Journal of Chromatography A 1637 (2021) 461848

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Dilemma on plasmid DNA purification: binding capacity *vs* selectivity

J.F.A. Valente[a,b], J.A. Queiroz[a], F. Sousa[a,*]

[a] CICS-UBI - Health Sciences Research Centre, Universidade da Beira Interior, Avenida Infante D. Henrique, 6200-506Covilhã, Portugal
[b] CDRSP-IPLEIRIA - Centre for Rapid and Sustainable Product Development, Instituto Politécnico de Leiria, Rua de Portugal - Zona Industrial, 2430-028Marinha Grande, Portugal

ARTICLE INFO

Article history:
Received 13 November 2020
Revised 21 December 2020
Accepted 23 December 2020
Available online 30 December 2020

Keywords:
Binding capacity
Chromatographic supports
DNA chromatography
Selectivity
Specific ligands

ABSTRACT

Plasmid DNA chromatography is a powerful field in constant development and evolution. The use of this technique is considered mandatory in the production of an efficient and safe formulation to be applied for plasmid-mediated gene therapy. Concerning this, the search for an ideal chromatographic support/ligand combination motivated scientist to pursue a continuous improvement on the plasmid chromatography performance, looking for a progression on the ligands and supports used.

The present review explores the different approaches used over time to purify plasmid DNA, ambitioning both high recovery and high purity levels. Overall, it is presented a critical discussion relying on the relevance of the binding capacity versus selectivity of the supports.

1. Introduction

Gene therapy has been attracting great interest in recent years namely, by using nucleic acids as active biopharmaceuticals to be applied in this therapy, as a way to correct genetic abnormalities or, in genetic vaccination to induce immune responses [1]. Non-viral vectors, like plasmid DNA (pDNA), are being investigated and considered in various current clinical trials and supercoiled (sc) plasmids are of particular interest due to their higher safety, integrity and biological efficiency [2].

The production of pDNA is usually performed in a recombinant *Escherichia coli (E. coli)* host through fermentation, accounting with around 3% w/w [3] of the global components of the *E. coli* extract (Fig. 1). Considering all the impurities present in the pDNA-containing lysate, regulatory agencies such as Food and Drug Administration (FDA, USA) and European Medicines Agency (EMA) impose several criteria that must be accomplished to prepare sc pDNA for therapeutic applications [4]. Thus, it is imperative to proceed to the total isolation of the pDNA from cellular debris and other impurities like RNA, proteins and genomic DNA (gDNA). Most of these impurities share some similar physicochemical characteristics with pDNA, like negative charge (RNA, gDNA and endotoxins), molecular mass (gDNA and endotoxins) and hydrophobicity (endotoxins) which highly restrains the isolation procedure [5]. Presently, the processes used for the pDNA recovery and purification comprise several sequential operations, which are combined to achieve a maximum sc pDNA recovery and purity level, minimizing the pDNA degradation (Fig. 2). This review is focused on the purification of the pDNA since this is one of the most important steps with great impact on the target biomolecule quality as well as on the efficiency, sustainability and robustness of the global biotechnological process. The application of different types of chromatography for pDNA purification has already been extensively reviewed. So, in a different perspective, this review intends to show how research efforts have been focused on the improvement of supports and the design of ligands as a way to overcome the pDNA purification challenges, trying to answer to the general dilemma related with the pursuit of higher binding capacities or enhanced selectivity.

2. pDNA purification by chromatography

Plasmid purification by chromatography deals with some concerns associated with the diversity of biomolecules present in the lysate extracts and their characteristics, such as size, shape, conformation, and rheological properties. Moreover, most of the impurities like RNA, gDNA or endotoxins, share with pDNA some properties which poses some challenges on their separation [7]. The elimination of such impurities is so critical that researchers try their elimination or reduction since the beginning of the downstream processing, even before the main purification process. The reduction of gDNA levels is usually achieved by denaturation, during the alkaline lysis and the RNA content can be reduced by the addition of RNA-digesting enzymes or including a precipitation step

* Corresponding author.
E-mail address: fani.sousa@fcsaude.ubi.pt (F. Sousa).

https://doi.org/10.1016/j.chroma.2020.461848
0021-9673/

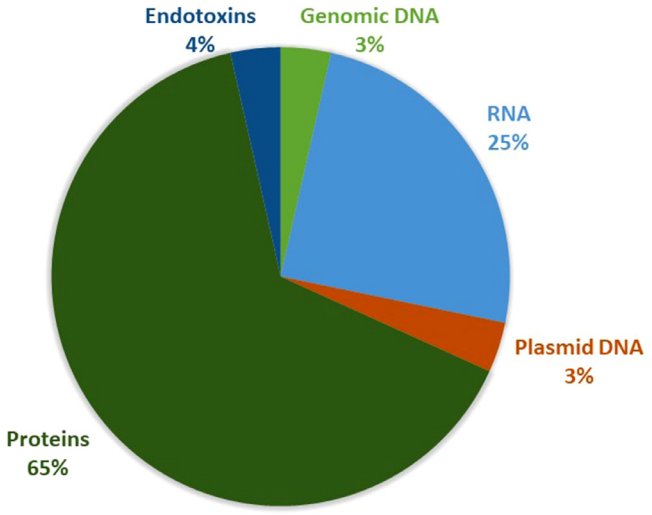


Fig. 1. Average composition of an *E. coli* lysate - adapted from [6].

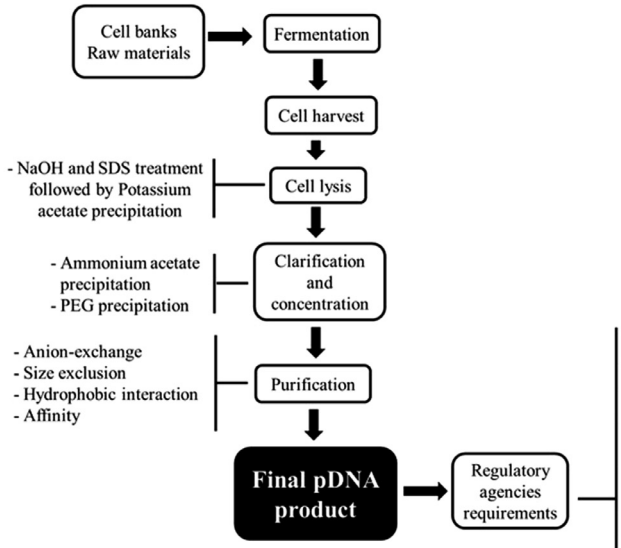
with salts, at the clarification stage. However, the RNA digestion by RNase will not be an option if the purification of biotherapeutics is intended, since, in accordance with the criteria of regulatory agencies, the use of animal-derived enzymes is not allowed [8]. Endotoxins removal is extremely important since this lipopolysaccharide component of *E. coli* can produce symptoms of toxic shock syndrome if present in sufficient quantities *in vivo* [9]. The elimination of endotoxins is commonly performed in the purification step, by chromatography.

Historically, the first methods used on pDNA purification were based on sucrose or cesium chloride–ethidium bromide density gradients ultracentrifugation. However, these methods are time-consuming and difficult to scale up, and also use toxic and mutagenic reagents making them undesirable for pDNA pDNA preparation [10]. To overcome these hurdles, liquid chromatography has been established as a central technique to guarantee the quality of the recovered pDNA products. Chromatography can explore properties like size, charge, hydrophobicity, accessibility of the nucleotide bases, topological features and/or affinity, promote the interaction between pDNA and the chromatographic matrices, expecting some selectivity and resolution, to finally recover the desirable sc pDNA species [11]. Initially, it was found a huge limitation related to the low capacity of the available matrices to bind large molecules, such as pDNA. Also, in the case of resins that presented higher capacities, they usually presented a lack of selectivity, due to the similarity between pDNA and impurities. This was the start point to define a strategy to reinvent the chromatographic supports.

3. Reinventing the chromatographic supports

Chromatography is a well-characterized and well-established method, which was initially exploited for the purification of small molecules and then, largely used by the pharmaceutical industry for proteins purification, since the 1950s [12]. The first matrices produced to perform the purification of proteins were constituted by particles with a mean size between 50 to 500 µm and a pore size surrounding the 30 nm. These dimensions were not suitable for pDNA purification, since the average pore size of a common chromatographic resin is usually smaller or of the same size of the radius of gyration of a pDNA molecule. This feature is responsible for the limited access of pDNA to the pores of standard chromatographic supports [7]. Over the years, the development of chromatographic supports for pDNA purification has tried to overcome the limitations associated to the diffusion of these molecules or with the binding capacities, culminating with the study of different supports, like the superporous matrices, the monoliths or adsorptive membranes [13–17].

The most frequently used method for primary capture of pDNA is anion-exchange chromatography (AEXC) [18] however, other chromatographic techniques also enable the isolation of pDNA



Specification	Approval criteria	Recommended assay
Plasmid		
Appearence	Clear, colourless solution	Visual inspection
Identity of plasmid	Restriction map as expected, sequence homology	Restriction mapping sequencing, PCR
Plasmid concentration	According to application	A260, HPLC, fluorescenece
Potency	According to application	Cell transfection
Impurities		
Proteins	Not detectabel, <0.01 µg/dose	Bicinchonic acid (BCA) assay, SDS-PAGE
RNA	Not detectable	0.8% agarose gel
gDNA	<0.05 µg/µg plasmid, <0.01 µg/dose	Hybridisation blots, PCR, fluorescence
Endotoxins	<0.1 EU/µg plasmid, <5 EU/ kg body weight	Limulus ameobocyte lysate (LAL) assay

Fig. 2. Generic upstream and downstream processing steps used for pDNA production- adapted from [2].

Table 1
Different chromatographic methods applied in pDNA purification.

Chromatographic methods	Description	Advantages	Disadvantages
Anion-exchange	Uses the electrostatic interactions between negatively charged plasmids and positively charged stationary phases	Ability to selectively isolate the sc pDNA from oc pDNA and also from other components of the lysate	Co-purification of biomolecules with charge and structure similar to pDNA like gDNA, endotoxins and some RNA, and also, the low capacity to bind pDNA.
Size Exclusion	Explores the different hydrodynamic sizes of the plasmids and their impurities to purify the target molecule	Good removal of RNA and proteins	Difficulty to remove gDNA (it elutes near the pDNA)
Hydrophobic Interaction	Uses the hydrophobic nature of the single-stranded nucleic acid impurities (RNA, denatured gDNA, and denatured pDNA) and endotoxins to promote the binding and retard the flow of these impurities through a hydrophobic support	Ability to isolate pDNA from endotoxins and single-stranded nucleic acids	Elution at high salt concentrations
Affinity Chromatography	Exploit the natural biological processes based on the molecular recognition for the selective purification of the sc pDNA ensuring that this sample is within the standards of regulatory agencies	One-step purification of pDNA from the lysate also, pure sc pDNA can be obtained	Use of biological ligands that can be unstable and are also associated with a low binding capacity

from lysates, such as the size exclusion chromatography (SEC) [19], the hydrophobic interaction chromatography (HIC) [20] and the affinity chromatography (AC) [4,8,21]. In Table 1 it is presented a summary of these chromatographic techniques, as well as the main advantages and disadvantages of each one.

As mentioned above, AEXC is the most used chromatographic technique namely in commercially available kits for pDNA purification, such as the ones available from Promega, Qiagen or NZYTech. However, it is important to refer that using AEXC, pDNA can co-elute with gDNA, high molecular weight RNA and endotoxins since these molecules have similar affinities for anion-exchange matrices [10]. To partially solve this problem, RNase A is commonly added to degrade RNA impurities, when pDNA is to be used in molecular biology protocols. Apart from the obvious costs involved, there are also concerns regarding RNase because it is purified from the bovine pancreas and, as previously mentioned, regulatory authorities recommend that bovine-derived materials should be avoided in the production of biotherapeutics [22].

The Q-Sepharose anion-exchange contains quaternary amine groups (Q) and has been largely used for globular proteins purification. At least, in theory, this support has not the most suitable characteristics for plasmids purification, mainly due to the small pore size, which limits these molecules to access to the pores. Besides, this kind of problem also can lead to low binding capacity (Table 1) [22]. To suppress this capacity limitation, matrices like Fractogel EMD DEAE from MERCK have been developed. This matrix is constituted by beads with large surface area, containing coupled polymer tentacles and large pores, which enables efficient capture of the pDNA culminating in higher binding capacities (Table 2) [23]. Moreover, there are also in the market matrices made of superporous agarose beads that can contain two sets of pores, the diffusion pores and so-called superpores or flow pores, in which the chromatographic flow can transport substances to the interior of each bead increasing the surface area of the bead and also increasing the binding capacity of these matrices [24]. The superporous agarose beads have large connecting flow pores with sizes that range from 1/4 to 1/20 of the overall bead diameter [24,25]. Superporosity not only improves the access of pDNA to the internal voids but may also allow convective pore flow to take place and consequently improve internal mass transfer. As an example, Deshmukh and collaborators developed in 2005 the CELBEADS that has approximately 3 µm of pore size and can achieve pDNA dynamic binding capacities of 1.4 mg/mL of adsorbent with recovery yields of 77% and 52% in batch and column modes, respectively (Table 2). The final plasmid product was also found to be free from RNA, gDNA, proteins and endotoxins [26]. Another example is the case of Cytopore (GE Healthcare, Sweden), also a superporous support with much larger pores, ranging the 30 µm, for which it was described a higher binding capacity, of about 13 mg/mL, but no information concerning the purity of the pDNA was available [27].

Particles with the possibility to adjust particle/pore diameters were also produced to adapt them to their purpose, for example, depending on their use in analytics or large-scale purification of nucleic acids [28]. Butyl-6PW and the octyl-6PW from Tosoh are examples of this versatility on the support production. These matrices were sequentially used, being the first support responsible for the retention of RNA and proteins and the second responsible for the adsorption of pDNA and gDNA [29]. In this approach, it was described as a capacity of 1.1 mg/mL and a pDNA recovery yield of 90%. The process was completed with a final polishing step with AEXC, to completely remove all impurities [28].

With the development of the new methodologies, membranes like Mustang from Pall Corporation or CIM monoliths from BIA Separations have been developed and applied in pDNA isolation and, with these systems, it was possible to reach high pDNA binding capacities (above 10 mg/mL) [30]. Concerning the monoliths, they display a higher porosity with large flow-through pores which enable the mobile phase to easily travel through the support and also, allow it to have an increased permeability and a lower back pressure in the chromatographic systems [31,32]. These unique characteristics allow fast and efficient separation in short processing times, reducing product degradation and buffer consumption, without diminishing the resolution and separation efficiency. Tarmann and collaborators in 2008 used different chromatographic supports to compare their performance with the CIM-DEAE monolith finding that this support presented the fastest adsorption rate and highest binding capacity (13 mg pDNA/mL) (Table 2) [33]. In a different approach, a pyridine-modified monolith was also used, focusing a HIC strategy, with interesting results concerning nucleic acids purification. In that research work, a pDNA binding capacity of 3 mg/mL was obtained, being also described the recovery of sc pDNA with homogeneity of 98% and recovery yield of 96% [20]. Nonetheless, some drawbacks are pointed to this kind of chromatography, namely because of the need of high salt concentration, and conjugation with other steps, thereby increasing the operation time and costs [11].

In the case of membranes application for the isolation of pDNA from lysates, Pereira and collaborators in 2010 were able to isolate the pVAX1-*LacZ* pDNA from impurities, particularly from RNA, us-

Table 2
Characterization of chromatographic supports used in pDNA purification.

Stationary phase		Basic material	Functional group	Bead size (μm)	Pore size (μm)	pDNA (kb)	Adsorption Capacity (mg pDNA/mL)	Ref.
Beads								
	Q-Sepharose Fast Flow	Agarose	Q	90	0.19	5.9	0.72	[22]
	Q- Ceramic Hyper D	Hydrogel/Ceramic	Q	50	0.3	5.9	>5.3	[22]
	Fractogel EMD DEAE	Methacrylate	DEAE	40-90	0.08	5.9	2.45	[22]
	Sephadex G	Dextran crosslinked with epichlorohydrin		50	-	6.1	-	[39]
	Source 30Q	Polystyrene divinylbenzene	Q	30	0.002-0.1	6.9	0.707	[23]
Superporous beads								
	POROS 50 HQ	Polystyrene divinylbenzene	PEI Q	-	<0.8	5.9	2.12	[22]
	CELBEADS	Cellulose		150-500	≈ 3	9.8	1.4	[26]
	Cytopore		DEAE	230	30	4	13	[27]
	Superporous Agarose	Agarose	Q	45-75	4	7	1-2	[40]
	Superporous Agarose	Agarose	PEI	45-75	2	7	3-4	[40]
Membranes								
	Mustang	Polyethersulfone	Q	-	0.8	6.1	10	[41]
	Natrix	Hydrogel	Q	-	0.45	6.4	12.4	[42]
	Sartobinds Epoxy 75	Cellulose	Linear alkyl	-	0.45	6.05	32.5	[34]
Monoliths								
	CIM DEAE	Methacrylate	DEAE	1.5	0.7-0.95	4.9	13.42	[33]
	CIM C4	Methacrylate	Pyridine	-	-	4.7	3	[20]
	CIM C4 HLD	Methacrylate	Butyl	0.95-1.1	2	4.7	≥2.5	[43]
Cryogels								
		PHEMAH	Cibacron Blue F3GA	-	10-100	4.3	32.4 mg/g polymer	[44]
			N-methacryloyl-(l)-histidine methyl ester (MAH)	-	10-100	4.3	13.5 mg/g polymer	[36]

ing a membrane modified with a linear alkyl chain ligand known for its hydrophobic behaviour [35]. However, it is important to refer that although this membrane could isolate the pDNA from the impurities, no attempt to specifically purify the sc pDNA species was mentioned. The plasmid was recovered in the flowthrough, with a yield of 73% and 60% of purity. The characterization of the binding capacity of these membranes revealed a high value, of about 32.5 mg/mL (Table 2). The method was described as fast, simple and effective enabling the reduction of the number of steps in purification processes of pDNA [34]. Therefore, it is important to note that the exploitation of hydrophobic interactions relies on the differences between the pDNA isoforms (namely the sc isoform) and more hydrophobic nucleic acids such as RNA, denatured gDNA, oligonucleotides and denatured pDNA forms [35]. This phenomenon occurs due to the presence of high concentration of a kosmotropic salt that will be responsible for the packaging and shielding of the hydrophobic bases of pDNA molecules, leading to a minimal interaction with the hydrophobic matrix. On the other hand, single-stranded nucleic acids show a higher exposure of the hydrophobic bases, and thus, strongly interact with the hydrophobic ligands. Concerning the endotoxins, as they are highly hydrophobic molecules, a stronger interaction occurs in hydrophobic columns, in comparison with pDNA [34].

Another emerged alternative for large molecules purification, such pDNA, are the cryogels. These materials present many advantages including their large pores and short diffusion path [36]. The large pores of cryogels, similarly to what was previously reported for superporous matrices, will allow penetration of large pDNA molecules to the internal surface area. High accessibility to binding sites and negligible internal mass transfer limitations are also characteristics of cryogels, making them attractive for pDNA binding studies [37,38]. However, as for some of the other supports, there are no reports of cryogels for the sc pDNA separation from the other pDNA isoforms.

Overall, to improve parameters such as selectivity, capacity, durability and cost-effectiveness, researchers are not only dedicated to the improvement of the base material of the chromatographic supports, but they are also paying a lot of attention to the design of ligands. Researchers are mainly trying to conjugate the optimization of the supports with specific ligands, independently on the chromatography type, to achieve the best chromatographic performance on pDNA purification (Fig. 3). Concerning the previously mentioned, the following topic will focus on the description of the specific ligands applied in pDNA chromatography.

4. Design of specific ligands for sc pDNA isolation

Current processes for pDNA purification, aiming its therapeutic application, require several chromatographic steps, which make pDNA production not only time-consuming but also costly [11]. Standard chromatographic methods present some limitations, when considering pDNA purification. In brief, AEXC can present poor selectivity towards pDNA due to their non-specific binding to the anion-exchange resin, while SEC has limited capacity and selectivity for pDNA, being mainly useful as polishing step, and although the efficient separation of pDNA from endotoxins and single-stranded nucleic acids in HIC, the pDNA elution occurs at high salt concentration and the isoforms separation is very difficult [5]. To suppress some of these limitations, AC employs a specific immobilized ligand to interact with the target biomolecule loaded under particular conditions that favour the binding to the support and also intends to promote the largest pDNA recovery level as possible. Due to the improved selectivity, this technique can achieve purification in a single process. However, the selectivity and specificity can only be achieved when ligands can recognize the molecule of interest which, in this case, is the sc pDNA [8,45]. Previous affinity strategies used on pDNA purification include a DNA binding protein, triplex DNA formation, aromatic affinity chromatography and amino-acid affinity chromatography [8,46].

Considering this approach, nucleic acids isolation was already established using a DNA-binding protein as a ligand. To the best of our knowledge, the first authors reporting the use of this chromatography was Woodgate and collaborators in 2002. They used a bifunctional zinc finger (ZNF) DNA-binding protein fused to Glutathione-S-transferase for the isolation of pDNA from a complex lysate, bearing the ZNF recognition. However, using this approach

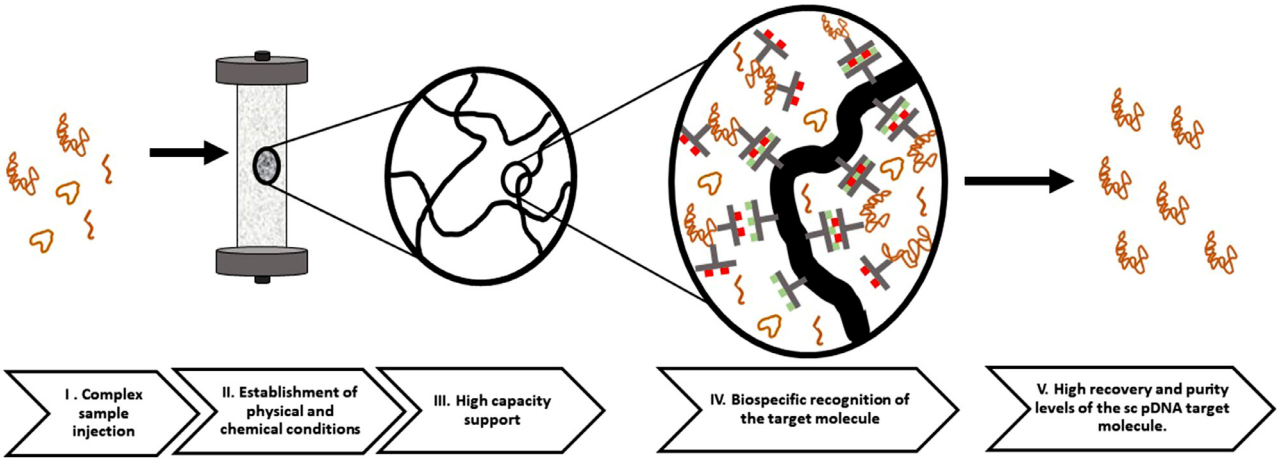


Fig. 3. Schematic representation of the ideal purification approach for sc pDNA. (I) Complex sample injection; (II) Establishment of physical and chemical conditions; (III) High capacity support; (IV) Biospecific recognition of the target molecule; (V) High recovery and purity levels of the sc pDNA target molecule.

it was only observed yield of 10% and no data about the elution of the pDNA was given [47]. This research group also used a different hetero functional protein, the LacI-His6-GFP, and in this case, they improved the recovery of pDNA to more than 80%, achieving a high purity in the final pDNA product that was free from detectable RNA and protein and with minimal genomic DNA contamination [48]. A different strategy is based on triple helix affinity, which involves the formation of Hoogsteen hydrogen bonds between thymine (T) and adenine (A) to form TAT triplexes, or between protonated cytosine (C+) that specifically recognizes guanine (G) to form CG-C+ triplexes [7]. To guarantee some stability during the use of these triplexes is imperative the use of acidic pH. The binding of pDNA occurs via an intermolecular triplex formation with a biotinylated oligonucleotide as a ligand, and the recovery occurs by loading the column with a mild alkaline buffer which is responsible for the destabilization of the Hoogsteen H-bonds. Sherman and collaborators in 1997 used this kind of chromatography on the purification of the plasmid pXL2563 which contained a poly-GAA sequence targeting the CTT oligonucleotide covalently bound to the Sepharose column used. The maximum plasmid recovery was 42% and the impurities were eliminated (as it is the case of RNA) or significantly reduced (for proteins and gDNA) [49]. A less explored, but still an important strategy, is related to the use of aromatic affinity ligands, being the most common the six-membered ring benzene [50]. These compounds could fully isolate the sc isoform being described purity levels between 98.8% and 100% and recovery yields of about 100% as is possible to see in Table 3.

Amino acids have been also used in the last years for affinity chromatography, as a way to mimic the natural interaction phenomena occurring between nucleic acids and proteins in biological organisms. Until now amino acids such as arginine, histidine, methionine among others have been successfully applied in the isolation of the sc pDNA isoform (Table 3) [4,51]. These specific ligands revealed to be a promising approach for pDNA purification, enabling the selective biorecognition of the sc pDNA isoform and allowing the elimination of the remaining pDNA isoforms and other impurities [52]. The main advantage of these affinity ligands is the multitude of interactions (hydrophobic, electrostatic, cation-π, van der Waals forces and/or hydrogen bond) that can be established with the sc pDNA, promoting this kind of affinity towards the target biomolecule, which consequently results in a more effective and selective separation. Also, with some of these ligands, there is a possibility to mainly favour some particular interactions through the adjustment of the binding and elution conditions, such as the temperature, flow rate and buffer composition (pH, ionic strength or presence of competitive agents), giving more versatility to the method [3]. When amino acids were immobilized on beads the sc pDNA purity levels ranged between 97 to 100% being the recovery yields between 40 to 70% [51–54]. It is also important to refer that, although selectivity is of high importance, and ideal chromatographic support should also be able to promote the isolation of high amounts of pure pDNA. Regarding this, some intents have been made to combine the selectivity of these ligands with the capacity of modern supports, by using, for example, monoliths. As presented in Table 3, when monoliths were modified with different ligands and used to obtain pDNA, the purity levels of this biomolecule ranged between 98.3 to 100% and the recovery was from 45.3 to 91.3% [21,53–57]. Also, when the capacity of the different supports is evaluated it is notorious that monoliths present a significantly higher pDNA binding capacity than the conventional beads-based chromatographic supports. For example, when histidine is immobilized on an agarose based matrix, the maximum DBC achieved was of about 0.5 mg/mL, while the immobilization of the same amino acid onto monolithic support yielded a DBC of around 11 mg/mL [53,57]. Also, arginine, a well-studied ligand for the sc pDNA isolation, was immobilized in both types of supports, and the higher DBC were found for the monoliths (5.18 mg/mL against 1.11 mg/mL in agarose support) [54].

The search for the most suitable ligands leads researchers to use multimodal ligands able to promote multiple and different elementary interactions with the target molecule. This type of ligands presents more than one active site which could enable simultaneously ionic and hydrophobic interactions, and these can be differently explored during the chromatographic run, by adjusting the experimental conditions and combining gradients [58,59]. This versatility usually increases the selectivity and specificity of the chromatographic process [60,61].

As an example, amino acids derivatives, such as histamine, were already exploited in this field, to accomplish the purification of sc pDNA. Černigoj and collaborators in 2013 exploited the hydrophobic (from the imidazole group) and also the ionic behaviour (from the amino group) of histamine to isolate pDNA from a sample made of oc and sc pDNA, combining a descending pH gradient with the use of $(NH_4)_2SO_4$ [50]. In another study, Silva-Santos and collaborators in 2016 used a commercially available multimodal matrix, the Capto adhere with an N-Benzyl-N-methyl ethanol amine ligand (from GE Healthcare Biosciences). In this work, hydrophobic interactions from the benzyl group and ionic interactions from the amine and hydroxyl groups of this ligand were exploited. The results revealed that 91.8% of the pDNA collected in

Table 3
The influence of different ligands and supports in the pDNA adsorption, recovery and purity.

		Ligand	pDNA (kb)	Main Conditions	pH	Capacity (mg/mL)	Recovery (%)	Purity (%)	Year	Ref.
BEADS	**Aromatic**	Phenyl		Hydrophobic	8		≈ 100	≈ 100	2005	[70]
		Phenyl boronate (3aPABA)	6.05	Hydrophobic	5.2	-	96	-	2011	[71]
		Mercaptopyrimidine	3.697	Hydrophobic	8	-	68.5	98.8	2013	[72]
		3,8-diamino-6-phenylphenanthridine (DAPP)	6.05	Ionic	5	0.337	74	100	2013	[73,74]
		N-Benzyl-N-methyl ethanol amine	3.7	Ionic	8	-	-	92.2	2016	[62]
		1,3-bis(4-phenylamidinium) triazene(Berenil)	6.05	Hydrophobic	8	-	87	99	2014	[75]
	Amino acid	Histidine	6.05	Hydrophobic	8	0.530	40	100	2006/2007	[53,63]
		Arginine	6.05	Ionic	8	-	79	97	2009	[52]
			8.702	Ionic	8	1.11	39.18	99	2013	[54]
			6.06	Ionic	8	1.7	43	92	2020	[76,77]
		Lysine	6.05	Ionic	8	-	45.5	100	2011	[78]
		Methionine	6.06	Hydrophobic	8	-	39 µg/ mL	97	2014	[4]
		Tyrosine	6.06	Hydrophobic	8	-	49.7	98.2	2019	[79]
	Triple helix	S-$(CTT)_7$	-	Hydrophobic	4.5	-	42	-	1997	[49]
		Polypyrimidineoligonucleotide	-	Ionic	5	0.028	38.8	-	1998	[80]
	Protein-DNA	LacI-His6-GFP	-	ionic	7.4	-	>80	-	2005	[48]
		Zinc Finger-Glutathione S-Transferase	-	-	8	-	10	-	2002	[47]
		LacI peptide	2.7	Ionic	7.4	0.022	81	92	2008	[45]
MONOLITHS	**Aromatic**	Carbonyldiimidazole(CDI)	6.05	Hydrophobic	8	-	74.4	100	2011	[81]
			14, 6.05 and 2.686	Hydrophobic	8	5.319, 5.478, 5.891	-	-	2014	[82]
	Amino acid	Histamine	5.1	-	variable	1.3	95	98	2013	[55,56]
				Hydrophobic	7.4	2.7	62	98.5		
				Ionic	5	4.2	-	-		
		Arginine	8.702	Ionic	7.9	5.18	83.5	100	2013/2015	[54]
			7.9	Ionic	7.5	2.53	-	93.3 - 98.5	2020	[83]
		Histidine	6.05	Hydrophobic	8	11.03	-	-	2015	[57]
		Agmatine	6.471	Ionic	9.6	-	45.3	99.6	2016	[21]
				Hydrophobic	8	3.2	51.8	98.3		

the first peak was in the oc form, whereas 92.2% of the pDNA collected in the second peak was supercoiled [62].

At the moment the pursuit of the ideal ligand motivates scientists to explore bioinformatic tools and combining them with molecular docking. All these technological tools move researchers to a new level of affinity ligands design ability since they can use combinatorial methods for systematic generation and screening of large numbers of novel compounds [63,64]. Concerning the combinatorial approach, there are already some studies where different scaffolds with different linkage molecules (like triazine or Ugi) were used to quickly and easily search for the suitable affinity ligand to be applied for the recovery of the target biomolecule [65]. Additionally, using these linkage molecules it could be possible to use and/or combine ligands such as DNA binding proteins, amino acids, nucleotides among others on the same chromatographic bead expecting to increase the sc pDNA recovery and purification yields. This strategy aims to modulate the specific interactions occurring between the ligand and the target molecule, combining electrostatic, hydrophobic interactions, van der Waals forces and/or hydrogen bonding. Nevertheless, from the best of our knowledge, there are no research works exploiting these techniques for pDNA chromatography. However, a diversity of studies simulating the DNA linkage to peptides or drugs for other kinds of applications are easily found what brings the opportunity to use these techniques in the specific ligand design to improve pDNA chromatography [66-69].

5. Concluding remarks and future perspectives

Selectivity and capacity in purification processes can present an opposite tendency, so researchers have been making a huge effort to counter this trend. Important progress on pDNA chromatography has been done improving drastically the suitability of the supports for large molecules. Concerning the described until now, it could be said that future will involve the use of supports with large surface contact areas and with large pores enabling not only a higher number of binding sites to the desired molecule but also promoting a better flow of the molecules within the support. With this approach, the support ability to bind and promote the recovery of large amounts of pDNA will increase, which will also enhance the economic feasibility of the chromatographic process applied on the recovery and purification of pDNA molecules. Furthermore, it is well known that the performance of chromatographic separations depends on several factors namely the flow of the mobile phase within the column, which will influence axial dispersion and peak widening, with impact on separation efficiency and purity. In this regard, scientific studies have shown that the use of ordered media provides significantly improved chromatographic performance [84]. Following the attempt to provide structural and morphological organization, additive manufacturing is beginning to be used in the production of chromatographic supports as a fast, highly accurate and reproducible methodology [85]. This method of manufacturing makes it possible to control the size, shape, position, alignment and configuration of the mono- or multi-material support structure to create complex structures that are impossible to produce conventionally. The use of additive manufacturing is expected to improve the performance of separation, resolution, productivity and cost-efficiency of the pDNA chromatographic supports and could be the future trend on the efficient pDNA chromatographic columns production.

Regarding the selectivity, continuous efforts must be done in ligands development, as they are crucial agents for the sc pDNA purification. In this regard, methodologies such as bioinformatic tools and molecular docking could help scientists to developed improved and highly targeted purification ligands. The mastery of this technology will allow the control of the chromatographic process,

reducing the amount of the required chemicals for impurities elimination and decreasing the chromatographic running time. Using these methodologies it is expected the establishment of chromatographic processes economically and environmentally sustainable.

Overall, science and technology start to work synergistically on the development of specific ligands, which could enable better recognition of the target molecule and, consequently could lead to high recovery and purity yields. The scientific community is more and more convinced on the relevance of focusing both on the improvement of the binding capacity of the supports and enhancement of selectivity through the design of more specific ligands, to globally improve the pDNA purification performance.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology (FCT), through the project UIDB/00709/2020, and by the project PTDC/BII-BBF/29496/2017 (PUREmiRSILs) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. J.F.A. Valente also acknowledges PhD fellowship (Ref SFRH/BD/96809/2013).

References

[1] A.B. Hill, M. Chen, C.K. Chen, B.A. Pfeifer, C.H. Jones, Overcoming gene-delivery hurdles: physiological considerations for nonviral vectors, Trend Biotech.34 (2) (2016) 91–105.
[2] D.M.F. Prazeres, G. Ferreira, Design of flowsheets for the recovery and purification of plasmids for gene therapy and DNA vaccination, Chem. Eng. Process. Intens.43 (5) (2004) 609–624.
[3] A. Sousa, F. Sousa, J.A. Queiroz, Differential interactions of plasmid DNA, RNA and genomic DNA with amino acid-based affinity matrices, J. Separat. Sci.33 (17–18) (2010) 2610–2618.
[4] J.F.A. Valente, A. Sousa, J.A. Queiroz, F. Sousa, Selective purification of supercoiled p53-encoding pDNA with l-methionine–agarose matrix, Anal. Biochem.459 (2014) 61–69.
[5] A.Sousa, F.Sousa, J.A.Queiroz, Differential interactions of plasmid DNA, RNA and genomic DNA with amino acid-based affinity matrices, 33(17-18) (2010) 2610-2618.
[6] J.Stadler, R.Lemmens, T.Nyhammar, Plasmid DNA purification, The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications 6(S1) (2004) S54-S66.
[7] A. Ghanem, R. Healey, F.G. Adly, Current trends in separation of plasmid DNA vaccines: a review, Anal. Chim. Acta760 (2013) 1–15.
[8] F. Sousa, D.M.F. Prazeres, J.A. Queiroz, Affinity chromatography approaches to overcome the challenges of purifying plasmid DNA, Trend Biotech.26 (9) (2008) 518–525.
[9] Z. Wei, W. Huang, J. Li, G. Hou, J. Fang, Z. Yuan, Studies on endotoxin removal mechanism of adsorbents with amino acid ligands, J. Chroma. B 852 (1–2) (2007) 288–292.
[10] G.N. Ferreira, G.A. Monteiro, D.M. Prazeres, J.M. Cabral, Downstream processing of plasmid DNA for gene therapy and DNA vaccine applications, Trend Biotech.18 (9) (2000) 380–388.
[11] M. Diogo, J.A. Queiroz, D.M.F. Prazeres, Chromatography of plasmid DNA, J. Chroma. A 1069 (1) (2005) 3–22.
[12] D.A.Egas, M.Wirth, Fundamentals of protein separations: 50 years of nanotechnology, and growing, 1 (2008) 833-855.
[13] D.I. Franco-Medrano, P. Guerrero-Germán, R.M. Montesinos-Cisneros, J. Ortega-López, A. Tejeda-Mansir, Plasmid pVAX1-NH36 purification by membrane and bead perfusion chromatography, Bioprocess Biosyst. Eng. 40 (3) (2017) 463–471.
[14] L.F. Amorim, R. Gaspar, P. Pereira, U. Černigoj, F. Sousa, J.A. Queiroz, Â. Sousa, Chromatographic HPV-16 E6/E7 plasmid vaccine purification employing L-histidine and 1-benzyl-L-histidine affinity ligands, Electrophoresis 38 (22-23) (2017) 2975–2980.
[15] A.T. Hanke, M. Ottens, Purifying biopharmaceuticals: knowledge-based chromatographic process development, Trend Biotech.32 (4) (2014) 210–220.
[16] C. Boi, Membrane chromatography for biomolecule purification, Curr. Trend Future Dev. (Bio-) Membr. (2019) 151–166.
[17] S. Cardoso, P. de Alcântara Pessoa Filho, F. Sousa, A.R. Azzoni, Arginine and di-arginine ligands for plasmid DNA purification using negative chromatography, Separat. Purif. Tech.202 (2018) 281–289.
[18] H.S. Barbosa, J.C. Marcos, in: Plasmid Purification, Therapeutic Applications, Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, 2009, pp. 1–22.
[19] L.Z. Li, Y. Liu, M.S. Sun, Y.M. Shao, Effect of salt on purification of plasmid DNA using size-exclusion chromatography, J. Chroma. A 1139 (2) (2007) 228–235.
[20] S. Cardoso, U. Černigoj, N.L. Krajnc, A. Štrancar, Chromatographic purification of plasmid DNA on hydrophobic methacrylate monolithic supports, Separat. Purif. Tech.147 (2015) 139–146.
[21] D. Bicho, C. Caramelo-Nunes, A. Sousa, F. Sousa, J.A. Queiroz, C. Tomaz, Purification of influenza deoxyribonucleic acid-based vaccine using agmatine monolith, J. Chroma. B 1012 (2016) 153–161.
[22] A. Eon-Duval, G. Burke, Purification of pharmaceutical-grade plasmid DNA by anion-exchange chromatography in an RNase-free process, J. Chroma. B 804 (2) (2004) 327–335.
[23] J. Urthaler, R. Schlegl, A. Podgornik, A. Strancar, A. Jungbauer, R. Necina, Application of monoliths for plasmid DNA purification: Development and transfer to production, J. Chroma. A 1065 (1) (2005) 93–106.
[24] P.E. Gustavsson, A. Axelsson, P.O. Larsson, Superporous agarose beads as a hydrophobic interaction chromatography support, J. Chroma. A 830 (2) (1999) 275–284.
[25] P.E. Gustavsson, P.O. Larsson, Superporous agarose, a new material for chromatography, J. Chroma. A 734 (2) (1996) 231–240.
[26] N. Deshmukh, A. Lali, Adsorptive purification of pDNA on superporous rigid cross-linked cellulose matrix, J. Chroma. B 818 (1) (2005) 5–10.
[27] P. Tiainen, M.R. Anower, P.O. Larsson, High-capacity composite adsorbents for nucleic acids, J. Chroma. A 1218 (31) (2011) 5235–5240.
[28] T. Kitamura, S. Nakatani, Separating plasmids from contaminants using hydrophobic or hydrophobic and ion exchange chromatography, U.S. Patent No 6 441 (2002) 160.
[29] M.C. Flickinger, Downstream industrial biotechnology: recovery and purification, John Wiley & Sons, 2013.
[30] V. Orr, L. Zhong, M. Moo-Young, C.P. Chou, Recent advances in bioprocessing application of membrane chromatography, Biotech. Adv.31 (4) (2013) 450–465.
[31] E.L. Pfaunmiller, M.L. Paulemond, C.M. Dupper, D.S. Hage, Affinity monolith chromatography: a review of principles and recent analytical applications, Anal. Bioanal. Chem.405 (7) (2013) 2133–2145.
[32] Z. Li, E. Rodriguez, S. Azaria, A. Pekarek, D.S. Hage, Affinity monolith chromatography: A review of general principles and applications, Electrophoresis 38 (22-23) (2017) 2837–2850.
[33] C. Tarmann, A. Jungbauer, Adsorption of plasmid DNA on anion exchange chromatography media, J. Separat. Sci.31 (14) (2008) 2605–2618.
[34] L.R. Pereira, D.M.F. Prazeres, M. Mateus, Hydrophobic interaction membrane chromatography for plasmid DNA purification: Design and optimization, J. Separat. Sci. 33 (9) (2010) 1175–1184.
[35] M. Diogo, J.A. Queiroz, D.M.F. Prazeres, Hydrophobic interaction chromatography of homo-oligonucleotides on derivatized Sepharose CL-6B: Application of the solvophobic theory, J. Chroma. A 944 (1–2) (2002) 119–128.
[36] I. Perçin, E. Sağlar, H. Yavuz, E. Aksöz, A. Denizli, Poly (hydroxyethyl methacrylate) based affinity cryogel for plasmid DNA purification, Int. J. Biol. Macromol. 48 (4) (2011) 577–582.
[37] G. Baydemir, N. Bereli, M. Andaç, R. Say, I.Y. Galaev, A. Denizli, Supermacroporous poly (hydroxyethyl methacrylate) based cryogel with embedded bilirubin imprinted particles, React. Funct. Polym.69 (1) (2009) 36–42.
[38] H. Alkan, N. Bereli, Z. Baysal, A. Denizli, Antibody purification with protein A attached supermacroporous poly (hydroxyethyl methacrylate) cryogel, Biochem. Eng. J.45 (3) (2009) 201–208.
[39] C. Kepka, J. Rhodin, R. Lemmens, F. Tjerneld, P.-E. Gustavsson, Extraction of plasmid DNA from Escherichia coli cell lysate in a thermoseparating aqueous two-phase system, J. Chroma. A 1024 (1–2) (2004) 95–104.
[40] P. Tiainen, P.-E. Gustavsson, A. Ljunglöf, P.-O. Larsson, Superporous agarose anion exchangers for plasmid isolation, J. Chroma. A 1138 (1–2) (2007) 84–94.
[41] M. Teeters, S. Conrardy, B. Thomas, T. Root, E. Lightfoot, Adsorptive membrane chromatography for purification of plasmid DNA, J. Chroma. A 989 (1) (2003) 165–173.
[42] L. Zhong, J. Scharer, M. Moo-Young, D. Fenner, L. Crossley, C.H. Honeyman, S.-Y. Suen, C.P. Chou, Potential application of hydrogel-based strong anion-exchange membrane for plasmid DNA purification, J. Chroma. B 879 (9–10) (2011) 564–572.
[43] F. Smrekar, A. Podgornik, M. Ciringer, S. Kontrec, P. Raspor, A. Štrancar, M. Peterka, Preparation of pharmaceutical-grade plasmid DNA using methacrylate monolithic columns, Vaccine 28 (8) (2010) 2039–2045.
[44] D. Çimen, F. Yılmaz, I. Perçin, D. Türkmen, A. Denizli, Dye affinity cryogels for plasmid DNA purification, Mater. Sci. Eng. C 56 (2015) 318–324.
[45] Y. Han, G.M. Forde, Single step purification of plasmid DNA using peptide ligand affinity chromatography, J. Chroma. B 874 (1–2) (2008) 21–26.
[46] M. Zhao, M. Vandersluis, J. Stout, U. Haupts, M. Sanders, R. Jacquemart, Affinity chromatography for vaccines manufacturing: Finally ready for prime time? Vaccine 37 (36) (2019) 5491–5503.
[47] J. Woodgate, D. Palfrey, D.A. Nagel, A.V. Hine, N.K. Slater, Protein-mediated isolation of plasmid DNA by a zinc finger-glutathione S-transferase affinity linker, Biotech. Bioeng.79 (4) (2002) 450–456.
[48] R.A. Darby, A.V. Hine, LacI-mediated sequence-specific affinity purification of plasmid DNA for therapeutic applications, FASEB J.19 (7) (2005) 801–803.
[49] P. Wils, V. Escriou, A. Warnery, F. Lacroix, D. Lagneaux, M. Ollivier, J. Crouzet, J. Mayaux, D. Scherman, Efficient purification of plasmid DNA for gene transfer using triple-helix affinity chromatography, Gene Therapy 4 (4) (1997) 323.

[50] C. Caramelo-Nunes, P. Almeida, J. Marcos, C. Tomaz, Aromatic ligands for plasmid deoxyribonucleic acid chromatographic analysis and purification: An overview, J. Chroma. A 1327 (2014) 1–13.
[51] F. Sousa, S. Freitas, A.R. Azzoni, D.M.F. Prazeres, J.A. Queiroz, Selective purification of supercoiled plasmid DNA from clarified cell lysates with a single histidine–agarose chromatography step, Biotech. Appl. Biochem.45 (3) (2006) 131–140.
[52] F. Sousa, D.M.F. Prazeres, J.A. Queiroz, Improvement of transfection efficiency by using supercoiled plasmid DNA purified with arginine affinity chromatography, J. Gene Med. 11 (1) (2009) 79–88.
[53] F. Sousa, D.M.F. Prazeres, J.A Queiroz, Dynamic binding capacity of plasmid DNA in histidine–agarose chromatography, Biomed. Chroma.21 (9) (2007) 993–998.
[54] A. Soares, J.A. Queiroz, F. Sousa, A. Sousa, Purification of human papillomavirus 16 E6/E7 plasmid deoxyribonucleic acid-based vaccine using an arginine modified monolithic support, J. Chroma. A 1320 (2013) 72–79.
[55] U. Černigoj, U. Vidic, M. Barut, A. Podgornik, M. Peterka, A. Štrancar, A multimodal histamine ligand for chromatographic purification of plasmid DNA, J. Chroma. A 1281 (2013) 87–93.
[56] A. Almeida, J.A. Queiroz, F. Sousa, A. Sousa, Optimization of supercoiled HPV-16 E6/E7 plasmid DNA purification with arginine monolith using design of experiments, J. Chroma. B 978 (2015) 145–150.
[57] L.F. Amorim, F. Sousa, J.A. Queiroz, C. Cruz, Â. Sousa, Screening of l-histidine-based ligands to modify monolithic supports and selectively purify the supercoiled plasmid DNA isoform, J. Mol. Recogn.28 (6) (2015) 349–358.
[58] A.R. Silva-Santos, C.P. Alves, D.M.F. Prazeres, A.M. Azevedo, A process for supercoiled plasmid DNA purification based on multimodal chromatography, Separ. Purif. Tech.182 (2017) 94–100.
[59] A.R. Silva-Santos, C.P. Alves, G. Monteiro, A.M. Azevedo, D.M.F. Prazeres, Multimodal chromatography of supercoiled minicircles: A closer look into DNA-ligand interactions, Separat. Purif. Tech. 212 (2019) 161–170.
[60] T. Matos, J.A. Queiroz, L. Bülow, Binding and elution behavior of small deoxyribonucleic acid fragments on a strong anion-exchanger multimodal chromatography resin, J. Chroma. A 1302 (2013) 40–44.
[61] A. Abdulrahman, A. Ghanem, Recent advances in chromatographic purification of plasmid DNA for gene therapy and DNA vaccines: a review, Anal. Chim. Acta1025 (2018) 41–57.
[62] A.R. Silva-Santos, C.P. Alves, D.M.F. Prazeres, A.M. Azevedo, Separation of plasmid DNA topoisomers by multimodal chromatography, Anal. Biochem.503 (2016) 68–70.
[63] N. Labrou, Design and selection of ligands for affinity chromatography, J. Chroma. B 790 (1–2) (2003) 67–78.
[64] A.C.A. Roque, C.R. Lowe, Advances and applications of de novo designed affinity ligands in proteomics, Biotech. Adv. 24 (1) (2006) 17–26.
[65] A. Pina, C. Lowe, A. Roque, Comparison of fluorescence labelling techniques for the selection of affinity ligands from solid-phase combinatorial libraries, Separat. Sci. Tech.45 (15) (2010) 2187–2193.
[66] B. Tang, F. Shen, D. Wan, B.H. Guo, Y.J. Wang, Q.Y. Yi, Y.J. Liu, DNA-binding, molecular docking studies and biological activity studies of ruthenium (II) polypyridyl complexes, RSC Adv.7 (56) (2017) 34945–34958.
[67] N. Shahabadi, M. Falsafi, Experimental and molecular docking studies on DNA binding interaction of adefovir dipivoxil: Advances toward treatment of hepatitis B virus infections, Spectrochim. Acta Part A Mol. Biomol. Spectros.125 (2014) 154–159.
[68] C.G. Ricci, P.A. Netz, Docking studies on DNA-ligand interactions: building and application of a protocol to identify the binding mode, J. Chem. Inform. Model.49 (8) (2009) 1925–1935.
[69] F. Jalali, P.S. Dorraji, Interaction of anthelmintic drug (thiabendazole) with DNA: Spectroscopic and molecular modeling studies, Arab. J. Chem.10 (2017) S3947–S3954.
[70] Y. Li, X.Y. Dong, Y. Sun, High-speed chromatographic purification of plasmid DNA with a customized biporous hydrophobic adsorbent, Biochem. Eng. J.27 (1) (2005) 33–39.
[71] A.G. Gomes, A.M. Azevedo, M.R. Aires-Barros, D.M.F. Prazeres, Studies on the adsorption of cell impurities from plasmid-containing lysates to phenyl boronic acid chromatographic beads, J. Chroma. A 1218 (48) (2011) 8629–8637.
[72] N. Bonturi, V.S.C.O. Radke, S.M.A. Bueno, S. Freitas, A.R. Azzoni, E.A. Miranda, Sodium citrate and potassium phosphate as alternative adsorption buffers in hydrophobic and aromatic thiophilic chromatographic purification of plasmid DNA from neutralized lysate, J. Chroma. B 919 (2013) 67–74.
[73] C. Caramelo-Nunes, P. Almeida, J. Marcos, C. Tomaz, Specific recognition of supercoiled plasmid DNA by affinity chromatography using the intercalator DAPP as ligand, J. Chroma. B 928 (2013) 121–124.
[74] C. Caramelo-Nunes, D. Bicho, P. Almeida, J. Marcos, C. Tomaz, Dynamic binding capacity and specificity of 3, 8-diamino-6-phenylphenanthridine-Sepharose support for purification of supercoiled plasmid deoxyribonucleic acid, J. Chroma. A 1307 (2013) 91–98.
[75] C. Caramelo-Nunes, M. Gabriel, P. Almeida, J. Marcos, C. Tomaz, Negative pseudo-affinity chromatography for plasmid DNA purification using berenil as ligand, J. Chroma. B 944 (2014) 39–42.
[76] G. Azevedo, J.F.A. Valente, A. Sousa, A. Pedro, P. Pereira, F. Sousa, J.A. Queiroz, Effect of chromatographic conditions on supercoiled plasmid DNA stability and bioactivity, Appl. Sci.9 (23) (2019) 5170.
[77] J.F.A. Valente, A. Sousa, G. Azevedo, J.A. Queiroz, F. Sousa, Purification of supercoiled p53-encoding plasmid using an arginine-modified macroporous support, J. Chroma. A (2020) 460890.
[78] A. Sousa, F. Sousa, J.A. Queiroz, Impact of lysine-affinity chromatography on supercoiled plasmid DNA purification, J. Chroma. B 879 (30) (2011) 3507–3515.
[79] J.F.A. Valente, A. Sousa, J.A. Queiroz, F. Sousa, DoE to improve supercoiled p53-pDNA purification by O-phospho-l-tyrosine chromatography, J. Chroma. B 1105 (2019) 184–192.
[80] T. Schluep, C.L. Cooney, Purification of plasmids by triplex affinity interaction, Nucleic Acids Res.26 (19) (1998) 4524–4528.
[81] A. Sousa, C. Tomaz, F. Sousa, J.A. Queiroz, Successful application of monolithic innovative technology using a carbonyldiimidazole disk to purify supercoiled plasmid DNA suitable for pharmaceutical applications, J. Chroma. A 1218 (46) (2011) 8333–8343.
[82] D. Bicho, Â. Sousa, F. Sousa, J.A. Queiroz, C. Tomaz, Effect of chromatographic conditions and plasmid DNA size on the dynamic binding capacity of a monolithic support, J. Separat. Sci. 37 (17) (2014) 2284–2292.
[83] A.M. Almeida, D. Costa, A.R. Simões, J.A. Queiroz, F. Sousa, Â. Sousa, Enhancement of a biotechnological platform for the purification and delivery of a human papillomavirus supercoiled plasmid DNA vaccine, New Biotech.59 (2020) 1–9.
[84] C.J. Fee, S. Dimartino, T. Huber, Separation Medium, U.S. Patent Appl.No 468 (2018) 16/063.
[85] M.T. Hearn, Trends in additive manufacturing of chromatographic and membrane materials, Curr. Opin. Chem. Eng.18 (2017) 90–98.