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EFFECTS OF DIFFERENT SURFACTANTS ON INDOMETHACIN MICROSPHERES FORMULATIONS

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ABSTRACT

Microencapsulation by the solvent evaporation technique was used to formulate Indomethacin-loaded poly(DL-lactide-co-caprolactone) microspheres with three different surfactants: Tween 80, Span 80, and Polyvinyl alcohol. Different formulations were prepared by changing drug masses, while keeping the quantities of the polymer and of the surfactant constant. The prepared microspheres were evaluated for drug content, particle size, morphology, drug-polymer interaction, stability, in vitro release, and cytotoxicity assays. Comparison was done to study the effects of the surfactant type on their characteristics. Microspheres presented a spherical and porous profile and were characterized by the stable character of the encapsulated drug. The usage of the Polyvinyl alcohol revealed the highest percent drug entrapment and drug loading, the biggest particles sizes, and the lowest drug release rate. It was the opposite in the case of Tween 80. A negligible cytotoxic effect was noted on Polyvinyl alcohol formulations having the highest drug content. Polymeric microspheres were used efficiently as a delivery system for Indomethacin. Changing the surfactant type had many advantages on drug encapsulation and release rate.

Keywords: Drug delivery; polymers; drugs; microencapsulation; in vitro release

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1. INTRODUCTION

Oral drug delivery systems (DDSs) are still the simplest and the most suitable way of drug administration in a controlled manner to a definite body site with a maximum therapeutic activity [1-4]. One of the most common techniques for the formulation of microspheres used as DDS is the solvent evaporation technique, which could be applied into different procedures[5,6]. These include the oil-in-water (o/w) co-solvent, the oil-in-oil (o/o) non-aqueous solvent evaporation, and the water-in-oil-in-water (w/o/w) double emulsion [5-7].

Because of their biocompatibility and their biodegradability, synthetic aliphatic polyesters have been extensively used in polymeric microspheres [6-8]. Among these polyesters, poly(DL-lactide-co-caprolactone) (PLC) is an amphiphilic copolymer with a high hydrophobic character. It can be easily degraded within the human body, through a non enzymatic hydrolytic cleavage of the ester bonds [8].

Indomethacin (Indo) is a non steroidal anti-inflammatory drug, poorly soluble in the fluids of the gastrointestinal tract revealing a low oral bioavailability [9]. Continuous Indo therapy could lead to severe adverse effects on the GI tract, such as bleeding, ulceration and perforation [10]. For this purpose, its incorporation into polymeric microspheres could be a way to reduce these problems.

A surfactant plays an important role in the preparation of polymeric microspheres by solvent evaporation technique. It allows easier spreading and mixing of the two phases [11]. Surfactants are amphiphilic in character and are characterized by their Hydrophile-Lipophile Balance (HLB) number [7]. Surfactants, with HLB number greater than 10, are hydrophilic; those with a HLB number lower than 10 are lipophilic surfactants [12].

In this study, Indo-loaded PLC microspheres were formulated using the solvent evaporation technique, by means of three different surfactants: Tween 80, Span 80, and Polyvinyl Alcohol (PVA). This was followed by characterization of these microspheres in terms of drug content, particle size, morphology, interaction between drug and polymer, stability, drug release and cytotoxicity.

2. MATERIALS AND METHODS

2.1. Chemicals

Poly(DL-lactide-co-caprolactone) (86 mol% DL-lactide), Indomethacin, Tween 80, Span 80, Polyvinyl Alcohol (PVA) (Mw 13000-23000, 87% hydrolyzed), dichloromethane (DCM), methanol (MeOH), hexane, and Phosphate Buffer Saline (pH=7.4, 25^{o}C, 0.01M) were purchased from Sigma-Aldrich Chemie Germany. All materials were of analytical grade and used as received.

2.2. Microspheres formulation

The o/w solvent evaporation technique is applied for the preparation of Tween 80 and PVA formulations, while the o/o solvent evaporation for Span 80 formulations. This change in experimental procedure is due to the hydrophobic character of Span 80. For each type of surfactant, drug masses range between 0.5 and 40 mg, the polymer quantity is fixed to 500 mg and the surfactant to 1% w/v. Tween 80, Span 80, and PVA are referenced “T”, “S” and “P” respectively.

- For Tween 80, Indo and PLC are dissolved in 14/6 ml DCM/MeOH to form the organic phase. This is added to a 250 ml aqueous phase containing the surfactant. The o/w emulsion is mixed continually for 6 hrs at 1400 rpm to allow the evaporation of the solvent. Microspheres are then recuperated by filtration. Finally they are washed with water and MeOH then dried.
- For PVA, the organic phase which is prepared in 7/3 ml DCM/MeOH, is added to the aqueous solution containing the surfactant. The latter is originally heated overnight at 40°C to enhance the solubility of PVA in water.
- For Span 80, the organic phase in which the drug and the polymer are dissolved in 14/6 ml DCM/MeOH, is added to 250 ml liquid Paraffin containing the surfactant. Microspheres are washed with hexane and MeOH.

2.3. Microspheres characterization

2.3.1. Drug content

7 mg microspheres are dissolved in 7/3 ml DCM/MeOH, and analyzed with UV/vis spectrophotometry (Microplate Spectrophotometer, Epoch, Biotek, USA) at 322 nm. The percentage of Drug Encapsulation (%DE) and Drug Loading (%DL) are calculated according to equations (1) and (2).

$$\%DE = \frac{\text{Encapsulated drug mass}}{\text{Introduced drug mass}} * 100 \qquad (1)$$

$$\%DL = \frac{\text{Encapsulated drug mass}}{\text{Microspheres mass}} * 100 \qquad (2)$$

2.3.2. Particle size

Laser diffraction granulometry is used to measure the particle size of microspheres (Horiba instrument Ltd., France). Microspheres are suspended in water, with few drops of Tween 80 added for a good dispersion. The average particle size is obtained in µm.

2.3.3. Morphological examination

Indo microspheres are analyzed for morphological characteristics by Scanning Electron Microscopy (SEM) (LYRA 3 XMU, TESCAN, USA). They are fixed to a carbon conductive tape then coated with platinum at 10 nm thicknesses.

2.3.4. FT-IR analysis

Spectra are recorded on a FT-IR spectrometer (Frontier, Perkin-Elmer, USA) using the ATR technique and KBr pellets technique to check the physico-chemical status of the polymer and the drug after microencapsulation.

2.3.5. Stability study

Microspheres are tested for stability under different storage conditions: at the powder state (4 ^{o}C, 25^{o}C, and 37^{o}C), in Phosphate Buffer Saline (PBS) (pH=7.4) solution at 37^{o}C and in acidic media (pH=2) at 37^{o}C. The physical appearance of these microspheres is then evaluated by the optical microscopy (LEICA DM LS2, Vashaw Scientific Inc., USA).

2.3.6. In vitro study

Microspheres are suspended in 25 ml PBS (pH=7.4) at 37^{o}C. At specific time intervals, 5 mL of the release medium is withdrawn and tested for its drug content at 322 nm, to monitor the release profile of the drug from microspheres.

2.3.7. Cytotoxicity assay [13]

The Human HaCaT Keratinocyte cells are used for this study. They are cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco-BRL, Paisley, Scotland), 1% 100 µg/ml penicillin-streptomycin, 1% 2mM L-Glutamine, and 1% Hepes. Cells are maintained in a 5% CO_2 humidified incubator at 37 ^{o}C. Cells are seeded into 96-well microtiter plates (Thermo Labsystems, Inc., USA) at a density of 2500 cells/well for 24 hours. They are then treated with different concentrations of Indo-loaded microspheres. Controls are prepared by adding blank polymer microspheres dissolved in 1% DMSO, or by adding the solvent media without microspheres or drug.

Cytotoxicity of microspheres on HaCaT cells is assayed using CytoTox 96 Non-radioactive Cytotoxicity Assay (Promega Corp., Madison, WI) at 24 hours. This method quantitatively measures the stable cytosolic enzyme, lactate dehydrogenase (LDH), which is released upon cell lysis. Released LDH in culture supernatants is measured with a 30-minute coupled enzymatic assay, which results in the conversion of a tetrazolium salt (INT) into a red formazan product. The amount of color formed is proportional to the number of lysed cells. The product is measured colorimetrically at 490 nm using an ELISA microplate reader (Multiskan Ascent, Thermo Labsystems, Inc., USA). The percentage viability of cells corresponds to 100−% of cytotoxicity. Every sample is assayed in triplicate to obtain the average absorbance.

3. RESULTS AND DISCUSSION

The solvent evaporation technique is used to encapsulate Indo within PLC microspheres. The preparation procedure is changed from one surfactant to the other in order to obtain well-shaped microspheres, and satisfactory particles sizes and drug content. A small quantity of organic mixture in the case of PVA is very important to avoid the formation of aggregates and allow good mixing of organic phase into the aqueous phase. The effect of surfactant type on microspheres is studied in terms of drug content, size, and drug release.

3.1. Drug content

Table 1 shows the results of %DE and %DL of the prepared formulations. Figures 1 and 2 illustrate the differences in %DE and %DL among the different formulations for each surfactant.

Table 1. %DE and %DL of the microspheres formulations

Microspheres Formulation	Quantity of Indo Introduced (mg)	%DE	%DL
T_1	0.5	24	0.02
T_2	1	53	0.10
T_3	3	25	0.15
T_4	10	19	0.38
T_5	20	11	0.44
S_1	0.5	35	0.03
S_2	1	56	0.11
S_3	3	29	0.17
S_4	10	22	0.44
S_5	20	18	0.72
P_1	0.5	67	0.07
P_2	1	75	0.15
P_3	3	41	0.25
P_4	10	39	0.78
P_5	20	35	1.4

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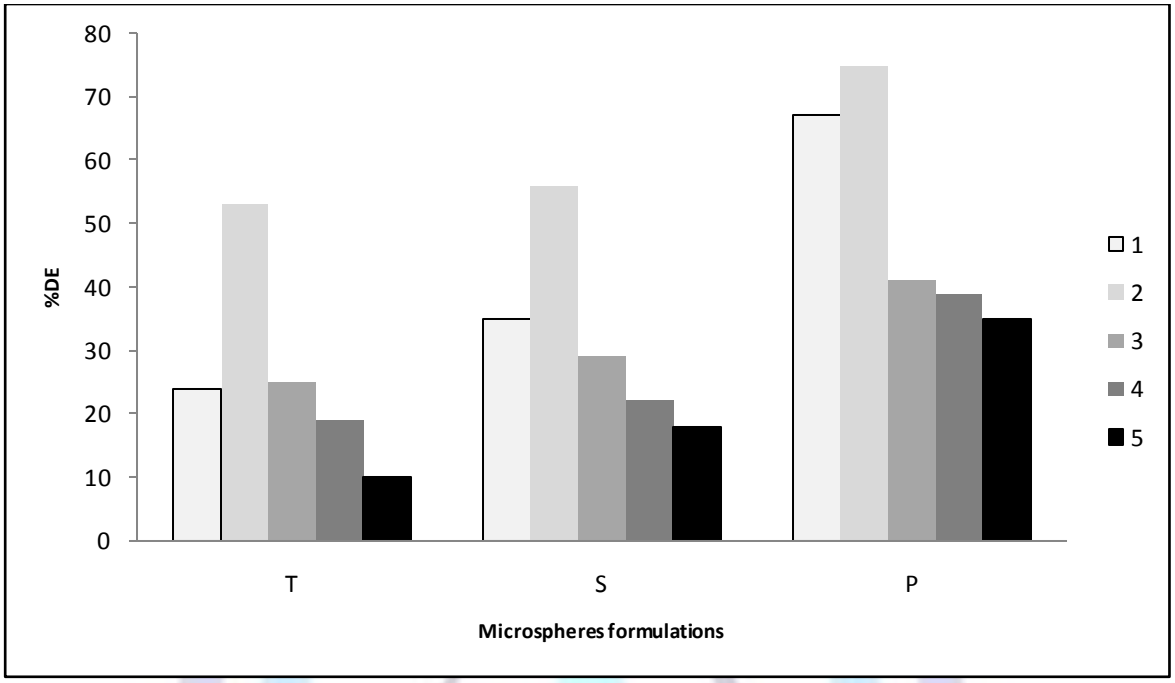

Fig 1: Comparison of %DE of the prepared microspheres

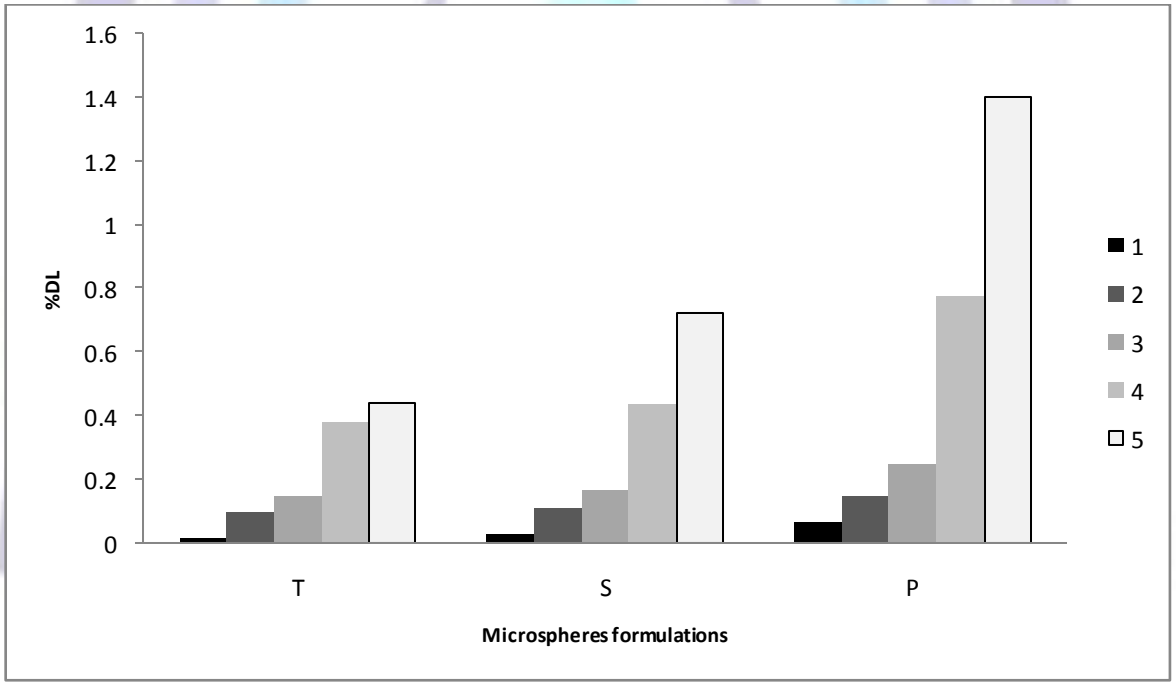

Fig 2: Comparison of %DL of the prepared microspheres

Comparison between T, S, and P formulations in Table 1, Figures 1 and 2, relatively to each quantity of Indo introduced show that P formulations have the highest %DE and %DL values, whereas T formulations have the lowest values. The high results obtained in the case of PVA could be confirmed by the fact that the encapsulation efficiency increases as the ratio between the Dispersed Phase and the Continuous Phase decreases (DP/CP) [14,15]. In fact, 10 ml DCM/MeOH were used instead of 20 ml during the preparation of microspheres using PVA yielding a DP/CP of 0.04, in opposite to a DP/CP of 0.08 for the other surfactants, which explains the results obtained. For formulations prepared with Tween 80, %DL increases from 0.02 to 0.1% as the %DE increase from 24% to 53% (Table 1). This reveals that as more drug mass is introduced, it is more included within microspheres. The same results are seen in the case of S and P formulations.

Concerning the formulations containing 40 mg drug, they have the lowest %DE and %DL among all the formulations and are negligible.

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3.2. Particle size

Among the different surfactants, the highest particle sizes are observed for P formulations (Table 2). The HLB value of the surfactant has a great effect on the particle size of microspheres; the lower the HLB value, the smaller the particle size (Table 2). A surfactant with a higher hydrophobic character creates a more stable emulsion with an organic dispersion media [11,12,16]. In addition, this could be related to the effect of heating the PVA solution during synthesis. The higher temperature increases the rate of solvent evaporation, thus the emulsion droplets solidify faster leading to the formation of larger particles [14].

Table 2. Effect of surfactants HLB values on the particles sizes

Surfactant Type	HLB	Microspheres Size Range (µm)
Tween 80	14.9	80-100
Span 80	4.3	225-300
PVA	18	390-450

On the other side, although the HLB value is bigger for Tween 80 than for Span 80, S formulations show larger particle sizes than T formulations. This could be explained by the high solubility of DCM in the continuous phase which is oily in the case of Span 80. DCM has a higher solubility in Paraffin oil than in water; this leads to a faster mass transfer between the two dispersed and continuous phases, thus to rapid precipitation of microspheres and larger particles [16].

3.3. Morphological examination

Figure 3 represents the morphological state of the prepared microspheres, as examined by SEM. Microspheres exhibit a spherical profile and a porous structure.

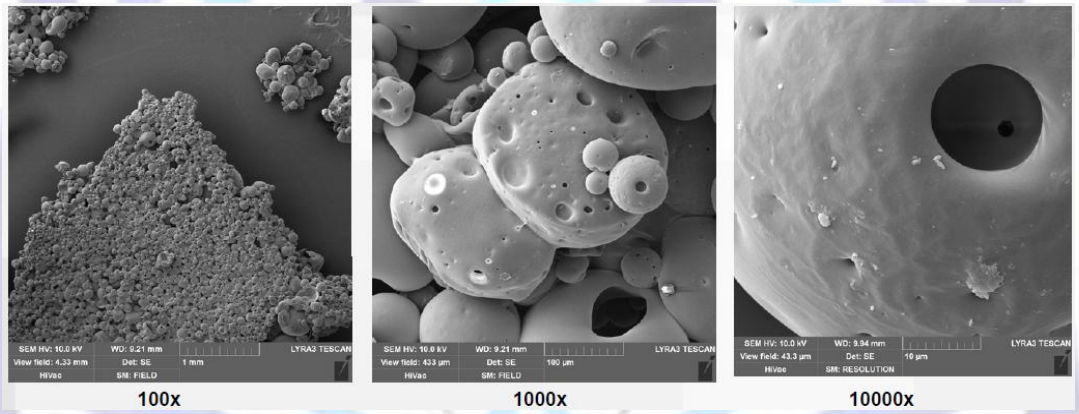

Fig 3: Microphotographs of Indo-loaded microspheres taken by SEM at different magnifications

3.4. FT-IR analysis

The FT-IR spectrum obtained for pure Indo is characterized by a significant broad band in the 3300-3500 cm^{-1} region assigned to –OH group of the drug (Figure 4), which is absent in the spectrum of PLC (Figure 5). Bands observed around 1600-1700 cm^{-1} (-C=O ketone), around 1450 cm^{-1} (-CH_3 aliphatic alkane), and 1100-1200 cm^{-1} (-C-O ester) are assigned to the polymer (Figure 6). The FT-IR spectrum of Indo-loaded PLC microspheres (Figure 6) show approximately the same distinctive absorption peaks as the FT-IR spectrum of PLC at the exception of a large band assigned to the -OH group of the drug. Consequently, the results indicate the absence of any chemical interaction between the drug and the polymer, and confirm the stability of the encapsulated drug.

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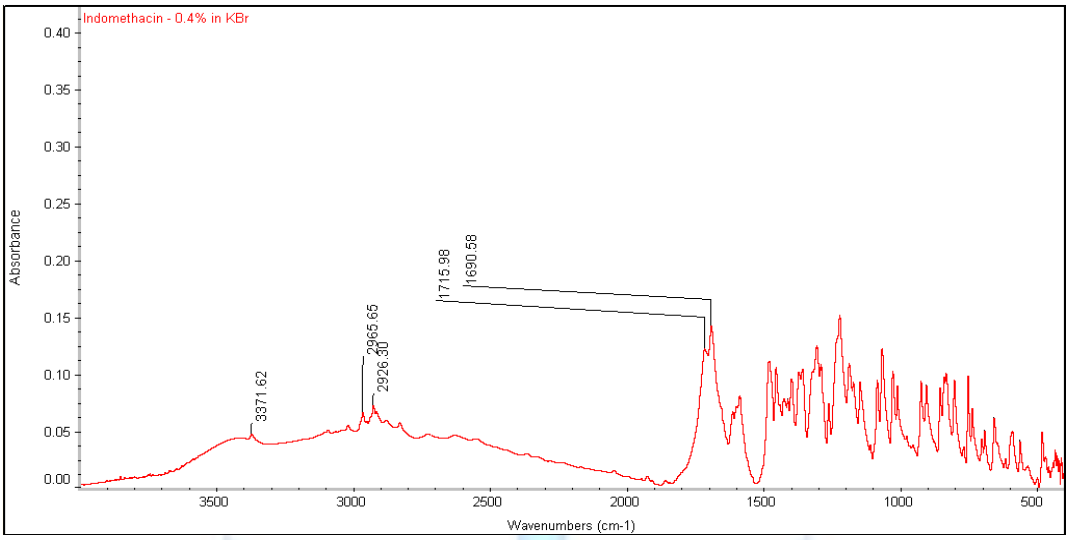

Fig 4: FT-IR spectrum of pure Indo

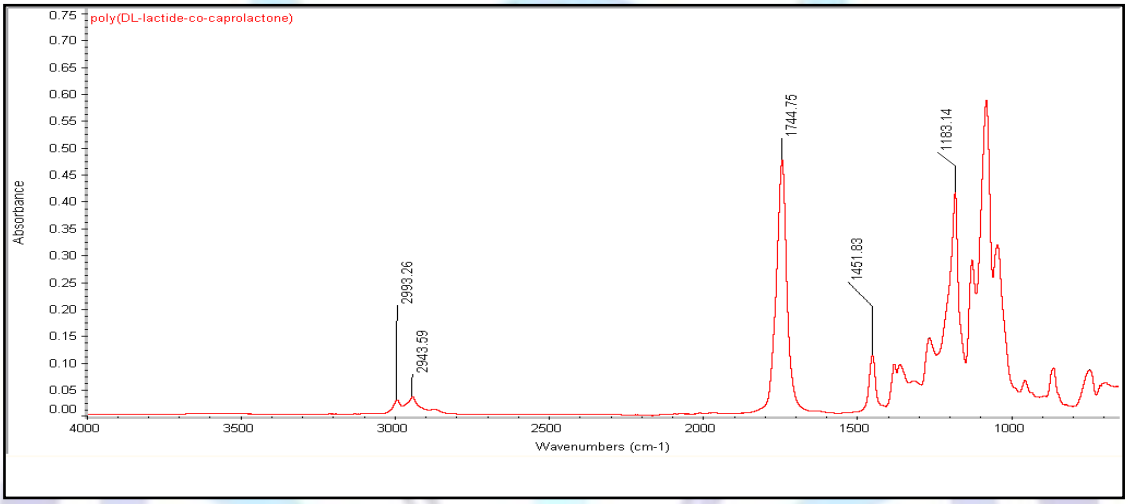

Fig 5: FT-IR spectrum of PLC

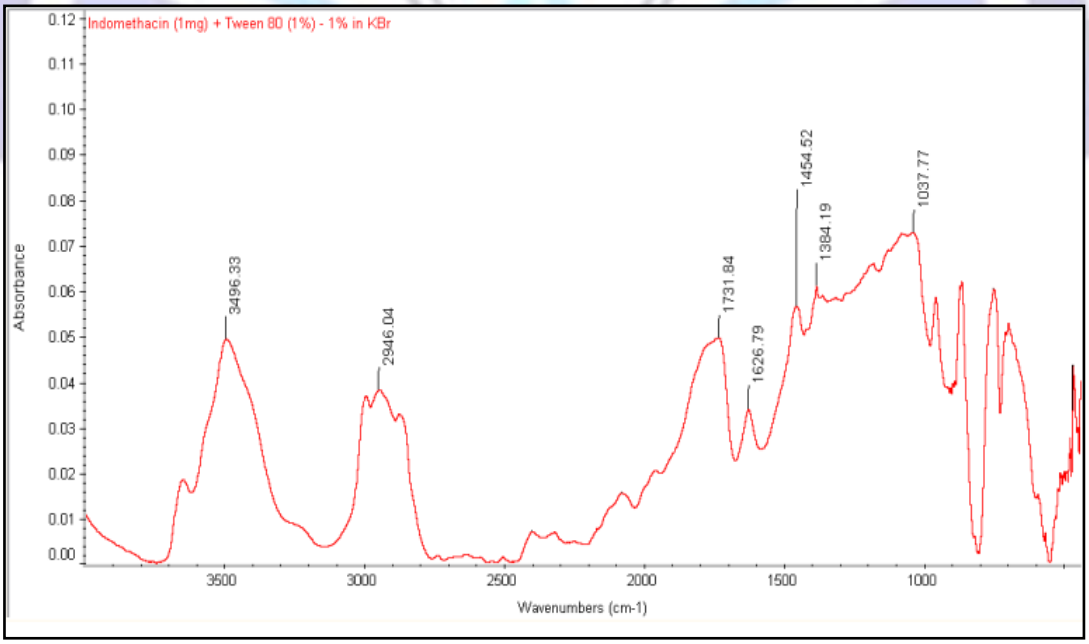

Fig 6: FT-IR Spectrum for Indo-loaded microspheres

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3.5. Stability study

After carrying out the stability test, no significant morphological changes in all the formulations (T, S, and P) at the powder state are detected for three months duration. Thus, the formulations remain stable in such storage conditions. On the other hand, microspheres stored in PBS and in acidic media don't show any physical change till the sixth week during which degradation started to occur (Figure 7). Degradation consists of a hydrolytic cleavage of ester bonds present in the polymer.

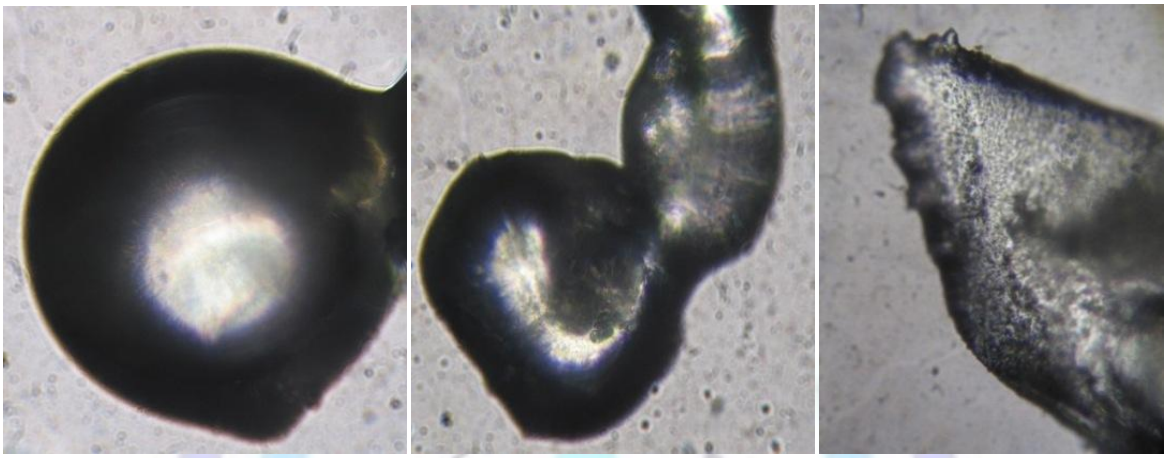

Fig 7 : Microphotographs of microspheres stored in PBS at 37°C

3.6. In Vitro study

The complete *in vitro* drug release time of T, S, and P formulations is presented in Table 3. The slowest release is observed for formulations having the highest %DL values. In addition, the release rate increases as the %DL decreases. In other words, a small quantity of drug loaded within the polymeric matrix requires a short duration to be completely releases. Drug release from biodegradable polymeric microspheres depends on the diffusion through the pores of the coating material and on the degradation of the polymer [17, 18].

Table 3. *In vitro* drug release time of Indo-loaded microspheres

Surfactant Type	Microspheres Size Range (µm)	Formulations	*In vitro* complete drug release time	%DL
Tween 80	80-100	T_1	10 min	0.02
		T_2	6 h	0.10
		T_3	8 h	0.15
		T_4	9 days	0.38
		T_5	12 days	0.44
Span 80	225-330	S_1	20 min	0.03
		S_2	8 h	0.11
		S_3	48 h	0.17
		S_4	10 days	0.44
		S_5	14 days	0.72
PVA	390-450	P_1	1 h	0.07
		P_2	30 h	0.15
		P_3	72 h	0.25
		P_4	12 days	0.78
		P_5	21 days	1.4

Besides, drug release by diffusion is kinetically controlled by the particles size. Usually microspheres with small particle sizes have fast release rates; while bigger microspheres have slower rates [19]. This could be explained by the decrease in the surface area to volume ratio of the drug encapsulated with the increase of the microspheres size, leading to modifications in the drug distribution within microspheres [20].

Compared with S and P formulations and regarding each drug quantity, T formulations have the highest drug release rates relative to their small sizes. In opposite, P formulations have the lowest release rates relatively to their large sizes. An increase in system size reduces drug release rates. This is due to the increase in the length of diffusion channels existing

in the polymeric matrix through which drug molecules are escaping [20, 21]. This in turn leads to the decrease in concentration gradients or drug transport rates, which are the driving forces for diffusion [22].

3.7. Cytotoxicity assay [13]

The cytotoxicity of PLC blank microspheres and drug-loaded microspheres prepared is evaluated using the HaCaT cell line. Microspheres prepared using PVA as surfactant are tested because they are exhibited the highest drug content among all the prepared formulations. Cells are seeded in a 96-well plate in the presence or absence of loaded microspheres for 24 hrs. PLC blank microspheres haven't shown any cytotoxic effect at different tested concentrations (0, 1, 2.5, 5, 10, and 20%) (Figure 8).

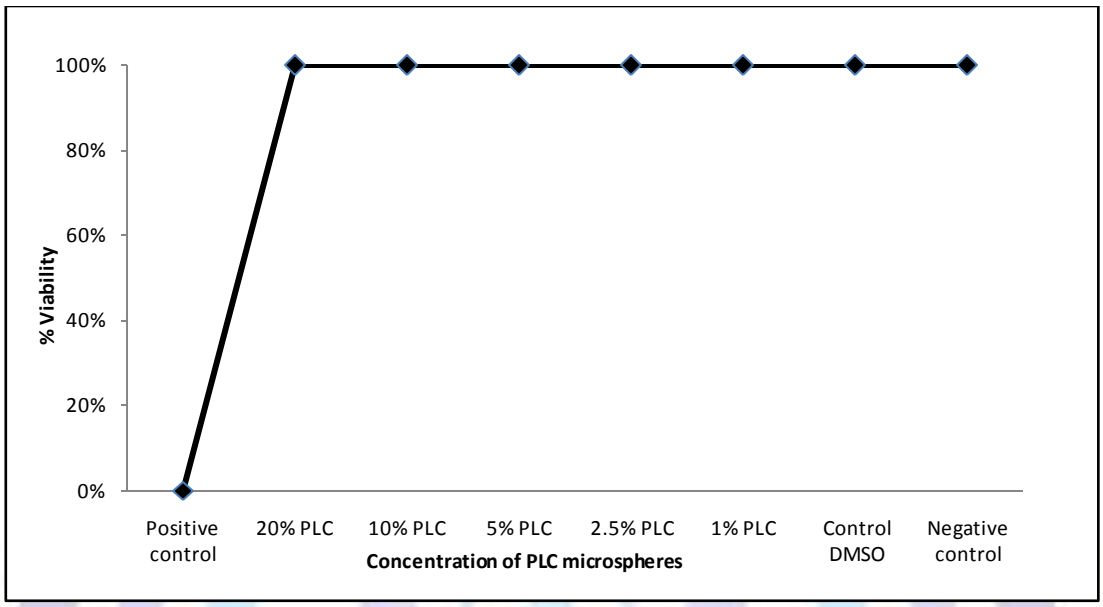

Fig 8: Percentage viability of different concentrations of PLC blank microspheres

Microspheres loaded with 0.5, 1, 3, 20 mg of Indo are assayed and compared to control microspheres. Viability of cells varied between 82-99 % at 24 hrs (Figure 9). A higher viability is observed for formulations with 20 mg Indo than for formulations with lower drug masses. This may be due to less absorption of microspheres.

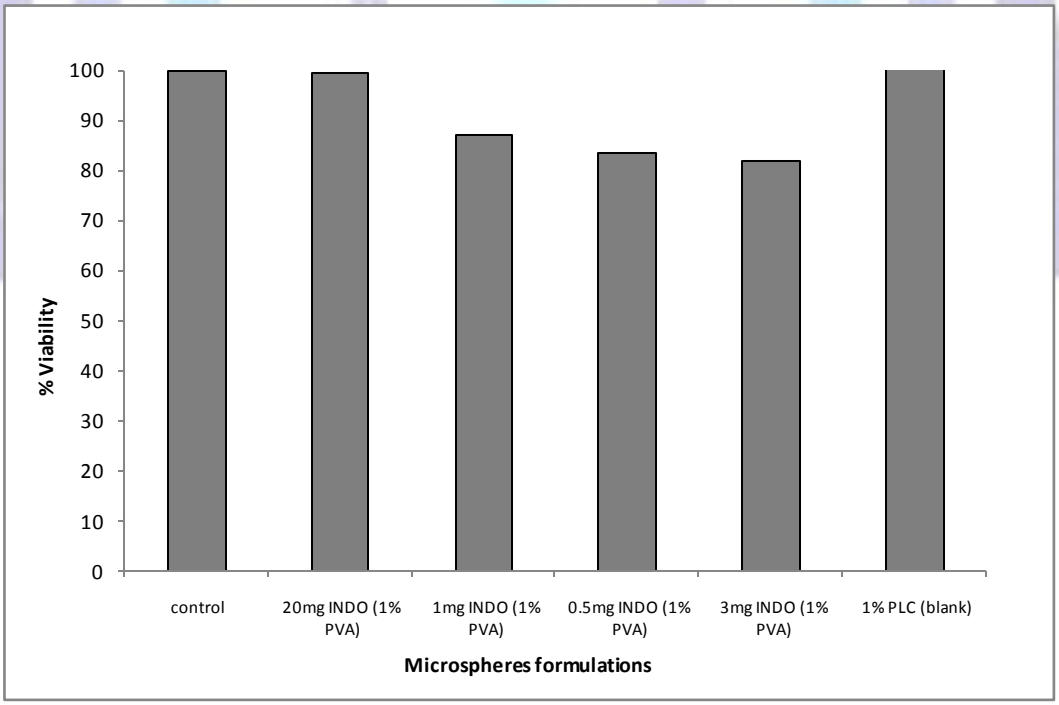

Fig 9: Percentage vability of cells at 24 h

4. CONCLUSION

The solvent evaporation technique was used to formulate Indo-loaded PLC microspheres by means of three different surfactants: Tween 80, Span 80, and PVA. All microspheres presented a spherical and porous profile and were characterized by the stable character of the drug encapsulated. The usage of the surfactant PVA gave the highest %DE and %DL, the biggest particles sizes, and the lowest drug release rates. As for the lowest percentages for drug content, the smallest particles sizes, and the highest drug release rates, they were obtained with formulations prepared with Tween 80. Employing PLC as a coating material proved successful in the microencapsulation of Indomethacin, with the ability to control the size of the prepared microspheres. Cytotoxic assays resulted in negligible effects on PVA formulations having the highest drug content.

5. ACKNOWLEDGEMENTS

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