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Preparation and study of poly(vinyl acetate) and poly(styrene) nanosized latex with indometacin

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During the last decade the nŭmber of investigations on the preparation and application of more effective drug release systems on the basis of nanocarriers from biocompatible and biodegradable polymers are considerably increasing. This is notably in force for practically water insoluble drugs to be applied in liquid forms (eye solutions for an example). The aim of the work presented was the preparation of model poly(vinyl acetate) and poly(styrene) nanosupports for indometacin and their potential inclusion in eye drops. The polymers are synthesized as nanosized latex by a radical polymerization of the monomers in the presence of indometacin. It is proved that the low polymerization temperature and initiator used do not influence indometacin structure and properties. The nanoparticles were characterized by attenuated total reflection Fourier transform infrared spectroscopic analyses, atomic force microscopy, scanning electron microscopy and transmission electron microscopy. The size of the latex particles was around 200 nm, determined by the scan electron microscopy. The indometacin delivery rate from the supports discussed in aqueous solutions was determined at pH 7.4. The change of this rate, in comparison with that for a pure drug substance, was established also as well as its dependence on the nature of the carrier.

1. Introduction

Indometacin (IMC), ([1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid) is a nonsteroidal anti-inflammatory drug, used to treat osteoarthritis, rheumatoid arthritis, bursitis, tendinitis, gout, ankylosing spondylitis and headaches (Sweetman 2007). It is practically insoluble in water, unstable in alkaline and acidic media and slightly soluble in alcohol (Lobenberg and Amidon 2000). Due to its insolubility in water, the drug formulations, which it is included, often show low and erratic bioavailability. Prolonged contact with the living of the stomach causes increasing irritation (Hirasawa et al. 2003; Alsaidan et al. 1998).

In ophthalmology, IMC is used in topical eye drops for prevention of miosis during cataract surgery, cystoid macular edema and conjunctivitis (Wickstrom 2008; Sweetman 2007). Its use in liquid formulations is limited due to insolubility in water, low bioavailability and ocular mucosa irritation.

In the last decade, research defines the use of nanoparticles of biocompatible and biodegradable polymers such as effective drug-release systems, which aim to increase the solubility, increased bioavailability and reduce the irritating effects of the drug (Motwani et al. 2008).

Nanoparticles were developed by emulsion radical polymerization based on copolymers of methyl methacrylate and glycidyl methacrylate with IMC (Nita et al. 2010).

Studies have been made on nanoparticles of cyclodextrin with IMC (Wongmekiat et al. 2006).

Poly(ε-caprolactonic) nanoparticles, nanocapsules and nanoemulsions of IMC were obtained, (average size 225 nm), via superficial cumulations, nanoprecipitation and spontaneous emulsification (Calvo et al. 1996)

The aim of this study was to develop an *in-situ* method of obtaining nanosized poly(vinyl acetate) (PVAc) and poly(styrene) latex (PSt) with indometacin for their application in ophthalmic formulations.

Table 1: Investigated nanosized latex with IMC

Designation	Type of polymer, including IMC and method of obtaining
IMC-PVAcL-1	PVAc latex, obtained by Method 1
IMC-PVAcL-2	PVAc latex, obtained by Method 2
IMC-PSt	PSt latex obtained by Method 1

2. Investigations and results

2.1. Preparation of nanoparticles

Nanosized poly(vinyl acetate) (PVAc) and poly(styrene) (PSt) latex with IMC was obtained by an radical polymerization of vinyl acetate or styrene, in presence of IMC. The investigation includes two models of nanosized latex, obtained by in-situ polymerization without agitation (Method 1) and polymerization with ultrasonic agitation (Method 2).

Table 1 shows the studied model latex and the method of their obtaining. Surfactants were not used during the process of polymerization or testing the latex.

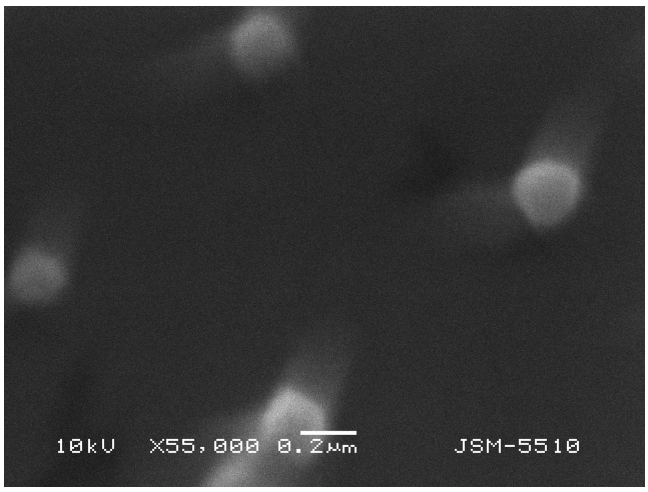


Fig. 1: Scanning electron microscopy of nanosized particles of IMC-PVAcL-1 obtained by Method 1

The approximate size of the latex particles was determined by scanning electron microscopy (SEM), made of nanosized latex IMC-PVAcL-1, obtained by Method 1. It shows that the average particle size was about 200 nm (Fig. 1).

2.2. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopic analyses and results

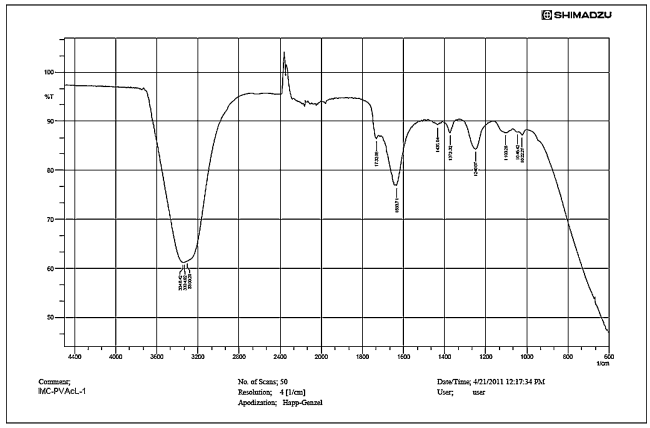
The IR-spectra of the latex IMC-PVAcL-1 (Fig. 2.a), IMC-PVAcL-2 (Fig. 2.b) and IMC-PSt (Fig. 2.c) do not show a similarity with the spectrum of IMC (Zlatkov et al. 2010), but its presence is proved when examining his release from PVAc and PSt carrier. Figures 2.a, 2.b and 2.c show similarity to each other, no matter the difference in the model carriers – PVAc and PSt. Displacements in the spectrum and the absence of vibrations of the characteristic groups of IMC, indicate the eventual existence of a complex between IMC and PVAc or PSt carrier (Casella et al. 1998).

2.3. Transmission Electron Microscopy

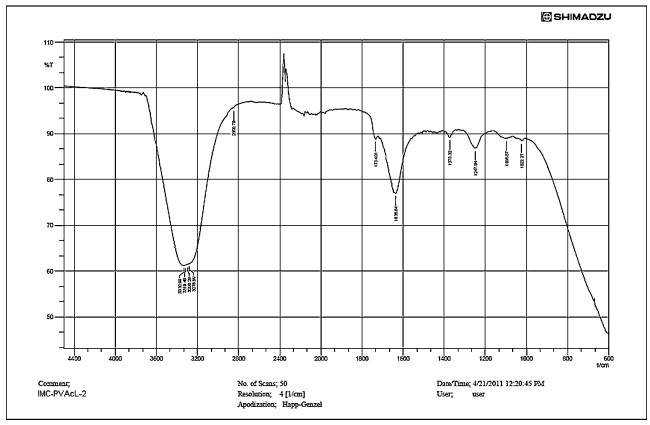
Figure 3.a shows a transmission electron microscopy (TEM) of IMC, which particles have an irregular shape and size under 20 nm. Figures 3.b and 3.c show TEM of IMC-PVAcL-1 and IMC-PSt. There are observed particles with spherical shape and a size under the observed with SEM 200 nm. All particles have a characteristic conformation, which indicates crystallization of the polymer (Fig. 3.d). This process can be explained by the crystallization of IMC itself, included in the nanosized particles.

2.4. Atomic Force Microscopy

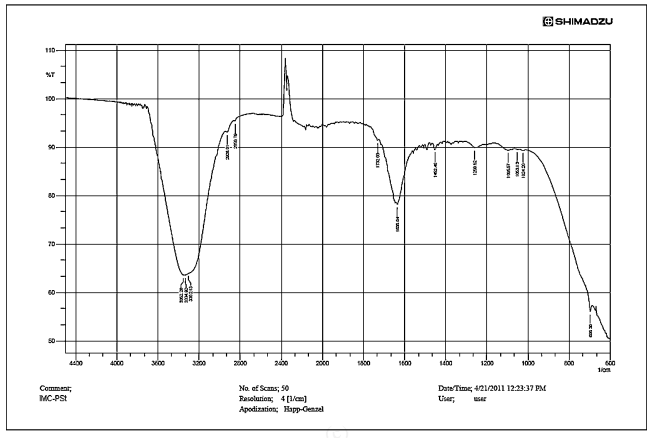
Figures 4.a and 4.b show a scanning probe microscopy (SPM) (AFM) of two of the samples examined - IMC-PVAcL-1 and IMC-PSt. This research proves again the spherical shape of the nanosized latex particles and their size, which is under 200 nm. Nanoparticles are tend to aggregate, which can be explained by the hydrophobic behavior of IMC, also of PSt and the absence of surfactant. The bigger is the aggregation of the particles at IMC-PSt, at IMC-PVAcL-1, even after this process of association, the height of the individual aggregates is over 200 nm (which can be seen by SEM).



(a)



(b)

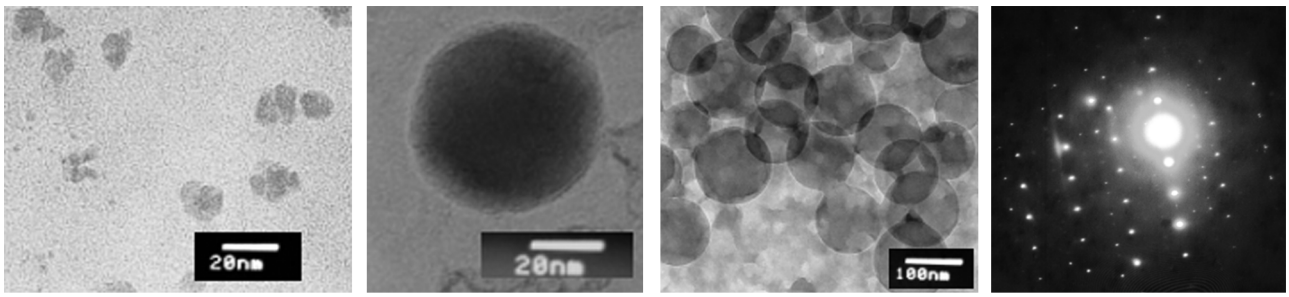


(c)

Fig. 2: IR-spectra of a) IMC-PVAcL-1, b) IMC-PVAcL-2, c) IMC-PSt

2.5. Release of IMC from model carriers

It is known that IMC is practically insoluble in water, unstable in alkaline media and precipitates at pH under 6.0 (AHFS 2001). Table 2 shows the release of IMC from the model nanosized latex in phosphate-phosphate buffer with pH 7.4, for 7 h. A comparison in % between the released IMC and the total quantity of the included indometacin, was made in equal volumes of the examined samples - IMC-PVAcL-1, IMC-PVAcL-2 and IMC-PSt. The release profile of IMC from different models indicate, that all of them shows a delayed release, with approximately the same speed. A significant difference we found at the initial quantity of released IMC in water (at ph 7.4); at IMC- PVAcL-1 the released IMC is around 3.5 times more, compared to IMC-PSt latex. Each survey was conducted 6 times.
This difference can be explained with the higher surface concentration of IMC on the PVAc nanoparticles, compared to those



(a) (b) (c) (d)

Fig. 3: TEM of a) IMC, b) IMC-PVAcL-1, c) IMC-PSt, d) SAED of IMC-PVAcD-3

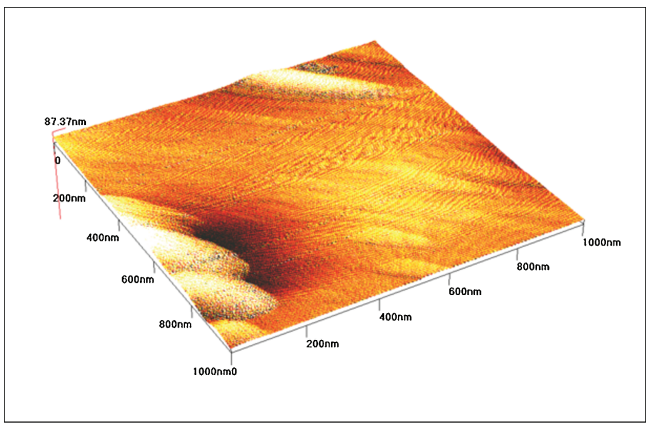
Table 2: Percentage ratio of the released IMC from the model carriers at pH 7.4 for 7 hours

Time of examination, [h]	0.5	1	1.5	2	2.5	3	3.5	4.5	5	5.5	6	7
Percent of released IMC by IMC-PVAcL-1, [%]	61.15	62.06	62.52	62.98	63.66	64.5	65.5	66.87	68.41	73.7	75.16	77.1
Percent of released IMC by IMC-PVAcL-2, [%]	34.74	34.74	34.97	34.97	35.2	35.2	35.2	35.2	35.2	35.2	36.3	36.57
Percent of released IMC by IMC-PSt, [%]	17.26	17.89	19.37	21.26	22.1	22.74	23.79	28.8	32	36.8	41.83	42.23

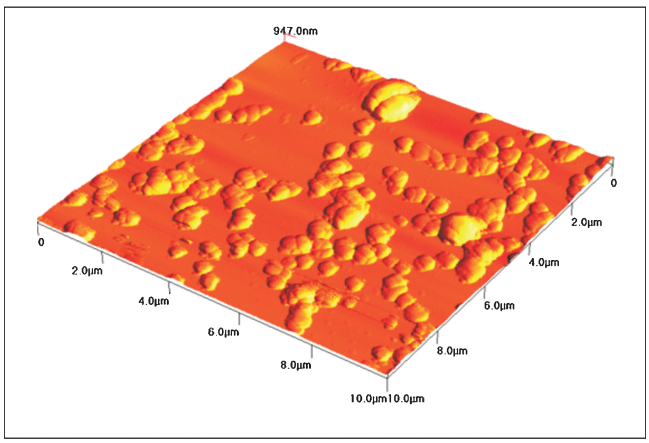
from PSt. The sorption of IMC on the PVAc surface is higher that this on the PSt surface.

To investigate the influence of the preparation method, on the release of IMC, and its stability in water (at ph 7.4), IMC-PVAcL-1 (obtained by Method 1), and IMC-PVAcL-2 (obtained by Method 2), are stored at ambient temperature for a period of 10 days (Table 3).

A comparison between the UV – spectra of IMC, IMC-PVAcL-1 and IMC-PVAcL-2, just prepared, and the same, but stored for 10 days, show a similarity at the UV-spectrums and the same absorption peaks. This result proves the stabilizing role of the PVAc – carrier.



(a)



(b)

Fig. 4: AFM of a) IMC-PVAcL-1 and b) IMC-PSt

3. Discussion

An interest can provoke the influence of agitation (via ultrasound) during the process of polymerization. For comparison PVAc latex was chosen. The change of the released IMC from IMC-PVAcL-2 is presented in Table 2, that in this case, the initial quantity of the released IMC from IMC-PVAcL2 is higher than that from PSt latex. During the examination, the percentage of the released IMC insignificantly changed by around 2%. This means, that under ultrasound stirring of the monomer - IMC mixture in water, IMC has a more homogeneous distribution in the emulsion drops, and after they became latex particles, it was much more harder to release the included IMC.

The model IMC-PVAcL-2, obtained by method 2, shows an increased quantity of the released IMC, for a long period of time (10 days) – Table 3. This result categorically demonstrates the influence of agitating on the kinetics of release and probably, by a controlled agitation it would be possible to control the release. The practical value of this result remains to be evaluated.

The results of our work proved, that IMC can be incorporated in PVAc and PSt nanosized latex particles via emulsive polymerization (*in-situ* inclusion). The resulting nanoparticles are complexes of PVAc and PSt with IMC and have a spherical shape with dimensions of 200 nm. Agitating during the polymerization is a crucial factor for the distribution of indometacin in the latex particles, therefore for the kinetic of its release. Latex obtained by stirring shows a delayed release of IMC compared to particles produced without stirring. IMC from IMC-PVAcL-2, obtained with stirring has a long release for more than 10 days. IMC, included in IMC-PVAc latex, is stable during the 10 day storage.

4. Experimental

4.1. Materials

In this research, the following materials were used: Indometacin, purum, ≥ 99.0%, Fluka BioChemika; Kaliumdihydrogenphosphat pro analysi, Merck; *di*-Natriumhydrogenphosphat pro analysi, Merck; Vinyl acetate, purum, ≥ 99.5% (GC), Fluka; Styrene, puriss., monomer, ≥ 99.5% (GC), Fluka; Ammonium persulfate, puriss. P.a., ACS reagent, ≥ 98% (RT), Fluka.

Table 3: Percentage ratio of the released IMC from IMC-PVAcL-1 and IMC-PVAcL-2 on the 1st, 5th and 10th day of the examination

Time of examination (days)	Percent of released IMC by IMC-PVAcL-1 (%)	Percent of released IMC by IMC-PVAcL-2 (%)
1st day	78.24	32.2
5th day	73.23	40.1
10th ден	48.78	47.4

4.2. Obtaining the nanosized poly(vinyl acetate) and poly(styrene) latex with indometacin

Obtaining the nanosized poly(vinyl acetate) and poly(styrene) latex was made by an radical polymerization of vinyl acetate or styrene, in presence of indometacin, at a ratio of monomers 10% (v/v), and the indometacin 1% (w/v). The polymerization was conducted in a nitrogen atmosphere and a temperature of 55 °C, for 90 min.
Ammonium persulphate in concentration 1% (w/v) was used as initiator. The conversion rate oh the monomers is 97–98% during the time of the polymerization (90 min). Two methods were applied:
Method 1 – Polymerization without agitation;
Method 2 – Polymerization made by agitation with ultrasonic generator.

4.3. Scanning Electron Microscopy

The size of the latex nanoparticles was determined by Scanning Electron Microscopy (SEM) (JEOL JSM-5510), using a device for cathodic pulverization and application of thin layers of gold (Fine Coater JEOL JFC-1200).

4.4. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopic analyses

ATR-FTIR was were performed using a IRAffinity-1 Spectrophotometer (Shimadzu) equipped with a MIRacle Attenuated Total Reflectance Attachment (diamond crystal) accessory (Pike, USA). The spectra were recorded from 4000 to 500 cm^{-1} using a DTLG detector. All spectra were corrected for H_2O and CO_2 using internal software.

4.5. Transmission Electron Microscopy scan (TEM)

Investigations were done on a transmission electron microscope JEOL JEM 2100 acceleration voltage 200 kV. The main parameters of the microscope were: tension: 80, 120, 160 и 200 kV; increase from 50 to 1 500 000 times; resolution 0.23 nm between two points and 0.14 nm between lines; modes: TEM, HRTEM (transmission electron microscopy with high resolution), SAED (selected area diffraction), NBD (nanodiffraction), CBED (convergent beam electron diffracttion).
Micro-quantities of the test substance were mixed in a test tube with distilled water. Place in an ultrasonic bath for 3 min and immediately thereafter, with pipette, suspension is dripped on pre-coated with carbon standard Cu grid. After air-drying in a dust free environment for several hours the grid is ready for observation in the microscope.

4.6. Atomic Force Microscopy (AFM)

A Q-Scope™ (Ambios Technology) scanner was used for scanning of the samples. The XY Range of scanner is 80 × 80 μm and Z Range is 8 μm. The Scan Type is Wavemode with Standard XY and Z Signal mode. The used Scan Rate is 0.5 Hz.

4.7. Indometacin release kinetcs

Examination on the release of IMC from the model nanosized latex was carried out in a termostated vessel, working volume for dissolution 100.0 ml; temperature 37 °C; stirring speed 100 min^{-1}.
Indometacin was quantitavely determined spectrophotometrically at λ = 320 nm on a UV/VIS spectrophotometer Ultrospec 3300 pro after filtering the samples through a filter Chromafil Xtra 0.45 μm. The measurement was made compared to the environment of examination – phosphate – phosphate buffer (Sorensen's phosphate buffer) at pH 7.4.

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