RESEARCH ARTICLE

Sugar-Phosphate Composite Glasses for Lysozyme: An Effective Technique for Lysozyme Preservation

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ABSTRACT

The Spray drying method was employed for the processing and storage preservation of lysozyme using sugar-phosphate glasses. While previous studies have reported the surfactants to reduce protein adsorption at the air/water interface, this study demonstrates that water-soluble inorganic phosphates effectively minimize lysozyme surface adsorption. Sugar glasses containing sucrose or mannitol, combined with water-soluble phosphates and glass formers such as PVP K30 or calcium lactate were prepared using hot plate drying. These sugar glasses were evaluated to confirm their glassy state using optical microscopy, infrared spectral analysis, X-ray diffraction (XRD), and thermal behavior studies (DSC).

The lysozyme mixtures with sugar glasses were processed using both spray drying and lyophilization. They were then analyzed for pH, moisture content, % yield, and % lysozyme activity. Physical changes in lysozyme were assessed using XRD. The batch exhibiting the highest lysozyme activity was selected for stability testing in accordance with ICH guidelines.

Results indicated that water-soluble phosphates, in combination with glass formers like PVP K 30 and calcium lactate, efficiently formed composite glasses during spray drying, whereas water-insoluble phosphates failed to produce a stable glassy matrix. XRD analysis of sodium phosphate monobasic and dibasic in the presence of glass formers confirmed an amorphous state during post-stability studies, with maximum lysozyme activity retained at room temperature.

The processing and storage stability of lysozyme was superior in spray-dried products compared to lyophilized ones. These findings validate the potential of sugar-phosphate composite glasses as an effective technique for lysozyme preservation, offering a promising approach to enhancing protein stability in pharmaceutical formulations.

Keywords: lysozyme, sugar phosphate glasses

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INTRODUCTION

Amorphous phases are essential in pharmaceutical solid dosage forms, affecting manufacturability, stability, shelf life, and drug delivery[1]. The amorphous state can enhance bioavailability due to its random molecular arrangement and lack of long-range order[2]. Common methods for producing amorphous phases include milling, rapid precipitation, and processes like lyophilization or spray drying, where increasing viscosity prevents crystallization. However, amorphous solids are thermodynamically unstable and tend to recrystallize over time, especially with exposure to moisture and heat, which increase molecular mobility and instability. The glass transition temperature (Tg) is a key parameter used to assess the stability of amorphous materials[3-5].

Proteins and polypeptides are typically prepared as dry powders using processes such as freeze-drying and spray-drying. These methods help mitigate the chemical degradation and physical instability of active molecules in solution while also addressing the challenges of crystallization. However, crystallizing biopharmaceuticals does not always guarantee long-term stability. For example, insulin is stable in its amorphous form than in its crystalline state during storage[5,6].

Excipients must remain in the glassy state, ensuring uniform entrapment of protein molecules within the matrix to ensure its stability. Maximizing the formulation's glass transition temperature (Tg) and preserving the protein's native structure post-processing are crucial steps for long-term stability. This is because molecular mobility persists even in the glassy state, potentially leading to chemical reactivity, crystallization, and structural collapse over time and with temperature changes[7,8].

Lysozyme is a naturally occurring enzyme with potent antimicrobial properties, primarily known for its ability to hydrolyze the peptidoglycan layer of bacterial cell walls. It has diverse applications in pharmaceuticals, food preservation, and biotechnology, serving as a preservative, therapeutic agent, and model protein for studying protein stability and folding. One common approach for preserving lysozyme is encapsulation within bio-erodible polymer matrices; however, this method often leads to enzyme inactivation[9-11]. The stabilization of lysozyme through sugar-phosphate glass complexation offers a promising

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Table 1: Morphology of sugars: Effect of glass former and Sodium phosphate

Calcium lactate	PVP K-30	Sodium Phosphate (monobasic)	Sucrose (5 %w/v)	Mannitol (5 %w/v)
1% w/v	-	-	Crystalline	Crystalline
3% w/v	-	-	Amorphous	Crystalline
5% w/v	-	-	Amorphous	Partly crystalline
-	1% w/v	-	Amorphous	Crystalline
-	3% w/v	-	Amorphous	Crystalline
-	5% w/v	-	Amorphous	Partly Amorphous
-	1% w/v	1% w/v	Amorphous	Crystalline
-	1% w/v	3% w/v	Amorphous	Crystalline
-	1% w/v	5% w/v	Amorphous	Crystalline
1% w/v	-	1% w/v	Partly crystalline	Crystalline
1% w/v	-	3% w/v	Amorphous	Crystalline
1% w/v	-	5% w/v	Amorphous	Crystalline

Table 2: Effect of additives on glass transition temperature (Tg) of Sucrose

S.No.	Mixture composition	Tg (^{0} c)
a	Sucrose 5% w/v	58
b	Sucrose + Sodium phosphate 5% w/v	75
c	Sucrose + Calcium lactate1 % w/v + Sodium phosphate 5% w/v	88
d	Sucrose + PVP K-30-1% w/v + sodium phosphate 5% w/v	112

solution to overcome temperature sensitivity. This approach enables the preservation of lysozyme's antimicrobial properties, rendering it a viable alternative to conventional antibiotics. The sugar-phosphate glass matrix provides a protective environment, shielding the enzyme from denaturation and degradation. This innovative strategy has potential applications in biomedical research and antimicrobial therapy development.

MATERIALS AND METHODS

Lysozyme (3X crystal) from egg white (Muramidase), HSN 35079099, was procured from Sisco Research Laboratories Pvt. Ltd. *Micrococcus lysodeikticus* (ATCC No. 4698) was obtained from Sigma-Aldrich. Sodium phosphate (monobasic and dibasic), disodium tetra borate, and calcium lactate were sourced from Merck Ltd. Ammonium phosphate was purchased from Loba Chemie Ltd. Mannitol and sucrose were obtained from M.B. Sugar and Pharmaceutical Ltd. Polyvinylpyrrolidone (PVK K-30) was supplied by ISP Ltd. Deionized and double-distilled water was used throughout the experiment.

Formulation of Hot-Plate Dried Sugar-Phosphate Glasses

A Multi-component mixture of solution (5% w/v) of sucrose or mannitol containing calcium lactate or polyvinylpyrollidone (PVP K-30) and sodium phosphate monobasic (1%, 3% and 5%w/v) were prepared separately. Aliquots of 100 µl of solution were dried on a glass slide at 40˚C using hot plate. The hot-plate dried mixtures were stored at 2-8˚C. The physical properties of dried mixtures were evaluated for optical microscopy, infrared spectral analysis, X ray diffraction pattern and thermal behavior[12-15].

Spray Drying of Lysozyme

A Multi-component aqueous solutions were prepared as per Table 1. 1mg/mL Lysozyme was first dissolved in double-distilled water, followed by the addition of the glass former, water-soluble and water-insoluble phosphates, and sucrose. The solutions were spray-dried under identical conditions. The feed solution was delivered through a 0.5 mm atomizing nozzle into the drying chamber via a peristaltic pump at a flow rate of 4–5 mL/min. Drying was carried out at an inlet temperature of 120 ± 2°C and an outlet temperature of 60 ± 3°C. The spray-dried powder was stored in vials at 4°C. The spray-dried mixtures were then evaluated for XRD and moisture content, pH and enzyme activity.

Freeze-Drying of Lysozyme

The Multi-component mixtures of sugar, phosphate along with glass formers were lyophilized at condenser temperature -50°C and product temperature -30°C, for 72 hours with pressure 0.06hPa. The lyophilized mixtures were evaluated for enzyme activity, moisture content, pH, XRD and stability studies[16-19].

Estimation of Lysozyme Activity

A *M. lysodeikticus* bacterial suspension and lysozymes (15µg/ml) were prepared by in phosphate buffer (0.067 M, pH 6.6). 0.5 ml of lysozyme solution was added to 2.5 ml of bacterial suspension. The absorbance was recorded at 450 nm. The activities of all sample were measured relative to that of a corresponding fresh sample. Each sample was assayed in triplicate[20,21].

$$\text{Activity (units /mg)} = \Delta\, 450\, \frac{\text{nm}}{\text{mm}} \,/\, 0.001 \text{ mg enzyme}$$

Stability Analysis of Optimized Lysozyme Mixtures

The spray dried mixture compositions of lysozyme with optimum activity were subjected to short term stability study as per ICH stability testing guidelines for biologicals. Four vials of each product containing 5gram of mixture were stored each separately at 25±2°C and 40±2°C / 75±5%RH respectively for two months. The mixtures were analyzed periodically for moisture content, pH and % enzyme activity. The XRPD analysis of stability batches was also performed at the end stability period[21,22]

RESULTS AND DISCUSSION

Formulation of Hot-Plate Dried Sugar-Phosphate Glasses

Table 3: The effect of inorganic phosphates with sucrose on preservation of lysozyme

Batch code	Composition	pH	Moisture content %	% Activity	% Yield
Water soluble phosphates					
Sodium Phosphate Monobasic (SPM)					
SPM-3	5 % S + 3 % SPM + L	4.24	4.82	70.32 ± 0.02	62
SPM-5	5 % S + 5 % SPM + L	4.13	3.26	81.61± 0.01	74
SPM-10	5 % S + 10 % SPM + L	3.79	4.23	60.40± 0.04	79
SPM-15	5 % S + 15% SPM + L	3.88	5.64	60.57± 0.03	82
Sodium Phosphate Dibasic (SPD)					
SPD-3	5 % S + 3 % SPD + L	8.44	5.54	54.30± 0.03	64
SPD-5	5 % S + 5 % SPD + L	8.42	2.88	83.81± 0.02	73
SPD-10	5 % S + 10 % SPD + L	8.41	3.72	47.48± 0.02	78
SPD-15	5 % S + 15 % SPD + L	8.40	4.57	41.36± 0.04	86
Potassium Phosphate Monobasic (PPM)					
PPM-3	5 % S + 3 % PPM + L	4.61	5.64	71.03± 0.03	66
PPM-5	5 % S + 5 % PPM + L	4.40	2.88	80.23± 0.01	71
PPM-10	5 % S + 10 % PPM + L	4.31	3.59	60.69± 0.03	76
PPM-15	5 % S + 15 % PPM + L	4.20	4.82	68.20± 0.03	81
Potassium Phosphate Dibasic (PPD)					
PPD-3	5 % S + 10 % PPD + L	9.19	4.29	40± 0.04	54
PPD-5	5 % S + 10 % PPD + L	9.18	2.92	48± 0.06	64
PPD-10	5 % S + 10 % PPD + L	9.21	6.10	23± 0.08	68
PPD-15	5 % S + 10 % PPD + L	9.20	5.23	20± 0.08	70
Ammonium Phosphate (AP)					
AP-3	5 % S + 3 % AP + L	4.25	--	--	--
AP-5	5 % S + 5 % AP + L	4.14	2.00	69.89± 0.02	78
AP-10	5 % S + 10 % AP + L	3.95	2.83	77.39± 0.03	80
AP-15	5 % S + 15 % AP + L	3.87	3.28	61.66± 0.02	84
Water insoluble phosphate					
Calcium Phosphate (CP)					
CP -3	5 % S + 3 % CP + L	2.4	5.68	32.41± 0.01	70
CP -5	5 % S + 5 % CP + L	2.6	3.40	37.94± 0.02	75
CP -10	5 % S + 10 % CP + L	2.3	3.27	30.43± 0.03	84
CP -15	5 % S + 15 % CP + L	2.4	2.51	21.47± 0.04	88
Tricalcium Phosphate (TCP)					
TCP -3	5 % S + 3 % TCP + L	6.2	5.28	36.73± 0.01	68
TCP -5	5 % S + 5 % TCP + L	6.6	4.49	35.28± 0.01	72
TCP -10	5 % S + 10 % TCP + L	6.4	3.51	28.96± 0.01	82
TCP -15	5 % S + 15 % TCP + L	6.2	2.50	25.63± 0.01	88
Where, S- sucrose, L- Lysozyme					

The effect of glass formers, calcium lactate and PVP K-30, alone and in combinations with sodium phosphate, on microscopic characteristics of mannitol and sucrose is shown in Table 1.

The IR analysis was used to analyze the interaction between sugar and phosphates in dried state. The figure 1 shows the IR graphs for sugar mixtures. The characteristics –OH stretching of sucrose and mannitol are at 3400 and 3325cm $^{-1}$.

The XRD pattern of mannitol and sodium phosphate mixtures shows crystalline behavior. The characteristic crystalline peaks were observed at 2Θ of 18 and 24 as shown in figure 2. The XRD pattern revealed that phosphates alone or mannitol with phosphate, along with glass former failed to inhibit the crystallization of mannitol. But, calcium lactate or PVP K-30 has effectively inhibited the crystallization of sucrose. Simultaneous effect of calcium lactate or PVP K-30 and sodium phosphate show crystalline nature of mannitol. A dried matrix of sucrose containing calcium lactate or PVP K-30 and sodium phosphate reveal amorphous nature. Thus the physical nature of mannitol-additive mixtures is crystalline. Calcium lactate (1%w/v) or PVP K-30 (1%w/v) and sodium phosphate (5%w/v) concentration has produced amorphous sucrose matrices.

The effect of additives on thermal properties of mannitol and sucrose is shown in Figure 3 and 4. The changes in glass transition temperature of sucrose due to sodium phosphate and in presence of mixture of PVP K-30-sodium phosphate or calcium lactate–sodium phosphate is shown in Table 2. The DSC thermogram of sucrose shows additive dependent effect on Tg of sugar. The presence of sodium phosphate increases the Tg of sucrose.

We found that glass formers alone, or in combination with sodium phosphate, failed to prevent mannitol crystallization. However, sodium phosphate facilitated

glass formation by acting as a crystallization inhibitor. IR analysis showed that the –OH stretching of mannitol remained unaltered, indicating that additives such as calcium lactate, PVP K-30, and sodium phosphate failed to form an effective network to inhibit its crystallization at higher temperatures. However, the –OH stretching of sucrose was significantly reduced (-50 to -140), suggesting strong molecular interactions. The analysis also revealed that the effect of sodium phosphate on the physical state of sucrose is concentration-dependent, with the initially amorphous sucrose matrix gradually transitioning to a crystalline state as phosphate concentration increases.

The DSC of a mixture of mannitol with calcium lactate or PVP K-30 and sodium phosphate show exothermic transition at about 70ºC. While Tg of sucrose was increases with presence of additives (112ºC). Thermal analysis showed that the additive effect of phosphate, PVP K-30, or calcium lactate enhances interactions with sucrose, helping to increase Tg of complex above to room temperature.

Spray Drying of Lysozyme

Table 3 summarizes the pH, % moisture content, % enzyme activity and % yield of spray dried lysozyme mixtures. The % lysozyme activity along with inorganic phosphates ranged from 0 to 83%, while the yield was from 54 to 88%. The Moisture content ranged from 2 to 6 %. The analysis for AP sample was not continued since it formed sticky mass.

The spray-dried products containing ammonium phosphate with sucrose, PVP K-30 and calcium lactate, each separately show crystalline peaks characteristics of sucrose (at ˚2θ of 18 to 24) as shown in figure 6.

Table 4 illustrates the impact of PVP K-30 and calcium lactate as glass formers, along with inorganic phosphates, on lysozyme preservation during spray drying. The

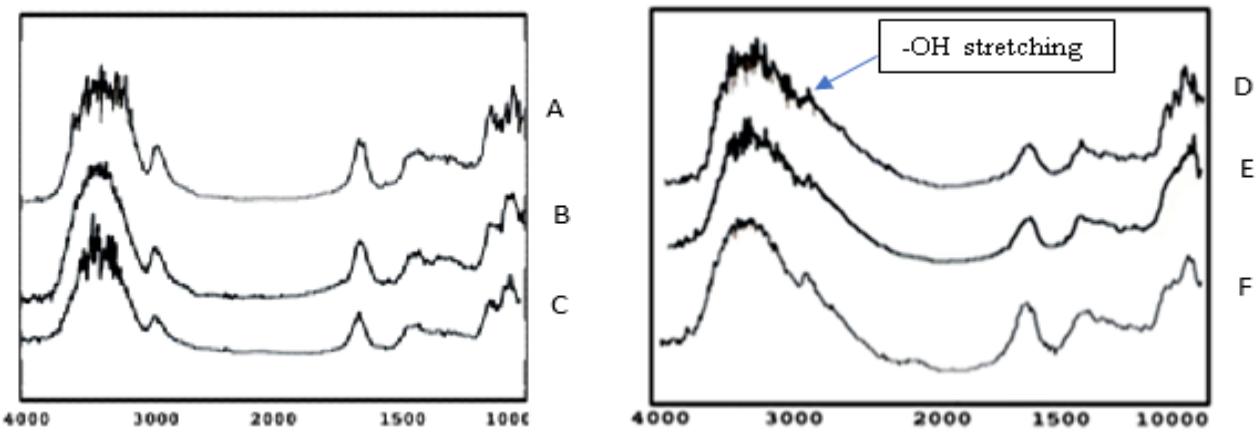

Figure 1: IR of sample A- 5% sucrose, B-5% sucrose+ 1% Calcium lactate, C- 5% sucrose+ 1% PVPK30, D- 5% sucrose+ 5% Sodium phosphate, E- 5% sucrose+1% PVPK30++ 5% Sodium phosphate, F- 5% sucrose+1% Calcium lactate+5% Sodium phosphate

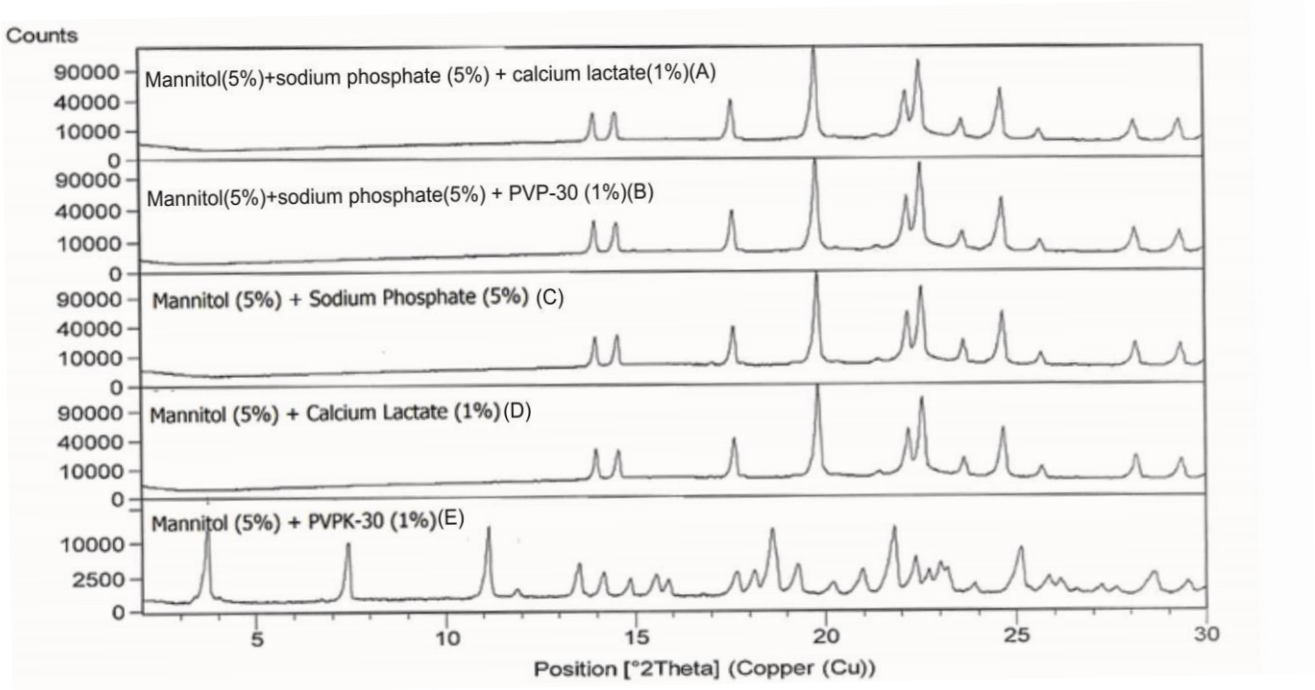

Figure 2: X ray diffraction pattern of mannitol a- 5% w/v Mannitol + 1% w/v calcium lactate + Sodium phosphate 5% w/v, b-5% w/v Mannitol + Sodium phosphate 5% w/v + 1% w/v PVP K-30, c- 5% w/v Mannitol + 5% w/v Sodium Phosphate, D- Sodium phosphate 5% w/v + 1% w/v Calcium lactate 1% w/v, E - 5% w/v Mannitol +1% w/v PVP K-30

presence of glass-forming agents improved the processing preservation efficiency of sucrose-phosphate mixtures, maintaining lysozyme activity above 95% as depicted in Table 4.

The figure. 5 confirmed that a 5% w/v phosphate concentration is most suitable for optimal % lysozyme activity. The highest lysozyme activity with phosphates alone was 81.61% in batch SPM-5, whereas batch SPMP

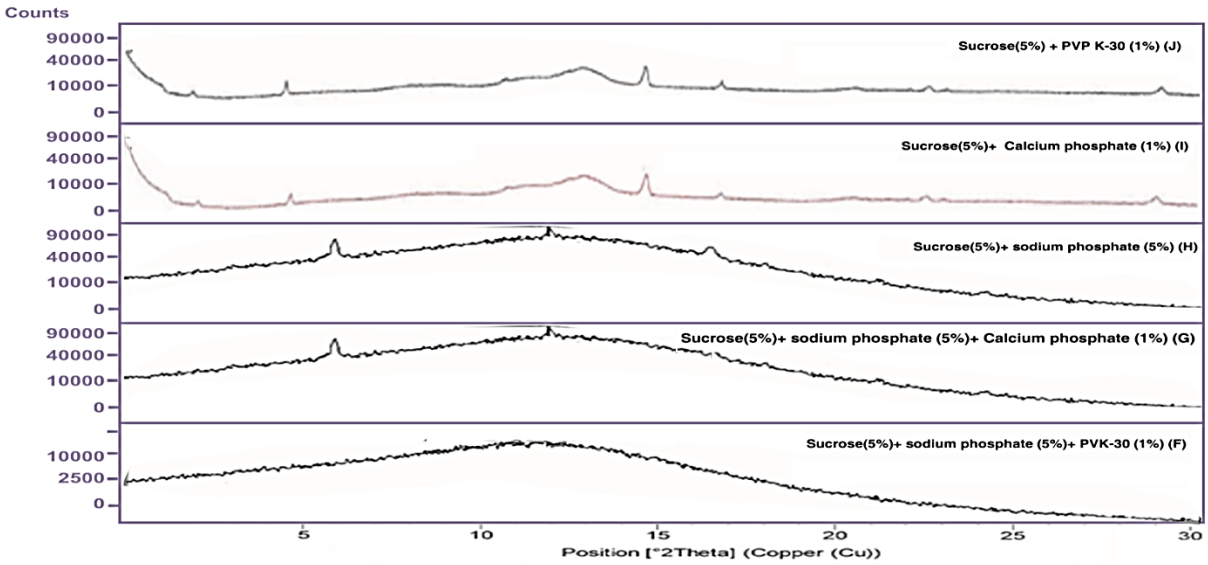

Figure 3: X ray diffraction pattern of Sucrose : F- 5% w/v Sucrose + Sodium phosphate 5% w/v + PVP K-30 1% w/v, G-5% w/v Sucrose + Sodium phosphate 5% w/v + 1% w/v calcium lactate, H- 5% w/v Sucrose + 5% w/v Sodium Phosphate, I- 5% w/v Sodium phosphate + 1% w/v Calcium lactate, J - 5% w/v Sucrose +1% w/v PVP K-30

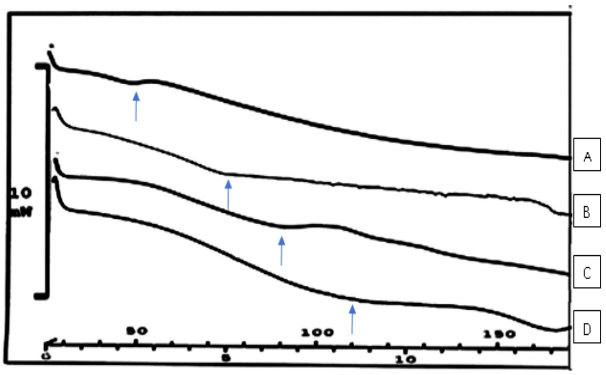

Figure 4a: Effect of additives on glass transition temperature (Tg) of sucrose A (Sucrose), B (5% w/v sucrose + 5% w/v Sodium phosphate), C (5% w/v sucrose+ 1% w/v calcium lactate + 5% w/v sodium phosphate) D (5% w/v sucrose+ 1% w/v PVP K-30 + 5% w/v sodium phosphate)

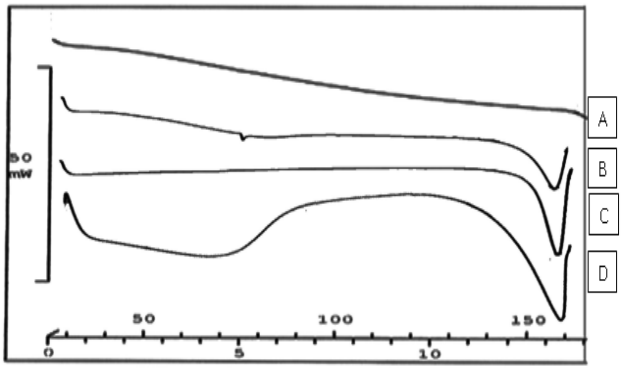

Figure 4b: Effect of additives on glass transition temperature (Tg) of mannitol A (Mannitol), B (5% w/v Mannitol + 5% w/v Sodium phosphate), C (5% w/v Mannitol+ 1% w/v calcium lactate + 5% w/v sodium phosphate) D (5% w/v Mannitol+ 1% w/v PVP K-30 + 5% w/v sodium phosphate)

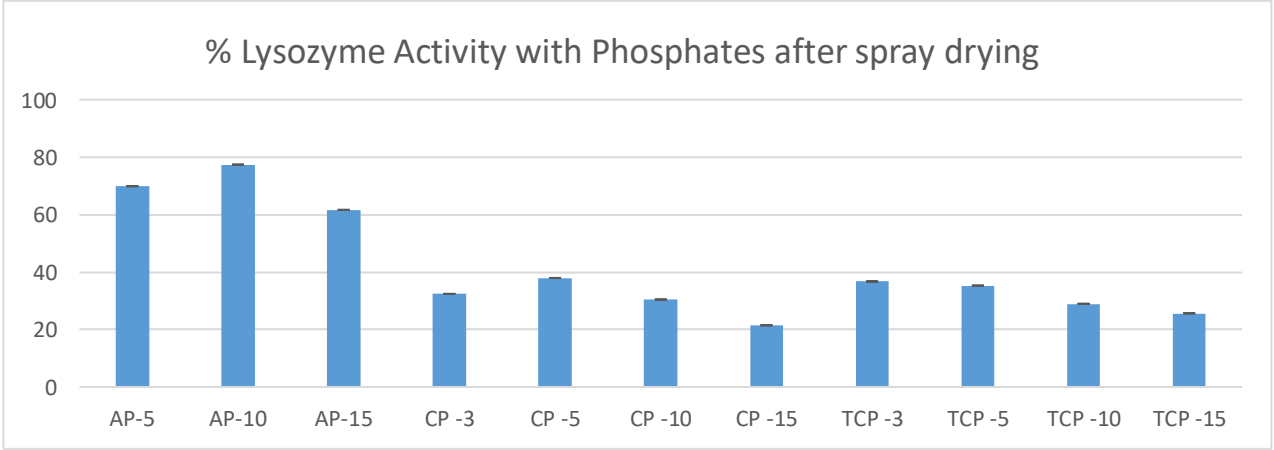

Figure 5: Lysozyme activity with phosphates after spray drying

Table 4: Effect of glass formers and phosphate on lysozyme after spray drying

Batch Code	Composition	pH	% Moisture Content	% Lysozyme Activity	% Yield
SPMC	5%w/v Sucrose + 5%w/v Sodium phosphate monobasic + 1%w/v Calcium lactate+ Lysozyme	4.8	2.82	98.35	86.1
SPMP	5%w/v Sucrose + 5%w/v Sodium phosphate monobasic + 1%w/v PVP K-30+ Lysozyme	4.13	3.01	98.91	89.7
SPDC	5%w/v Sucrose + 5%w/v Sodium phosphate dibasic + 1%w/v Calcium lactate+ Lysozyme	8.44	2.97	95.39	88.5
SPDP	5%w/v Sucrose + 5%w/v Sodium phosphate dibasic + 1%w/v PVP K-30+ Lysozyme	8.42	3.12	98.32	85.4
PPMC	5%w/v Sucrose + 5%w/v Potassium phosphate monobasic + 1%w/v Calcium lactate+ Lysozyme	4.22	3.21	97.02	77.9
PPMP	5%w/v Sucrose + 5%w/v Potassium phosphate monobasic + 1%w/v PVP K-30 + Lysozyme	4.40	0.81	98.57	83.1
PPDC	5%w/v Sucrose + 5%w/v Potassium phosphate dibasic + 1%w/v Calcium lactate+ Lysozyme	9.18	2.84	49.65	68.4
APC	5%w/v Sucrose + 5%w/v Ammonium phosphate dibasic + 1%w/v Calcium lactate+ Lysozyme	4.10	2.57	86.47	80.6
APP	5%w/v Sucrose + 5%w/v Ammonium phosphate dibasic + 1%w/v PVP K-30 + Lysozyme	4.08	2.74	88.58	86.5

achieved 98.61%. This indicates that higher concentrations of phosphates alone led to crystallization, which adversely affected lysozyme activity. The efficiency of sucrose-phosphate mixtures was enhanced by the presence of glass-forming agents, as evidenced in figure 7, with lysozyme activity remaining above 95%. However, ammonium phosphate and potassium phosphate dibasic, when combined with both glass formers, resulted in significant lysozyme activity loss. The higher lysozyme activity is attributed to stronger interactions between sucrose and additives. PVP K-30 has been reported to form hydrogen bonds with sucrose[12], while calcium lactate exhibits network-forming ability. Additionally, inorganic phosphates such as sodium and potassium phosphate dibasic contributed to preserving lysozyme activity during spray drying. The optimal pH range for maximum lysozyme activity is 60–80%, with a moisture content of 3-6%. However, despite these constraints, spray-dried lysozyme-sugar phosphate glass mixtures maintained high enzymatic activity.

The study confirmed that ammonium phosphate failed to form an amorphous glassy composite with sucrose, likely due to the acidic pH of the mixtures before spray drying. Potassium phosphate monobasic effectively preserved the native structure of lysozyme during the process. While crystallinity was significantly inhibited, a fully amorphous glass was not achieved. Sodium showed a higher affinity than potassium for complexion with sucrose. Spray drying with sodium phosphate (mono- or dibasic), PVP K-30, and calcium lactate resulted in a completely amorphous matrix. Variations in the physical state of these mixtures are expected to impact lysozyme's activity

Freeze-Drying of Lysozyme

The spray dried mixture of sucrose-sodium phosphate showing maximum % lysozyme activity (SPMC and SPMP) were subjected for lyophilization. The XRD data revealed the crystallization of sucrose during lyophlization in figure 8 and 9.

The moisture content and % lysozyme activity of lyophilized products is shown in Table 5. The lysozyme activity was found to be in range of 48 to 64%.

The XRD data from figure 8 and 9 confirmed that phosphate could not produce stable sucrose glass during lyophilization. The significant loss of lysozyme activity is

Table 5: Lysozyme activity of lyophilized batch

Batch code	Composition	Condition	pH	Moisture content (%)	Lysozyme activity (%)
LSPMC	5 % S+ 5 %SPM +1 % CL+ L	Initial	4.8	3.14	75.12%
		2 M /ACC	6.2	7.14	48.12%
LSPMP	5 % S +5 %SPM+ 1 % PVP K-30+ L	Initial	4.12	3.05	81.14%
		2 M /ACC	6.5	6.05	64.19%

attributed to crystallization of sucrose during lyophilization. The comparative results reveal that spray drying is better than lyophilization to produce stable sucrose- phosphate glassy matrix.

Stability Analysis of Optimized Lysozyme Mixtures

The stability study data comprising of % moisture content and % lysozyme activity is shown in figure 10 and 11. The maximum enzyme activity was retained with SPMC, SPMP, SPDC and SPDP batches confirming formulation of phosphate glass formers for preservation of lysozyme.

Moisture absorption in ammonium phosphate mixtures increased over time, leading to a gradual loss of lysozyme activity due to their hygroscopic nature. Although ammonium phosphate provided maximum processing preservation, it did not form a glassy matrix with sucrose. The absorbed moisture (3–6%) increased molecular mobility, causing protein unfolding and accelerating lysozyme degradation under storage conditions.

Potassium phosphate monobasic exhibited some crystallinity during storage, and its moisture absorption

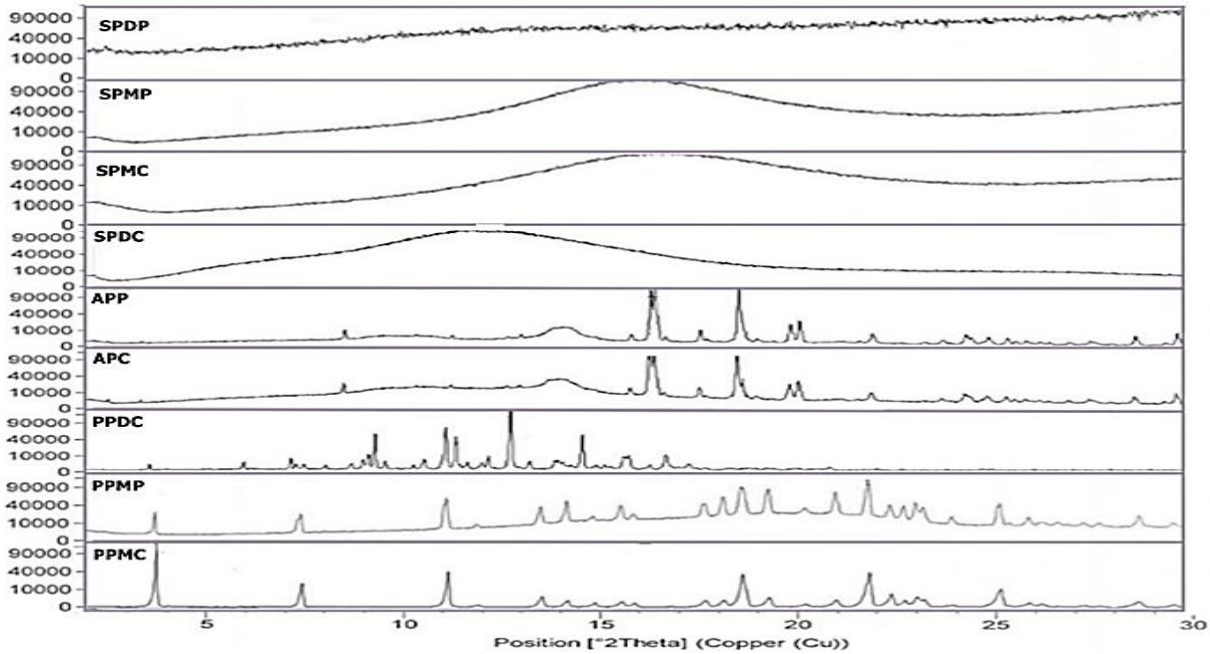

Figure 6: XDR for lysozyme with glass formers and phosphates

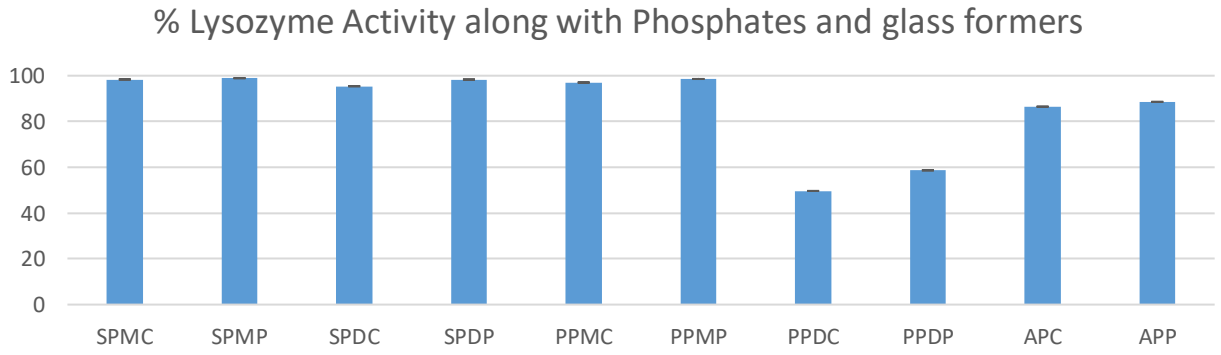

Figure 7: Lysozyme activity with glass formers and phosphates after spray drying

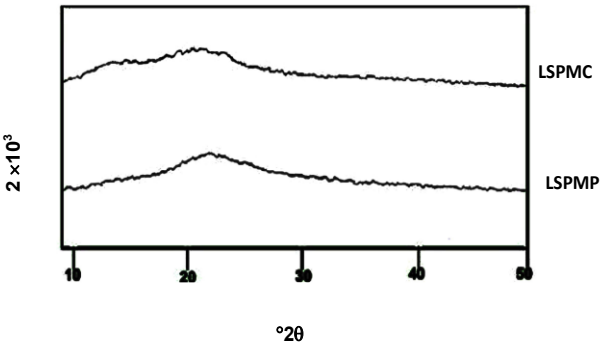

Figure 8: XRD pattern of lyophilized batch before stability

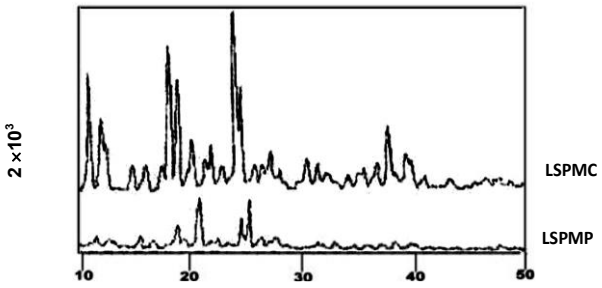

Figure 9: XRD pattern of lyophilized batch after stability

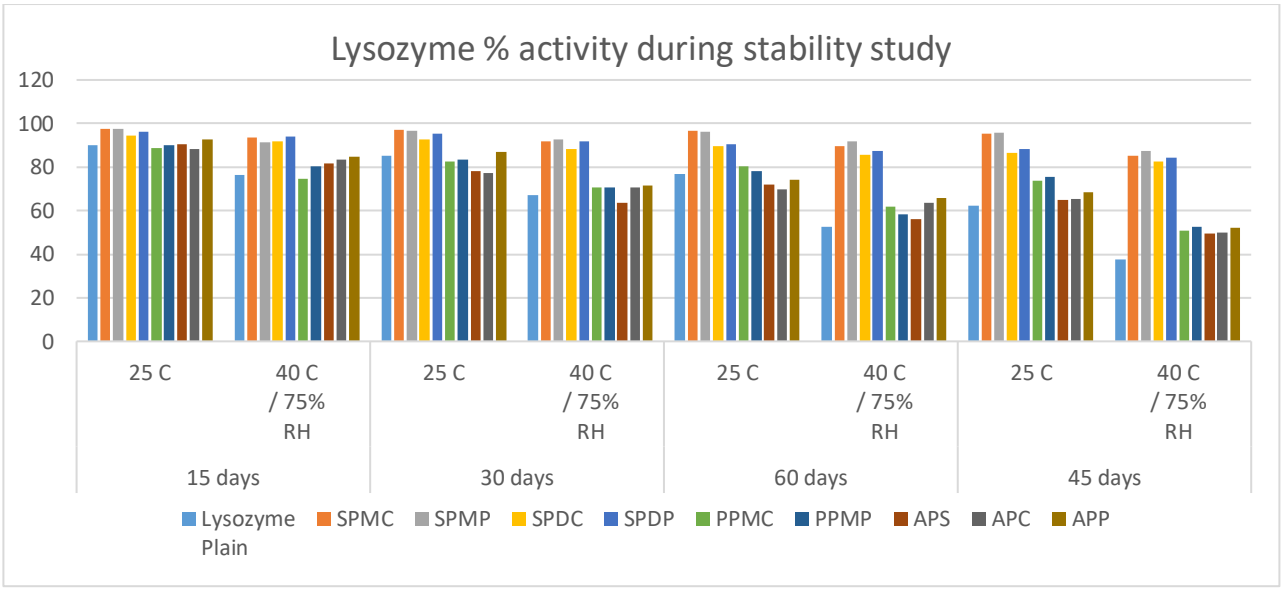

Figure 10: Lysozyme activity (%) of spray dried lysozyme sugar phosphate mixtures along with glass formers during stability study

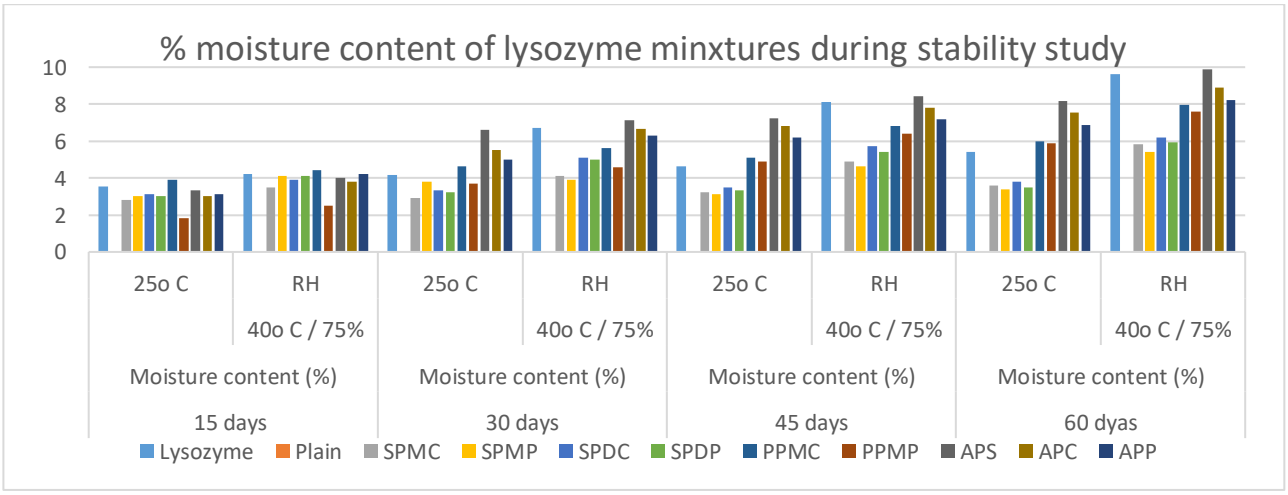

Figure 11: % moisture content of spray dried lysozyme sugar phosphate mixtures along with glass formers during stability study

further enhanced sucrose crystallization by lowering the glass transition temperature, weakening the glassy structure, and contributing to enzyme activity loss.

In contrast, sodium phosphate (mono or dibasic) products remained amorphous and absorbed significantly less moisture, maintaining content below 3.5% at room temperature and 5.2% at 40°C/75% RH. Post-storage XRD analysis confirmed the amorphous nature of all sodium phosphate batches, which retained lysozyme activity above 90%. The results suggest that the sucrose-sodium phosphate with glass former matrix effectively preserved lysozyme during processing by maintaining its structure even in the presence of moderate moisture levels.

CONCLUSION

The processing and storage stability of lysozyme is better in spray-dried products than that in lyophilized ones. The stabilization of lysozyme through sugar-phosphate glass complexion offers a promising solution to overcome temperature sensitivity. This approach enables the preservation of lysozyme's antimicrobial properties, rendering it a viable alternative to conventional antibiotics. The sugar-phosphate glass matrix provides a protective environment, shielding the enzyme from denaturation and degradation. These sugar-phosphate glass former mixtures are stable at higher temperature. The concept can be exploited for making different dosage form such Tablets or powder for oral solution. The formation of such a glass at a moderate temperature is interesting and has not been reported earlier. This innovative strategy has potential applications in biomedical research and antimicrobial therapy.

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