**Manuscript entitled: "Effect of Formulation Parameters on Encapsulation Efficiency and Release Behavior of *p*-aminobenzoic acid (PABA)-Loaded Ethylcellulose Microspheres" (JSCS-PM-2449) evaluated by referees.**

**The responses to Reviewers are stated below:**

**Response for Referee 1:**

1. The abbreviation used in abstract should be introduced first.

**Response:**

For the line 55 we state the abbreviation PABA:EC ratios because full names were given in the abstract.

**From 2 to 7, the corrections were applied.**

1. X-RD should be written as XRD.
2. In line 19 and 58 as well as through the whole text before the “gastric medium…” a word “simulated” should be written.
3. In the Experimental Part, subsection Preparation of Microspheres, amount of dichloromethane phase, water phase as well as the concentration of EC in organic phase should be given. The last point is of particular importance, since later in the text some characteristics of the microspheres are explained with increased viscosity of dichloro-methane phase, i.e. increased polymer concentration, during preparation phase.
4. In line 87 instead “The content…” it should be written “The starting composition”.
5. In the equation (1) and (2) instead of “.” The multiplication sign should be used, e.g. “x”.
6. In “Scanning Electron Microscopy” section “University of Bordeaux” should be deleted.
7. In the line 116, in the text “Thermal analysis curves…” the word “curves” should be deleted. In this section (DSC) the atmosphere in which experiments were performed should be given.

**Response:**

In the section (DSC), The DSC experiments were performed as described in DSC section in hermitically sealed aluminum pans and we have not used an inert atmosphere.

1. In the line 124 the word “theta” should be replaced with corresponding Greek letter for diffraction angle and the word “degree” with corresponding unit for diffraction angle. From the Fig. 5 it seems that these measurements were performed form 2θ = 5 o, not from 2θ = 1 o.

**9. The corrections were applied.**

1. From the text in the line 133: “and replaced in the reactor” it is
not clear whether the same sample used in the UV measurements is returned
back into the reactor, or the fresh buffer is added instead.

**Response:**

 10- ‘’and replaced in the reactor’’, that mean that the same sample used in the UV

 measurements is returned back into the reactor.

**From 11 to 20, the corrections were applied.**

1. The size characterization (SEM), XRD, FTIR and DSC characterization of the microspheres should be all organized under one subtitle “Microspheres Characterization”, i.e. there is no need for the subtitle “Micrometric Characteristics of the Microspheres”
2. In the line 143 the word “shell” should be replaced by the word “surface”. From the Fig1d. it seems that these microspheres are just not perfectly spherical, it does not look as if they were “ovoid”.
3. Through the whole text the “size distribution” should be used instead of “dispersion”.
4. It would be convenient to give also a mean diameter and a standard deviation from the mean diameter values, beside the given *d32* and *δ*.

**Response:**

 The mean diameter and standard deviation from mean diameter values are given in Table I.

1. The whole text below the TABLE II should be given at some other place, e.g. in the experimental part (SEM characterization)

**Response:** It is given in the experimental part Particle size.

1. It would be more appropriate if the microphotographs a and b in the Fig2 could be given with same magnification.
2. In the line 179 the text “with invisible pores” should be replaced with “without any visible pores”.
3. In the line 181 instead of “decreasing PABA:EC ratio..”, it would be more convenient to use “with decreasing amount of PABA” or “with increasing amount of EC”. This holds also for the whole text, like in the line 278.
4. In the line 205 delete word “curves”.
5. In the line 208 the word “rounded” should be replaced with “wide”.
6. The questions about DSC and XRD analysis:

Is there any explanation of the endothermic peak positioned at 250 °C in the Fig4a? The endothermic peak in the Fig4c is centered at around 170°C, not at 180 °C, as stated in the text.

It is speculative to claim that “there is a significant reduction of PABA crystallinity”. The change in the peak position can be a consequence of some interaction between polymer matrix and the drug. It is not clear how the Tg was determined from Fig4b and needs to be explained. What is the origin of exothermic peak at around 190 °C in the DSC curve of EC? Can this process interfere with the melting peak from the drug in the microspheres? The diffraction pattern of the loaded microspheres show sharp, well resolved peaks from the drug, meaning that it is in crystalline state. The statement “diffraction pattern, which was less intense as compared to pure PABA” (given in the line 226) can be a consequence of the amount of the substance investigated, not of reduced crystallinity. DSC and XRD together show that the drug is in crystalline state, and it seems too speculative to state that there is a reduction in crystallinity, based on the presented data.

**Response:**

We have observed a sharp endothermic peak at 191°C corresponding to the melting point of PABA (Fig. 4a) accompanied by an endothermic peak positioned at 250 °C. This endothermic peak positioned at 250 °C in the Fig. 4 (a) and (c) accompanying PABA and appears to be a compound derived from eventual degradation of PABA at a temperature higher than its melting point at 191 °C. The thermogram of the loaded microspheres (Fig. 4 (c)) showed a wide endothermic peak at around 170 °C, indicating the melting point of PABA in the presence of EC. This change in the peak position can be a consequence of some interaction between polymer matrix EC and the drug.

The thermal transition of the polymer EC in the ethylcellulose microspheres (blank test) was observed at ~85 °C which corresponding to the glass transition temperature of EC. The Tg was difficult to detect, it is generally depending on the molecular weight and the degree of substitution of EC used. It was noticed by other authors31-33 that the variability of the thermal transition temperature Tg is thoroughly depending on the structure of EC and the addition of some compounds like plasticizer in order to increasing the porosity of the microspheres32. Further, the elongation, flexibility and Tg decreased significantly with the increase of EC ethoxyls groups31. Observation of Fig. 4b indicates that the Tg involves a subtle baseline change, indicating that EC is a strong glass former which exhibits limited rheological and heat capacity change through the transition, rendering it more difficult to characterize. The exothermic peak at around 190 °C is associated with oxidative degradation of ethylcellulose, generally observed for the pure EC. Such phenomenon is also suggested by H. L. Lai et al. working on the thermal properties of ethylcellulose31.

**For the statements 22 and 23, the corrections were applied.**

22. In the line 221 the text “during the emulsion and solvent evaporation process” should be deleted.

23. In the line 225 the 1 should be replaced with 5.

24. The text “The presence of X-ray diffractograms which was much decreased the loaded microspheres indicated …” in the line 227 has to be reconstructed.

**Response:**

The diffraction pattern of the loaded microspheres show sharp, well resolved peaks from the drug, meaning that it is in crystalline state in the polymer matrix EC.

25. The subsections “Data kinetics” and “In vitro release study” should be merged into the subsection.

 **Response:**

 The subsections Data kinetics and *in vitro* release study were merged into the subsection.

 26. The text “This process is related with phenomenon of mass transfer controlled by diffusion. Thus a vertical tangent is observed at the beginning of the process with a linear effect at short time.” should be reconstructed.

**Response:**

The text was deleted.

 27. Some conclusion about the drug transport from obtained n values in the KP model should be given. Also obtained values of rate constants are not explicitly discussed.

**Response:**

For Higuchi model, the obtained values of rate constants (*k*H) were decreased by varying the process parameters such as: increasing the amount of polymer, decreasing surfactant concentration and stirring speed (Table III).

Divers values of *n* for cylinders and spheres are described by Ritger and Peppas.37,38 The values of *n* for the microspheres prepared by varying the process parameters are ranging from 0.29 to 0.39 (Table III), indicating the shift of Fickian transport. For the Korsmeyer-Peppas model, the values of rate constants were depended on the obtained *n* values.

**For the statements 28 and 33, the corrections were applied.**

28. The TABLE IV should be excluded from the text, since it is a repetition of the description given in the text.

29. In the caption of the Fig6 and 7, the word “carrying” should be replaced with “having”. In the Fig6 the Greek letter τ should be replaced with t as in corresponding equation.

30. In the line 276 the text “release rate at equilibrium” is not clear and needs to be changed. Also, in the same sentence the text “the increase in the organic phase viscosity” should be deleted.

31. Figure 9 with corresponding explanation in the manuscript should be given in the supplementary material.

**Response:**

Figure 9 is given in the supplementary material in the Title Fig. 10 (after the corrections, Figure 9 corresponds for a new added graph, given also in supplementary material).
32. There are a lot of technical errors in the axes titles in presented diagrams. In all the Figures the axes titles should be changed to stick to the Journal style.
33. The text needs extensive language corrections.

**Response for Referee 2:**

1. The authors stated well the purpose of skin application in the introduction section, but why is this important for this paper?

**Response:**

1. Despite of the new biological properties of PABA cited in the introduction section, its microencapsulation was performed only in the case of solar filter to protect the skin, we have found in the literature pharmaceutical formulations of PABA for oral administration but not microparticles, for these reasons, we have cited the microcapsules of PABA used for skin application (to mention the PABA was microencapsulated only in this field).
2. Page 3, line 72: it is the test solution, not buffer solution (by definition). Leave both for the sake of future mentioning.

**Response:**

2- The corrections were applied.

1. Were the samples spatter coated prior to Scanning Electron Microscopy utilization, or the

scanning was performed under either low-vacuum or ESEM mode? Please state.

**Response:**

1. The morphology and surface topography of the prepared microspheres were examined without coating using Scanning Electron Microscopy (SEM Quanta 200 FEI) at 50 Pa s under 12 kV of accelerated tension. The microspheres were mounted on double-scotched carbon film fixed on a metal support.
2. The PABA-loaded particle sizes from Table II are not correlated to the statements given in the lines 155-167, and lines 191-193. How the authors explain the decrease in EE with reduction of the particle size, and how is this correlated with the increase in the total surface area of the obtained microparticles? Please explain.

**Response**

Each factor (Surfactant type and stirring speed) has a significant effect on particle size and EE

but not with the same manner:

Lines 155-167

It was stated that the lower encapsulation efficiency obtained with Tween 80 is due to the structure, the porosity and the surface of the microspheres.

All the prepared microspheres have porous surface but the Tween 80 microspheres are porous but with different structure and surface topography. Tween 80 microspheres have bigger pores as shown in Fig. 1d. Furthermore, using PVA as surfactant may result in a more stable emulsion which hinders the mass transfer of PABA to the continuous phase and thus improve its entrapment.

Lines 191-193

Decrease in EE with the reduction of particle size

The results in Table II confirmed that the microparticle mean size decreased with an increase in the stirring speed, which is a well-documented effect. The force of higher stirring speed distributes the internal phase into smaller droplets, resulting in the formation of smaller microspheres. The decrease in EE with the reduction of particle size can be explained by the fact that increase in the stirring speed delivers greater energy to the system, resulting in an increased breakdown of the forming microparticles and lower encapsulation efficiency.

i.e., the reduction of particle size caused by the increase in stirring speed results in the decreases in encapsulation efficiency.

The decrease in EE with the reduction of particle size can also be ascribed to the larger surface area of smaller microparticles. These microparticles have an enhanced contact with the aqueous phase during the emulsification process which may result in an increase in the

loss of PABA.

1. It would be more pronounced if the authors present the cumulative PABA release as normalized values, since the percentage was affected by the amount of initially encapsulated model compound. These graphs could be given as Supplementary Material.

**Response**

The cumulative PABA release was presented as normalized values. The Table of values of mt of cumulative PABA release (values of the graph of Fig. 9) is given in the next page. mt is the amount of PABA released at the time t



**Fig.9. Cumulative release of PABAH+ (mt) from the microspheres in pH 1.**2 at 37 °C havingdifferent PABA:EC ratios (1:1, 1:2,1:3 and 1:4 with **1% PVA and 600 rpm constants)**

The Table of values of mt of cumulative PABA release : values of the graph of Fig. 9 where mt is the amount of PABA released at the time t.

We have presented the cumulative PABA release as normalized values in the graph given as supplementary materials in the Title field ‘’Fig. 9’’. If not, Please explain more.

 Table of values of mt of cumulative PABA release

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | F11 | F12 | F13A | F14 |
| t (min) | mt (g) | mt (g) | mt (g) | mt (g) |
| 2 | 0,00222 | 0,00182 | 0,00124 | 4,57E-4 |
| 3 | 0,00287 | 0,00187 | 0,0014 | 5,54E-4 |
| 5 | 0,00339 | 0,00202 | 0,0015 | 6,98E-4 |
| 7 | 0,00352 | 0,0022 | 0,00159 | 6,52E-4 |
| 9 | 0,00372 | 0,00226 | 0,00163 | 8,28E-4 |
| 11 | 0,00404 | 0,00235 | 0,00177 | 8,61E-4 |
| 13 | 0,00449 | 0,00252 | 0,00186 | 9,13E-4 |
| 15 | 0,00457 | 0,00267 | 0,00189 | 9,91E-4 |
| 20 | 0,00463 | 0,0028 | 0,00202 | 0,00111 |
| 25 | 0,00509 | 0,003 | 0,00224 | 0,00124 |
| 37 | 0,00515 | 0,00326 | 0,00241 | 0,00131 |
| 42 | 0,00561 | 0,00357 | 0,00255 | 0,00137 |
| 47 | 0,00616 | 0,00369 | 0,00267 | 0,0015 |
| 57 | 0,00652 | 0,00385 | 0,00273 | 0,00161 |
| 67 | 0,00737 | 0,00391 | 0,00287 | 0,00157 |
| 77 | 0,00809 | 0,00415 | 0,00304 | 0,00168 |
| 87 | 0,0077 | 0,00437 | 0,00313 | 0,00176 |
| 99 | 0,00796 | 0,00457 | 0,00326 | 0,00188 |
| 107 | 0,00822 | 0,00474 | 0,00361 | 0,00202 |
| 127 | 0,00881 | 0,0052 | 0,00372 | 0,00196 |
| 152 | 0,009 | 0,00528 | 0,00383 | 0,00218 |
| 163 | 0,0092 | 0,00556 | 0,00398 | 0,00228 |
| 179 | 0,00946 | 0,00577 | 0,0042 | 0,00239 |
| 199 | 0,00985 | 0,006 | 0,00424 | 0,00254 |
| 214 | 0,01005 | 0,00607 | 0,00457 | 0,00258 |
| 234 | 0,01057 | 0,00642 | 0,00464 | 0,00267 |
| 254 | 0,01076 | 0,00652 | 0,0047 | 0,00282 |
| 284 | 0,01096 | 0,00678 | 0,00479 | 0,00287 |
| 299 | 0,01103 | 0,00665 | 0,00489 | 0,003 |
| 344 | 0,01115 | 0,00717 | 0,00509 | 0,00324 |
| 359 | 0,01138 | 0,00731 | 0,00522 | 0,00333 |
| 389 | 0,01182 | 0,00744 | 0,00529 | 0,00346 |
| 419 | 0,012 | 0,00751 | 0,00545 | 0,00357 |
| 450 | 0,01233 | 0,00766 | 0,00574 | 0,00385 |
| 479 | 0,01254 | 0,00783 | 0,00583 | 0,00405 |
| 1200 | 0,016 | 0,01038 | 0,008 | 0,00598 |
| 1260 | 0,01626 | 0,01057 | 0,00802 | 0,0062 |
| 1320 | 0,01631 | 0,01077 | 0,00818 | 0,00639 |
| 1360 | 0,0166 | 0,011 | 0,00835 | 0,00652 |
| 1420 | 0,01702 | 0,01109 | 0,00855 | 0,00672 |
| 1480 | 0,01689 | 0,01123 | 0,00861 | 0,00698 |
| 2580 | 0,01742 | 0,0117 | 0,00913 | 0,00737 |
| 2640 | 0,01735 | 0,01181 | 0,00926 | 0,00741 |
| 2700 | 0,01722 | 0,01152 | 0,00922 | 0,0073 |
| 2760 | 0,01717 | 0,01174 | 0,0093 |  |

1. Was the FT-IR spectrum of pure PABA taken in a wet (dissolved) form? Please explain in details, and state it for all samples. Then, if the wet mode was performed, explain in details how you managed to cover the surface of the whole diamond area using wet particles of such a small size (even when applying T13A and T13B particles).

**Response:**

6- No, the samples pure PABA, blank ethylcellulose microspheres and the loaded microspheres in solid state were analyzed by Infra-red spectroscopy without any prior preparation.

The solid sample (enough) is placed onto the small crystal area ensuring good contact. Once

the solid has been placed on the crystal area, the pressure arm should be positioned over the crystal/sample area. Force is applied to the sample, pushing it onto the diamond surface. Then, the spectrum is measured after just one click.

**Minor changes 1 to 3 were applied.**

Some minor changes are listed below:
1. Page 1, line 27: move references to the end of sentence.
2. Please italicize the constants throughout the Manuscript.
3. Please find some typos and minor changes within the corrected pdf document.