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Research Article

PROTEIN BINDING: A STUDY OF INTERACTION BETWEEN DICLOFENAC SODIUM AND BOVINE SERUM ALBUMIN

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ABSTRACT

The study was designed to examine the binding of Diclofenac sodium with bovine serum albumin (BSA) (Ex vivo) at different concentration levels of Bovine serum albumin (BSA) under controlled temperature 37^{0}C and phosphate buffer pH7.4 conditions. The protein binding behavior of Diclofenac sodium (sodium[o-(2,6-dichloroanilino)phenyl]acetate) in BSA was investigated by U.V spectroscopy Analysis. Spectroscopic estimations of drug release were made with a constant Diclofenac Sodium concentration while varying the concentration of BSA. The drug protein binding is distinguishable at different levels of BSA (mg) as there was a mark decrease in percentage of Diclofenac Sodium release from 77.5 % (Control) to 71.78%. Comparable results were obtained with different BSA concentrations indicating that albumin is probably the responsible protein which decreases the release of drug. The difference in extent of binding of BSA with similar drug samples were significantly showing the promising effect of BSA over Diclofenac Sodium release when drug dissolution profile was observed.

Key words: *Bovine serum Albumin, Diclofenac sodium, Protein binding*

INTRODUCTION

A drug's efficiency may be affected by the degree to which it binds to the proteins within blood plasma. The less bound a drug is, the more efficiently it can traverse cell membranes or diffuse. Common blood proteins that drugs bind to are human serum albumin [1], lipoprotein, glycoprotein [2-4], α, β, γ globulins and immune globulins [5]. The plasma protein binding of drugs has shown significant effects on pharmacokinetics such as liver metabolism rate, renal clearance, Biomembrane permeation rate and steady state distribution volume [6, 7]

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A drug in blood exists in two forms: bound and unbound. Depending on a specific drug's affinity for plasma protein, a proportion of the drug may become bound to plasma proteins, with the remainder being unbound. If the protein binding is reversible, then a chemical equilibrium will exist between the bound and unbound states, such that:

Protein + drug ⇌ Protein-drug complex

Notably, it is the unbound fraction which exhibits pharmacologic effects when the drug undergoes metabolism in the liver where as the bounded drug will accumulate and distribute into the tissues leading to a decrease in plasma concentration profile. [9]

Diclofenac is an analgesic, antipyretic, anti-rheumatic medicament comes under category of non-steroidal-anti-inflammatory-drug (NSAID) (Fig. 1). It is related to the subcategory of NSAID that are derivatives of

the phenylacetic acid and its chemical name is 2-(2,6- dichloroanilino) phenylacetic acid. Its usually available forms are sodium or potassium salt. Diclofenac is a weak acid with a pKa 4.0, it has a protein-binding ability of more than 99% and it is light sensitive [10].

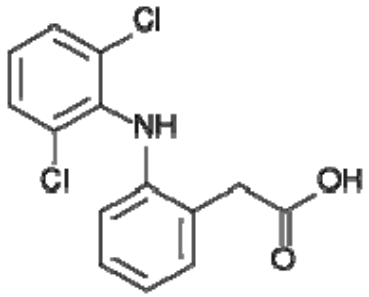

Figure. 1. The chemical structure of Diclofenac

The exact mechanism of its actions is not entirely known, but it is thought that the primary mechanism is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). COX1 and 2 are the most explored COX isoenzymes and COX1 is a constitutive enzyme involved i.e. in the protection of the gastrointestinal mucosa and COX2 is an inducible enzyme that upregulates the inflammatory response. Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side effect of Diclofenac. Diclofenac has a low to moderate preference to block the COX2-isoenzyme (approximately 10-fold) and therefore it is said to have a somewhat lower incidence of gastrointestinal complications than noted with other NSAIDs i.e. aspirin [10,11].

Human serum albumin (HSA) is a carrier for several drugs i.e. Diclofenac and by this it plays an important role in the regulation of plasma concentrations of these, including both endogenous and exogenous compounds. HSA shares 76% homology with Bovine serum albumin (BSA) and as this is easily accessible, of low cost and of medical importance it can therefore be used in this project [10, 12,13]. BSA consists of amino acid chains forming a single polypeptide with a well-known sequence, which contains three homologous _-helixes domains (I–III). Each domain contains 10 helixes which are divided into anti-parallel six-helixes and four subdomains. HAS contains 585 amino acid residues with only one tryptophan located at position 214 along the chain. The BSA molecule is formed by 582 amino acid residues; with tryptophan at position 134 and 212 [10, 14].

The present work was designed to perform an exhaustive study to evaluate the effect of concentration of BSA on Diclofenac Sodium using U.V spectrography.

MATERIAL AND METHODS

Diclofenac Sodium was obtained from Bengal Dr. Johns lab Pvt Ltd, Haridwar as a generous gift. Whereas BSA was obtained from college lab (Merck).

Determination of Ex Vivo BSA influenced Diclofenac release: The release profile of Diclofenac sodium (control) was carried out in phosphate buffer solution (*p*H7.4) and in presence of different concentration of BSA i.e 10-30 mg in phosphate buffer solutions (*p*H7.4) at 272 nm using UV-VIS spectrophotometer (Shimadzu)

Release Profile of Diclofenac Sodium (control):

Time in min.	*Absorbance*	*Conc. in mcg/ml*	*Conc. in mcg/5ml*	*Cumulative conc. in mcg/5ml*	*Conc. in mcg/200ml*	*Cumulative conc. in mcg/200ml*	*Cumulative conc. in mg/200ml*	*% Cumulative release*
0	0	0	0	0	0	0	0	0
5	0.342	5.5295	27.6475	27.6475	1105.9	1105.9	1.1059	55.29
10	0.468	5.6555	28.2775	55.925	1131.1	1158.7475	1.1587475	57.93
15	0.911	6.0985	30.4925	86.4175	1219.7	1275.625	1.275625	63.78
30	1.325	6.5125	32.5625	118.98	1302.5	1388.9175	1.3889175	69.44
60	1.721	6.9085	34.5425	153.5225	1381.7	1500.68	1.50068	75.03
90	1.795	6.9825	34.9125	188.435	1396.5	1550.0225	1.5500225	77.50

Release Profile of Diclofenac Sodium with 10 mg BSA:

Time in min.	*Absorbance*	*Conc. in mcg/ml*	*Conc. in mcg/5ml*	*Cumulative conc. in mcg/5ml*	*Conc. in mcg/200ml*	*Cumulative conc. in mcg/200ml*	*Cumulative conc. in mg/200ml*	*% Cumulative release*
0	0	0	0	0	0	0	0	0
5	0.263	5.4505	27.2525	27.2525	1090.1	1090.1	1.0901	54.50
10	0.422	5.6095	28.0475	55.3	1121.9	1149.1525	1.1491525	57.45
15	0.938	6.1255	30.6275	85.9275	1225.1	1280.4	1.2804	64.02
30	1.288	6.4755	32.3775	118.305	1295.1	1381.0275	1.3810275	69.05
60	1.468	6.6555	33.2775	151.5825	1331.1	1449.405	1.449405	72.47
90	1.592	6.7795	33.8975	185.48	1355.9	1507.4825	1.5074825	75.37

Release Profile of Diclofenac Sodium with 20 mg BSA:

Time in min.	*Absorbance*	*Conc. in mcg/ml*	*Conc. in mcg/5ml*	*Cumulative Conc. in mcg/5ml*	*Conc. in mcg/200ml*	*Cumulative Conc. in mcg/200ml*	*Cumulative Conc. in mg/200ml*	*% Cumulative Release*
0	0	0	0	0	0	0	0	0
5	0.238	5.4255	27.1275	27.1275	1085.1	1085.1	1.0851	54.25
10	0.425	5.6125	28.0625	55.19	1122.5	1149.6275	1.1496275	57.48
15	0.71	5.8975	29.4875	84.6775	1179.5	1234.69	1.23469	61.73
30	0.988	6.1755	30.8775	115.555	1235.1	1319.7775	1.3197775	65.98
60	1.298	6.4855	32.4275	147.9825	1297.1	1412.655	1.412655	70.63
90	1.481	6.6685	33.3425	181.325	1333.7	1481.6825	1.4816825	74.08

Release Profile of Diclofenac Sodium with 30 mg BSA:

Time in min.	*Absorbance*	*Conc. in mcg/ml*	*Conc. in mcg/5ml*	*Cumulative Conc. in mcg/5ml*	*Conc. in mcg/200ml*	*Cumulative Conc. in mcg/200ml*	*Cumulative Conc. in mg/200ml*	*% Cumulative Release*
0	0	0	0	0	0	0	0	0
5	0.242	5.4295	27.1475	27.1475	1085.9	1085.9	1.0859	54.29
10	0.4	5.5875	27.9375	55.085	1117.5	1144.6475	1.1446475	57.23
15	0.678	5.8655	29.3275	84.4125	1173.1	1228.185	1.228185	61.41
30	0.812	5.9995	29.9975	114.41	1199.9	1284.3125	1.2843125	64.21
60	0.971	6.1585	30.7925	145.2025	1231.7	1346.11	1.34611	67.31
90	1.265	6.4525	32.2625	177.465	1290.5	1435.7025	1.4357025	71.78

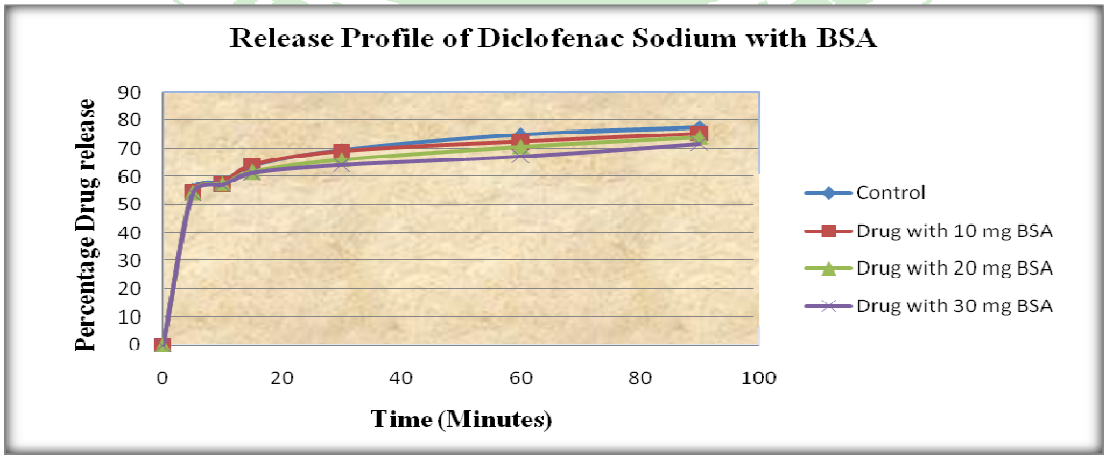

Figure 2: Release Profile of Diclofenac Sodium with BSA

RESULTS AND DISCUSSION

The release profile of Diclofenac sodium (control) was carried out in phosphate buffer solution (*p*H7.4) and in presence of different concentration of BSA i.e 10-30 mg in phosphate buffer solutions (*p*H7.4) at 272 nm using UV-VIS spectrophotometer (Shimadzu). The drug release profile study with different concentrations of BSA has shown protein binding of Diclofenac sodium with BSA whereas it was not so in without BSA (Control). The bound fraction of the drug is increased with increase in the concentration of the BSA. So that the drug release get decreased from 77.50% to 71.78% which furnish evidence of protein binding between BSA and Diclofenac Sodium. Here by it can be assumed that protein binding (a prominent interaction between drug and protein) may lead to decrease in drug release up to certain extent that may affect the drug dose response in the body. So that in the selection dose of the drug protein binding of drug is an important criteria and it should be taken in keen interest.

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