

Table: Factorial design set (a) and results (b); established working range (c) for the methoda) 2⁴-cube + star Statistical design for optimising of chromatographic conditions

Set	-x	-1	0	+1	+x
Variable					
Start acetonitrile (%)	6	8	10	12	14
End acetonitrile (%)	92	94	96	98	100
Flow-rate (ml/min)	0.6	0.8	1.0	1.2	1.4
Temperature (°C)	30	35	40	45	50

b) Results of selected experiments

Exp. no.	Res* T-TP	Res* TP-TE	Res* TE-TUD	Sym T	Sym TP	Sym Te	Sym TUD
1	5.516	6.108	7.193	1.269	1.247	1.328	1.097
11	5.73	5.093	6.189	1.379	1.284	1.229	1.211
12	4.902	5.638	6.844	1.391	1.373	1.349	1.306
13	6.825	5.328	5.808	1.140	1.272	1.163	1.101
26	6.178	4.779	5.16	1.184	1.31	1.128	1.174

Res* – Resolution

Sym – Symmetry

Boldface-optimum

c) working range (W) of the method, retention time (R), Slope (S) and correlation coefficient (C) for the calibration curve

Substance	R (min)	W (ng/10 µl injection)	S (µV · s/µg/ml)	C
T	4.10	0.1012–0.6072	7061	0.9997
TP	4.59	0.1016–0.6096	5382	0.9997
TE	5.02	0.1042–0.6252	2091	0.9991
TUD	5.53	0.1004–0.6024	1098	0.9974

in the literature [5, 6]. Short reequilibration time of the column and a variable rinse time for sample clean-up with 10% acetonitrile in water prefers the method for build-in in automated analysis systems.

References

- 1 Täuber, U.; Schröder, K.; Düsterberg, B.; Matthes, H.: J. Drug Metab. Pharmacokin. **11**, 145 (1986)
- 2 Nieschlag, E.: Clin. Endocrinol. (Oxf.) **45**, 261 (1996)
- 3 Coert, A.; Geelen, J.; de Visser, J.: Acta Endocr. **79**, 789 (1975)
- 4 Nieschlag, E.; Behre, H. M.: Testosterone: Action, Deficiency, Substitution, p. 332, Springer-Verlag, Heidelberg 1998
- 5 Karunanithy, R.; Becket, G.; Leung, S. L.; Ng, S. E.: J. Pharm. Biomed. Anal. **13**, 375 (1985)
- 6 Carignan, G.; Lodge, B.; Shakum, W.: J. Pharm. Sci. **69**, 1214 (1980)
- 7 Matlin, S. A.; Chan, L.; Prazeres, M. A.; Mehani, S.; Cass, Q. B.: J. High Resolut. Chromatogr. **10**(4), 186 (1987)
- 8 Najavas, R.; Imaz, C.; Carrera, D.; Garcia, M.; Perez, M.; Rodriguez, C.: J. Chromatogr. **673**, 159 (1995)

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Development of biodegradable poly(α-methylmalate) microspheres

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During the recent years some novel biodegradable oligoesters based on tartaric, malic and other acids have been used for the development of biodegradable drug delivery systems apart from conventional poly(lactic acid) (PLA), poly(D,L-lactide-co-glycolide) (PLGA), polycaprolactone (PCL), and their co-polymers [1]. Some of our previous papers reported the possibilities to prepare chemically crosslinked drug delivery systems based on malic acid via polycondensation [2]. Being biocompatible and biodegradable is the main advantage of the polymalates as drug carriers [3]. Their degradation results in malic acid which is a natural metabolite in the Krebs Cycle. Thus Belcheva et al. [4] implemented a polycondensation method to prepare a series of polymalates of Mw > 5000 with potential application as biodegradable drug carriers. Wada et al. [5] used low molecular weight PLA oligomers for achieving drug release rates higher than those provided by high molecular weight PLA.

The above mentioned studies provoked our investigations aiming at the preparation of drug loaded microspheres based on a novel biodegradable poly(α-methyl-malate) polymer (PMM). Microspheres were prepared by a simple solvent evaporation method. Comparatively, microspheres from traditionally used PLGA co-polymer were obtained under the same process conditions. Isopropylantipyrine was selected as a model agent for incorporation because it is a lowly soluble compound and release could be followed easily by spectrophotometry.

PMM microspheres were obtained with a drug loading of 30% and PLGA microspheres were loaded with 28% drug. A negligible amount of drug was wasted in the external aqueous phase since the drug is slightly soluble in water. Scanning electron microscopy (SEM) showed that both samples of microspheres have spherical shape and smooth surfaces. No drug crystals are observed on the microsphere surfaces. Microspheres obtained from PMM seem to be on average smaller (5–80 µm) than those obtained from PLGA (30–80 µm). The reason for this difference might be the higher viscosity of the inner organic phase of the PLGA emulsion. This leads to the formation of larger drops during emulsification and respectively to

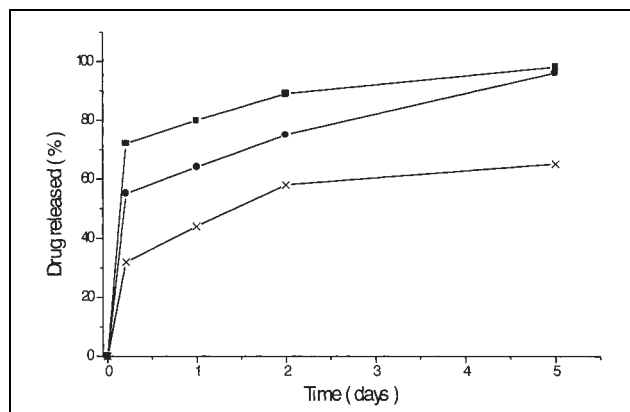


Fig. 1: In vitro release of isopropylantipyrine from: lyophilized PMM microspheres (■); non-lyophilized PMM microspheres (●); and PLGA-microspheres (×)

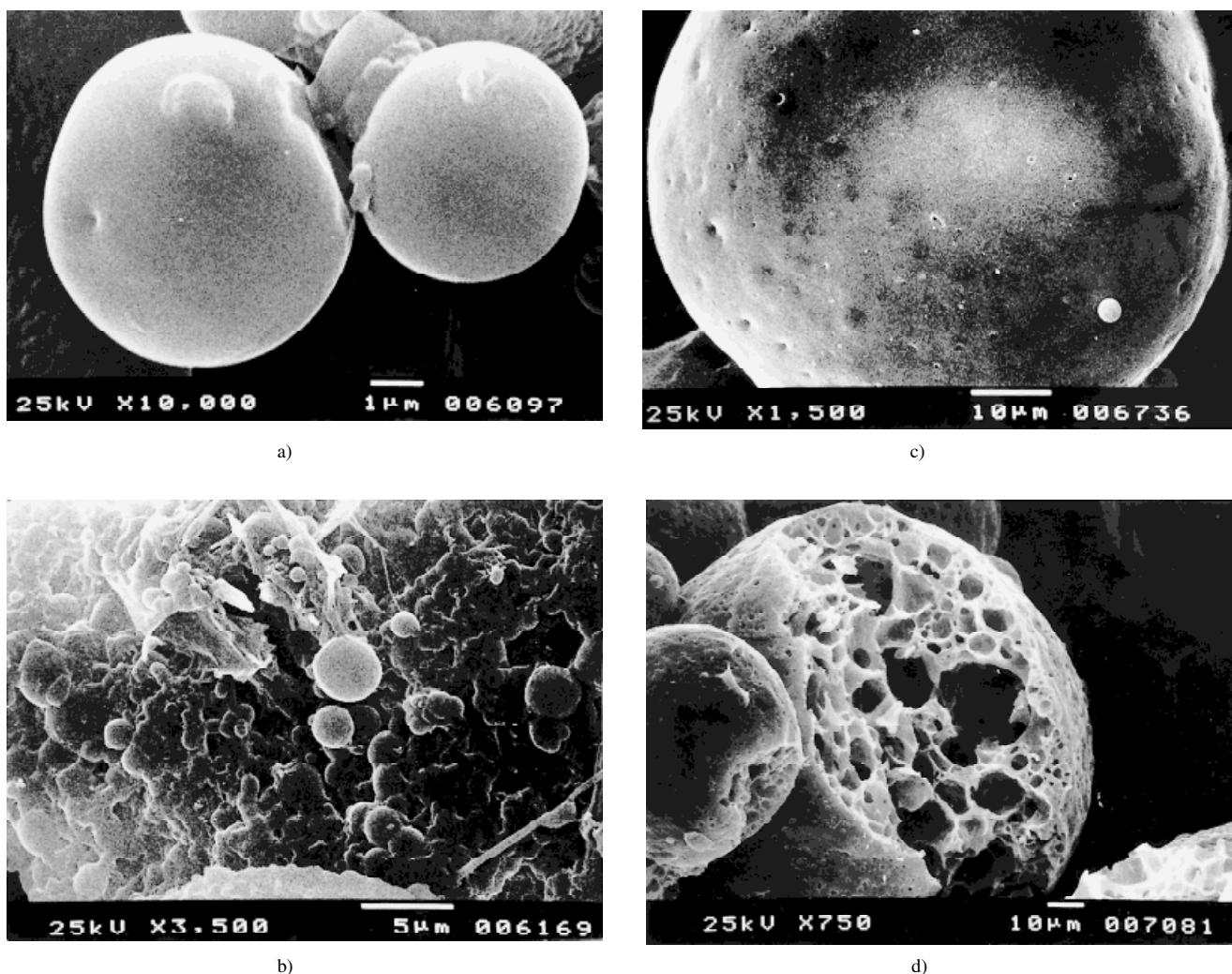


Fig. 2: Scanning electron micrographs of microspheres during release:

- a) PMM microspheres before release;
- b) PMM microspheres 2 days after release;
- c) PLGA microspheres before release;
- d) PLGA microspheres 20 days after release

microspheres of larger size. Polyvinyl alcohols (PVA 1500 and PVA 80000) were used as stabilizers but the difference in their molecular weights does not affect the characteristics of the microspheres obtained.

The *in vitro* release profiles of the model drug from PMM and PLGA microspheres are illustrated in Fig. 1. The model drug was released from PMM microspheres in a biphasic manner, characterized by an initial "burst" effect (50–60% in the first hours) followed by a continuous and slower release thereafter. The degree of the "burst" effect depends on the method used for drying the microspheres. The effect discussed is better pronounced in the case of lyophilized PMM microspheres than when using vacuum dried PMM microspheres.

Investigations on insulin loaded microspheres [6] gave the same results which is an evidence of the influence of lyophilisation on the release process. Both samples release in the beginning at a high rate which then remains relatively constant and is lower after the second day (3% per day for the lyophilized PMM microspheres and 7% for those vacuum dried).

A comparison of the release rates shows that drug release from PLGA microspheres is much slower. About 90% of the loaded drug are released within 30 days. PMM microspheres release the model drug much more rapid than

PLGA microspheres due to the lower molecular weight and faster degradation of the PMM polymer, a consequence from the more pronounced hydrophilic properties of PMM polymer if compared to those of PLGA co-polymer.

Electron microscope studies of PMM microspheres during release reveal that the samples change in size chiefly and no cracks and pores occur over their surface (Fig. 2, b). The latter fact suggests the release from PMM microspheres to proceed as a result from surface erosion unlike the release from PLGA microspheres which do not change their size but increase the porosity of the matrix (Fig. 2, d).

In conclusion, the biodegradable PMM microspheres prepared release the model hydrophobic drug completely for about 5 days. The release is described by a biphasic manner corresponding to a foremost burst effect which can be avoided by optimizing the microencapsulation conditions. It could be concluded that poly(α -methylmalate) polymer can be used as a novel biodegradable drug carrier.

Experimental

1. Materials

Poly(α -methyl-malate) (Mw = 3000) was supplied by Institute of Polymers, Bulgarian Academy of Sciences (Sofia, Bulgaria). Poly(D,L-lactide-

co-glycolide) (PLGA) with a D,L-lactide: glycolide molar ratio of 50:50 was supplied by Boehringer Ingelheim (Ingelheim, Germany). Isopropylantipyrine (1,2-dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenyl, 3 *H*-pyrazol-3-one) was obtained from Sopharma (Sofia, Bulgaria). Polyvinyl alcohol (PVA 15000 and PVA 80000), (Fluca). All other reagents were of analytical grade (Fluca).

2. Methods

2.1. Preparation of microspheres

Isopropylantipyrine-loaded microspheres were prepared using a solvent-evaporation method. Briefly, given amounts of model drug (1.0 g) and polymer (PMM or PLGA, respectively) were dissolved in 10 ml CH₂Cl₂ and emulsified in 100 ml PVA aqueous solution (2% wt.) by sonication (Ultrasonic disintegrator, UD-20, 22 ± 1.65 kHz). The emulsion was agitated at room temperature until CH₂Cl₂ was evaporated completely. The microspheres were collected by centrifugation conducted at 10000 rpm and 0 °C for 10 min, washed with distilled H₂O and lyophilized or vacuum dried.

2.2. Determination of surface morphology and microsphere size

The shape and surface characteristics of the formulations were examined by scanning electron microscopy (SEM) (Stereoscan, Cambridge Instruments, Cambridge, UK).

2.3. Drug loading

A weighed quantity of microspheres was incubated in 10 ml of 1 N NaOH solution at room temperature for 24 h. After suitable dilution with 0.1 N NaOH solution the absorbance was measured spectrophotometrically at λ = 266 nm (Hewlett Packard 8452 A diode array spectrophotometer equipped with a HP Vectra 386/25 computer). The amount of drug was determined from a standard plot of isopropylantipyrine prepared under similar conditions.

2.4. In vitro drug release

In vitro drug release was conducted in a shaker bath at 37 °C (150 rpm), in phosphate buffer (pH = 7.4). The amount of isopropylantipyrine was measured spectrophotometrically (λ = 266 nm) (Hewlett Packard 8452 A).

References

- Wada, R.; Hyon, S.-H.; Ikada, Y.: J. Biomater. Sci. Polymer Edn. **8**, 715 (1996)
- Lamboy, N.; Peikov, P.; Belcheva, N.; Gesheva, K.; Zlatkov, A.; Danchev, N.; Smid, J.: J. Controlled Rel. **45**, 193 (1997)
- Braud, C.; Bunel, C.; Vert, M.: Polym. Bull. **13**, 293 (1985)
- Belcheva, N.; Tsvetanov, Ch.; Panayotov, I.; Lazarova, St.: Macromol. Chem. **191**, 213 (1990)
- Wada, R.; Hyon, S.-H.; Ikada, Y.: J. Pharm. Sci. **10**, 919 (1990)
- Haackel, S.; Sachse, A.; Albayrak, C.; Muller, R.: Proc. 2nd World Meeting APGI/APV, Paris, 507 (1988)

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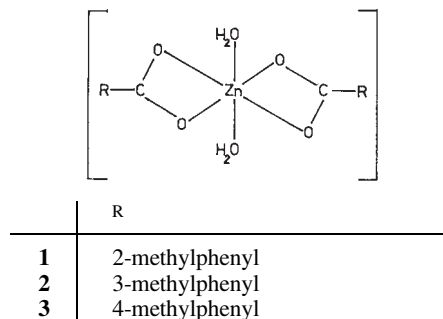
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Antiinflammatory activity of diaquabis(cresoxyacetato)zinc(II) complexes

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In numerous groups of (carboxylato)zinc(II) complexes their antiinflammatory, antiradical and antimicrobial activities have been studied [1–4]. It is known, that low molecular complexes of the [Zn(H₂O)₂(ROCH₂COO)₂] type may be beneficial in affecting the inflammation process in animal models [1, 3]. On the basis of these findings and the study of antiphlogistic activity of uncomplexed cresoxyacetic acids as well as their copper(II) salts [5], this paper is focused on the study of antiinflammatory activities of the mononuclear diaquabis(cresoxyacetato)zinc(II) complexes with the general formula [Zn(H₂O)₂(ROCH₂COO)₂], where R = 2-methylphenyl (complex **1**), 3-methylphenyl (complex **2**) and 4-methylphenyl (complex **3**), including the corresponding isomeric cresoxyacetic acids **1a–3a**.

All studied Zn(II) complexes belong to the group of mononuclear (carboxylato)zinc(II) complexes with tetragonally-distorted octahedral stereochemistry [6], as it was found for the structure of the diaquabis(phenoxyacetato)zinc(II) complex by X-ray analysis [7]. Thus, their polyhedron is formed by aryloxyacetate ion chelate coordination via two oxygen atoms of RCOO[–] anions to the Zn(II) central atom and by axial water molecule coordination.



Using a routine plethysmometric method, the evaluation of antiinflammatory activity of all compounds was carried out in rat paw carrageenan-induced edema (Table). The effects of the complexes **1–3** were compared to those of the free isomeric cresoxyacetic acids **1a–3a**. In general, all diaquabis(cresoxyacetato)zinc(II) complexes are more effective than the acids. The average antiphlogistic activities of the Zn(II) complexes expressed as a measure of edema volume reduction decreased in the following order: **1/1a** (70/26%), **2/2a** (68/50%), **3/3a** (58/48%). The results obtained indicate the involvement of Zn²⁺ ions (administered in the form of complexes) in the modulation of the animal inflammation model. Because the activity of complexes has not correlated with their metal content, it is very likely that the bioavailability of these ions is closely related to the aquation processes of [Zn(H₂O)₂(ROCH₂COO)₂] species and to the formation of low molecular complexes under *in vivo* conditions. Collectively, the results support the idea that Zn(II) complexes can be used to create the pharmacoactive forms containing Zn(II) [1, 8]. From this point of view the complexes **1** and **2** might appear as potential antiphlogistics.