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Silica-Gelatin Composite Materials for Prolonged Desorption of Bioactive Compounds

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The set of gelatin and silica-gelatin films with incorporated bioactive compounds of different chemical nature was prepared. The swelling behavior of the films and desorption of active ingredients into aqueous media were studied. For all the silica-gelatin films we observed considerable improvement of the materials water stability and retardation of the release of bioactive compounds as compared to gelatin samples. The results obtained make it possible to consider silica-gelatin composite materials as promising disintegrating dosage forms for prolonged desorption of bioactive compounds.
Keywords: composite materials, gelatin, silica, bioactive compounds, controlled release.

1. INTRODUCTION

Gelatin (Scheme 1) is a protein derived by denaturation of collagen; it consists of varying amounts of 18 amino acids, of which glycine, proline and hydroxyproline are most abundant [1]. In the sol state, the gelatin molecules are the separate, randomly oriented coils [2]. As the temperature of gelatin solution decreases below 35 °C, the hydrogen bonds that were broken during denaturation begin to rehabilitate, the original structure of collagen partially restores and a polymer network is formed. According to [2, 3], the junction zones of the network are composed of triple helices, which are stabilized by interchain hydrogen bonds of two types: either between C = O and N–H groups or via a water molecule between two C = O groups or between C = O and N–H groups.

Gelatin is known to be extensively used in pharmaceutical industry for the preparation of disintegrating dosage forms [4 – 6]. The active substances in such forms may be enclosed in water soluble gelatin capsules [4, 5] or incorporated in pores of the polymer films [6], with both gelatin capsules and films being usually obtained from gelatin gels of varied composition. Upon entry into an aqueous medium, gelatine capsule or medicine-containing film absorb water, swell and release the active ingredient; over time, they dissolve completely. When it is necessary to increase the time of dissolution of gelatin materials and decelerate the desorption of medicines, "physical" or "chemical" cross-linking agents are introduced into parent gelatin solution. These agents form hydrogen or covalent bonds with polymer molecules, thus decreasing the materials swelling [6 – 9].

Silica has recently drawn attention as a potentially interesting cross-linking agent [1, 10, 11] owing to high surface concentration of ≡SiO$^-$ and ≡SiOH groups, which can participate in electrostatic interactions with positively charged sites of gelatin molecules and in the formation of hydrogen bonds with carboxyl or amino groups of the polymer. Silica-gelatin composite materials described in [1, 10, 11] were prepared by the sol-gel method using a gelatin sol and a solution of sodium silicate. Silica particles were formed in such systems from silicate anions simultaneously with the formation of the gel network. No decrease in swelling of the composites compared with pure gelatin samples was observed [10].

In our previous study [11] we investigated the silica-gelatin composite materials based on gelatin and pyrogenic silica. It was found that adding the silica nanoparticles to gelatin sol decelerates significantly the swelling and dissolution of the resulting materials making them potentially interesting dosage form for controlled drugs release. Based on literature data [1, 2] and on the results of our rheological and thermogravimetric studies [12, 13], we suggested that the improvement of water stability of silica-gelatin films may be caused by such factors as formation of additional bonds between gelatin molecules and silica silanol groups, adsorption of gelatin on silica surface and a denser packing of the polymer in silica-gelatin suspension, screening the polar sites of gelatin molecules by silica silanol groups and decrease in the material water affinity.

Addition of active ingredients to parent gelatine sol and silica-gelatin suspension may influence the gelatin-gelatin and the silica-gelatin interactions, thus affecting the properties of composite materials. For example, in accordance with our preliminary results, high concentrations of vitamin B1 in silica-gelatin suspension influenced adsorption of gelatin on silica surface, lowered pH of the suspension and increased swelling of silica-gelatin films [13]. Contrary, according to [14], active ingredient of gatifloxacin-loaded gelatin nanoparticles acted simultaneously as cross-linking agent, providing its own sustained slow release into aqueous medium. Thus, the objective of this work was to study the properties of gelatin and silica-gelatin materials containing biologically active substances of various chemical nature and to estimate opportunities of using the silica-gelatin composites for prolonged desorption of bioactive compounds.

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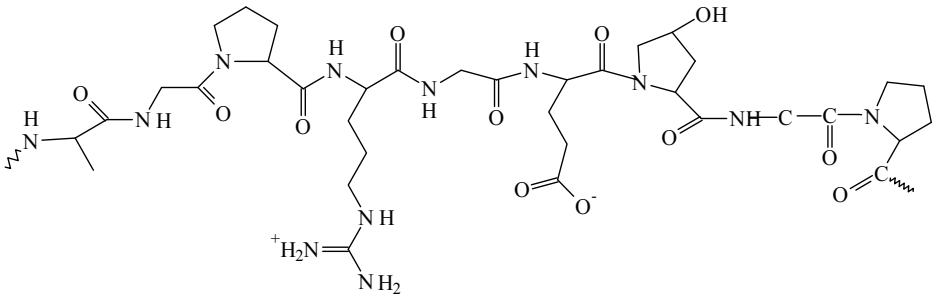

Scheme 1. A basic unit of gelatin molecule [1]

2. EXPERIMENTAL

Gelatin and silica-gelatin materials were prepared using gelatin from Fluka and highly dispersed silica of A300 trade mark with a specific surface area of 250 m^2/g. Distilled water, conventional buffer solutions (pH 1.68, 3.56, 6.86, 9.18 and 12.45), mixture of 70 % ethanol with distilled water or with pH 6.86 buffer solution were the auxiliary components used for materials preparation. Commercially available ("Merck") vitamins B1 (thiamine hydrochloride), B6 (pyridoxine hydrochloride) and PP (nicotinamide), synthetic antioxidant flavonol (3-hydroxyflavone, FL), complex of flavonol with zinc ([FL-Zn]) and extracts of leaves of *Magnolia sieboldy* (MS) and *Magnolia kobus* (MK) in 70 % ethanol were used as bioactive ingredients. Flavonol was synthesized as described earlier [15], its complex with Zn was prepared by mixing of 1.0 mM solutions of flavonol and zinc acetate ($Zn(CH_3COO)_2$, Sigma-Aldrich Corp.) in 1 : 1 ratio. Extracts of *Magnolia sieboldy* (MS) and *Magnolia kobus* (MK) were obtained from dried magnolias leaves by conventional procedure [16]. According to our previous data [16], the extracts contained such bioactive compounds as rutin, quercetin, quercetrin, caffeic acid and other with equivalent antioxidant concentration of 3.7 mM and 3.0 mM of ascorbic acid, respectively.

Gelatin and silica-gelatin materials were prepared in the form of the films with thickness of about 0.1 mm; such thin films were used, for example, in dentistry as a biodegradable device for controlled release of antioxidant meloxicam into periodontal pocket [6]. To produce the films, 2 ml of film forming gelatin solution (FFGS) or silica-gelatin suspension (FFSGS) was poured on a polymer substrate as a thin layer and dried at room temperature for several days. The film forming solutions / suspensions were prepared as follows.

Step 1. Preparation of gelatin solution. 500 mg of gelatine were placed in glass, 5 ml of distilled water/ /conventional buffer solution/ethanol-water or ethanol-buffer mixture were added and the mixture was heated in a water bath with stirring for 20 min to dissolve gelatin.

Step 2. Preparation of silica suspension. 200 mg of silica were admixed with 5 ml of distilled water/conventional buffer solution/ ethanol-water or ethanol-buffer mixture or with 5 ml of active substance solution in distilled water/ conventional buffer solution/ethanol-water or ethanol-buffer mixture. Then the resulting suspension was stirred using a magnetic stirrer for 20 min.

Step 3. Preparation of FFSGS for silica-gelatin films production. 5 ml of gelatin solution and 5 ml of silica suspension, both prepared as mentioned above, were admixed with each other. The resulting suspension was stirred for 5 min.

Step 4. Preparation of FFGS for gelatin films production. 5 ml of gelatin solution was admixed with 5 ml of water/conventional buffer solution/ethanol-water or ethanol-buffer mixture or with 5 ml of active substance solution in distilled water/ buffer solution/ethanol-water or ethanol-buffer mixture. Again, the mixture was additionally stirred for 5 min.

Concentration of gelatine in all FFGS / FFSGS was 50 mg/ml, silica to gelatin ratio in silica-gelatin suspensions was equal to 0.4.

To explore the role of pH, the conventional buffer solutions, pH 1.68, 3.56, 6.86, 9.18, 12.45, were used for the sample preparation. The film forming solutions/ /suspensions of different pH value were also prepared using distilled water, with pH of FFGS/FFSGS being adjusted by adding HCL or NaOH solutions.

Vitamins B1, B6, PP were introduced into FFGS/ /FFSGS as a solution in distilled water or in pH 6.86 buffer, while flavonol, flavonol-zinc complex and extracts of magnolias leaves were initially dissolved in 70 % ethanol and then admixed with water or pH 6.86 buffer in 2 : 8 ratio. The quantity of bioactive substances in resulting films was (2 – 10) mkmole per 1 g of gelatin.

To study swelling of the films in aqueous medium, the films were weighed, placed into water, and, in certain time intervals, removed from it to measure weight increase. The data on materials swelling are given as a quantity of absorbed water $M(H_2O)$ per gram of gelatin M(Gel) in a dry film. For $M(H_2O)/M(Gel)$ parameter, standard error of the mean was 3 % – 4 % of its value; scattering of the data corresponding to 90 % confidence interval did not exceed 10 %.

In order to explore desorption of bioactive compounds, the weighed films were placed into the glasses, added with fixed volume of distilled water and shaken up for 0.05 h – 5 h at room temperature. The amounts of thiamine, pyridoxine, nicotinamide, flavonol or components of magnolia leaves extracts, which were released from films into water, was calculated from the changes in the UV absorption spectra of appropriate solutions at 267, 324, 313, 343 and 330 nm, respectively. The data are presented as a time-dependent ratio of desorbed part of active substance M_{des} to its overall amount M in the film.

3. RESULTS AND DISCUSSION

Preparation of gelatin materials with incorporated bioactive substances implies inclusion of some supplementary elements into film forming solutions/ /suspensions. This may be bioactive compounds themselves or some auxiliary components, for example, solvents. In turn, these ingredients may influence the gelatin-gelatin and silica-gelatin interaction and effect the composite properties.

Addition of bioactive compounds to film forming solutions/suspensions can, in particular, change pH of FFGS / FFSGS. To explore potential effect of pH of solutions/suspensions on the materials properties, gelatin and silica-gelatin films were prepared from FFGS / FFSGS with varied pH value.

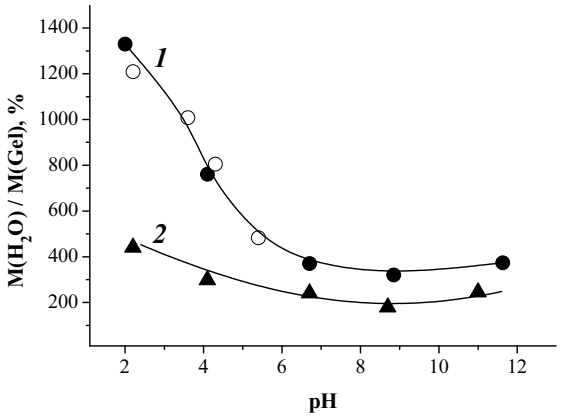

Fig. 1. Effect of pH of FFGS / FFSGS on swelling of gelatin (1) and silica-gelatin (2) films. Swelling time is 1 h

Curves 1 and 2 on Fig. 1, solid symbols, give swelling of gelatin and silica-gelatin films prepared by using standard buffer solutions. The data obtained show the strong influence of pH of FFGS on the swelling of corresponding gelatin films. The effect was especially dramatic over pH range <4; the films from gelatin solutions with pH ~ 2.0 were found to be dissolved completely as early as in 1.5 h – 2 h after immersion them into water. The similar results were obtained when FFGS were prepared by using distilled water and pH value of FFGS were adjusted by adding small amounts of HCl or NaOH solutions (curve 1, open circles). The strong dependence of the swelling of gelatin films on the pH of FFGS makes one to assume the important contribution of electrostatic interactions to gelation process. Lowering the pH of gelatin solution appears to decrease the fraction of dissociated carboxyl COO^- groups (pK ~ 4, [17]) which can attract histidine NH_2^+ groups (pK ~ 12.5, [17]), thus weakening the interaction of gelatin molecules.

Presence of silica reduces the effect of pH on the films swelling (Fig. 1, curve *2*) that appears to be due to doping the suspension with additional negatively charged $\equiv SiO^-$ groups. Silica can also adsorb the cations, which originate in buffer solutions and can prevent interaction of positively and negatively charged groups of gelatin molecules, or silica can influence the gel properties by means of polymer adsorption. Whatever mechanism is responsible for this effect, it should be noted that silica addition decreases significantly the swelling of the materials prepared in wide range of pH value.

Using of solvent like ethanol for films preparation may be necessary to introduce water insoluble bioactive

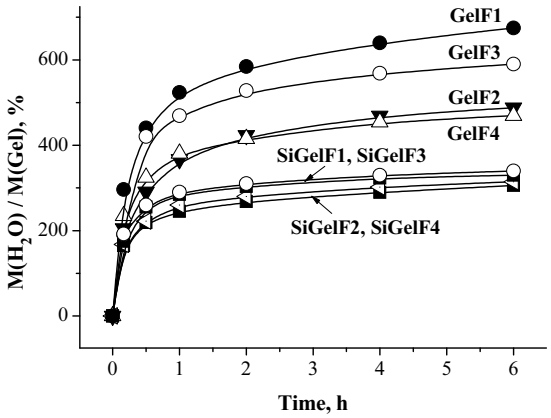

Fig. 2. Effect of addition of ethanol to FFGS/FFSGS on swelling of gelatin and silica-gelatin films

Table 1. Designation of gelatin and silica-gelatin films and composition of FFGS / FFSGS used for preparation of the films with incorporated bioactive compounds

Film type	Name of gelatin / silica-gelatin film	Composition and pH of film forming gelatin solution/ silica-gelatin suspension		
		Active ingredient	Other components	pH
F1	GelF1 / SiGelF1	–	H_2O	5.1 / 5.1
	GelF1-B1 / SiGelF1-B1	Vitamin B1	H_2O	4.7 / 4.9
	GelF1-B6 / SiGelF1-B6	Vitamin B6	H_2O	4.9/ 5.0
F2	GelF2 / SiGelF2	–	pH 6.86 buffer	6.9 / 6.8
	GelF2-B1 / SiGelF2-B1	Vitamin B1	pH 6.86 buffer	6.9 / 6.9
	GelF2-PP / SiGelF2-PP	Vitamin PP	pH 6.86 buffer	6.9 / 6.9
F3	GelF3 / SiGelF3	–	70 % ethanol : H_2O = 2 : 8	5.0 / 5.1
	GelF3-M1 / SiGelF3-MS	Extract of *Magnolia sieboldy*	70 % ethanol : H_2O = 2 : 8	5.0 / 5.0
	GelF3-M2 / SiGelF3-MK	Extract of *Magnolia kobus*	70 % ethanol : H_2O = 2 : 8	5.0 / 5.0
F4	GelF4 / SiGelF4	-	70 % ethanol : pH 6.86 buffer = 2 : 8	6.9 / 6.9
	GelF4-FL / SiGelF4-FL	Flavonol	70 % ethanol : pH 6.86 buffer = 2 : 8	6.9 / 6.9
	GelF4-[FL-Zn] / SiGelF4-[FL-Zn]	Fl-Zn complex	70 % ethanol : pH 6.86 buffer = 2 : 8	6.9 / 6.9

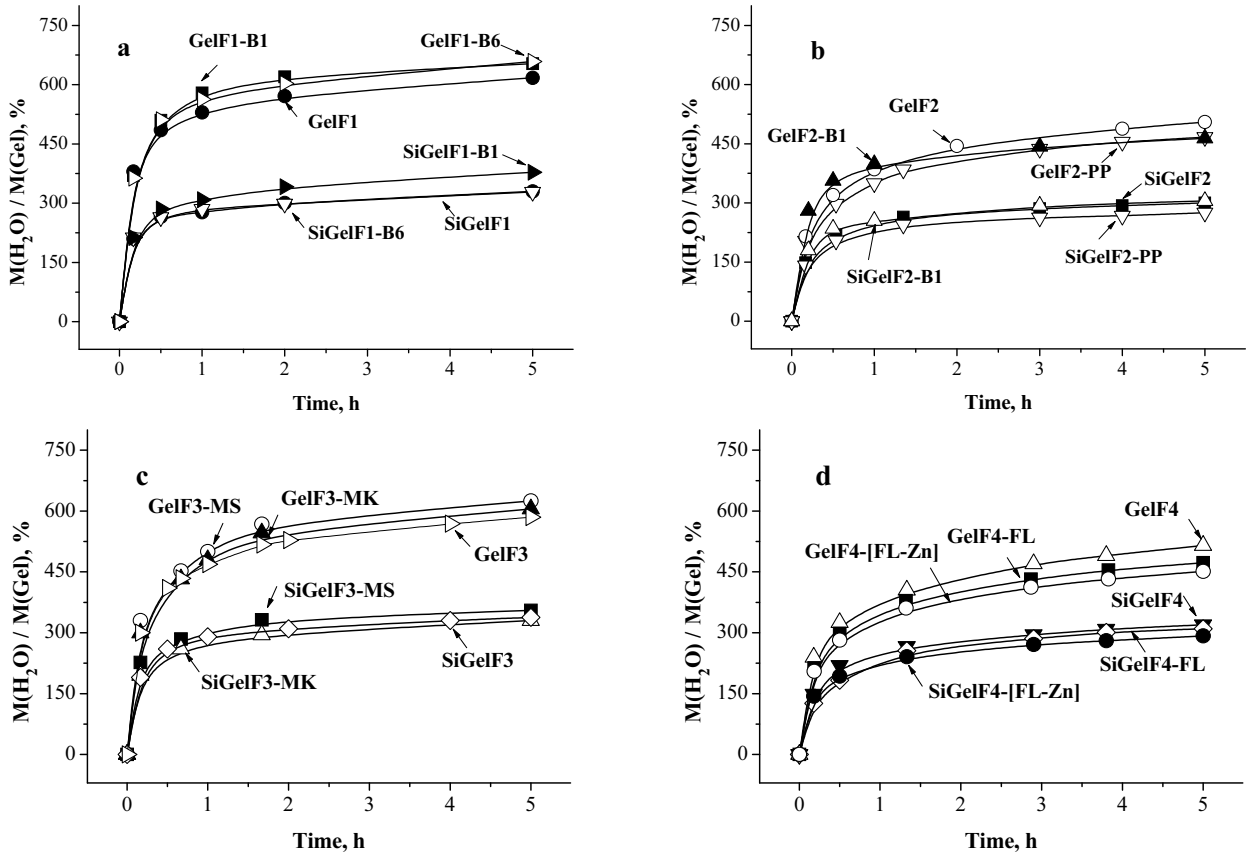

Fig. 3. Swelling of F1 – F4 (a – d) gelatin and silica-gelatin films with incorporated bioactive compounds. Designation of the samples as in Table 1

compounds into gelatin materials. To explore the effect of ethanol addition on the properties of gelatin materials, we compared the swelling of F1 – F4 films prepared from four types of film forming solution / suspension (Table 1). The control films of F1 and F2 types were prepared using distilled water and pH 6.86 buffer as a solvent while F3 and F4 films were obtained on the basis of water-ethanol and buffer-ethanol mixtures.

Fig. 2 illustrates effect of ethanol addition on the swelling of gelatin and silica-gelatin films. Comparing the swelling of GelF1 and GelF3 films, one can conclude that presence of ethanol give rise to some decrease in water absorption for gelatin film from water-based FFGS (by ~ 11 %). One can suggest that ethanol can reinforce electrostatic interactions of gelatin molecules in this solution by means of changing conformation of the polymer and lowering the dielectric permeability of the medium [18]. In buffer-based gelatin solution and in silica-gelatin suspensions electrostatic gelatin-gelatin and silica-gelatin interactions are already realized owing to higher pH value of FFGS (see Fig. 1) and due to presence of $\equiv SiO^-$ groups, thus effect of ethanol addition proves to be comparatively slight. The data of Fig. 2 also show the improvement of water stability for gelatin and silica-gelatin samples prepared from buffer-based FFGS / FFSGS and confirm the deceleration of the materials swelling for silica-gelatin films as compared to gelatin ones.

As it was mentioned above, the most stable gelatin and silica-gelatin films were obtained from FFGS / FFSGS with pH of about 6 – 8. Based on this result, we prepared the films with incorporated bioactive compounds using pH 6.86 buffer (films of F2 type, Table 1) and the buffer-ethanol mixture (films of F4 type). Several films were also obtained using solutions of bioactive compounds in distilled water (F1 films) and in ethanol-water mixture (F3 films). The designation of the films and resulting composition and pH values of film forming solutions and suspensions are given in Table 1.

Fig. 3 gives the water absorption data for gelatin / silica-gelatin materials with incorporated bioactive compounds and for control films without active ingredients. As one can conclude from the Fig. 3, there are no significant distinctions in swelling behavior of the films differing from each other only by availability of bioactive substance. It only worth to note some increase in swelling of SiGelF1-B1 film as compared with SiGelF1 and SiGelF1-B6 ones (by ~ 15 %, Fig. 3, a); as it was mentioned above, this may be caused by effect of vitamin B1 on silica-gelatin interaction [13]. Generally, presence of silica and pH of film forming solutions / suspensions (see Table 1) remain the major factors affecting the films swelling.

The penetration of water into gelatin films and the diffusion of the film constituents into water are known to be the two sides of the same process of dissolution of gelatin materials occurring upon their contact with aqueous medium [19]. Therefore, the deceleration of materials swelling observed for silica-gelatin composites should also lead to a slower release of incorporated bioactive compounds. Indeed, as Fig. 4, a – c, show, desorption of vitamins B1, B6, PP and extracts of magnolia leaves from silica-gelatin materials occurs much more slowly than from

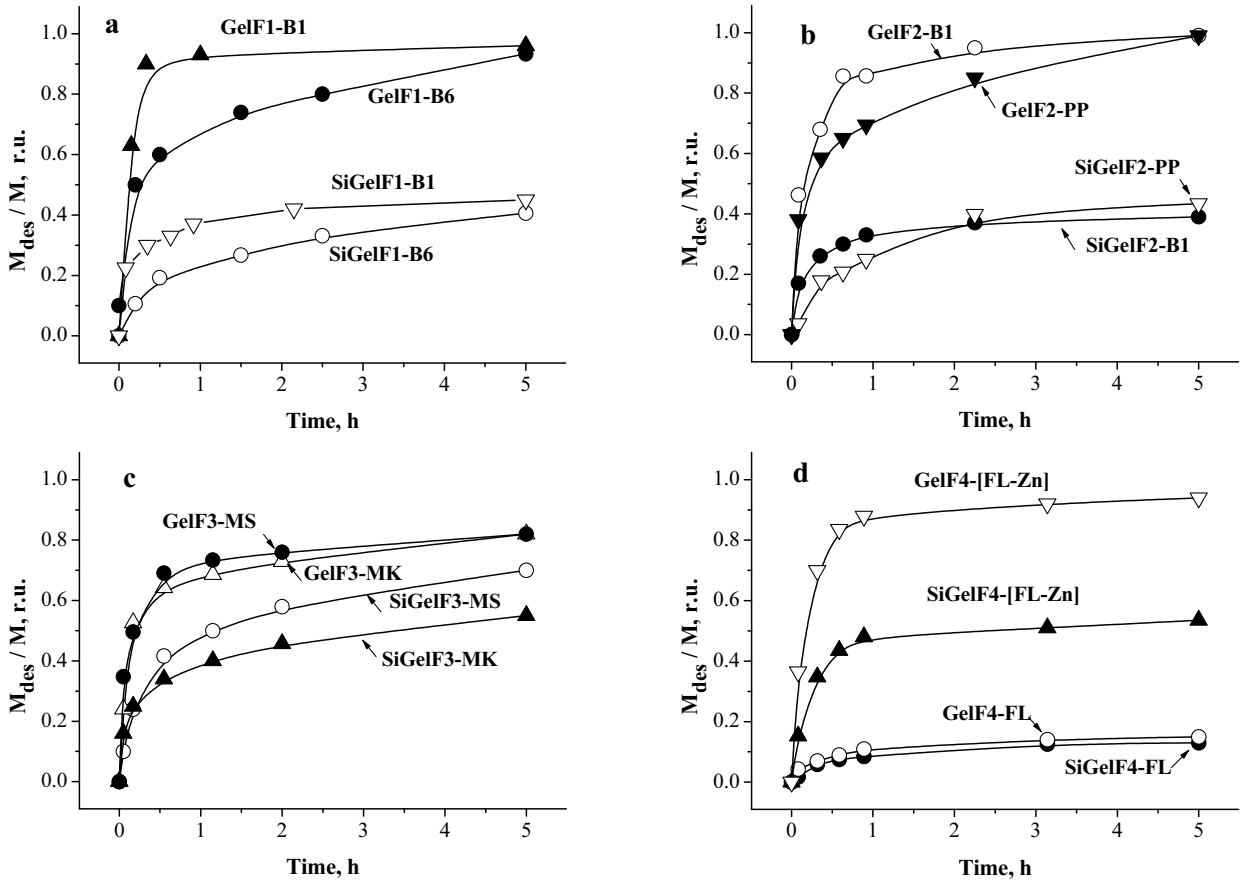

Fig. 4. Desorption of vitamins B1 and B6 (a), vitamins B1 and PP (b), extracts of *Magnolia sieboldy* and *Magnolia kobus* (c), flavonol and FL-Zn complex (d) from gelatin and silica-gelatin films of F1 – F4 types (a – d, respectively)

gelatin ones. Desorption of vitamins B1 from water-based GelF1-B1 and SiGelF1-B1 films (Fig. 4, a) was slightly faster than from buffer-based GelF2-B1 and SiGelF2-B1 films (Fig. 4, b), which also correlated with the swelling behavior of the films.

In contrast to the films swelling behavior, the release of bioactive compounds was also affected by the nature of active ingredient. For every pair of compounds or extracts incorporated in the films of F1-F3 type, noticeable distinctions in their release profile were stated. For example, we observed faster desorption of vitamin B1 as compared with vitamins B6 and PP from both gelatin and silica-gelatin F1-F2 films (Fig. 4, a, b). Release of extract of *Magnolia sieboldy* from SiGelF3 film distinguished from desorption of extract of *Magnolia kobus* (Fig. 4, c). These observations are in agreement with literature data [20 – 22], revealing the dependence of drugs release on such factors as molecular weight and water solubility of the compounds, interaction of the substances with polymer matrix et cetera.

The rate of desorption of flavonol from both gelatin and silica-gelatin films was very low (Fig. 4, d) and was controlled, apparently, by poor water solubility of this compound rather than by the properties of gelatin and silica-gelatin materials. To improve flavonol solubility.and to increase the amount of the antioxidant which can be released from the films into water, we introduced flavonol into FFGS / FFSGS in the form of FL-Zn complex. Zinc was chosen for complex preparation as it is known to be usually included in many vitamins and cosmetic compositions. Fig. 4, d, show that desorption of flavonol in the form of FL-Zn complex increases from both gelatin and silica-gelatin films. As in the case of other active ingredients, the silica-gelatin composite provides a slower release of FL-Zn complex as compared to gelatin film.

4. CONCLUSIONS

Using film forming gelatin solutions and silica-gelatin suspensions of varied composition gelatin and silica-gelatin thin film with/without bioactive ingredients were prepared, swelling behaviour of the films and desorption of bioactive substances into aqueous medium were studied.

It was found that the properties of gelatin and silica-gelatin films are affected by pH of film forming solutions/suspensions, with the most stable film being obtained from solutions / suspensions with pH 6 – 8. Presence of silica reduced the dependence of films properties on the solution pH value, increasing the stability of the films prepared in wide range of pH. The additional introduction into solutions/suspensions of another solvent (ethanol) or bioactive compounds of various chemical nature (vitamins B1, B6, PP, flavonol, Fl-Zn complex, extracts of *Magnolia sieboldy* and *Magnolia kobus*) caused no significant effect on the films swelling.

The data on the swelling of the films with incorporated bioactive compounds correlated with the data on the rate of the release of active ingredients into water. For all the compounds investigated, the appropriate silica-gelatin films differed from gelatin ones by lower swelling

in aqueous medium and by slower desorption of bioactive ingredients. It was found that desorption of active ingredient from gelatin and silica-gelatin films is also affected by chemical nature of the compounds and, to a lower extent, by type of film-forming gelatin solution or silica-gelatin suspension. With all this going on, it was the presence of silica that played dominant role in improvement of the materials stability and in retardation of the release of active ingredient. Thus, the results obtained make it possible to consider silica-gelatin composite materials as promising disintegrating dosage forms for prolonged desorption of bioactive compounds.

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