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Research Article

Enhancement of Water Solubility of Poorly Water-Soluble Drug using Milk Protein as Carrier

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ABSTRACT

Introduction: The two key challenges for formulation scientists in developing therapeutic delivery are the dissolution and solubility of a low aqueous-soluble drug. Many powerful drug molecules do not have therapeutic effects owing to solubility concerns, yet they can be dangerous when administered in large dosages. Solid dispersion technology is a good method for increasing solubility & dissolution, along with bioavailability. Material & Methods: solid dispersion of atorvastatin calcium (ATC) was developed using casein, infant formula, and poly-ethylene glycol 6000 by conventional fusion method and characterized for several characterization parameters. Conclusion: Solid dispersion of atorvastatin was efficiently developed. The dissolution of atorvastatin solid dispersion was discovered to be noticeably increased as compared to atorvastatin API, according to the current investigation, SD of atorvastatin was a superior alternative for increasing the dissolution of weakly soluble therapeutic agent.

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INTRODUCTION

Pharmaceutical experts are continually researching innovative techniques to increase drug dissolution rates, allowing for the efficient oral administration of drugs that are weakly aqueous-soluble. To increase the solubility of drugs that are weakly aqueous soluble, several techniques have been studied extensively, including solid dispersions, solid lipid nanoparticles, inclusion complexation, emulsion-based drug administration, hydrotrophy & etcetera (Madan JR, et al, (2015), Hiremath SN., et al, (2010), Dua K., et al,(2014), Dua K., et al, (2016), Dua K., et al, (2014)). In past several years, important Efforts in research have been devoted toward the formation of permeable carriers for enhancing the solubility of relatively hydrophobic drug API (Pathak K., et al, (2009)).

Protein contained inn milk is casein that is responsible for transferring nutrients from the mother to her kids. As a result, it is a natural transporter of bioactive substances and may be utilised to transport compounds of focus. It is an intrinsically unstructured protein (IUP) because it lacks disulphide links, is proline-rich, as well as having inadequate secondary and tertiary structures. It is composed of four phosphate-rich amphiphilic components that self-assemble into a micellar structure with sizes ranging from 50 to 300 nm. Several in vivo investigations have found that casein-based carriers, such as defatted milk, improve the bioavailability of weakly water-soluble drugs (Thomas Eason., et al, (2022)).

These drugs are a kind of drug that are cholesterol-lowering, has a limited bioavailability and dissolution rate, driving the creation of innovative preparations (Pathak K., et al, (2011), Jouyban A., et al, (2013)).

Atorvastatin calcium is a (BCS) Biopharmaceutics Classification System Class II drug & 1 of the best-selling world's statins, with a 12% (% F) absolute oral bioavailability from a forty mg oral dose form. Oral bioavailability is primarily hampered by dissolution rate

and solubility rate. As a result, employing milk protein as a carrier to improve Atorvastatin solubility was proposed.

MATERIALS AND METHODS

Atorvastatin solubility

Atorvastatin's saturation solubility in distilled water (DW) and (pbs) Phosphate buffer pH 7.2 is checked. Before usage, the solution had been degassed. Both the each of the media had been prepared, and an excess of atorvastatin had been added to each of them before being shaken in an incubator at 200 rotation per minute for 24 hours at thirty-seven degree celsius. The solution was then centrifuged for 15 minutes at 2000 rotation per minute after 24 hours. The supernatants had been diluted using the appropriate medium UV- visible spectrophotometer Spectrophotometer (Shimadzu, uv-1780, series) was used to detect the absorbance at 250 nm, and the solubility was calculated (Jagwani Y., et al, (2011), Morais AP., et al, (2012)).

Phase solubility study

This technique investigates the influence of carrier on the dissolution of ATC. Measurement of solubility were performed using phosphate buffer (pH 7.2), as described by Higuchi & Connors. In six beakers, 20 milligram of atorvastatin was solubilized in methanol solution. Carriers were added to all beaker in the amounts shown in Table 2. In the beaker, 10 millilitres of pbs was added, and solution was agitated for 24 hours before being filtered using Whatman filter paper. Absorbance at 250 nm was measured with a UV visible spectrophotometer (SHIMADZU, UV 1780 series), and the solubility of atorvastatin in all the different matrix forms was determined & compared to standard drug dissolution in 7.2 pH phosphate buffer.

Table 1: Shows results of phase solubility study.

Batches	Ratio of Drug: Carrier		Solubility	
	1:1	1:2	1:1	1:2
Amount of ATC (mg)	20		0.098±0.01	
Amount of methanol	1.5	1.5	-	-
PEG6000 (mg)	20	40	0.493±0.06	0.526±0.07
Infant formula (mg)	20	40	0.412±0.04	0.425±0.06
Casein (mg)	20	40	0.517±0.06	0.561±0.02

Preparation of solid dispersion

Polyethylene glycol was used as a carrier to create solid dispersions, Infant formula, and casein by technique of fusion solvent modification. PEG 6000 was melted at its respective melting point over a thermostatically controlled magnetic stirrer, and API was integrated into the molten carrier material.

The casein and infant formula were dissolved in water and combined with the PEG 6000 and active pharmaceutical ingredient. Before being flash cooled in an ice bath, the entire mixture was held at the correct melting temperature for 10 minutes. Hardened mixture was than mashed in a mortar-pestle, sieved # 44, & stored in a desiccator.

Table 2: Formulation and release profile

SD Batches	PEG X1	Infant formula X2	Casein X3
F1	+(20)	- (10)	+(20)
F2	- (10)	+(20)	+(20)
F3	-(10)	- (10)	+(20)
F4	- (10)	+ (20)	- (10)
F5	- (10)	- (10)	- (10)
F6	+ (20)	+ (20)	- (10)
F7	+(20)	+(20)	+(20)
F8	+(20)	-(10)	- (10)

*X1: PEG 6000, X2: Infant formula and X3: Casein, weight in (mg)

Design of Experimental

DOE is applied to investigate influence of 3 independent factors on the in-vitro dissolution profile of atorvastatin, a solid dispersion was created using the (2^3) factorial design. The factorial design used eight batches to examine three components at two levels (F1-F8). During the investigation, three parameters were examined (Table 2) the (X1) PEG 6000 concentration, the Infant formula (X2), & the concentration of Casein (X3).

Characterization of Atorvastatin Solid dispersion

The methods differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy(FTIR), & phase solubility study were used to examine the atorvastatin solid dispersion.

Fourier transform infrared spectroscopy (FTIR).

Spectrophotometer was used to capture the Fourier-transform infrared spectra of atorvastatin and carrier across a range of 4000-400 cm1 (shimadzu affinity-1). These scans were analyzed for the existence of atorvastatin main peaks, shifting and masking atorvastatin peaks due to carriers like casein, infant formula and PEG 6000 and the appearance of new peaks.

Differential scanning colorimetry analysis (DSC)

Differential scanning calorimetry (*DSC*) is a thermo-analytical technique used for DSC thermograms of pure atorvastatin, carrier, and drug solid.

The instrument was calibrated using indium (156^0C), tin (232^0C) & zinc (419.5^0C) as internal standards. Empty aluminium pan was utilized as a reference. Each sample was precisely weighed and packed in an aluminium pan. The probes were heated from 40-3500 degrees Celsius at a rate of 100 degrees Celsius per minute in a nitrogen environment.

In-vitro dissolution studies

The releases study of solid dispersions tablets was carried out using USP dissolution apparatus (Type 2). The dissolving media, 900 ml of Pbs 7.2 ph was added to flask while maintained temperature at 37±0.5ºC. Study was performed on different SD containing an equivalent amount of drug. 5 ml aliquots were removed at predetermined time intervals & replaced with the equivalent quantity of new dissolving medium. The samples collected were analyzed at 250 nm using Ultraviolet visible spectrophotometer (shimadzu, uv-1780, series)**.**

One-way ANOVA

One way ANOVA testing of all batches were done at ($p < 0.05$) and resulting optimized batches to be kept for stability testing.

Stability study

The stability of the optimized formulation (SD8) was tested as described (Mathews BR., et al, (1999)). The improved SD was wrapped in aluminium paper and stored for 6 months in the stability chamber at 75±5% RH and 40±2o. Following the conclusion of the storage period, collected samples were subjected to in-vitro dissolution and analysis. The paired t-test was used to determine whether there was a significant difference at $p = 0.05$. The similarity index (F2) was used to measure the similarity between before & after storage, which demonstrates the stability of the improved formulation.

RESULTS AND DISCUSSION

Solubility determination

The solubility of atorvastatin in (DW) distilled water & (pbs) phosphate buffer was discovered to be 0.026±0.08 mg/ml and 0.098±0.01 mg/ml, respectively.

Phase solubility study

The findings of phase solubility investigations are discussed in Table 2. When ATC is loaded in casein, its solubility in phosphate buffer increases. When compared to pure ATC ($0.9 \times 10\text{-}1 \pm 0.01$mg/ml), the matrix comprising ATC: Casein at 1: 2 ratios had the maximum solubility ($5.61 \times 10\text{-}1 \pm 0.02$ mg/ml).

Full factorial design

Preliminary research was conducted to identify the elements influencing SD formulation. 3 factors i.e., carrier (PEG 6000, infant formula & Casein) was studied at 2 levels (+1 and –1) & 8 batches (F1-F8) of SD were formulated using (2^3) factorial design (Table 1). DSC, FTIR, and in-vitro release tests were performed on all batches initially.

Characterization of matrix

Fourier transform infra-red spectroscopy (FTIR)

This analysis was used to define the potential interaction in the ATC-Casein, ATC-Infant formula & ATC-PEG6000 as carrier. Figure 1 depicts the FTIR spectra of ATC and Carriers. The combined individual characteristic peaks of carrier and ATC in the FTIR spectrum of ATC-carriers indicated that ATC was physically rather than chemically adsorbed on surface of carrier.

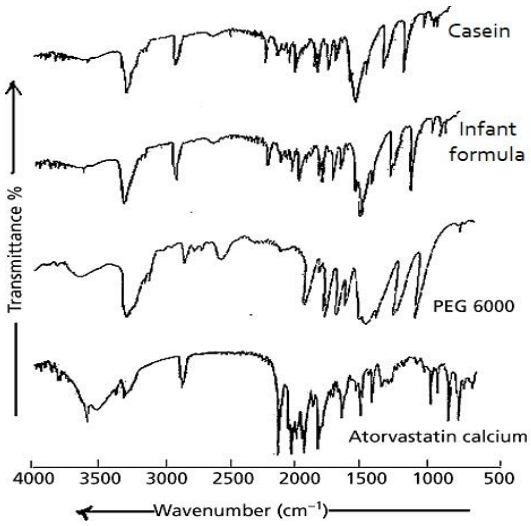

Figure 1. Spectrumofatorvastatin(a), Atorvastatin - Carrier (c) Carrier(b).

Differential scanning calorimetry analysis (DSC)

Figure 2a depicts thermogram of atorvastatin. The graph depicted the onset temperature. Atorvastatin thermogram reveals an at 158 °C, there is an endothermic melting peak. Carrier was amorphous, with no discernible apex. Figure 2b depicts the DSC thermogram of the ATC-Carrier matrix. The thermogram shows that ATC has completely lost its crystalline state due to the creation of the ATC-

Carrier matrix.

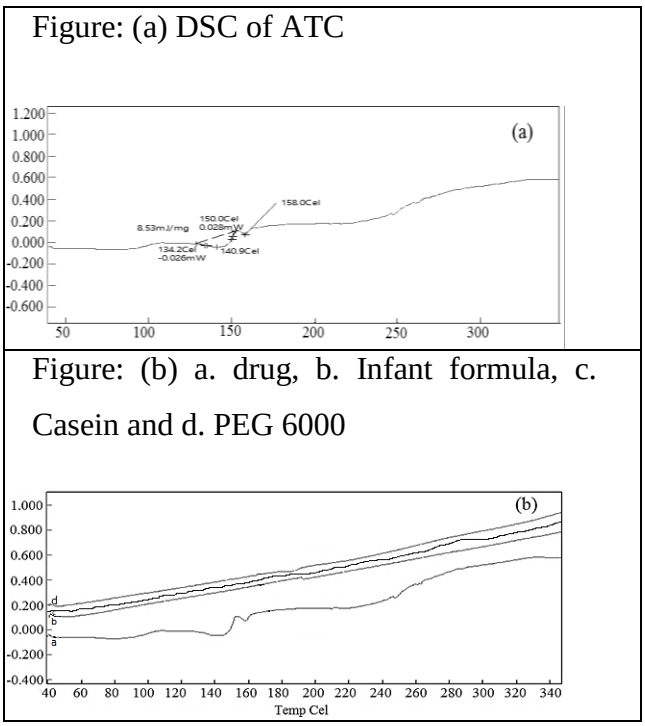

Figure 2: (a) DSC of atorvastatin and (b) mixture (Atorvastatin- Carrier matrix), drug (Atorvastatin) & excipient (Carrier).

Phase solubility

Results are presented in Table 2. The data indicate a rise in solubility of Atorvastatin in pbs 7.2 ph when loaded in casein. The maximum solubility was achieved in the matrix containing ATC: Casein at 1: 2 ratio in compared to pure Atorvastatin i.e.; ($5.61 \times 10^{-1} \pm 0.02$ mg/ml) ($0.9 \times 10^{-1} \pm 0.01$mg/ml).

In-vitro dissolution studies

The tablets containing Casein solid dispersion showed maximum release of ATC compared with tablets containing Infant formula & PEG 6000, marketed & conventional atorvastatin tablets. Optimized formulation (F8) loaded with casein showed promising improvement in ($p<0.05$) drug release (99.08±1.35% in 20 min) compared with formulation (F5) containing PEG 6000 (93.67±1.12% in 20 min) and conventional (47.31±0.74% in 20 min). The release pattern of atorvastatin from various formulations is shown in Figure 3.

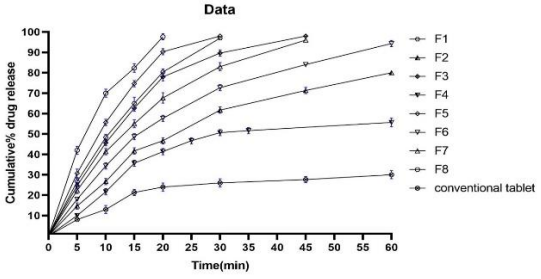

Figure 3. ATC drug release % from varied formulations.

One-way ANOVA

All formulation batches show significant difference in one way ANOVA testing. Optimized batch obtained was F8 showing positive release pattern.

Stability testing

Optimised formulation stability (F8) was evaluated at elevated condition. Assay & In-vitro dissolution studies were performed after the completion of storage period. Similarity index value was found to be 89.62. Date from Table shows there was no significant variation i.e., $p>0.05$ in concentration of drug determined by paired t-test.

CONCLUSION

From the present study it was investigated there is possibility of improving solubility of atorvastatin using milk protein as carrier by solid dispersion technique. The DSC and FTIR studies demonstrated the formulation of SD. Solubility and dissolution rate of atorvastatin was found successfully enhanced. Formulation was found to be stable at ambient temperature conditions. The pharmacological evaluation of the formulation revealed significant increase in its hypo-lipidemic effect as compared to pure drug. Therefore, the present methodology can be regarded as a novel and commercially feasible technique for improving the in-vitro and in-vivo performance of atorvastatin.

Table 3: Stability study data of Atorvastatin containing F8.

Time (min)	Before storage	After 6 months Storage	t-test at 0.05 LS	Similarity Factor (f2)
0	0.00±0.00	0.00±0.00		
5	42.18±1.71	41.20±1.09	Not Significant	89.62
10	70.49±1.16	69.38±1.11		
15	82.12±1.08	80.20±1.40		
20	96.08±1.20	94.12±1.25		
% Assay	96.34±1.18	95.28±1.34	Not Significant	--

*(Mean ± SD, n=3)

CONFLICT OF INTEREST

All the contributing authors declare no conflict of interest.

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