CHEMISTRY, BIOLOGICAL PROPERTIES AND ANALYTICAL METHODS FOR ESTIMATION OF PROBENECID: AN UPDATED REVIEW

Shilpa Sahu, Jyoti Verma, Bina Gidwani, Shiv Shankar Shukla *

Columbia Institute of Pharmacy, Raipur (C.G.), India

***Corresponding author**

Prof. Shiv Shankar Shukla

Professor, Department of Pharmaceutical Quality Assurance

Columbia Institute of Pharmacy,

Chhattisgarh Swami Vivekanand Technical University, Bhilai (C.G.),

India, Pin - 493111

Abstract

In recent decades, gout treatment has advanced quickly, and numerous medications have been developed for both acute and long-term maintenance. Colchicine, febuxostat, and pegloticase are three drugs used to treat gout. Pharmacokinetics, pharmacodynamics, population-specifics, benefits, and contraindications are all crucial considerations when prescribing these medications. Each drug's pharmacokinetic variables include factors related to absorption, distribution, metabolism, and elimination. Probenecid's, indicating new potential therapeutic uses in medicine. Probenecid is currently used as an adjuvant to improve the bioavailability of many medications in the central nervous system (CNS). As demonstrated by its impact on neurological and neurodegenerative disorders, numerous studies also indicate that this medication possesses significant neuroprotective, antiepileptic, and anti-inflammatory qualities. Even though probenecid's clinical use has decreased over time, preclinical research suggests that it could be helpful in enhancing traditional psychiatric treatments where the medications used have low bioavailability due to either a high efflux from the central nervous system, a high urine clearance, or a poor passage through the blood–brain barrier.

Probenecid's history, pharmacological characteristics, and new research applications are outlined in this review, along with its potential pharmacological intervention for neuro degeneration resulting from neuro inflammation. In order to assess renal proximal tubule organic anion transport function in kidney illness, we also suggest a probenecid stress test.

Keywords: Probencid, neuro inflammation, neurogenration, gout treatment.

INTRODUCTION

Lowering blood uric acid levels is the primary treatment for hyperuricemia and gout [1]. Gout also referred to as "a disease of kings," is a chronic condition. In 1848, Sir Alfred Baring Garrod made the discovery [2]. Probenecid, sulfinpyrazone, oral colchicine, and allopurinol were among the anti-gout medications. Since glucocorticoids and nonsteroidal anti-inflammatory medications are less specific for the treatment of gout or hyperuricemia than the chosen therapies, they were not regarded as outcomes of interest in this investigation [3]. Due in part to the widespread belief that gout is a well-understood condition that is simple to detect and treat and frequently brought on by food and lifestyle excesses, gout has gotten little review attention for many years despite being one of the most prevalent rheumatic diseases [4]. Many persons with hyperuricemia do not develop gout or even create UA crystals, despite though it is the primary pathogenic defect in gout. In actuality, gout only occurs in 5% of patients with hyperuriceamia greater than 9 mg/dL. As a result, it is believed that gout is influenced by a number of additional factors, including hereditary predisposition [5, 6].Gout is typically treated with uricosuric medications, which raise uric acid excretion in the urine, or XO (xanthine oxidase) inhibitors, which hinder the last stage of uric acid production and reduce plasma uric acid Reducing and keeping the serum urate at the normal level (<6.0 mg/d) is the levels [7]. In general, three strategies are used to accomplish this goal [8]. The purpose of the second one is to increase the excretion of uric acid. The third tactic involves converting uric acid to allantoin by administering recombinant uricases [9].

CLASSIFICATION

Gout is classified as acute and chronic depending upon the severity. Acute gout is a painful ailment that frequently only affects one joint. Glucocorticoids, colchicine, and former NSAIDs are the medications utilised. Chronic gout is characterised by recurrent flare-ups of inflammation and discomfort. Multiple joints may be impacted by substances like probencid, sufinpyazone, and allopurinol. The Classification of Anti-gout drugs are shown in figure 1.

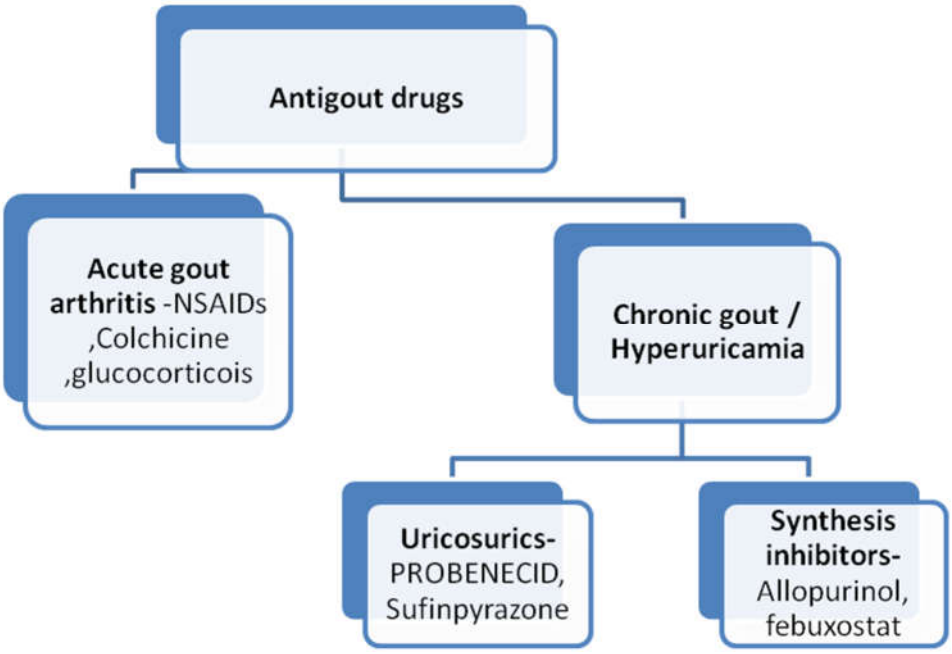


Fig.1- Classification of Anti-Gout Drugs

According to WHO probenecid (PBN) is a fat-soluble derivative of benzoic acid, in 1949. Since 1950, it was first introduced to the market as Benemid [10]. In 1949, probenecid, also known as p-(di-n-propylsulphamyl)-benzoic acid, was created with the intention of lowering penicillin's renal clearance. Probenecid significantly increased serum exposure when penicillin's renal clearance was reduced, indicating that lower drug dosages were needed to achieve comparable, (PK/PD) target attainment. As the ability to generate more varied, affordable, and safe β-lactam

antibiotics quickly increased in the post-war era, probenecid's impact on penicillin clearance shifted mostly to academia[11].One Competitive inhibition of organic anion transporters, which are in charge of the excretion of organic drugs like penicillin, is how it works [12]. According to certain research conducted between the 1950s and 1960s, PBN prevents active transport across the blood–brain barrier (BBB). Numerous studies in the field of psychiatry have been conducted as a result of its ability to reduce the excretion of the acidic metabolites of serotonin (5-hydroxy indole acetic acid) and dopamine (homovanillic acid) [13]. Probenecid and zidovudine could be used together to decrease the daily dosage and increase the time between doses, improving convenience and lowering expenses [14]. In this context, even though the majority of PROB's protective effect in the brain have been primarily linked to its ability to accumulate the inhibitory metabolite kynurenic acid, which further inhibits glutamate-related excite-toxicity in various animal models of neurological disorders, the fundamental of PROB's pharmacokinetics and mechanisms of action are discussed in this review [15]. There are many probenecid-sensitive transporters, including those in plants [16]. Probenecid was historically created in the 1940s to lessen penicillin's renal excretion [17]. Probenecid was initially used to treat gonorrhoea, Lyme disease, mycobacterial infections, and Haemophilus influenza infections [18]. A uricosuric medication called probenecid is prescribed to treat hyperuricemia linked to gout and gouty arthritis. Because it increases and prolongs the plasma levels of some antibiotics by inhibiting renal excretion, it was also employed as an adjuvant for therapy with antibiotics such methicillin, ampicillin, and penicillin [19].

Synonyms - Benecid, Benemid,Benurly, Pro-cid,Probecid, Probenecid, Probenecid Weimer.

Chemistry - Chronic gout has long been treated with probenecid ,a synthetic sulphonamide that has the dual properties of increasing uric acid excretion and inhibiting penicillin excretion [20].However, PRO is classified as a BCS II medication with weak water solubility and good permeability by the biopharmaceutical categorisation system (BCS) [21].The literature shows that it is only 72.2 ug/ml soluble in water at 37 degree Celsius[22].Studies on the elastic crystals created when PRO co-precipitates with 4,4-azopyridine,[23] 4,4-bipyridine [24], azacytidine, and piperazine have recently been conducted. 1,2- bis (4- pyridyl)

ethane 2.43 times increase) [25]. By creating PRO salts, we want to increase the solubility of PRO molecules, which have a carboxyl group that can dissociate.[26] .Finally, we discovered that pro can combine with 4-aminopyridine (4DAP) to generate a new phase. 4AMP and 4DAP, which are frequently used basic conformers [27] have been used to create salts with Furantoin[28], Piroxicam and Meloxicam[29], and Ibuprofen [30]. The structure of probenecid is shown in figure 2.

Chemical structure of Probenecid

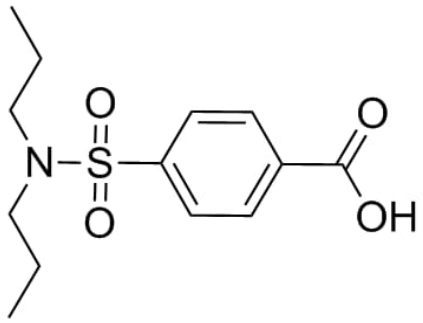


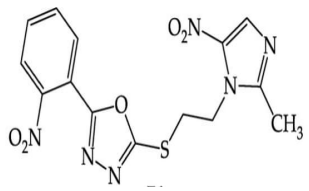
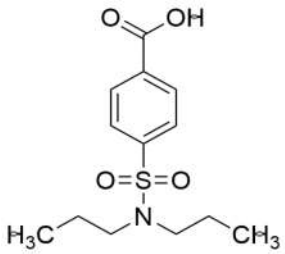
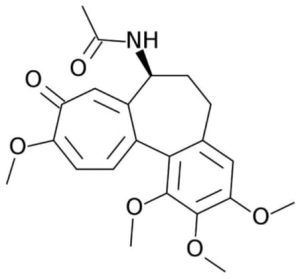
Fig. 2 – Structure of Probenecid

DERIVATIVES OF PROBENECID

The oxadiazole, amide, amino and pro drug derivatives of Probenecid are listed in table 1.

Table 1 - Derivatives of Probenecid along with their use

Derivative	Description	Use
Oxadiazole derivatives(furamizole, raltegravir,)	The transformation of probenecid into its oxadiazole derivatives and studies of their biological functions have received somewhat less attention. However, the ability of 1, 3, 4-oxadiazole–phthalimide hybrids generated from probenecid to	HIV , microbial infection, anticancer

	inhibit dengue virus NS2B/NS3 protease has been studied [31].	
Amide derivatives 	The human carbonic anhydrase (hCA, EC 4.2.1.1) transmembrane isoforms hCA IX and XII were found to be strongly and selectively inhibited by novel amide derivatives of Probenecid. Complete loss of hCA I and II inhibition resulted from the suggested chemical conversion of the carboxylic acid into an amide group (Kis>10,000 nM).	Hyperuricemia
Amino derivative 	By altering probenecid, amino acid derivatives that make use of the L-type amino acid transporter 1 (LAT1) for cellular uptake can be produced. These derivatives can be made to be stable and act as new derivatives, or they can be made to act as prodrugs by releasing the parent substance following chemical or enzymatic conversion.	Gout ,hyperuricemia
Prodrug derivative	Certain probenecid derivatives can be made into prodrugs, which are substances that are carried across cell membranes and then changed into the active ingredient in the body.	Anticancer, hypouricemic, anti-inflammatory

PHYSICAL PROPERTIES –

The physical properties like solubility, PKa value are listed in table 2.

Table 2 – Physical properties of Probenecid

Physical property	**Condition**
Chemical name	P [(Dipropyl amino) sulfonyl] benzoic acid,
Molecular weight	285.36 g/mol
Molecular formula	C13H19NO4S
State	Crystaline powder
Colour	White
Solubility	Soluble in Acetone, slightly soluble in ethanol or chloroform ,but insoluble in water
Odor	Odorless
Taste	Bitter
Melting point	194-196 C
Boiling point	438.0±47.0 C
Pka	5.8
Stability	Stable, but light may be sensitive
Form	Neat

SYNTHESIS

The synthesis of multi-component crystal (such as co-crystal, salts and solvates)[32], of active pharmaceutical ingredients (APIS)to improve their physicochemical properties, such as solubility, dissolution rate, bioavailability , stability , and finding widespread application in the pharmaceutical industry [33].Notably, 40% of currently marketed medications have poor water solubility [34], and salt production is the favoured method for treating the low solubility of medicinal molecules[35].“The substance formed by the combination of cations and anions “is how the IUPAC defines a salt. An ionised API and another counter-ion are necessary for the synthesis of a therapeutic molecule as a salt. Thus, the synthesis of salt is a well-established technique for improving the physiochemical characteristics of medication with ionisation ability. To create the diazonium salt, PABA undergoes a controlled reaction with sodium nitrite and hydrochloric acid. P-carboxyl benzene sulfonyl chloride is created when the diazonium salt reacts with sulphur dioxide in diluted

hydrochloric acid. Probenecid is created when dipropylamine and p-carboxyl benzene sulfonyl chloride combine [36]. The synthesis is shown in figure 3.

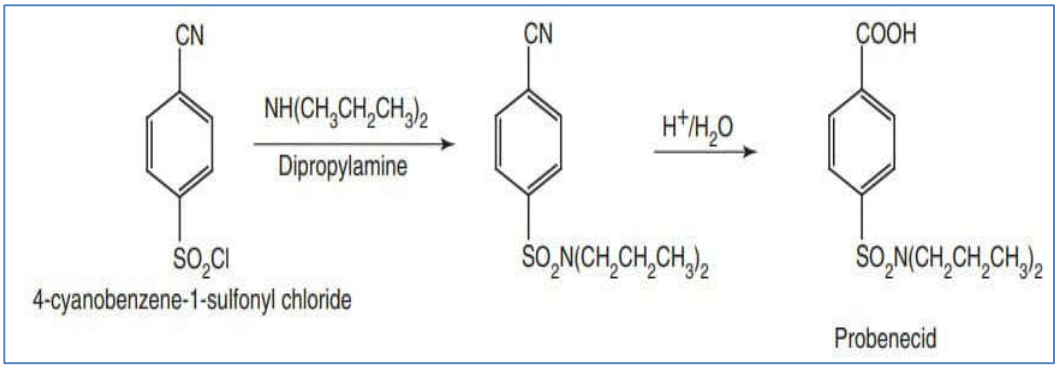


Fig.3. –Chemical synthesis of probenecid

MECHANISM OF ACTION - For many years, probenecid has been used to treat gout. The medicine works by inhibiting a renal tubular transporter, which prevents reuptake and facilitates the excretion of uric acid that causes the illness [37].The basolateral membrane of the renal proximal tubule expresses human OAT, a plasma membrane protein belonging to the solute carrier family that mediates drug and toxicant disposition [38]. This protein is in charge of the tubular absorption of blood cations, neutral substances, and organic anions [39]. While OAT3 (organic anion transport) is found in the choroid plexus and the blood-brain barrier (BBB) [40]. OAT1 is expressed in cortical and hippocampus neurones as well as the ependymal cell layer of the choroid plexus [41]. The mechanism is explained in figure 4.

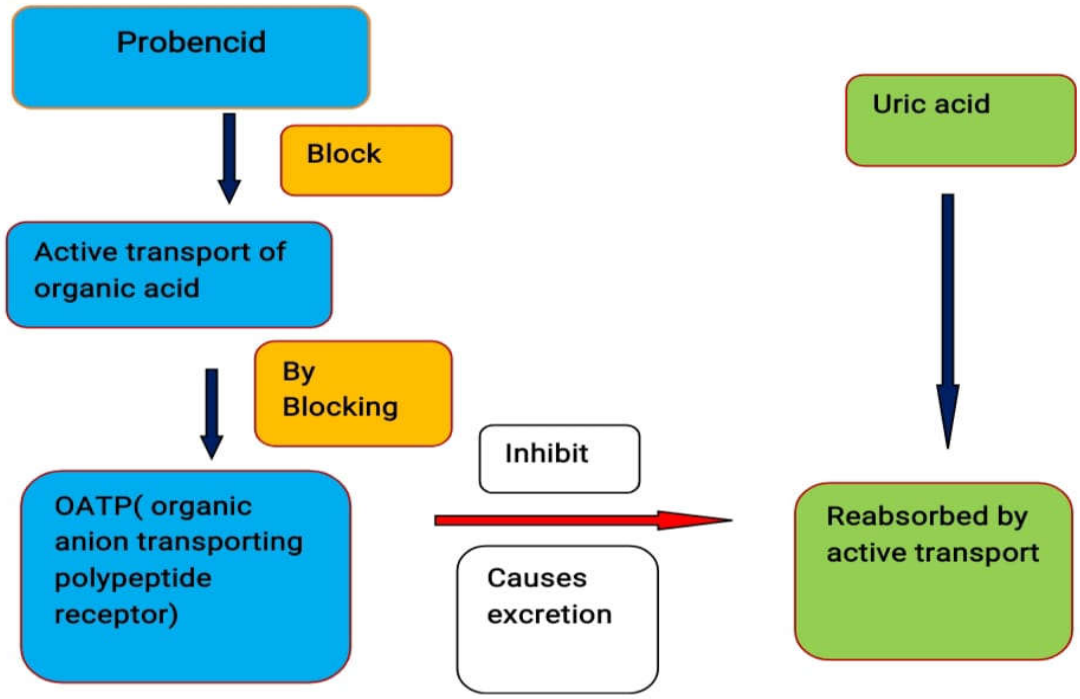


Fig.4 - Mechanism of action of Probenecid

PK AND PD / PHARMACOLOGICAL PROPERTY OF PROBENECID

Absorption

PROB has a low oil:water partition ratio (log10D 0.06 at pH 7) and is a weak acid (pKa 3.7). The maximal dosage for PROB in adults is 3 g, or 2 g taken orally in one dose (around 25 mg/kg) [42]." Healthy adults have a dose-dependent half-life of 2–12 hours, a small apparent volume of distribution (0. 003-0. 014 L/kg), 85–95% binding to plasma albumin, and efficient absorption following oral doses, with a peak in plasma concentrations occurring at 1–5 hours at concentrations of 20–200 Fg/mL [43]. It can be given orally or intravenously. The digestive tract absorbs it entirely after oral treatment, with a maximum dose of 3g per day [44].

Distribution

Because PBN binds 85-95% to albumin and has a volume of distribution of 0.003-0.014 L/kg, it has a high percentage of binding to plasma proteins. This medication has half-life of two to twelve hours, depending on the dosage. Because of its high lipid solubility, PBN diffuses freely across the blood-brain barrier and is actively transported out of the brain.Probenecid's early applications were identified without a

precise understanding of its molecular targets. Three commonly established in vivo renal targets of probenecid are mainly expressed in the proximal tubule: uric acid transporter 1 (SLC22A12/URAT1), organic anion transporters 1 and 3 (SLC22A6/OAT1, SLC22A8/OAT3).Probenecid enters the bloodstream quickly after oral treatment and is strongly linked to albumin [45].

Metabolism

In the liver, PROB is broken down via glucuronide conjugation (- 20%) and alkyl side chain oxidation (- 70%). These metabolites may be responsible for the uricosuric action of PROB in humans and appear to be just as effective as PROB at preventing tubular production of organic acids. Compared to PROB, they are less lipid-soluble, have lower binding affinities to plasma proteins, and are eliminated by the kidney more quickly [46].

Elimination

Both products have a lesser binding affinity to plasma proteins yet impede the tubular secretion of organic acids, they are eliminated more quickly than PBN. A small portion of the drug's by products, which make up 75-85% of total PBN, are expelled unaltered through the urine. A higher alkaline pH promotes excretion [47].

In contrast to other models, a two-compartment open model best described the PK of probenecid in healthy dogs based on the AIC and the plasma concentration-time profile. Since the administered dose may be insufficient to fully saturate probenecid's high-affinity plasma protein binding [48].

DRUG INTERACTION-

Captopril, indomethacin, ketoprofen, ketorolac, naproxen, cephalosporins, quinolones, penicillins, methotrexate, zidovudine, ganciclovir, lorazepam, and acyclovir are a few of the significant clinical interactions that probenecid has with these medications. Probenecid reduces the excretion of these medications in all of these interactions, which may result in higher amounts of these medications Drug Interactions of Probenecid. Probenecid is a medication used to treat gout and hyperuricemia. It can interact with other drugs, affecting their efficacy and safety.

Here are some drug interactions of probenecid [44]. The description of drug interaction is listed in table 3.

Table 3- Drug interaction of probenecid

Interacting Drug	Effect of Interaction	Reference
Penicillin antibiotics	Probenecid increases penicillin levels by reducing renal clearance.	49
Cephalosporin antibiotics	Probenecid increases cephalosporin levels by reducing renal clearance.	50
Non-steroidal anti-inflammatory drugs (NSAIDs)	Increased risk of gastrointestinal side effects.	51
Salicylates (e.g., aspirin)	Reduced uricosuric effect of probenecid.	52
Methotrexate	Increased methotrexate levels, potentially leading to toxicity.	53
Dapsone	Increased dapsone levels, potentially leading to toxicity.	54

MARKETED FORMULATION

The conventional available marketed formulation of probenecid along with its combination drugs and patents are listed in table 4.

Table 4 – marketed formulations of probenecid

Drug name	Generic name	Brand Name	Category	Dosage form	Use
Probenecid	Probenecid and	benemid	Uricosuric agent.	**Tablet (500)**	Treatment of gout, hyperuricemia
Colbenemid	Probenecid+ colchicine	Colbene mid	Uricosuric agent, anti-	Tablet (500 mg	Treatment of gouty arthritis.

			gout.	probenecid + 0.5 colchicine	
Probampacin	Probenecid + ampicillin	Probampacin	Uricosuric agent, antibiotic	Tablets or capsules	Treatment of hyperuricemia, infection
Probenecid	Probenecid	None	Uricosuric agent,	Tablets	Treatment of hyperuricemia

PATENT OF PROBENECID

Patent no.	**Inventor**	**Title of patent**	**Year**
US-3655742-A	Morgans David J; Bavitz Joseph F;Castello Robert A	Process for producing magnesium probenecid tetrahydrate	1969
US-9827279-B2	Yamamoto Naoki,Watanabe Isamu, Suzuki Atsushi.	Xanthine oxidase inhibitor and uric acid production inhibitor	2008
US-3705946-A	Dyke Richard W; Sweeney Martin J	Method treating hyperuricemia	1971
US-4510322-A	Blaine Edward H; Cragoe Jr Edward J; Hirschmann Ralph F	Indacrinone having enhanced uricosuric	1981
US-9827279-B2	Yamamoto Naoki,Watanabe Isamu, Suzuki Atsushi.	Xanthine oxidase inhibitor and uric acid production inhibitor	2008

COMBINATION FORM OF PROBENECID

S. no.	**Combination form**	**Use**
1.	Probenecid + colchicine	Treat gout
2.	Probenecid + penicillin (penicillin G or V)	Inhibit renal excretion
3.	Pobenecid + amoxicillin	Infection like gonrrhea
4.	Probenceid + meropenem	Slowing renal clearance
5.	Probenecid + cidofovir	Antiviral

MICRO AND NANO FORMULATIONS

Nanotechnology is a field where the research is at a boon to improve the physical and chemical properties of drug molecule. This technology overcomes the limitations associated with conventional delivery system. As per the findings; research on probenecid contributed to the formation of various nano and micro based delivery systems as listed in table 5.

Table 5 – micro and nano delivery system of probenecid

Sr. No	Year	Author	Title	Outcome of work	Name of journal	Reference
1.	**2012**	S. P. Vyas, A. K. Jain, N. K. Jain	Formulation and Evaluation of Probenecid Microcapsules	The study formulated and evaluated probenecid microcapsules using different polymers, demonstrating improved release characteristics and bioavailability.	Journal of Microencapsulation	55
2.	**2015**	P. K. Lakshmi, A. S. Kumar, G. S. Kumar	Development of Probenecid Microspheres for Sustained Release	The study developed and optimized probenecid microspheres using different polymers, resulting in sustained release and improved therapeutic efficacy.		56

3.	**2018**	S. S. Katiyar, P. K. Gupta, A. Kushwah, V. S. Belgamwar	Microencapsulation of Probenecid for Enhanced Bioavailability	The study developed and optimized microencapsulated probenecid using ethyl cellulose and Eudragit RS 100, resulting in improved bioavailability and sustained release	Journal of Microencapsulation	**57**
4.	**2020**	R. K. Verma, S. K. Singh, A. K. Singh	Design and Evaluation of Probenecid-Loaded Microemulsions	The study successfully formulated and evaluated probenecid-loaded microemulsions, demonstrating improved solubility and bioavailability.	AAPS PharmSciTech	**58**
5.	**2017**	Liu Y, Wang L, Zhao Y, Zhang L.	Nanocrystal Formulation of Probenecid for Enhanced Solubility	The study formulated and evaluated probenecid nanocrystals, demonstrating improved solubility and dissolution rate.	International Journal of Pharmaceutics	**59**
6.	**2018**	Patel P, Parmar K, Patel J, Shah D.	Solid Lipid Nanoparticles for Probenecid Delivery	The study developed and optimized solid lipid nanoparticles (SLNs) for probenecid delivery, resulting in improved solubility and bioavailability	Journal of Nanopharmaceutics and Drug Delivery	**60**

7.	**2019**	Reddy MS, Pappu S, Kommineni N, Madhusudhan B.	Nanostructured Lipid Carriers for Probenecid Delivery	The study developed and optimized nanostructured lipid carriers (NLCs) for probenecid delivery, resulting in improved solubility and bioavailability.	AAPS PharmSciTech	**61**
8.	**2020**	Singh PK, Sharma N, Sharma S, Kumar V.	Probenecid-Loaded Polymeric Nanoparticles for Enhanced Bioavailability	The study formulated and evaluated probenecid-loaded polymeric nanoparticles, demonstrating improved bioavailability and sustained release.	Journal of Pharmaceutical Sciences	**62**

ANALYTICAL AND BIO-ANALYTICAL OF PROBENECID DRUG

Over the past three decades, significant advancements have been made in the development and validation of bioanalytical methods. Numerous analytical methods, including ion-monitary, fluoremetric, reverse phase high performance liquid chromatography, derivative spectroscopy, and high performance liquid chromatography, have been documented for Probenecid quantitative analysis, while some of these techniques are more complicated, expensive, and time-consuming. Spectrophotometric analysis is a quick, promising, and trustworthy technique for quantitative analysis of probenecid that can be utilised to get over all of these issues [63, 64]. Probenecid analysis involves various analytical methods to determine its presence and concentration in biological samples. Some of these methods are listed in table 6.

Sr. No	Technique	Year	Authors	Description	Outcome	Reference
1.	*HPLC*	2018	Zhang Y, Li X, Wang Y, Li Z.	Reverse-phase HPLC (RP-HPLC) with UV detection was for probenecid's chromophoric properties	*The mobile Phase used was* Acetonitrile: water (50:50, v/v) and s*tationary Phase* was C18 column (250 mm x 4.6 mm, 5 µm). The method was successfully applied to the analysis of probenecid in human plasma, demonstrating good accuracy and precision.	65, 66
2.	LC-MS/MS	2020	Patel P, Parmar K, Patel J, Shah D.	A rapid and sensitive LC-MS/MS method was developed for the quantification of probenecid in human plasma.	LC-MS/MS system with electrospray ionization (ESI) source *Mobile Phase used was* Acetonitrile: water (70:30, v/v) with 0.1% formic acid and *Stationary Phase was* C18 column (100 mm x 2.1 mm, 3 µm)	67, 68
3.	GC	2020	Singh PK, Sharma N, Sharma S, Kumar V.	A sensitive and selective GC-MS method was developed for the determination of probenecid in human plasma.	GC-MS system with electron ionization (EI) source having m*obile Phase as* Helium gas and *Stationary Phase as* DB-5MS column (30 m x 0.25 mm, 0.25 µm) was used	69
4.	*Capillary Electrophoresis*	2017	Liu Y, Wang L, Zhao Y,	The method was successfully applied to the analysis of	CE system with UV detector was used. The m*obile Phase was* 50 mm phosphate buffer (pH	70

			Zhang L.	probenecid in human plasma, demonstrating good accuracy and precision	7.0) and s*tationary Phase was* Fused-silica capillary (50 cm x 50 µm)	
5.	*Thin layer chromatography*			TLC plate (Silica gel 60 F254) was used. The m*obile Phase was* Toluene: ethylacetate: formic acid (6:4:1, v/v/v) and s*tationary phase was* Silica gel 60 F254. The *Rf Valu was found to be 0*.58	A sensitive and selective TLC method was developed for the determination of probenecid in pharmaceutical formulations	71, 72
6.	HPTLC	2019	Patel P, Parmar K, Patel J, Shah D	HPTLC system with UV detectors was used. The m*obile Phase was* Toluene: ethylacetate: formic acid (6:4:1, v/v/v) and s*tationary Phase was* Silica gel 60 F254 plates. The Rf *Value:* 0.58	The method was successfully applied to the analysis of probenecid in pharmaceutical formulations, demonstrating good accuracy and precision	73
7.	FTIR	2020	Patel et al.	FTIR spectroscopy was used to characterize the molecular structure of probenecid.	The FTIR spectrum of probenecid showed characteristic peaks corresponding to its functional groups, confirming its molecular structure	74

8.	TEM/DLS	2020	Singh et al.	TEM or DLS was used to characterize the size and morphology of probenecid nanoparticles	The TEM or DLS analysis showed that the probenecid nanoparticles had a uniform size distribution and spherical morphology	75

CONCLUSION

Probenecid is a medication with a rich history and diverse applications. Its chemistry, biological properties, and analytical methods have been extensively studied, making it a valuable treatment option for various conditions. Probenecid's chemical structure and mechanism of action enable it to effectively treat gout and hyperuricemia by inhibiting uric acid reabsorption in the kidneys. Its potential applications in neuroprotection and cardiovascular disease treatment are also being explored, highlighting its versatility as a therapeutic agent. The analytical methods for probenecid, including high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and ultraviolet (UV) spectrophotometry, provide a robust framework for detecting and quantifying the drug in biological samples. These methods have been optimized for various applications, including pharmacokinetic studies and therapeutic drug monitoring. In conclusion, probenecid's chemistry, biological properties, and analytical methods make it a valuable medication with a range of applications. Its effectiveness in treating gout and hyperuricemia, combined with its potential uses in neuroprotection and cardiovascular disease treatment, highlight its importance in the field of medicine.Probenecid's therapeutic implications are significant, particularly in the treatment of gout and hyperuricemia. Its ability to inhibit uric acid reabsorption in the kidneys makes it an effective treatment option for patients with these conditions. Additionally, its potential applications in neuroprotection and cardiovascular disease treatment may provide new avenues for therapeutic intervention.

Conflict of interest - NONE

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