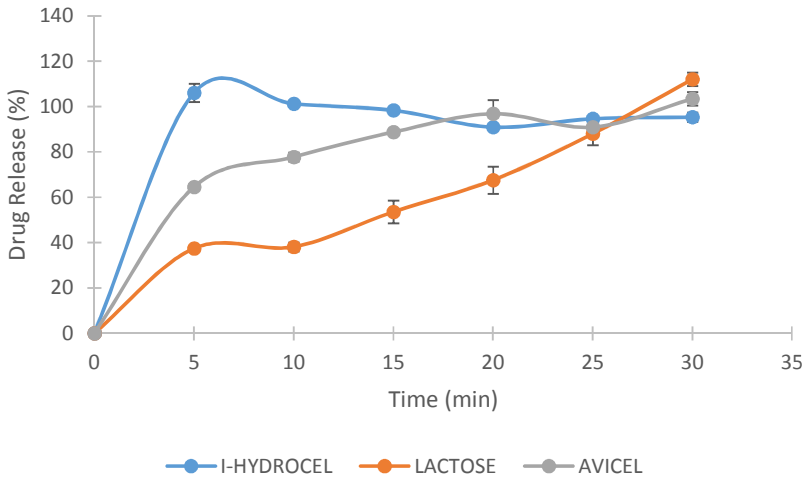

Fig. 3. Dissolution profiles of the tablets prepared with *I-hydrocel*, avicel PH 101 or lactose

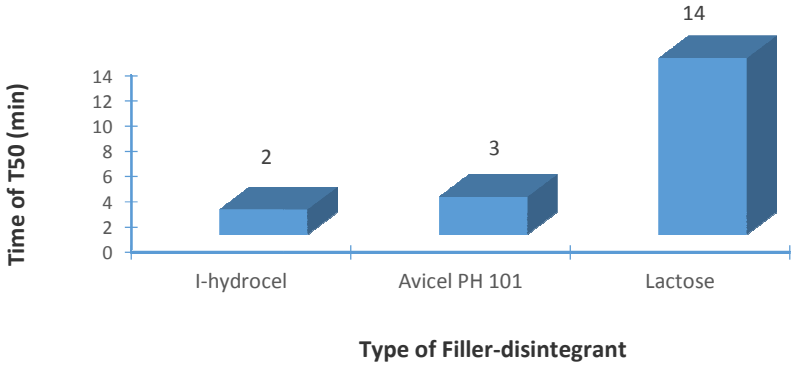

Fig. 4. Bar chart showing the time of release of 50% of piroxicam from the various matrices

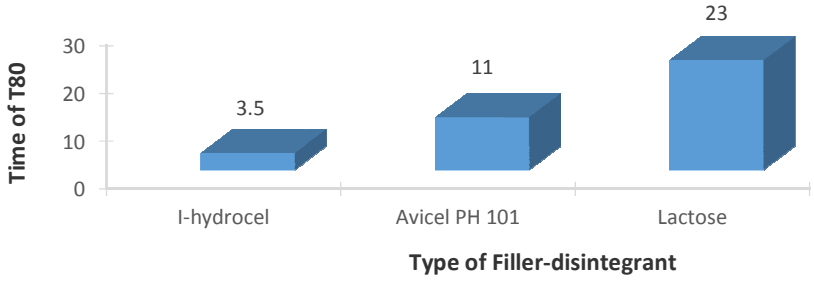

Fig. 5. Bar chart showing the time of release of 80% of piroxicam from the various matrices

show that the tablets formulated with *I-hydrocel* attained both T_{50} and T_{80} in the shortest time, followed by those containing avicel PH 101 while the batches prepared with lactose took the longest time (*I-hydrocel* < avicel PH 101 < lactose). These depicted the ability of *I-hydrocel* to rank highest in terms of the DE.

4. CONCLUSION

The study evaluated the usefulness of *I-hydrocel*, a novel hydrophilic cellulose matrix as a filler-disintegrant in the formulation of piroxicam orally dispersible tablets in comparison with avicel PH 101 and lactose. Tablets possessing higher mechanical strength and dissolution efficiency, DE were obtained with *I-hydrocel* compared to avicel PH 101 and lactose. The high value of DE, low T_{50} and T_{80} for the tablets containing *I-hydrocel* is suspected to be due to its hydrophilic nature which is believed to have enhanced the solubility of piroxicam noted for its poor water solubility [12]. Therefore, *I-hydrocel*, being a new excipient could serve as an economic and efficient filler-disintegrant in the formulation of piroxicam orally dispersible tablets.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Srikanth P, Narayana R, Wasim RS, Brito RS. A review on oral controlled drug delivery. Int. J. Advanced Pharmaceutics. 2013;3(1):51-58.
2. Sastry SV, Nyshdham JR, Fix JA. Recent technological advances in oral drug delivery: A review. Pharmaceutical Science and Technology Today. 2000;3:138-45.
3. Seager H. Drug-delivery products and the zydis fast-dissolving dosage form. Journal of Pharmacy and Pharmacology. 1998; 50(4):375-82.
4. Lindgren S, Janzon L. Dysphagia; Prevalence of swallowing complaints and clinical findings. Medical Clinics of North America. 1993;77:3-5.
5. Velmurugan S, Sundar V. Oral disintegrating tablets: An overview. International Journal of Chemical and Pharmaceutical Sciences. 2010;1(2):1-12.
6. Fu Y, Yang S, Jeong SH, Kimura S, Park K. Orally fast disintegrating tablets: Developments, technologies, taste-masking and clinical studies. Critical Review in Therapeutic Drug Carrier System. 2004;21:433-76.
7. Brown D. Orally disintegrating tablets-taste over speed. Drug Delivery Technology. 2003;3:58-61.
8. Ahmed IS, Nafadi MM, Fatahalla FA. Formulation of a fast-dissolving ketoprofen tablet using freeze-drying in blisters technique. Drug Development and Industrial Pharmacy. 2006;32(4):437-42.
9. Makino T, Yamada M. Kikuta J. Fast dissolving tablet and its production. 1993; European Patent. 0553777 A2.
10. Abdelbary G, Prinderre P, Eouani C, Joachim J, Reynier JP, Piccerelle P. The preparation of orally disintegrating tablets using a hydrophilic waxy binder, International Journal of Pharmaceutics. 2004;278:423-33.
11. Ugoeze KC, Nwachukwu N, Anumaka FC, Ezioka GN. Studies on a hydrophilic cellulose matrix derived from *Ipomoea batatas* tubers I: Processing and physicochemical properties. Indo American Journal of Pharmaceutical Research. 2017;7(08):638-645.
12. Karataş A, Yüksel N, Baykara T. Improved solubility and dissolution rate of piroxicam using gelucire 44/14 and labrasol. Farmaco. 2005;60(9):777-82.
13. Brogden RN, Heel RC, Speight TM, Avery GS. Piroxicam: A reappraisal of its pharmacology and therapeutic efficacy. Drugs. 1984;28(4):292-323.
14. Carstensen JT, Chan FC. Flow rates and repose angle of wet-processed granulations. J. Pharm. Sci. 1977;66: 1235.
15. Odeku OA, Awe OO, Popoola B, Odeniyi MA, Itiola OA. Compression & mechanical properties of tablet formulations containing corn, sweet potato and cocoyam starches as binders. Pharm. Technol. 2005;29(4): 82-90.
16. Carr R. Classifying flow of solids. Chem. Eng. 1965;72:69-72.
17. Hausner HH. Friction conditions in a mass of metal powder. Int. J. Powder Metal. 1967;3:7-13.
18. British pharmacopeia. Her Majesty's Stationery Office, London; 2012.
19. Fell JT, Newton JM. Determination of tablet strength by the diametral compression test. J. Pharm. Sci. 1970;59: 688-691.

20. Stulzer HK, Tagliari MP, Cruz AP, Silva MAS, Laranjeira MCM. Compatibility studies between piroxicam and pharmaceutical excipients used in solid dosage forms. Pharmaceutical Chemistry Journal. 2008;42(4):215-219.
21. Neumann BS. Advances in pharmaceutical sciences. Bean, H.S.; Beckett, A.H. & Carless, J.E. (eds.), Academic Press, London. 1967;2:181.
22. Lachman L, Liberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy, 3rd ed., Varghese Publishing House, Bombay. 1987;430-453.
23. Kapil C, Bharat P, Abhishek C, Varun T. Formulation and evaluation of fast dissolving tablets of telmisartan. Int. Journal of Pharmaceutical Sciences and Research. 2013;4(4):1514-1520.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21751