**Responses for Referee 2**

**1.** Page 1, lines 16-18: the authors state that “The in vitro release kinetics of the cation of 2-ABZT were established at 37°C in simulated gastric medium pH 1.2…” but this has to be explained why the release was monitored for the period more than an hour (as recommended by Pharmacopeia) and therefore, why not in simulated gastrointestinal simulated medium. Please explain.

**Response:**

The *in vitro* release kinetics of the predominate cation 2-ABZTH+ with a pKa = 2.51 were established at 37°C in a simulated gastric medium pH 1.2. Indeed, the release behavior of 2-ABZTH is governed according to Fick’s law. Then, in our case, the release studies was monitored and maintained for the period beyond 100 to 300 minutes (more than an hour) until the equilibrium level of the phenomenon was reached. This will be required in the future studies concerning the *in vivo* tests to evaluate the therapeutic activity of the 2-ABZTH+ cation.

Thus, the diffusion coefficient of any active agent in the body (plasma concentration) depends on its equilibrium mass (long times). That is why we realized the release kinetics at long times (more than an hour). Thereby, we need a monitoring of release was monitored for the period more than an hour.

The kinetic modeling of this phenomenon of diffusion through different galenic forms has been extensively studied (model of Cranck37).

In a complementary work, the behavior of 2-ABZTH+ will be studied in simulated gastrointestinal medium at pH = 8.

37. J. Cranck, *The mathematics of diffusion*, 2nd Ed., Clarendon, Oxford, United Kingdom, 1976, 85 (ISBN 0 19 853344 6).



Scheme 1. The form of 2-aminobenzothiazole (2-ABZT) in simulated gastric medium pH 1.2.

**2.** The authors could follow strict conditions suggested by Pharmacopeia, since they only investigated the release from the prepared devices in acidic medium. This will show the audience the detailed behavior and mechanism of the matrices used as potential drug carriers.

1. Ethylcellulose (EC) is used as an encapsulating retardant material for the controlled and sustained release microspheres. It is a non-biodegradable and biocompatible polymer, is the non-ionic, pH insensitive cellulose ether and water-insoluble polymer40. This combination of properties makes it useful in enteric coating of microparticles because it is resistant to the acid condition of the stomach.

In this mechanistic model, the release of the drug involves transfer of the dissolved molecule through water-filled pores. In previous researches41,42, the surface study of the EC microspheres using Scanning Electron Microscopy (SEM) after the release experiment showed bigger pores suggesting that the drug was released through water-filled pores and the mechanism of drug release was diffusion controlled.

Many researchers like Mura *et al*.43, Friedman and Golomb44, Soskolne *et al.*45, have demonstrated the ability of EC to sustain the release of drugs.

1. The effect of the matrix type on the release rate was studied
by introducing in a single case the cellulose acetate butyrate (CAB)
for a single formulation MS4 , it is just one formulation of the 6 formulations.

CAB polymer is being used for the enteric coating for preparing controlled-release formulations. This matrix is insoluble in water at physiological pH values and capable of swelling.

The CAB polymer contains the short acetyl side groups and the long butyryl side groups. The looser and more disordered arrangement of CAB macromolecules provides CAB with much more free volume and elevated molecular mobility. Thereby, by comparing the two matrices, CAB exhibit slower release rate than the EC polymer. On contact with water, the CAB polymer starts to swell and the hydrogel layer starts to grow around the dry core of the microparticle. The hydrogel presents a diffusional barrier for water molecules penetrating into the polymer matrix and the drug molecules being released46.

**References :**

40. S. Kamel, N. Ali, K. Jahangir, S. M. Shah, A. A. El-Gendy, *eXPRESS Polymer Let.* **2** (2008) 758

41. I. Abdelmalek, I. Svahn, S. Mesli, G. Simonneaux, A. Mesli, *J. Mater. Environ. Sci*. **5** (2014) 1799

42. M. K. Das, K. Rama Rao*. Acta Pol. Pharm.* **63** (2006)141

43. P. Mura, M. T. Faucci, A. Manderioli, G. Bramanti, P. Parrini, **25** (1999) 257

44. M. Friedman, G. Golomb, **17** (1982) 323

45. W. A. Soskolne, G. Golomb, M. Friedman, M. N. Sela, *J. Periodontal Res.,* **18** (1983) 330

46. A. M. Lowman, N. A. Peppas: Hydrogels. in Encyclopedia of Controlled Drug Delivery, (Ed.: Mathiowitz E.) Wiley, New York, 2000, 397–417

 **3/** Page 3, line 88: the authors state “Then, the obtained dispersion was filtered…”, but the washing of emulsion system is extremely though. This has to be explained in details and justified.

 **a/ Yes, the washing of emulsion system is obligatory and though:**

We have forgotten with an error to note that the microspheres were washed several times with deionized water after the filtration.

The following sentence is missing:

The solidified microspheres were filtered, washed several times with deionized water and dried under vacuum in a desiccator containing CaCl2.

 **b/ To justify the response given above, the microencapsulation process used for the preparation of the microspheres is explained in details as the following steps:**

For insoluble or poorly water-soluble drugs, the oil-in-water (o/w) method is frequently used. It consists of four major steps (Fig. 6):

(1) dissolution of the hydrophobic drug in an organic solvent containing the polymer;

(2) emulsification of this organic phase, called dispersed phase, in an aqueous phase called continuous phase;

(3) extraction of the solvent from the dispersed phase by the continuous phase, accompanied by solvent evaporation, transforming droplets of dispersed phase into solid particles; and **(4) the formed microspheres were vacuum filtered and washed several times, recovery and drying of microspheres to eliminate remaining emulsifier the residual solvent.**

 

Figure 6. The steps of the microencapsulation by solvent evaporation process.

**4/.** Figure 4 does not show standard deviations. The authors need to state with how many samples the release studies were performed, and the standard deviations have to be presented in Figure 4.

**Responses:**

The *in vitro* release studies were performed once time. Therefore, we have realized one experiment study of drug release, which meaning one sample for each formulation sample at predefined times. So, for this reason, Figure 4 does not show standard deviations.

**5/.** **a.** In page 2, line 49: the authors state “So, its formulation for oral controlled release requires a specific coating able to float over the gastric fluid with a prolonged period. But their release experiment showed the release in the gastric medium for the period of 25 hours much longer than the cellulose devices would potentially stay when being digested. Please explain.

**Responses:**

**5.a.** Many papers have evaluated microencapsulated controlled release preparations using EC47,48 and CAB polymers49 as retardant materials with an extended release up to 18-20 h. Furthermore, V. Ramesh Babu et al. have demonstrated that the microspheres containing CAB polymer could be retained in the gastric environment for more than 12 h, which would help to improve the bioavailability of the drug49-51.

Evolution of pharmaceutical technology has lead to the development of newer methods of drug administration as well as the design and application of controlled release (CR) formulations for the effective targeting of certain drugs to the site of action. In particular, the use of polymeric systems provides a way to develop CR dosage formulations to achieve the desired therapeutic results to target site as well as optimization of CR of the drug to obtain the maximum dose regimen with minimum of side effects. The release of a drug from a polymeric matrix occurs to transport the solute molecules (drug) to the medium that surrounds the system by a molecular diffusion phenomenon through the polymeric microspheres.

**References :**

47. K. R. [Rao](https://www.ncbi.nlm.nih.gov/pubmed/?term=Rao%20KR%5BAuthor%5D&cauthor=true&cauthor_uid=16423758) , P. [Senapati](https://www.ncbi.nlm.nih.gov/pubmed/?term=Senapati%20P%5BAuthor%5D&cauthor=true&cauthor_uid=16423758), M. K. [Das](https://www.ncbi.nlm.nih.gov/pubmed/?term=Das%20MK%5BAuthor%5D&cauthor=true&cauthor_uid=16423758) ., [*J Microencapsul.*](https://www.ncbi.nlm.nih.gov/pubmed/16423758) **22** (2005) 863

48. Z. El Bahri, J.-L. Taverdet, *Powder Technol.* **172** (2007) 30

49. V. Ramesh Babu, K. S. V. Krishna Rao, Y. Lee, Polym. Bull. **65** (2010) 157

50. [Ajit P. Rokhade](https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Rokhade%2C+Ajit+P), [Sangamesh A. Patil](https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Patil%2C+Sangamesh+A), [Anagha A. Belhekar](https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Belhekar%2C+Anagha+A) , [Shivaraj B. Halligudi](https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Halligudi%2C+Shivaraj+B), *J Appl Polym Sci.* **105** (2007)

51. Varshosaz, Jaleh Taymouri, S. Jafari, E. Jahanian-Najafabadi, A.Taheri, Azade. *J. Drug Deliv. Sci. and Technol*. **48** (2018) 9.

**5/. b.** Moreover, in page 9, lines 224-229: this discussion has less sense since, according to Pharmacopeia, the release kinetics in such acidic medium (i.e. pH 1.2) has to be conducted for only an hour, therefore, the authors have to justify why the dissolution tests were monitored and presented for the period of around 25 hours, while in real testing conditions, the microspheres with the drug loaded would spend about an hour in gastric tract.

**Response:**

**5.b.** The *in vitro* release kinetics of the predominate cation 2-ABZTH+ with a pKa = 2.51 were established at 37°C in a simulated gastric medium pH 1.2. Indeed, the release behavior of 2-ABZTH+ is governed according to Fick’s law. Then, in our case, the release studies was monitored and maintained for the period beyond 100 to 300 minutes (more than an hour) until the equilibrium level of the phenomenon was reached. This will be required in the future studies concerning the *in vivo* tests to evaluate the therapeutic activity of the 2-ABZTH+ cation.

Thus, the diffusion coefficient of any active agent in the body (plasma concentration) depends on its equilibrium mass (long times). That is why we realized the release kinetics at long times (more than an hour). Thereby, we need a monitoring of release was monitored for the period more than an hour. Moreover, the kinetic modeling of this phenomenon of diffusion through different galenic forms has been extensively studied (model of Cranck37).

Microparticulate drug delivery systems are continuously investigated to study the controlled release (CR) of orally administered drugs. The microspheres provide the prolonged release of a single dose, thereby minimizing the frequent administration and hence reducing the side effects. These microparticles could be retained in the gastric environment for more than 20 h, which would help to improve the bioavailability of the drug with a controlled and sustained release.

37. J. Cranck, *The mathematics of diffusion*, 2nd Ed., Clarendon, Oxford, United Kingdom, 1976, 85 (ISBN 0 19 853344 6).

6. The authors did not make any conclusion which of the samples tested would be a choice for further analysis, neither what would be the future steps in developing such systems, nor and more importantly, why this study was important and thereafter conducted. This has to be addressed in the Manuscript and in Conclusion section.

**Responses:**

All the prepared formulations would be chosen for further analysis studies concerning the *in vivo* tests to evaluate the therapeutic activity of the 2-ABZTH+ cation. Beside the *in vitro* release experiments, the *in vivo* studies permit to determine which of the samples tested will assure the prolonged release with efficacy in the therapeutic zone (plasma concentration).

In conclusion, the attempt to prepare controlled release microspheres loaded with 2-ABZT with high encapsulation efficiency (EE) was successful.Then, in a complementary work, the behavior of 2-ABZTH+ will be studied in simulated gastrointestinal medium at pH = 8. Indeed*, in vivo* tests will be required in the future studies to evaluate the therapeutic activity of the 2-ABZTH+ cation.

**Conclusion (Conclusion has been modified as recommended by the referee 2).**

Herewith, I also suggest the list of typos and minor changes:
1. Page 1, lines 9-10: the authors state that “based on 2-aminobenzothiazole-loaded cellulose derivatives”, but the prepared devices are not based on drug loaded, but on polymer used for its preparation, since it causes prolonged (or not) release. Please correct it.
2. Page 1, line 16-17: the authors stated “SEM analysis showed spherical microspheres”. SEM images show that obtained microparticles are spherical in shape. Please clarify it.
3. Page 1, line 17: please write the full drug name before using the abbreviation.
4. Page 2, line 49. Please state the correct pK constant. pKa or pKb.
5. Page 2, line 59: the authors state “of 2-ABZT microspheres”, but this should be “microspheres loaded with 2-ABZT”. Please correct it. In line 62 it is written “loaded by”, in fact, the system is “loaded with”.

**Reponses:**

The remarks cited above were applied and the text in the research article was clarified (in a red color).